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**Factor VIII complex with XTEN and von Willebrand Factor protein, and uses thereof**

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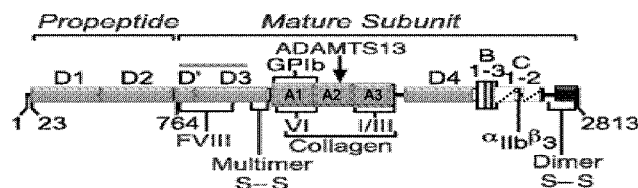
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[Continued on next page]

(54) Title: FACTOR VIII COMPLEX WITH XTEN AND VON WILLEBRAND FACTOR PROTEIN, AND USES THEREOF

Figure 1D. Von Willebrand Factor



➤ ~ 250 kDa protein, forms multimers (> 20 MDa) by disulfide bonding

➤ Associates with FVIII (95-98%) in non-covalent complex

- Protects FVIII from protease cleavage/activation
- Stabilizes heavy & light chain
- Prevents clearance of FVIII by scavenger receptors

Extends half-life

- Clearance of FVIII-vWF complex through vWF-receptors
- Prevents pinocytosis and recycling of rFVIII-Fc?

Limits half-life

(57) Abstract: The present invention includes a chimeric protein comprising a VWF protein with D' domain and D3 domain of VWF, one or more XTEN sequence, and a FVIII protein, wherein the VWF fragment, the XTEN sequence, or the FVIII protein are linked to or associated with each other. The chimeric protein can further comprise one or more Ig constant region or a portion thereof (e.g., an Fc region). A polypeptide chain of a VWF fragment is associated with a FVIII polypeptide chain linked to an XTEN sequence. The VWF fragment polypeptide chain can prevent or inhibit binding of endogenous VWF to FVIII protein linked to the XTEN sequence. By preventing or inhibiting binding of endogenous VWF to FVIII protein, VWF fragment can extend half-life of chimeric protein comprising FVIII protein. The invention includes nucleotides, vectors, host cells, use of VWF fragment, or chimeric proteins.



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FACTOR VIII COMPLEX WITH XTEN AND VON WILLEBRAND FACTOR  
PROTEIN, AND USES THEREOF

## BACKGROUND OF THE INVENTION

[0001] Haemophilia A is a bleeding disorder caused by defects in the gene encoding coagulation factor VIII (FVIII) and affects 1-2 in 10,000 male births. Graw *et al.*, *Nat. Rev. Genet.* 6(6): 488-501 (2005). Patients affected with hemophilia A can be treated with infusion of purified or recombinantly produced FVIII. All commercially available FVIII products, however, are known to have a half-life of about 8-12 hours, requiring frequent intravenous administration to the patients. *See* Weiner M.A. and Cairo, M.S., *Pediatric Hematology Secrets*, Lee, M.T., 12. Disorders of Coagulation, Elsevier Health Sciences, 2001; Lillicrap, D. *Thromb. Res.* 122 Suppl 4:S2-8 (2008). In addition, a number of approaches have been tried in order to extend the FVIII half-life. For example, the approaches in development to extend the half-life of clotting factors include pegylation, glycopegylation, and conjugation with albumin. *See* Dumont *et al.*, *Blood*. 119(13): 3024-3030 (Published online Jan. 13, 2012). Regardless of the protein engineering used, however, the long acting FVIII products currently under development are reported to have limited half-lives – only to about 1.5 to 2 hours in preclinical animal models. *See id.* Consistent results have been demonstrated in humans, for example, rFVIII<sup>Fc</sup> was reported to improve half-life up to ~ 1.7 fold compared with ADVATE® in hemophilia A patients. *See Id.* Therefore, the half-life increases, despite minor improvements, may indicate the presence of other T<sub>1/2</sub> limiting factors. *See* Liu, T. *et al.*, 2007 ISTH meeting, abstract #P-M-035; Henrik, A. *et al.*, 2011 ISTH meeting, abstract #P=MO-181; Liu, T. *et al.*, 2011 ISTH meeting abstract #P-WE-131.

[0002] Plasma von Willebrand Factor (VWF) has a half-life of approximately 12 hours (ranging from 9 to 15 hours). [http://www.nhlbi.nih.gov/guidelines/vwd/2\\_scientificoverview.htm](http://www.nhlbi.nih.gov/guidelines/vwd/2_scientificoverview.htm) (last visited October 22, 2011). The VWF half-life may be affected by a number of factors: glycosylation pattern, ADAMTS-13 (a disintegrin and metalloprotease with thrombospondin motif-13), and various mutations in VWF.

[0003] In plasma, 95-98% of FVIII circulates in a tight non-covalent complex with full-length VWF. The formation of this complex is important for the



maintenance of appropriate plasma levels of FVIII in vivo. Lenting *et al.*, *Blood*. 92(11): 3983-96 (1998); Lenting *et al.*, *J. Thromb. Haemost.* 5(7): 1353-60 (2007). The full-length wild-type FVIII is mostly present as a heterodimer having a heavy chain (MW 200kD) and a light chain (MW 73kD). When FVIII is activated due to proteolysis at positions 372 and 740 in the heavy chain and at position 1689 in the light chain, the VWF bound to FVIII is removed from the activated FVIII. The activated FVIII, together with activated factor IX, calcium, and phospholipid ("tenase complex"), induces the activation of factor X, generating large amounts of thrombin. Thrombin, in turn, then cleaves fibrinogen to form soluble fibrin monomers, which then spontaneously polymerize to form the soluble fibrin polymer. Thrombin also activates factor XIII, which, together with calcium, serves to crosslink and stabilize the soluble fibrin polymer, forming crosslinked (insoluble) fibrin. The activated FVIII is cleared fast from the circulation by proteolysis.

[0004] Due to the frequent dosing and inconvenience caused by the dosing schedule, there is still a need to develop FVIII products requiring less frequent administration, *i.e.*, a FVIII product that has a half-life longer than the 1.5 to 2 fold half-life limitation.

#### BRIEF SUMMARY OF THE INVENTION

[0005] The present invention is directed to a chimeric protein comprising (i) a von Willebrand Factor (VWF) fragment comprising the D' domain and the D3 domain of VWF, (ii) an XTEN sequence, and (iii) a FVIII protein, wherein the VWF fragment and the XTEN sequence are linked by an optional linker and wherein the VWF fragment or the XTEN sequence is linked to or associated with the FVIII protein. The chimeric protein can comprise a single polypeptide chain comprising the VWF fragment, the XTEN sequence, and the FVIII protein, or two polypeptide chains, a first chain comprising the VWF fragment and the second chain comprising the FVIII protein, wherein the XTEN polypeptide is linked either to the VWF fragment or the FVIII protein.

[0006] In one embodiment, the chimeric protein of the invention comprises a formula comprising:

(a) V-X-FVIII,

- (b) FVIII-X-V,
- (c) V-X:FVIII,
- (d) X-V:FVIII,
- (e) FVIII:V-X, or
- (f) FVIII:X-V,

wherein V comprises a VWF fragment,

X comprises one or more XTEN sequences, and

FVIII comprises a FVIII protein. The hyphen (-) can be a peptide bond or a linker, *e.g.*, a cleavable linker, while the colon (:) represents a chemical association or a physical association between the polypeptides, for example a covalent or non-covalent bond.

**[0007]** In another embodiment, the chimeric protein further comprises (iv) an immunoglobulin (Ig) constant region or a portion thereof (also indicated as F1 or a first Ig constant region or a portion thereof) linked to the VWF fragment, the XTEN sequence, the FVIII protein, or any combinations thereof. In other embodiments, the chimeric protein further comprises an additional Ig constant region or a portion thereof (also indicated as F2 or a second Ig constant region or a portion thereof). The first Ig constant region or a portion thereof can be linked to the VWF fragment or the XTEN sequence, and the second Ig constant region can be linked to the FVIII protein. The first Ig constant region, the second Ig constant region or a portion thereof, or both can extend the half-life of the FVIII protein.

**[0008]** In some embodiments, the second Ig constant region or a portion thereof (F2) is linked to the VWF fragment by a linker, *e.g.*, a processable linker. In other embodiments, the second Ig constant region or a portion thereof (F2) is associated with the (first) Ig constant region or a portion thereof (F1). The second Ig constant region or a portion thereof (F2) and the first Ig constant region or a portion thereof (F1) can be identical or different. The second Ig constant region or a portion thereof can be associated with the Ig constant region or a portion thereof by a covalent bond, *e.g.*, a disulfide bond. The VWF fragment linked to the first Ig constant region or a portion thereof may also be associated with the FVIII protein linked to the second Fc region by a non-covalent bond. In certain embodiments, the FVIII protein can further comprise one or more additional XTEN sequences which are linked to the C-terminus or N-terminus of the FVIII

protein or inserted immediately downstream of one or more amino acids in the FVIII protein (*e.g.*, one or more XTEN insertion sites). In some embodiments, the half-life of the FVIII protein is extended, compared to wild type FVIII or a FVIII protein without the VWF fragment.

**[0009]** In some embodiments, the chimeric protein comprises a formula comprising:

- (g) V-L2-X-L1-F1:FVIII-L3-F2;
- (h) V-L2-X-L1-F1:F2-L3-FVIII;
- (i) F1-L1-X-L2-V:FVIII-L3-F2;
- (j) F1-L1-X-L2-V:F2-L3-FVIII;
- (k) V-L2-X-L1-F1-L4-FVIII-L3-F2;
- (l) F2-L3-FVIII-L4-F1-L1-X-L2-V;
- (m) FVIII-L3-F2-L4-V-L2-X-L1-F1; or
- (n) F1-L1-X-L2-V-L4-F2-L3-FVIII,

wherein V comprises a VWF fragment,  
 each of L1, L2, and L3 comprises an optional linker, *e.g.*, a cleavable linker,  
 L4 is an optional linker, *e.g.*, a processable linker,  
 FVIII comprises a FVIII protein,  
 X comprises one or more XTEN sequences,  
 F1 comprises an optional first Ig constant region or a portion thereof,  
 F2 comprises an optional second Ig constant region or a portion thereof, and  
 (:) is a covalent bond or non-covalent bond.

**[0010]** The present invention is also directed to a chimeric protein comprising (i) a FVIII protein, (ii) an XTEN sequence, and (iii) an Ig constant region or a portion thereof, wherein the XTEN sequence is linked to the FVIII protein by an optional linker at the N-terminus or C terminus of the FVIII protein or inserted immediately downstream of one or more amino acids in the FVIII protein (*e.g.*, one or more insertion sites) and wherein the Ig constant region or a portion thereof is linked to or associated with the FVIII protein or the XTEN sequence. In one embodiment, the Ig constant region or a portion thereof useful for the chimeric protein comprises a first Fc region. In another embodiment, the chimeric protein further comprises an additional Ig constant region or a portion thereof. The additional Ig constant region or a portion thereof useful for the invention can

comprise a second Fc region, which is linked to or associated with the first Fc region, *e.g.*, by a covalent bond. In other embodiments, the first Fc region is linked to the second Fc region by a linker, *e.g.*, a processable linker.

**[0011]** In other aspects, a chimeric protein comprises (i) a FVIII protein, (ii) an XTEN sequence, (iii) a VWF fragment, and (iv) an Ig constant region or a portion thereof, which comprises the D' domain and the D3 domain of VWF, wherein the XTEN sequence is linked to the FVIII protein by an optional linker at the N-terminus or C terminus of the FVIII protein or inserted immediately downstream of one or more amino acids in the FVIII protein (*e.g.*, one or more insertion sites), the VWF fragment is linked to or associated with the FVIII protein or the XTEN sequence, and the Ig constant region or a portion thereof is linked to the FVIII protein, the XTEN sequence, the VWF fragment, or any combinations thereof. Non-limiting examples of the chimeric proteins may comprise a formula, which comprises:

- (1) FVIII(X1)-L1-F1:V-L2-X2-L3-F2;
- (2) FVIII(X1)-L1-F1:F2-L3-X2-L2-V;
- (3) F1-L1-FVIII(X1):V-L2-X2-L3-F2;
- (4) F1-L1-FVIII(X1):F2-L3-X2-L2-V;
- (5) FVIII(X1)-L1-F1-L4-V-L2-X2-L3-F2;
- (6) FVIII(X1)-L1-F1-L4-F2-L3-X2-L2-V;
- (7) F1-L1-FVIII(X1)-L4-V-L2-X2-L3-F2, or
- (8) F1-L1-FVIII(X1)-L4-F2-L3-X2-L2-V,

wherein FVIII(X1) comprises a FVIII protein and one or more XTEN sequences, wherein one or more of the XTEN sequences are linked to the N-terminus or C-terminus of the FVIII protein or inserted immediately downstream of one or more amino acids in the FVIII protein (*e.g.*, one or more XTEN insertion sites); each of L1, L2, or L3 comprises an optional linker, *e.g.*, a cleavable linker; L4 is a linker, a processable linker;

X2 comprises one or more XTEN sequences;

F1 comprises an Ig constant region or a portion thereof;

F2 comprises an optional additional Ig constant region or a portion thereof, and

V comprises a VWF fragment;

(-) is a peptide bond or one or more amino acids; and

(:) comprises a covalent bond or a non-covalent bond.

**[0012]** One aspect of the invention is that the VWF fragment useful for the chimeric protein does not bind to a VWF clearance receptor, which prevents or inhibits interaction of the FVIII protein with endogenous VWF. The chimeric protein comprising the VWF fragment thus has reduced clearance or is not cleared through a VWF clearance pathway. Another aspect of the invention is that the VWF fragment is capable of protecting the FVIII protein from one or more protease cleavages, protecting the FVIII protein from activation, stabilizing the heavy chain and/or the light chain of the FVIII protein, or preventing clearance of the FVIII protein by one or more scavenger receptors.

**[0013]** Because of the VWF fragment's ability to prevent or inhibit interaction between the FVIII protein and endogenous VWF, the half-life of the FVIII protein, is extended compared to a FVIII protein without the VWF fragment. In one embodiment, the half-life of the FVIII protein is extended at least about 1.5 times, at least about 2 times, at least about 2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than wild type FVIII. In another embodiment, the half-life of the FVIII protein is at least about 10 hours, at least about 11 hours, at least about 12 hours, at least about 13 hours, at least about 14 hours, at least about 15 hours, at least about 16 hours, at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours.

**[0014]** The Ig constant region or a portion thereof useful for the chimeric protein comprises a first Fc region, which is linked to the VWF fragment by an optional linker, *e.g.*, a cleavable linker. The chimeric protein can further comprise an additional Ig constant region or a portion thereof, which is linked to the FVIII protein or the XTEN sequence, the Ig constant region or a portion thereof, the VWF fragment, or any combinations thereof by an optional linker. In one embodiment, the additional Ig constant region or a portion thereof is linked to the

FVIII protein by an optional linker. The additional Ig constant region or a portion thereof can comprise a second Fc region.

**[0015]** The Ig constant region or a portion thereof useful in the present invention and the additional Ig constant region or a portion thereof useful in the present invention are identical or different.

**[0016]** In some aspects, the FVIII protein is linked to an XTEN sequence at the C-terminus or the N-terminus of the FVIII protein or inserted immediately downstream of one or more amino acids in mature native human FVIII (*e.g.*, one or more insertion sites) or any combinations thereof. One or more insertion sites in the FVIII protein can be located within one or more domains of the FVIII protein selected from the group consisting of the A1 domain, the a1 acidic region, the A2 domain, the a2 acidic region, the A3 domain, the B domain, the C1 domain, the C2 domain, and any combinations thereof or between one or more domains of the FVIII protein selected from the group consisting of the A1 domain and a1 acidic region, the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, the A3 domain and C1 domain, the C1 domain and C2 domain, and any combinations thereof or between two domains of the FVIII protein selected from the group consisting of the A1 domain and a1 acidic region, the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, the A3 domain and C1 domain, the C1 domain and C2 domain, and any combinations thereof.

**[0017]** In one embodiment, the one or more insertion sites are located immediately downstream of one or more amino acids in mature native human FVIII (*e.g.*, SEQ ID NO: 4 [mature FVIII sequence-full length]) selected from the group consisting of the amino acid residues in Table 7, 8, 9, 10, 11, or any combinations thereof.

**[0018]** In another embodiment, the one or more insertion sites are located in one or more permissive loops of mature native human FVIII. In other embodiments, the one or more insertion sites are located in the a3 region of mature native human FVIII. For example, an XTEN sequence can be inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 4 (full length mature FVIII). In other embodiments, a FVIII protein is linked to at least two XTEN sequences, a

first XTEN sequence inserted within the a3 region, and a second XTEN sequence inserted within a permissive loop in the FVIII protein (*e.g.*, A1-1, A1-2, A2-1, A2-2, A3-1, or A3-2). In still other embodiments, a FVIII protein is linked to at least three XTEN sequences, a first XTEN sequence inserted within the a3 region and a second XTEN sequence and a third XTEN sequence inserted within one or two permissive loop in the FVIII protein (*e.g.*, A1-1, A1-2, A2-1, A2-2, A3-1, or A3-2).

**[0019]** In certain embodiments, the one or more insertion sites for one or more XTEN insertions are immediately downstream of one or more amino acids (numbered relative to mature FVIII sequence) selected from the group consisting of:

- |                       |                       |                      |
|-----------------------|-----------------------|----------------------|
| (1) amino acid 3,     | (2) amino acid 18,    | (3) amino acid 22,   |
| (4) amino acid 26,    | (5) amino acid 32,    | (6) amino acid 40,   |
| (7) amino acid 60,    | (8) amino acid 65,    | (9) amino acid 81,   |
| (10) amino acid 116,  | (11) amino acid 119,  | (12) amino acid 130, |
| (13) amino acid 188,  | (14) amino acid 211,  | (15) amino acid 216, |
| (16) amino acid 220,  | (17) amino acid 224,  | (18) amino acid 230, |
| (19) amino acid 333,  | (20) amino acid 336,  | (21) amino acid 339, |
| (22) amino acid 375,  | (23) amino acid 399,  | (24) amino acid 403, |
| (25) amino acid 409,  | (26) amino acid 416,  | (26) amino acid 442, |
| (28) amino acid 487,  | (29) amino acid 490,  | (30) amino acid 494, |
| (31) amino acid 500,  | (32) amino acid 518,  | (33) amino acid 599, |
| (34) amino acid 603,  | (35) amino acid 713,  | (36) amino acid 745, |
| (37) amino acid 1656, | (38) amino acid 1711, | (39)                 |
| amino acid 1720,      |                       |                      |
| (40) amino acid 1725, | (41) amino acid 1749, | (42)                 |
| amino acid 1796,      |                       |                      |
| (43) amino acid 1802, | (44) amino acid 1827, | (45)                 |
| amino acid 1861,      |                       |                      |
| (46) amino acid 1896, | (47) amino acid 1900, | (48)                 |
| amino acid 1904,      |                       |                      |
| (49) amino acid 1905, | (50) amino acid 1910, | (51)                 |
| amino acid 1937,      |                       |                      |

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 amino acid 2111,  
 (55) amino acid 2120, (56) amino acid 2171, (57)  
 amino acid 2188,  
 (58) amino acid 2227, (59) amino acid 2277, and  
 (60) two or more combinations thereof.

**[0020]** In some embodiments, one XTEN is inserted in the FVIII protein. In some embodiments, two XTENs are inserted in the FVIII protein. In some embodiments, 3 XTENs are inserted in the FVIII protein.

**[0021]** In a particular example, a first XTEN is inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 4, and a second XTEN is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 4 (full-length mature FVIII). In another example, a first XTEN is inserted immediately downstream of amino acid 403 corresponding to SEQ ID NO: 4, and a second XTEN is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 4. In some examples, a first XTEN is inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 4, and a second XTEN is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 4. In other examples, a first XTEN is inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 4, a second XTEN is inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 4, and a third XTEN is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 4. In yet other embodiments, a first XTEN is inserted immediately downstream of amino acid 403 corresponding to SEQ ID NO: 4, a second XTEN is inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 4, and a third XTEN is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 4. In still other embodiments, a first XTEN is inserted between amino acids 403 and 404 corresponding to SEQ ID NO: 4, a second XTEN is inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 4, and a third XTEN is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 4. In certain embodiments, a first XTEN is inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 4 (full-



length mature FVIII), a second XTEN is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 4, and a third XTEN is inserted immediately downstream of amino acid 1900 corresponding to SEQ ID NO: 4. In some embodiments, a first XTEN is inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 4, a second XTEN is inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 2, a third XTEN is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 4, and a fourth XTEN is inserted immediately downstream of amino acid 1900 corresponding to SEQ ID NO: 4. In another example, an XTEN is inserted immediately downstream of amino acid 745 corresponding to SEQ ID NO: 4. In an additional example, a first XTEN is inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 4 and a second XTEN is inserted immediately downstream of amino acid 1900 corresponding to SEQ ID NO: 4. In some embodiments, a first XTEN is inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 4, a second XTEN is inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 4, and a third XTEN is inserted immediately downstream of amino acid 1900 corresponding to SEQ ID NO: 4. In another example, a first XTEN is immediately inserted downstream of amino acid 403 corresponding to SEQ ID NO: 4 and a second XTEN is inserted immediately downstream of amino acid 745 corresponding to SEQ ID NO: 4. In some embodiments, a first XTEN is inserted immediately downstream of amino acid 745 of corresponding to SEQ ID NO: 4, and a second XTEN is inserted immediately downstream of amino acid 1900 corresponding to SEQ ID NO: 4. In some embodiments, a first XTEN is inserted immediately downstream of amino acid 18 corresponding to SEQ ID NO: 4, and a second XTEN is inserted immediately downstream of amino acid 745 corresponding to SEQ ID NO: 4.

**[0022]** In some embodiments, the FVIII protein is a dual chain FVIII isoform. In some embodiments, the FVIII protein is a single chain FVIII isoform.

**[0023]** In some embodiments, the XTEN that is inserted is SEQ ID NO: 39 (AE288). In some examples, the XTENS that are inserted are SEQ ID NOs: 38 and 37 (AG144 and AE144). In some examples, the XTENS that are inserted are SEQ ID NOs: 37, 38 and 37 (AE144, AG144, and AE144). In some

embodiments. the XTENs that are inserted are SEQ ID NOs: 37 and 40 (AE144 and AE288). In some embodiments, the XTENs that are inserted are AE42 (SEQ ID NO: 36), AE72 (SEQ ID NO: 127), AE144\_2A (SEQ IDNO: 128), AE144\_3B (SEQ ID NO: 129), AE144\_4A (SEQ ID NO: 130), AE144\_5A (SEQ IDNO: 131), AE144\_6B (SEQ IDNO: 132), AG144\_A (SEQ ID NO: 133), AG144\_B (SEQ IDNO: 134), AG144\_C (SEQ ID NO: 135), AG144\_F (SEQ IDNO: 136), AE864 (SEQ ID NO: 43), AE576 (SEQ ID NO: 41), AE288 (SEQ IDNO: 39), AE288\_2 (SEQ ID NO: 137), AE144 (SEQ ID NO: 37), AG864 (SEQ ID NO: 44), AG576 (SEQ ID NO: 42), AG288 (SEQ ID NO: 40), AG144 (SEQ ID NO: 38), and any combinations thereof.

**[0024]** The FVIII protein useful in the invention can comprise B domain or a portion thereof, *e.g.*, SQ B domain deleted FVIII. In one embodiment, the FVIII protein comprises single chain FVIII. In another embodiment, the single chain FVIII contains at least one amino acid substitution at a residue corresponding to residue 1648, residue 1645, or both of full-length mature Factor VIII polypeptide (SEQ ID NO: 4) or residue 754, residue 751, or both of SQ BDD Factor VIII (SEQ ID NO: 6). In other embodiments, the amino acid substitution is an amino acid other than arginine. In some embodiments, the FVIII protein comprises a heavy chain of FVIII and a light chain of FVIII, wherein the heavy chain and the light chain are associated with each other by a metal bond.

**[0025]** The FVIII protein can have a low affinity to or does not bind to a low-density lipoprotein receptor-related protein (LRP), *e.g.*, by containing at least one amino acid substitution that lowers the affinity to or eliminates the binding to the LRP. Such at least one amino acid substitution can be at a residue corresponding to residue 471, residue 484, residue 487, residue 490, residue 497, residue 2092, residue 2093 or two or more combinations thereof of full-length mature FVIII. In a particular embodiment, the amino acid substitution at residue 471, 484, or 497 is an amino acid other than arginine, the amino acid substitution at residue 487 is an amino acid other than tyrosine, the amino acid substitution at residue 2092 is an amino acid other than lysine, or the amino acid substitution at residue 2093 is an amino acid other than phenylalanine.

**[0026]** In some embodiments, the FVIII protein contains at least one amino acid substitution, which induces the FVIII protein to be more stable than a FVIII

protein without the substitution. Such substitutions can be located in the A2 domain and the A3 domain of the FVIII protein, *e.g.*, at a residue corresponding to residue 664, residue 1826, residue 662, residue 1828, or two or more combinations thereof of full-length mature FVIII.

**[0027]** The VWF fragment useful for the present invention comprises a D' domain and D3 domain, which together are capable of binding to FVIII. The VWF fragment can comprise the amino acid sequence of the D' domain is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 866 of SEQ ID NO: 2 and/or the amino acid sequence of the D3 domain is at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 867 to 1240 of SEQ ID NO: 2. In one embodiment, the VWF fragment is a monomer. In another embodiment, the VWF fragment comprises at least two VWF fragments, at least three VWF fragments, at least four VWF fragments, at least five VWF fragments, or at least six VWF fragments. In one embodiment, the two or more VWF fragments may be identical or they may be different. The VWF fragment can comprise an amino acid at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 1240 of SEQ ID NO: 2. The VWF fragment may consist essentially of or consist of amino acids 764 to 1240 of SEQ ID NO: 2. In certain embodiments, the VWF fragment can contain at least one amino acid substitution at a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 of SEQ ID NO: 2. In other embodiments, the VWF fragment further comprises the D1 domain, the D2 domain, or the D1 and D2 domains of VWF.

**[0028]** The VWF fragment may further comprise a VWF domain selected from the group consisting of the A1 domain, the A2 domain, the A3 domain, the D4 domain, the B1 domain, the B2 domain, the B3 domain, the C1 domain, the C2 domain, the CK domain, one or more fragments thereof, and any combinations thereof. For example, the VWF fragment can consist essentially of or consist of: (1) the D' and D3 domains of VWF or fragments thereof; (2) the D1, D', and D3 domains of VWF or fragments thereof; (3) the D2, D', and D3 domains of VWF or fragments thereof; (4) the D1, D2, D', and D3 domains of VWF or fragments thereof; or (5) the D1, D2, D', D3, and A1 domains of VWF or fragments thereof.

In some embodiments, the VWF fragment further comprises a signal peptide of VWF or FVIII which is operably linked to the VWF fragment.

**[0029]** One or more of the linkers useful in the invention have a length of at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1200, 1400, 1600, 1800, or 2000 amino acids. In some embodiments, one or more of the linkers have a length of about 1 to about 2000 amino acids. In one embodiment, one or more of the linkers have a length of at least about 20, 35, 42, 48, 73, 75, 95, 98, 144, 288, 324, 333, 576, or 864 amino acids. In another embodiment, one or more of the linkers comprise a gly/ser peptide, an XTEN sequence, or both. Examples of the gly/ser peptide include, but are not limited to, a formula of (Gly<sub>4</sub>Ser)<sub>n</sub> (SEQ ID NO: 139) or S(Gly<sub>4</sub>Ser)<sub>n</sub> (SEQ ID NO: 140), wherein n is a positive integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. For example, the (Gly<sub>4</sub>Ser)<sub>n</sub> linker can be (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO: 63) or (Gly<sub>4</sub>Ser)<sub>4</sub> (SEQ ID NO: 138). In one embodiment, the linker comprises at least one first cleavage site at the N-terminus of the linker, at least one second cleavage site at the C-terminus of the linker, or both. In another embodiment, the linker comprises 20 amino acids, 35 amino acids, 48 amino acids, 73 amino acids, or 95 amino acids thrombin cleavable linker. The cleavable linkers can comprise one or more of the cleavage sites by a protease selected from the group consisting of factor XIa, factor XIIa, kallikrein, factor VIIa, factor IXa, factor Xa, factor IIa (thrombin), Elastase-2, Granzyme-B, TEV, Enterokinase, Protease 3C, Sortase A, MMP-12, MMP-13, MMP-17, and MMP-20, *e.g.*, TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 8). Non-limiting examples of one or more of the cleavage sites comprise an amino acid sequence selected from the group consisting of RRRR (SEQ ID NO: 9), RKRRKR (SEQ ID NO: 10), RRRRS (SEQ ID NO: 11), TQSFNDFTR (SEQ ID NO: 12), SVSQTSKLTR (SEQ ID NO: 13), DFLAEGGGVR (SEQ ID NO: 14), TTKIKPR (SEQ ID NO: 15), LVPRG (SEQ ID NO: 16), ALRPR (SEQ ID NO: 17), KLTRAET (SEQ ID NO: 18), DFTRVVG (SEQ ID NO: 19), TMTRIVGG (SEQ ID NO: 20), SPFRSTGG (SEQ ID NO: 21), LQVRIVGG (SEQ ID NO: 22), PLGRIVGG (SEQ ID NO: 23), IEGRTVGG (SEQ ID NO: 24), LTPRSLLV (SEQ ID NO: 25), LGPVSGVP (SEQ ID NO: 26), VAGDSLEE (SEQ ID NO: 27),

GPAGLGGA (SEQ ID NO: 28), GPAGLRGA (SEQ ID NO: 29), APLGLRLR (SEQ ID NO: 30), PALPLVAQ (SEQ ID NO: 31), ENLYFQG (SEQ ID NO: 32), DDDKIVGG (SEQ ID NO: 33), LEVLFGQP (SEQ ID NO: 34), and LPKTGSES (SEQ ID NO: 35). In some embodiments, the first cleavage site and the second cleavage site are identical or different.

**[0030]** The XTEN sequence useful for the invention can be selected from the group consisting of AE42 (SEQ ID NO: 36), AE144 (SEQ ID NO: 37), AG144 (SEQ ID NO: 38), AE288 (SEQ ID NO: 39), AG288 (SEQ ID NO: 40), AE576 (SEQ ID NO: 41), AG576 (SEQ ID NO: 42), AE864 (SEQ ID NO: 43), AE72 (SEQ ID NO: 127), AE144\_2A (SEQ ID NO: 128), AE144\_3B (SEQ ID NO: 129), AE144\_4A (SEQ ID NO: 130), AE144\_5A (SEQ ID NO: 131), AE144\_6B (SEQ ID NO: 132), AG144\_A (SEQ ID NO: 133), AG144\_B (SEQ ID NO: 134), AG144\_C (SEQ ID NO: 135), AG144\_F (SEQ ID NO: 136), AE288\_2 (SEQ ID NO: 137), or AG864 (SEQ ID NO: 44). In a particular embodiment, the XTEN sequence comprises AE288 or AG288.

**[0031]** The chimeric protein of the invention can be polysialylated, pegylated, or hesylated.

**[0032]** The present invention is also directed to a polynucleotide or a set of polynucleotides encoding the chimeric protein. The polynucleotide can further comprise a polynucleotide chain, which encodes PC5 or PC7. The invention is also directed to a vector comprising the polynucleotide or the set of polynucleotides and one or more promoter operably linked to the polynucleotide or the set of polynucleotides. The vector can further comprise an additional vector, which comprises a polynucleotide chain encoding PC5 or PC7. The invention is also drawn to a host cell comprising the polynucleotide or the vector. The host cell can be a mammalian cell, *e.g.*, HEK293 cell, CHO cell, or BHK cell. In some embodiments, the PC5 or PC7 of the host cell cleaves the D1D2 domains of VWF.

**[0033]** The invention is also directed to a pharmaceutical composition comprising the chimeric protein, the polynucleotide, the vector, or the host cell, and a pharmaceutically acceptable carrier. The composition of the invention thus has an extended half-life compared to wild type FVIII protein. The half-life of the FVIII protein is extended at least about 1.5 times, at least about 2 times, at least about

2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than wild type FVIII. The half-life of Factor VIII is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours.

**[0034]** The composition of the present invention can be administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration. In one embodiment, the composition is administered via parenteral administration, *e.g.*, intravenous or subcutaneous administration. The composition of the invention is useful to treat a bleeding disease or condition in a subject in need thereof. The bleeding disease or condition is selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the iliopsoas sheath and any combinations thereof. In one embodiment, the subject treated with the chimeric protein is scheduled to undergo a surgery. In another embodiment, the treatment is prophylactic or on-demand.

**[0035]** The invention is also directed to a method of preventing or inhibiting binding of a FVIII protein with endogenous VWF comprising adding an effective amount of the chimeric protein, the polynucleotide vector, the host cell, or the composition to a subject in need thereof, wherein the VWF fragment binds to the FVIII protein and thus prevents or inhibits binding of endogenous VWF. The

present invention is further directed to a method of extending or increasing the half-life of the FVIII protein, wherein the method comprises administering an effective amount of the chimeric protein, the polynucleotide, the vector, the host cell, or the composition to a subject in need thereof, wherein the VWF fragment binds to the FVIII protein and thus extends or increases the half-life of the FVIII protein. Also provided is a method of preventing or inhibiting clearance of a FVIII protein from a cell, wherein the method comprises administering an effective amount of the chimeric protein, the polynucleotide, the vector, the host cell, or the composition to a cell comprising a FVIII protein or a polynucleotide encoding the FVIII protein, wherein the protein having VWF activity binds to the FVIII protein. The subject useful for the present methods is an animal, e.g., a human, e.g., a patient suffering from hemophilia A.

[0036] The present invention also provides a method of treating a bleeding disease or disorder in a subject in need thereof comprising administering an effective amount of the chimeric protein, the polynucleotide, the vector, the host cell, or the composition, wherein the bleeding disease or disorder is selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, and bleeding in the iliopsoas sheath. The treatment can be prophylactic or on-demand. In one embodiment, the effective amount is 0.1 µg/kg to 500 mg/kg.

[0037] The invention also includes a method of making a chimeric protein, comprising transfecting one or more host cell with the polynucleotide or the vector and expressing the chimeric protein in the host cell.

[0037a] Definitions of the specific embodiments of the invention as claimed herein follow.

[0037b] According to a first embodiment of the invention, there is provided a chimeric protein comprising (i) a von Willebrand Factor (VWF) fragment comprising the D' domain and the D3 domain of VWF, (ii) an extended length polypeptide (XTEN) sequence, and (iii) a factor VIII (FVIII) protein, wherein the VWF fragment and the

XTEN sequence are linked by an optional linker and wherein the VWF fragment is linked to or associated with the FVIII protein by a covalent bond.

**[0037c]** According to a second embodiment of the invention, there is provided a chimeric protein comprising a first polypeptide chain and a second polypeptide chain, wherein the first polypeptide chain comprises a VWF fragment, a first XTEN sequence, and a first Ig constant region or a portion thereof and the second polypeptide chain comprises a FVIII protein, a second XTEN sequence, and a second Ig constant region or a portion thereof, wherein the first polypeptide chain is linked to or associated with the second polypeptide chain through a covalent bond, and wherein the second XTEN is linked to the C-terminus or N-terminus of the FVIII protein or is inserted immediately downstream of one or more insertion sites in the FVIII protein.

**[0037d]** According to a third embodiment of the invention, there is provided a polynucleotide or a set of polynucleotides encoding the chimeric protein of the first or second embodiments.

**[0037e]** According to a fourth embodiment of the invention, there is provided a vector comprising the polynucleotide or the set of polynucleotides of the third embodiment and one or more promoters operably linked to the polynucleotide or the set of polynucleotides.

**[0037f]** According to a fifth embodiment of the invention, there is provided a host cell comprising the polynucleotide or the set of polynucleotides of the third embodiment or the vector of fourth embodiment.

**[0037g]** According to a sixth embodiment of the invention, there is provided a pharmaceutical composition comprising the chimeric protein of the first or second embodiments, the polynucleotide or the set of polynucleotides of the third embodiment, the vector of the fourth embodiment, or the host cell of the fifth embodiment, and a pharmaceutically acceptable carrier.

**[0037h]** According to a seventh embodiment of the invention, there is provided a method of treating a bleeding disease or disorder in a subject in need thereof comprising administering an effective amount of the chimeric protein of the first or second embodiments, the polynucleotide or the set of polynucleotides of the third embodiment, the vector of the fourth embodiment, the host cell of the fifth embodiment, or the composition of the sixth embodiment,



wherein the bleeding disease or disorder is selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, and bleeding in the iliopsoas sheath.

**[0037i]** According to an eighth embodiment of the invention, there is provided a method of making a chimeric protein, comprising culturing the host cell of fifth embodiment under suitable medium and expressing the chimeric protein in the host cell.

#### BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

**[0038]** Figure 1A-D. Schematic diagrams of VWF fragments. Fig. 1 A shows three exemplary VWF fragments useful for the invention, e.g., VWF-002, VWF- 010, and VWF-013. VWF-002 contains amino acids 1 to 477 of SEQ ID NO: 124 (amino acids 764 to 1240 of SEQ ID NO: 2) and is synthesized without the pre/propeptide sequences. VWF-010 contains the D1D2 domains in addition to the D'D3 domains. VWF-013 contains the D1D2D'D3 domains in addition to

**[Text continues on page 17.]**

alanine residues substituting cysteines at residues 336 and 379 of SEQ ID NO: 123 Fig. 1B shows VWF-031, which contains the D1D2D'D3 domains fused to an Ig constant region or a portion thereof, *e.g.*, an Fc region, by a cleavable linker, *e.g.*, a 48 amino acids thrombin cleavable linker. Fig. 1C shows VWF-025, which is a nucleotide sequence encoding D1D2D'D3 domains contained in pLIVE vector, and VWF-029, which is a nucleotide sequence encoding D1D2D'D3 domains with two amino acid substitutions, C336A and C379A, in pLIVE vector. Fig. 1D shows full-length VWF fragment comprising propeptide (the D1 and D2 domains) and mature subunits (the D', D3, A1, A2, A3, D4, B1-3, C1-2 domains). The VWF fragment is about 250 kDa protein and forms multimers (> 20 MDa) by disulfide bonding. The VWF fragment associates with FVIII (95-98%) in non-covalent complex and then extends half-life of FVIII by protecting FVIII from protease cleavage/activation, stabilizing heavy & light chain, and preventing clearance of FVIII by scavenger receptors. The VWF fragment also can limit half-life of FVIII by clearance of FVIII-VWF complex through VWF receptors and preventing pinocytosis and recycling of rFVIII-Fc.

**[0039]** Figure 2. Pharmacokinetic profile of rFVIII-XTEN (rFVIII-AE288 or rFVIII-288AE) in VWF D'D3 expression mice or in FVIII and VWF double knockout (DKO) mice. Figure 2A shows the timeline of hydrodynamic injection (HDI) of the D'D3 domain encoding plasmid DNA (VWF-025) (day -5), intravenous dosing of rFVIII-XTEN AE288 (day 0), and PK sample collection (day 5). Figure 2B shows FVIII activity measured by a FVIII chromogenic assay after IV dosing of rFVIII-XTEN288 in D1D2D'D3 mice (inverted triangle) and rFVIII-XTEN288 in DKO mice (diamond). Fig. 2C shows the D'D3 plasma level (ng/mL) after administration of VWF-025. The X axis represents time in hours.

**[0040]** Figure 3. Schematic diagram of exemplary VWF:FVIII heterodimer constructs. The constructs have the common structure represented as formula FVIII-F1-L1-V-X-L2-F2, but contain examples of different variable linkers. The construct (FVIII-161) shown contains a heterodimeric FVIII (the heavy chain and the light chain are associated by a metal bond) linked to a first Fc region and a VWF fragment, which is the D' and D3 domains of VWF (*i.e.*, amino acids 1 to 477 of SEQ ID NO: 2 with amino acid substitutions C336A and C379A) linked to an XTEN sequence, which is further linked to a cleavable linker and a second Fc

region. The XTEN sequence contained in FVIII-161 is an XTEN AE288 sequence, and the linker is a thrombin cleavable linker, which has 35 amino acids. In FVIII-161, the FVIII protein linked to the first Fc region is linked to the VWF fragment by a processable linker. Upon expression, the processable linker can be cleaved by an intracellular processing enzyme, thus making the construct three polypeptide chains associated with each other.

**[0041]** Figure 4 is schematic diagrams of FVIII-VWF heterodimer or monomer examples. FVIII-168, FVIII-175, FVIII-172, FVIII-174, and FVIII-170. Construct FVIII-168 comprises a single chain FVIII sequence (having an alanine residue substitute the arginine residues at residues 1645 and 1648) linked to a first Fc region, which is then fused to a VWF fragment linked to a second Fc region by a thrombin cleavable linker, which has 48 amino acids. AE288 XTEN is inserted in the B domain of the single chain FVIII sequence. The linkage between the first Fc region and the VWF fragment comprises a linker that is capable of being cleaved by an intracellular processing enzyme, *i.e.*, processable linker. Construct FVIII-175 comprises a single chain FVIII (having an alanine residue substitute the arginine residues at residues 1645 and 1648) linked to AE288 XTEN and a first Fc region, which is linked to a second Fc region by a linker, *e.g.*, a processable linker. AE288 XTEN is inserted in the B domain of the single chain FVIII sequence. Construct FVIII-172 comprises two polypeptide chains, a first chain comprising a heavy chain FVIII sequence fused to AE288 XTEN, a second chain comprising a light chain FVIII sequence, a first Fc region, a linker (*e.g.*, a processable linker), a VWF fragment, a thrombin cleavable linker (*e.g.*, 48 amino acids), and a second Fc region. Construct FVIII-174 comprises two polypeptide chains, a first chain comprising a heavy chain FVIII sequence fused to AE288 XTEN and a second chain comprising a light chain FVIII, a first Fc region, a linker (*e.g.*, a processable linker), and a second Fc region. Construct FVIII-170 comprises a VWF fragment, AE288 XTEN, a linker (*e.g.*, a thrombin cleavable linker, which is 35 amino acids in length), and a single chain FVIII sequence.

**[0042]** Figure 5. Pharmacokinetic profile of FVIII/VWF heterodimers containing an XTEN sequence in combination with an Fc region. Constructs FVIII-161, FVIII-168, and FVIII-172 were administered to FVIII:VWF double knockout (DKO) mice by Hydrodynamic injection (HDI) at 100ug/mouse dose. Construct

FVIII-170 was administered to FVIII:VWF DKO mice by HDI at 50 µg/mouse dose. The post-HDI plasma FVIII activity was analyzed by FVIII chromogenic assay for 24 hr post-HDI. The FVIII activity of the FVIII:VWF heterodimers containing an XTEN sequence and Fc domains was compared with the FVIII activity of BDD-FVIII without the VWF fragment, XTEN sequence, and Fc domains.

**[0043]** Figure 6. Schematic diagrams of FVIII-VWF heterodimer examples co-transfection system. Fig. 6A. Construct FVIII-169 contains the full-length FVIII sequence (with an alanine residue substituting the arginine residues at 1645 and 1648 and with an XTEN sequence inserted in the single chain FVIII sequence), which is linked to an Fc region. VWF-031 contains the D1D2D'D3 fragment (with an alanine residue substituting the cysteine residues at 336 and 379) which is linked to another Fc region with a 48 thrombin cleavable linker. After intracellular processing, construct FVIII-169 produces a full length single chain FVIII (SCFVIII) fused to one Fc fragment and an XTEN sequence, and construct VWF-031 produces a 477 amino acid D'D3 fragment linked to another Fc fragment. Two covalent bonds can be formed between the Fc fragments that are linked to the SC FVIII or the D'D3 fragment, this in turn allows a non-covalent association of FVIII and D'D3. Fig. 6B. Construct FVIII-173 contains a heterodimeric FVIII sequence, a heavy chain FVIII sequence linked to an XTEN sequence and a light chain FVIII sequence linked to an Fc region. VWF-031 is described above. After intracellular processing, construct FVIII-173 produces a heterodimeric protein, a heavy chain FVIII fused to an XTEN sequence, a light chain FVIII fused to one Fc fragment, and construct VWF-031 produces a 477 amino acid D'D3 fragment linked to another Fc fragment. Two covalent bonds can be formed between the Fc fragments that are linked to the light chain FVIII or the D'D3 fragment, this in turn allows a non-covalent association of FVIII and D'D3.

**[0044]** Figure 7. Binding Affinity of Exemplary FVIII:VWF containing an XTEN sequence and Fc domains to immobilized hVWF in Octet assay. The binding affinity for FVIII-169/VWF-031 and FVIII-057 (rFVIII-Fc) fused to immobilized hVWF was tested using biolayer interferometry based measurements (Octet assay). Figure 7A shows binding response in nanomoles of FVIII169 and FVIII-Fc

drug substance (a positive control) to immobilized hVWF. Figure 7B shows binding response of human IgG1 (a negative control) to immobilized human VWF.

**[0045]** Figure 8. Pharmacokinetic (PK) profile of FVIII-169 in HemA and FVIII:VWF double knockout (DKO) mice. Figure 8A shows the PK profile of FVIII-169/VWF-031 and FVIII-Fc in HemA mice. HemA mice were treated with a single intravenous dose of FVIII-169/VWF-031 at 200 IU/kg. Plasma samples collected from the mice were tested by FVIII chromogenic assay. Half-life of FVIII-169/VWF-031 was calculated using WinNonlin program. Figure 8B shows the PK profile of FVIII-169/VWF-031, FVIII-169/Fc, and FVIII-Fc in FVIII/VWF DKO mice.

**[0046]** Figure 9. PK profile of FVIII-XTEN variants in D'D3 expressing FVIII/VWF DKO mice. Figure 9A shows comparison of the PK profile of the FVIII-XTEN variants, FVIII with one XTEN, FVIII with two XTENs, and FVIII with three XTENs. One, two, or three XTENs were inserted in various portions of FVIII including C-terminus and B-domain. CT indicates that an XTEN is linked to the C-terminus of FVIII. Insertion site B/CT indicates that one XTEN is inserted between amino acid residue 745 and amino acid residue 746 of the FVIII protein and another XTEN is linked to the C-terminus of the FVIII protein. The amino acid residue numbering corresponds to the SQ BDD FVIII protein sequence. Insertion site 1900/B/CT indicates that a first XTEN is inserted between amino acid residue 1900 and amino acid residue 1901 of FVIII, a second XTEN is inserted between amino acid residue 745 and amino acid residue 746 of FVIII, and a third XTEN is linked to the C-terminus of FVIII. The mouse strain used to administer the FVIII-XTEN variants is a DKO mouse strain expressing D'D3 domains. Figure 9B shows the PK profile of FVIII-XTEN with three XTEN insertions. The FVIII-XTEN (1900/B/CT) variant was administered to either the FVIII/VWF DKO mice or HemA mice. The half-life of FVIII-XTEN (1900/B/CT) is compared.

**[0047]** Figure 10. FVIII activity of FVIII-Fc (hollow triangle), FVIII169:Fc (filled circle), and FVIII169:VWF31 (hollow triangle) in mouse DKO plasma measured by chromogenic assay. FVIII:Fc contains a dual-chain FVIII (Heavy chain and Light chain) fused to an Fc dimer (*i.e.*, monomer-dimer hybrid). FVIII169 is

described above (containing AE288 in the B domain, immediately downstream of amino acid 745 corresponding to mature FVIII sequence). FVIII169:Fc contains FVIII169 fused to an Fc dimer. FVIII169:VWF31 contains VWF31 in addition to the Fc dimer, FVIII169 fused to the first Fc region and VWF31 fused to the second Fc region, wherein the first Fc region and the second Fc region form a covalent bond, *e.g.*, one or more disulfide bonds.

**[0048]** Figure 11. Effects of Fc, XTEN, and VWF-D'D3 fragments on FVIII half-life extension. BDD-FVIII (REFACTO®) (square), FVIIFc (circle), FVIII169/Fc (triangle), and FVIII169/VWF031 (inverted triangle) were administered to FVIII and VWF double knockout (DKO) mice. The FVIII activity was measured by chromogenic assay, and the half-life was calculated using the WinNonlin-Phoenix program. X-axis shows time, and the Y-axis shows the FVIII plasma activity in mU/mL.

**[0049]** Figure 12A-C. Effects of different XTENs in rFVIII-XTEN/VWF heterodimer in HemA mice. Figure 12A shows the FVIII plasma activity normalized to 5 min value (%) of two XTENs inserted immediately downstream of residues 1900 and 1656 corresponding to mature FVIII sequence (*i.e.*, FVIII-195 (dual chain FVIII isoform) and FVIII-199 (single chain FVIII isoform)), compared to FVIII-169 containing an XTEN immediately downstream of residue 745 corresponding to mature FVIII sequence. FVIII-169/VWF-031 (filled circle), FVIII-199/VWF-031 (filled square), and FVIII-195/VWF031 (hollow square) were administered in HemA mice to measure the FVIII plasma activity. Figure 12B shows the half-life extension effect of the second XTEN insertion immediately downstream of residues 403 (A2 domain) and 745 (B domain) (*i.e.*, FVIII-203) and residues 745 (B domain) and 1900 (A3 domain) (FVIII-204) corresponding to mature FVIII sequence compared to FVIII-169 (an XTEN insertion in B domain only). FVIII-204/VWF031 (filled triangle), FVIII-169/VWF-031 (filled circle), FVIII-203/VWF-031 (filled square), and scBDD-FVIII (hollow diamond) were administered to HemA mice. The X-axis shows FVIII plasma activity normalized to 5 min value (%), and the y-axis shows time in hours. Figure 12C shows the half-life extension effect of the two XTEN insertions immediately downstream of residues 18 (A1 domain) and 745 (B domain) (*i.e.*, FVIII-205) compared to FVIII-169 (a single XTEN insertion in the B domain) and

single chain FVIII without any Fc regions or any XTENS (*i.e.*, FVIII-207). Figure 12C additionally shows the half-life extension effect of three XTEN insertions incorporated immediately downstream of residues 26 (A1 domain), 1656 (A3 domain), and 1900 (A3 domain) (*i.e.*, FVIII-201) compared to FVIII-169 (a single XTEN insertion immediately downstream of residue 745). FVIII-205/VWF-031 (filled square), FVIII-201/VWF-031 (inverted triangle), FVIII-169/VWF-031 (filled circle), and FVIII-207 (hollow diamond) were administered to HemA mice. The FVIII plasma activity normalized to 5 min value (%) (X-axis) was measured over time in hours (Y-axis).

**[0050]** Figure 13. FVIII activity of rFVIII-XTEN/VWF-XTEN heterodimer in FVIII/VWF DKO mice. FVIII activity of plasma samples was analyzed by FVIII chromogenic assay, and the regression curve of plasma FVIII activity (X-axis) as a function of time (Y-axis) was plotted. FVIII-155 (scFVIII-Fc without any XTENS) was co-expressed with VWF-034 (VWF-Fc with AE 288 XTEN plus a 35 residue thrombin cleavable linker). The half-life of FVIII-155/VWF-034 was compared with that of FVIII-169/VWF-031, which has a AE 288 XTEN inserted into the B domain junction (immediately downstream of residue 745 corresponding to mature FVIII polypeptide) of FVIII.

**[0051]** Figure 14A-H. Schematic diagrams of various rFVIII-XTEN/VWF constructs. These constructs are also described in other sections herein. Fig. 14A shows single chain B domain deleted FVIII protein (sometimes indicated herein as scBDD-FVIII). The scBDD-FVIII constructs contain two substitutions at residues 1645 and 1648 from Arg to Ala. Figure 14B shows two polypeptide chain construct (FVIII155/VWF031), the first chain comprising single chain FVIII linked to an Fc region without any XTENS and the second chain comprising the VWF D'D3 fragment linked to an Fc region. This construct is used as a control. Fig. 14C shows two polypeptide chain construct (FVIII199/VWF031), the first chain comprising single chain FVIII linked to an Fc region, in which a first XTEN is inserted immediately downstream of residue 1900 corresponding to mature FVIII sequence and a second XTEN is inserted immediately downstream of residue 1656 corresponding to mature FVIII sequence, and the second chain comprising the VWF D'D3 fragment linked to an Fc region. Fig. 14D shows two polypeptide chain construct (FVIII201/VWF031), the first chain comprising single

chain FVIII protein linked to an Fc region, in which a first XTEN is inserted immediately downstream of residue 26 corresponding to mature FVIII sequence, a second XTEN is inserted immediately downstream of residue 1656 corresponding to mature FVIII sequence, and a third XTEN is inserted immediately downstream of residue 1900 corresponding to mature FVIII sequence, and the second chain comprising the VWF D'D3 fragment linked to an Fc region. Fig. 14E shows two polypeptide chain constructs (FVIII169/VWF031), the first chain comprising single chain FVIII protein linked to an Fc region, in which an XTEN is inserted immediately downstream of residue 745 (indicated as "B") corresponding to mature FVIII sequence, and the second chain comprising the VWF D'D3 fragment linked to an Fc region. Fig. 14F shows two polypeptide chain construct (FVIII203/VWF031), the first chain comprising single chain FVIII protein, in which a first XTEN is inserted at residue 745 ("B") corresponding to mature FVIII sequence and a second XTEN is inserted at residue 1900 corresponding to mature FVIII sequence, and the second chain comprising the VWF D'D3 fragment linked to an Fc region. Fig. 14G shows two polypeptide chain construct (FVIII204/VWF031), the first chain comprising single chain FVIII protein linked to an Fc region, in which a first XTEN is inserted immediately downstream of residue 403 corresponding to mature FVIII sequence and a second XTEN is inserted immediately downstream of residue 745 ("B") corresponding to mature FVIII sequence, and a second chain comprising the VWF D'D3 fragment linked to an Fc region. Fig. 14H shows two polypeptide chain construct (FVIII205/VWF031), the first chain comprising single chain FVIII, in which a first XTEN is inserted immediately downstream of residue 18 corresponding to mature FVIII sequence and a second XTEN is inserted immediately downstream of residue 745 ("B") corresponding to mature FVIII sequence, and the second chain comprising the VWF D'D3 fragment linked to an Fc region.

**[0052]** Figure 15. FVIII activity of rFVIII-XTEN/VWF and BDD-FVIII in FVIII/VWF DKO mice. FVIII activity of plasma samples was analyzed by FVIII chromogenic assay, and the regression curve of plasma FVIII activity (X-axis) as a function of time (Y-axis) was plotted. The half-life of rFVIII-XTEN/VWF (FVIII-205/VWF-031) was compared with that of BDD-FVIII and rFVIII-Fc.



[0053] Figure 16. Efficacy of FVIII-XTEN-Fc:VWF-Fc heterodimers in HemA mice using tail clip bleeding model. The HemA mice tail clip bleeding model was used to compare the efficacy of FVIII169/VWF034, FVIII205/VWF031, and BDD-FVIII. The median blood loss in ml for 200 IU/kg of FVIII169/VWF034 and FVIII205/VWF031 is compared with 200 IU/kg of BDD-FVIII, 65 IU/kg of BDD-FVIII, 20 IU/kg of BDD-FVIII, and vehicle.

## DETAILED DESCRIPTION OF THE INVENTION

### DEFINITIONS

[0054] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0055] The term "polynucleotide" or "nucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA) or plasmid DNA (pDNA). In certain embodiments, a polynucleotide comprises a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid" refers to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a Factor VIII polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) from other polynucleotides in a solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides of the present invention. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid can include regulatory elements such as promoters, enhancers, ribosome binding sites, or transcription termination signals.

**[0056]** As used herein, a "coding region" or "coding sequence" is a portion of polynucleotide which consists of codons translatable into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is typically not translated into an amino acid, it may be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl terminus of the resulting polypeptide. Two or more coding regions of the present invention can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. It follows, then, that a single vector can contain just a single coding region, or comprise two or more coding regions, *e.g.*, a single vector can separately encode a binding domain-A and a binding domain-B as described below. In addition, a vector, polynucleotide, or nucleic acid of the invention can encode heterologous coding regions, either fused or unfused to a nucleic acid encoding a binding domain of the invention. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

**[0057]** Certain proteins secreted by mammalian cells are associated with a secretory signal peptide which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that signal peptides are generally fused to the N-terminus of the polypeptide, and are cleaved from the complete or "full-length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, a native signal peptide or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, *e.g.*, a human tissue plasminogen activator (TPA) or mouse  $\beta$ -glucuronidase signal peptide, or a functional derivative thereof, can be used.

**[0058]** The term "downstream" refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In certain embodiments, downstream nucleotide

sequences relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

**[0059]** The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence. In certain embodiments, upstream nucleotide sequences relate to sequences that are located on the 5' side of a coding region or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.

**[0060]** As used herein, the term "regulatory region" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding region, and which influence the transcription, RNA processing, stability, or translation of the associated coding region. Regulatory regions may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites and stem-loop structures. If a coding region is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

**[0061]** A polynucleotide which encodes a gene product, *e.g.*, a polypeptide, can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. In an operable association a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory regions in such a way as to place expression of the gene product under the influence or control of the regulatory region(s). For example, a coding region and a promoter are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the gene product encoded by the coding region, and if the nature of the linkage between the promoter and the coding region does not interfere with the ability of the promoter to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can also be operably associated with a coding region to direct gene product expression.

[0062] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit  $\beta$ -globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

[0063] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0064] The term "expression" as used herein refers to a process by which a polynucleotide produces a gene product, for example, an RNA or a polypeptide. It includes without limitation transcription of the polynucleotide into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product, and the translation of an mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation or splicing, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

[0065] A "vector" refers to any vehicle for the cloning of and/or transfer of a nucleic acid into a host cell. A vector may be a replicon to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. A "replicon" refers to any genetic element (*e.g.*, plasmid, phage, cosmid, chromosome, virus) that functions as an autonomous unit of replication *in vivo*,

*i.e.*, capable of replication under its own control. The term "vector" includes both viral and nonviral vehicles for introducing the nucleic acid into a cell *in vitro*, *ex vivo* or *in vivo*. A large number of vectors are known and used in the art including, for example, plasmids, modified eukaryotic viruses, or modified bacterial viruses. Insertion of a polynucleotide into a suitable vector can be accomplished by ligating the appropriate polynucleotide fragments into a chosen vector that has complementary cohesive termini.

**[0066]** Vectors may be engineered to encode selectable markers or reporters that provide for the selection or identification of cells that have incorporated the vector. Expression of selectable markers or reporters allows identification and/or selection of host cells that incorporate and express other coding regions contained on the vector. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, kanamycin, hygromycin, bialaphos herbicide, sulfonamide, and the like; and genes that are used as phenotypic markers, *i.e.*, anthocyanin regulatory genes, isopentanyl transferase gene, and the like. Examples of reporters known and used in the art include: luciferase (Luc), green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), -galactosidase (LacZ), -glucuronidase (Gus), and the like. Selectable markers may also be considered to be reporters.

**[0067]** The term "plasmid" refers to an extra-chromosomal element often carrying a gene that is not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear, circular, or supercoiled, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

**[0068]** Eukaryotic viral vectors that can be used include, but are not limited to, adenovirus vectors, retrovirus vectors, adeno-associated virus vectors, and poxvirus, *e.g.*, vaccinia virus vectors, baculovirus vectors, or herpesvirus vectors. Non-viral vectors include plasmids, liposomes, electrically charged lipids (cytofectins), DNA-protein complexes, and biopolymers.

- [0069] A "cloning vector" refers to a "replicon," which is a unit length of a nucleic acid that replicates sequentially and which comprises an origin of replication, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. Certain cloning vectors are capable of replication in one cell type, *e.g.*, bacteria and expression in another, *e.g.*, eukaryotic cells. Cloning vectors typically comprise one or more sequences that can be used for selection of cells comprising the vector and/or one or more multiple cloning sites for insertion of nucleic acid sequences of interest.
- [0070] The term "expression vector" refers to a vehicle designed to enable the expression of an inserted nucleic acid sequence following insertion into a host cell. The inserted nucleic acid sequence is placed in operable association with regulatory regions as described above.
- [0071] Vectors are introduced into host cells by methods well known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter.
- [0072] "Culture," "to culture" and "culturing," as used herein, means to incubate cells under *in vitro* conditions that allow for cell growth or division or to maintain cells in a living state. "Cultured cells," as used herein, means cells that are propagated *in vitro*.
- [0073] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups,

proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a natural biological source or produced recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

**[0074]** An "isolated" polypeptide or a fragment, variant, or derivative thereof refers to a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can simply be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for the purpose of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

**[0075]** Also included in the present invention are fragments or variants of polypeptides, and any combination thereof. The term "fragment" or "variant" when referring to polypeptide binding domains or binding molecules of the present invention include any polypeptides which retain at least some of the properties (*e.g.*, FcRn binding affinity for an FcRn binding domain or Fc variant, coagulation activity for an FVIII variant, or FVIII binding activity for the VWF fragment) of the reference polypeptide. Fragments of polypeptides include proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein, but do not include the naturally occurring full-length polypeptide (or mature polypeptide). Variants of polypeptide binding domains or binding molecules of the present invention include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants can be naturally or non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions.

**[0076]** The term "VWF fragment" or "VWF fragments" used herein means any VWF fragments that interact with FVIII and retain at least one or more properties that are normally provided to FVIII by full-length VWF, *e.g.*, preventing

premature activation to FVIIIa, preventing premature proteolysis, preventing association with phospholipid membranes that could lead to premature clearance, preventing binding to FVIII clearance receptors that can bind naked FVIII but not VWF-bound FVIII, and/or stabilizing the FVIII heavy chain and light chain interactions.

**[0077]** A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, if an amino acid in a polypeptide is replaced with another amino acid from the same side chain family, the substitution is considered to be conservative. In another embodiment, a string of amino acids can be conservatively replaced with a structurally similar string that differs in order and/or composition of side chain family members.

**[0078]** As known in the art, "sequence identity" between two polypeptides is determined by comparing the amino acid sequence of one polypeptide to the sequence of a second polypeptide. When discussed herein, whether any particular polypeptide is at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). BESTFIT uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is



calculated over the full-length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

**[0079]** As used herein, an "amino acid corresponding to" or an "equivalent amino acid" in a VWF sequence or a FVIII protein sequence is identified by alignment to maximize the identity or similarity between a first VWF or FVIII sequence and a second VWF or FVIII sequence. The number used to identify an equivalent amino acid in a second VWF or FVIII sequence is based on the number used to identify the corresponding amino acid in the first VWF or FVIII sequence.

**[0080]** As used herein, the term "insertion site" refers to a position in a FVIII polypeptide, or fragment, variant, or derivative thereof, which is immediately upstream of the position at which a heterologous moiety can be inserted. An "insertion site" is specified as a number, the number being the number of the amino acid in mature native FVIII (SEQ ID NO:4) to which the insertion site corresponds, which is immediately N-terminal to the position of the insertion. For example, the phrase "a3 comprises an XTEN at an insertion site which corresponds to amino acid 1656 of SEQ ID NO: 4" indicates that the heterologous moiety is located between two amino acids corresponding to amino acid 1656 and amino acid 1657 of SEQ ID NO: 4.

**[0081]** The phrase "immediately downstream of an amino acid" as used herein refers to position right next to the terminal carboxyl group of the amino acid. Similarly, the phrase "immediately upstream of an amino acid" refers to the position right next to the terminal amine group of the amino acid. Therefore, the phrase "between two amino acids of an insertion site" as used herein refers to a position in which an XTEN or any other polypeptide is inserted between two adjacent amino acids. Thus, the phrases "inserted immediately downstream of an amino acid" and "inserted between two amino acids of an insertion site" are used synonymously with "inserted at an insertion site."

**[0082]** The terms "inserted," "is inserted," "inserted into" or grammatically related terms, as used herein refers to the position of an XTEN in a chimeric polypeptide relative to the analogous position in native mature human FVIII. As used herein the terms refer to the characteristics of the recombinant FVIII polypeptide relative to native mature human FVIII, and do not indicate, imply or infer any methods or

process by which the chimeric polypeptide was made. For example, in reference to a chimeric polypeptide provided herein, the phrase "an XTEN is inserted into immediately downstream of residue 745 of the FVIII polypeptide" means that the chimeric polypeptide comprises an XTEN immediately downstream of an amino acid which corresponds to amino acid 745 in native mature human FVIII, *e.g.*, bounded by amino acids corresponding to amino acids 745 and 746 of native mature human FVIII.

**[0083]** A "fusion" or "chimeric" protein comprises a first amino acid sequence linked to a second amino acid sequence with which it is not naturally linked in nature. The amino acid sequences which normally exist in separate proteins can be brought together in the fusion polypeptide, or the amino acid sequences which normally exist in the same protein can be placed in a new arrangement in the fusion polypeptide, *e.g.*, fusion of a Factor VIII domain of the invention with an Ig Fc domain. A fusion protein is created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship. A chimeric protein can further comprises a second amino acid sequence associated with the first amino acid sequence by a covalent, non-peptide bond or a non-covalent bond.

**[0084]** As used herein, the term "half-life" refers to a biological half-life of a particular polypeptide *in vivo*. Half-life may be represented by the time required for half the quantity administered to a subject to be cleared from the circulation and/or other tissues in the animal. When a clearance curve of a given polypeptide is constructed as a function of time, the curve is usually biphasic with a rapid  $\alpha$ -phase and longer  $\beta$ -phase. The  $\alpha$ -phase typically represents an equilibration of the administered Fc polypeptide between the intra- and extra-vascular space and is, in part, determined by the size of the polypeptide. The  $\beta$ -phase typically represents the catabolism of the polypeptide in the intravascular space. In some embodiments, FVIII and chimeric proteins comprising FVIII are monophasic, and thus do not have an alpha phase, but just the single beta phase. Therefore, in certain embodiments, the term half-life as used herein refers to the half-life of the polypeptide in the  $\beta$ -phase. The typical  $\beta$ -phase half-life of a human antibody in humans is 21 days.

**[0085]** The term "linked" as used herein refers to a first amino acid sequence or nucleotide sequence covalently or non-covalently joined to a second amino acid sequence or nucleotide sequence, respectively. The first amino acid or nucleotide sequence can be directly joined or juxtaposed to the second amino acid or nucleotide sequence or alternatively an intervening sequence can covalently join the first sequence to the second sequence. The term "linked" means not only a fusion of a first amino acid sequence to a second amino acid sequence at the C-terminus or the N-terminus, but also includes insertion of the whole first amino acid sequence (or the second amino acid sequence) into any two amino acids in the second amino acid sequence (or the first amino acid sequence, respectively). In one embodiment, the first amino acid sequence can be linked to a second amino acid sequence by a peptide bond or a linker. The first nucleotide sequence can be linked to a second nucleotide sequence by a phosphodiester bond or a linker. The linker can be a peptide or a polypeptide (for polypeptide chains) or a nucleotide or a nucleotide chain (for nucleotide chains) or any chemical moiety (for both polypeptide and polynucleotide chains). The term "linked" is also indicated by a hyphen (-).

**[0086]** As used herein the term "associated with" refers to a covalent or non-covalent bond formed between a first amino acid chain and a second amino acid chain. In one embodiment, the term "associated with" means a covalent, non-peptide bond or a non-covalent bond. This association can be indicated by a colon, *i.e.*, (:). In another embodiment, it means a covalent bond except a peptide bond. For example, the amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a thiol group on a second cysteine residue. In most naturally occurring IgG molecules, the CH1 and CL regions are associated by a disulfide bond and the two heavy chains are associated by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system). Examples of covalent bonds include, but are not limited to, a peptide bond, a metal bond, a hydrogen bond, a disulfide bond, a sigma bond, a pi bond, a delta bond, a glycosidic bond, an agnostic bond, a bent bond, a dipolar bond, a Pi backbond, a double bond, a triple bond, a quadruple bond, a quintuple bond, a sextuple bond, conjugation, hyperconjugation, aromaticity, hapticity, or antibonding. Non-limiting examples

of non-covalent bond include an ionic bond (*e.g.*, cation-pi bond or salt bond), a metal bond, an hydrogen bond (*e.g.*, dihydrogen bond, dihydrogen complex, low-barrier hydrogen bond, or symmetric hydrogen bond), van der Waals force, London dispersion force, a mechanical bond, a halogen bond, aurophilicity, intercalation, stacking, entropic force, or chemical polarity.

**[0087]** The term "monomer-dimer hybrid" used herein refers to a chimeric protein comprising a first polypeptide chain and a second polypeptide chain, which are associated with each other by a disulfide bond, wherein the first chain comprises a clotting factor, *e.g.*, Factor VIII, and a first Fc region and the second chain comprises, consists essentially of, or consists of a second Fc region without the clotting factor. The monomer-dimer hybrid construct thus is a hybrid comprising a monomer aspect having only one clotting factor and a dimer aspect having two Fc regions.

**[0088]** As used herein, the term "cleavage site" or "enzymatic cleavage site" refers to a site recognized by an enzyme. Certain enzymatic cleavage sites comprise an intracellular processing site. In one embodiment, a polypeptide has an enzymatic cleavage site cleaved by an enzyme that is activated during the clotting cascade, such that cleavage of such sites occurs at the site of clot formation. Exemplary such sites include, *e.g.*, those recognized by thrombin, Factor XIa or Factor Xa. Exemplary FXIa cleavage sites include, *e.g.*, TQSFNDFTR (SEQ ID NO: 45) and SVSQTSKLTR (SEQ ID NO: 46). Exemplary thrombin cleavage sites include, *e.g.*, DFLAEGGGVR (SEQ ID NO: 47), TTKIKPR (SEQ ID NO: 48), LVPRG (SEQ ID NO: 49) and ALRPR (amino acids 1 to 5 of SEQ ID NO: 50). Other enzymatic cleavage sites are known in the art.

**[0089]** As used herein, the term "processing site" or "intracellular processing site" refers to a type of enzymatic cleavage site in a polypeptide which is a target for enzymes that function after translation of the polypeptide. In one embodiment, such enzymes function during transport from the Golgi lumen to the trans-Golgi compartment. Intracellular processing enzymes cleave polypeptides prior to secretion of the protein from the cell. Examples of such processing sites include, *e.g.*, those targeted by the PACE/furin (where PACE is an acronym for Paired basic Amino acid Cleaving Enzyme) family of endopeptidases. These enzymes are localized to the Golgi membrane and cleave proteins on the carboxyterminal

side of the sequence motif Arg-[any residue]-(Lys or Arg)-Arg. As used herein the "furin" family of enzymes includes, *e.g.*, PCSK1 (also known as PC1/Pc3), PCSK2 (also known as PC2), PCSK3 (also known as furin or PACE), PCSK4 (also known as PC4), PCSK5 (also known as PC5 or PC6), PCSK6 (also known as PACE4), or PCSK7 (also known as PC7/LPC, PC8, or SPC7). Other processing sites are known in the art.

**[0090]** In constructs that include more than one processing or cleavage site, it will be understood that such sites may be the same or different.

**[0091]** The term "Furin" refers to the enzymes corresponding to EC No. 3.4.21.75. Furin is subtilisin-like proprotein convertase, which is also known as PACE (Paired basic Amino acid Cleaving Enzyme). Furin deletes sections of inactive precursor proteins to convert them into biologically active proteins. During its intracellular transport, pro-peptide of VWF can be cleaved from mature VWF molecule by a Furin enzyme. In some embodiments, Furin cleaves the D1D2 from the D'D3 of VWF. In other embodiments, a nucleotide sequence encoding Furin can be expressed together with the nucleotide sequence encoding a VWF fragment so that D1D2 domains can be cleaved off intracellularly by Furin.

**[0092]** In constructs that include more than one processing or cleavage site, it will be understood that such sites may be the same or different.

**[0093]** A "processable linker" as used herein refers to a linker comprising at least one intracellular processing site, which is described elsewhere herein.

**[0094]** Hemostatic disorder, as used herein, means a genetically inherited or acquired condition characterized by a tendency to hemorrhage, either spontaneously or as a result of trauma, due to an impaired ability or inability to form a fibrin clot. Examples of such disorders include the hemophilias. The three main forms are hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency or "Christmas disease") and hemophilia C (factor XI deficiency, mild bleeding tendency). Other hemostatic disorders include, *e.g.*, Von Willebrand disease, Factor XI deficiency (PTA deficiency), Factor XII deficiency, deficiencies or structural abnormalities in fibrinogen, prothrombin, Factor V, Factor VII, Factor X or factor XIII, Bernard-Soulier syndrome, which is a defect or deficiency in GPIb. GPIb, the receptor for VWF, can be defective and lead to lack of primary clot formation (primary hemostasis) and increased bleeding

tendency), and thrombasthenia of Glanzman and Naegeli (Glanzmann thrombasthenia). In liver failure (acute and chronic forms), there is insufficient production of coagulation factors by the liver; this may increase bleeding risk.

**[0095]** The chimeric molecules of the invention can be used prophylactically. As used herein the term "prophylactic treatment" refers to the administration of a molecule prior to a bleeding episode. In one embodiment, the subject in need of a general hemostatic agent is undergoing, or is about to undergo, surgery. The chimeric protein of the invention can be administered prior to or after surgery as a prophylactic. The chimeric protein of the invention can be administered during or after surgery to control an acute bleeding episode. The surgery can include, but is not limited to, liver transplantation, liver resection, dental procedures, or stem cell transplantation.

**[0096]** The chimeric protein of the invention is also used for on-demand treatment. The term "on-demand treatment" refers to the administration of a chimeric molecule in response to symptoms of a bleeding episode or before an activity that may cause bleeding. In one aspect, the on-demand treatment can be given to a subject when bleeding starts, such as after an injury, or when bleeding is expected, such as before surgery. In another aspect, the on-demand treatment can be given prior to activities that increase the risk of bleeding, such as contact sports.

**[0097]** As used herein the term "acute bleeding" refers to a bleeding episode regardless of the underlying cause. For example, a subject may have trauma, uremia, a hereditary bleeding disorder (*e.g.*, factor VII deficiency) a platelet disorder, or resistance owing to the development of antibodies to clotting factors.

**[0098]** Treat, treatment, treating, as used herein refers to, *e.g.*, the reduction in severity of a disease or condition; the reduction in the duration of a disease course; the amelioration of one or more symptoms associated with a disease or condition; the provision of beneficial effects to a subject with a disease or condition, without necessarily curing the disease or condition, or the prophylaxis of one or more symptoms associated with a disease or condition. In one embodiment, the term "treating" or "treatment" means maintaining a FVIII trough level at least about 1 IU/dL, 2 IU/dL, 3 IU/dL, 4 IU/dL, 5 IU/dL, 6 IU/dL, 7 IU/dL, 8 IU/dL, 9 IU/dL, 10 IU/dL, 11 IU/dL, 12 IU/dL, 13 IU/dL, 14 IU/dL, 15 IU/dL, 16 IU/dL, 17

IU/dL, 18 IU/dL, 19 IU/dL, or 20 IU/dL in a subject by administering a chimeric protein or a VWF fragment of the invention. In another embodiment, treating or treatment means maintaining a FVIII trough level between about 1 and about 20 IU/dL, about 2 and about 20 IU/dL, about 3 and about 20 IU/dL, about 4 and about 20 IU/dL, about 5 and about 20 IU/dL, about 6 and about 20 IU/dL, about 7 and about 20 IU/dL, about 8 and about 20 IU/dL, about 9 and about 20 IU/dL, or about 10 and about 20 IU/dL. Treatment or treating of a disease or condition can also include maintaining FVIII activity in a subject at a level comparable to at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% of the FVIII activity in a non-hemophiliac subject. The minimum trough level required for treatment can be measured by one or more known methods and can be adjusted (increased or decreased) for each person.

#### CHIMERIC PROTEINS

[0099] The present invention is directed to extending the half-life of a Factor VIII protein using a VWF fragment and an XTEN sequence by preventing or inhibiting a FVIII half-life limiting factor, *i.e.*, endogenous VWF, from associating with the FVIII protein. Endogenous VWF associates with about 95% to about 98% of FVIII in non-covalent complexes. While endogenous VWF is a FVIII half-life limiting factor, endogenous VWF bound to a FVIII protein is also known to protect FVIII in various ways. For example, full length VWF (as a multimer having about 250 kDa) can protect FVIII from protease cleavage and FVIII activation, stabilize the FVIII heavy chain and/or light chain, and prevent clearance of FVIII by scavenger receptors. But, at the same time, endogenous VWF limits the FVIII half-life by preventing pinocytosis and by clearing FVIII-VWF complex from the system through the VWF clearance pathway. It is believed, while not bound by a theory, that endogenous VWF is a half-life limiting factor that prevents the half-life of a FVIII protein fused to a half-life extender from being longer than about two-fold that of wild-type FVIII. Therefore, the present invention is directed to preventing or inhibiting interaction between endogenous VWF and a FVIII protein using a VWF fragment, thereby increasing a half-life of the FVIII protein by using an XTEN sequence alone or an XTEN sequence in combination with an Ig constant region or a portion thereof. The

XTEN sequence can be linked to the FVIII protein or the VWF fragment. The FVIII protein associated with the VWF fragment is thus cleared from the circulation more slowly by one or more VWF clearance receptors and then can have the full half-life extension of the XTEN sequence or the XTEN sequence in combination of the Ig constant region, as compared to wild type FVIII or a FVIII protein without the VWF fragment.

**[0100]** In one embodiment, a VWF fragment is associated (or linked) with the FVIII protein by a covalent or a non-covalent bond. In some instances, however, the physical blockage or chemical association (*e.g.*, non-covalent bonding) between the VWF fragment and the FVIII protein may not be strong enough to provide a stable complex comprising the FVIII protein and the VWF fragment in the presence of endogenous VWF. For example, a VWF fragment forming a non-covalent bond with a FVIII protein without any other connections may readily be dissociated *in vivo* from the FVIII protein in the presence of endogenous VWF, replacing the VWF fragment (*e.g.*, recombinant VWF, *i.e.*, rVWF) with endogenous VWF. Therefore, the FVIII protein non-covalently bound to endogenous VWF would undergo the VWF clearance pathway and be readily cleared from the system. In order to prevent the dissociation of the VWF fragment with the FVIII protein, in some embodiments, the association or linkage between the FVIII protein and the VWF fragment is a covalent bond, *e.g.*, a peptide bond, one or more amino acids, or a disulfide bond. In certain embodiments, the association (*i.e.*, linkage) between the adjunct moiety and the FVIII protein is a peptide bond or a linker between the FVIII protein and the VWF fragment ("FVIII/VWF linker"). Non-limiting examples of the linker are described elsewhere herein. In some embodiments, the VWF fragment is a polypeptide comprising, consisting essentially of, or consisting of at least about 10, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2500, 3000, or 4000 amino acids. Non-limiting examples of the VWF fragment are described elsewhere herein.

**[0101]** In certain embodiments, the VWF fragment chemically (*e.g.*, non-covalently) binds to or physically blocks one or more VWF binding sites on a FVIII protein. The VWF binding site on a FVIII protein is located within the A3 domain or the C2 domain of the FVIII protein. In still other embodiments, the



VWF binding site on a FVIII protein is located within the A3 domain and C2 domain. For example, the VWF binding site on a FVIII protein can correspond to amino acids 1669 to 1689 and/or 2303 to 2332 of SEQ ID NO: 4 [full-length mature FVIII].

**[0102]** The invention also provides a chimeric protein (comprising a FVIII protein and a VWF fragment) further comprising one or more XTEN sequences, which provide additional half-life extension properties. The one or more XTEN sequences can be inserted within the FVIII protein or the VWF fragment or linked to the N-terminus or the C-terminus of the FVIII protein or the VWF fragment. The invention also includes a FVIII protein linked to an XTEN sequence (a first half-life extending moiety) and an Ig constant region or a portion thereof (a second half-life extending moiety) so that the two half-life extending moieties extend the half-life of the FVIII protein through two different mechanisms.

**[0103]** In some embodiments, a chimeric protein comprises a FVIII protein linked to a first Ig constant region or a portion thereof (*e.g.*, a first FcRn binding partner), a VWF fragment linked to a second Ig constant region or a portion thereof (*e.g.*, a second FcRn binding partner), and one or more XTEN sequences inserted or linked to the FVIII protein or the VWF fragment, wherein the VWF fragment prevents the FVIII half-life limiting factor (*e.g.*, endogenous VWF) from binding to the FVIII protein, wherein the first and second Ig constant regions or portions thereof forms a covalent bond, *e.g.*, a disulfide bond, and the one or more XTEN sequences extends the half-life of the FVIII protein.

**[0104]** In certain embodiments, a chimeric protein of the invention comprises a FVIII protein linked to a VWF fragment by an optional linker (*i.e.*, FVIII/VWF linker) and one or more XTEN sequences inserted or linked to the FVIII protein or the VWF fragment, wherein the VWF fragment prevents the FVIII half-life limiting factor (*e.g.*, endogenous VWF) from binding to the FVIII protein and the one or more XTEN sequences extends the half-life of the FVIII protein. In one aspect, the optional linker (FVIII/VWF linker) comprises a sortase recognition motif. In another aspect, the optional linker (FVIII/VWF linker) comprises a cleavable site. Examples of the cleavage linker (*i.e.*, linker containing one or more cleavage site) are described elsewhere herein.

[0105] The chimeric protein of the present invention includes, but is not limited to:

- (1) a VWF fragment comprising a D' domain and a D3 domain, an XTEN sequence, and FVIII, wherein the XTEN sequence is linked to the VWF fragment;
- (2) a FVIII protein, an XTEN sequence, and an Ig constant region or a portion thereof, wherein the FVIII protein is linked to an XTEN sequence and the Ig constant region or a portion thereof, or
- (3) a FVIII protein, an XTEN sequence, and a VWF fragment, wherein the XTEN sequence is linked to the FVIII protein at the C-terminus or N-terminus or inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites) of FVIII, and the VWF fragment and the FVIII protein are associated with each other.

**(1) Von Willebrand Factor (VWF) fragment linked to XTEN, and FVIII**

[0106] The present invention is directed to a chimeric protein comprising (i) a VWF fragment comprising a D' domain and a D3 domain of VWF, (ii) an XTEN sequence, and (iii) a FVIII protein, wherein (i), (ii), and (iii) are linked to or associated with each other. The VWF fragment linked to the XTEN sequence, as a part of a chimeric protein in the present invention, associates with the FVIII protein, thus preventing or inhibiting interaction between endogenous VWF and the FVIII protein. In certain embodiments, the VWF fragment, which is capable of preventing or inhibiting binding of the FVIII protein with endogenous VWF, can at the same time have at least one VWF-like FVIII protecting property. Examples of the VWF-like FVIII protecting properties include, but are not limited to, protecting FVIII from protease cleavage and FVIII activation, stabilizing the FVIII heavy chain and/or light chain, and preventing clearance of FVIII by scavenger receptors. As a result, the VWF fragment can prevent clearance of the FVIII protein through the VWF clearance pathway, thus reducing clearance of FVIII from the circulatory system. In some embodiments, the VWF fragments of the present invention bind to or are associated with a FVIII protein and/or physically or chemically block the VWF binding site on the FVIII protein. The FVIII protein associated with the VWF fragment is thus cleared from the circulation more slowly, as compared to wild type FVIII or FVIII not associated with the VWF fragment.

**[0107]** In one embodiment, the invention is directed to a chimeric protein comprising (i) a VWF fragment comprising the D' domain and the D3 domain of VWF, (ii) an XTEN sequence, and (iii) a FVIII protein, wherein the XTEN sequence is linked to the VWF fragment (*e.g.*, (a1) V-X or (a2) X-V, wherein V comprises a VWF fragment and X comprises an XTEN sequence), and the VWF fragment is linked to or associated with the FVIII protein. In another embodiment, the VWF fragment and the XTEN sequence can be linked by a linker (*e.g.*, (a3) V-L-X or (a4) X-L-V) or a peptide bond. The linker can be a cleavable linker, *e.g.*, a thrombin cleavable linker, which can be cleaved at the site of coagulation. In other embodiments, the VWF fragment, the XTEN sequence, and the FVIII protein are placed in a single polypeptide chain. In still other embodiments, the chimeric protein comprises two polypeptide chains, a first chain comprising the VWF fragment and the XTEN sequence and a second chain comprising the FVIII protein. In yet other embodiments, the chimeric protein comprises three polypeptide chains, a first chain comprising the VWF fragment and the XTEN sequence, a second chain comprising a light chain of FVIII and a third chain comprising a heavy chain of FVIII, wherein the first chain and the second chain are associated with each other (*e.g.*, covalent bond, *e.g.*, disulfide bond), and the second chain and the third chain are associated with each other (*e.g.*, metal bond). In still other embodiments, the XTEN sequence can be linked to the N-terminus or the C-terminus of the VWF fragment or inserted immediately downstream of one or more amino acids in the VWF fragment.

**[0108]** In certain embodiments, a chimeric protein of the invention comprises a formula comprising:

- (a) V-X-FVIII,
- (b) FVIII-X-V,
- (c) V-X:FVIII,
- (d) X-V:FVIII,
- (e) FVIII:V-X,
- (f) FVIII:X-V, or
- (a5) X-V-FVIII,

wherein V comprises a VWF fragment,  
X comprises one or more XTEN sequences,

FVIII comprises a FVIII protein;

(-) represents a peptide bond or one or more amino acids; and

(:) is a chemical association or a physical association. In one embodiment, (:) represents a chemical association, *e.g.*, at least one non-peptide bond. In another embodiment, the chemical association, *i.e.*, (:) is a covalent bond. In other embodiments, the chemical association, *i.e.*, (:) is a non-covalent interaction, *e.g.*, an ionic interaction, a hydrophobic interaction, a hydrophilic interaction, a Van der Waals interaction, or a hydrogen bond. In other embodiments, (:) is a non-peptide covalent bond. In still other embodiments, (:) is a peptide bond. In yet other embodiments, (:) represents a physical association between two sequences, wherein a portion of a first sequence is in close proximity to a second sequence such that the first sequence shields or blocks a portion of the second sequence from interacting with another moiety, and further that this physical association is maintained without allowing the second sequence to interact with other moieties. The orientation of the polypeptide formulas herein is listed from N-terminus (left) to C-terminus (right). For example, formula V-X-FVIII means formula NH<sub>2</sub>-V-X-FVIII-COOH. In one embodiment, the formulas described herein can comprise any additional sequences between the two moieties. For example, formula V-X-FVIII can further comprise any sequences at the N-terminus of V between V and X, between X and FVIII, or at the C-terminus of FVIII unless otherwise specified. In another embodiment, the hyphen (-) indicates a peptide bond.

**[0109]** In other embodiments, a chimeric protein of the invention comprises a formula comprising:

- (a) V(X1)-X2-FVIII,
- (b) FVIII-X2-V(X1),
- (c) V(X1):FVIII,
- (d) FVIII:V(X1), or
- (a5) X2-V(X1)-FVIII,

wherein V(X1) comprises a VWF fragment and a first XTEN sequence (X1),

wherein the XTEN sequence is inserted immediately downstream of one or more amino acids in the VWF fragment,

X2 comprises one or more optional XTEN sequences,

FVIII comprises a FVIII protein;

(-) is a peptide bond or one or more amino acids; and

(:) is a chemical association or a physical association.

**[0110]** In some embodiments, a chimeric protein comprises (i) a VWF fragment comprising a D' domain and a D3 domain of VWF, (ii) an XTEN sequence, (iii) a FVIII protein, (iv) a first optional linker, and (v) a second optional linker, wherein the XTEN sequence is linked to the VWF fragment and/or to the FVIII protein by the linker. In certain embodiments, a chimeric protein comprises a formula comprising:

- (b1) V-L1-X-L2-FVIII,
- (b2) FVIII-L2-X-L1-V,
- (b3) V-L1-X:FVIII,
- (b4) X-L1-V:FVIII,
- (b5) FVIII:V-L1-X,
- (b6) FVIII:X-L1-V,
- (b7) X-L1-V-L2-FVIII, or
- (b8) FVIII-L2-V-L1-X,

wherein V comprises a VWF fragment,

X comprises one or more XTEN sequences,

FVIII comprises a FVIII protein,

L1 comprises a first optional linker, *e.g.*, a first cleavable linker,

L2 comprises a second optional linker, *e.g.*, a second cleavable linker or an optional processable linker;

(-) is a peptide bond or one or amino acids; and

(:) is a chemical association or a physical association. In one embodiment, (:) represents a chemical association, *e.g.*, at least one non-peptide bond. In another embodiment, the chemical association, *i.e.*, (:) is a covalent bond. In other embodiments, the chemical association, *i.e.*, (:) is a non-covalent interaction, *e.g.*, an ionic interaction, a hydrophobic interaction, a hydrophilic interaction, a Van der Waals interaction, or a hydrogen bond. In other embodiments, (:) is a non-peptide covalent bond. In still other embodiments, (:) is a peptide bond. In yet other embodiments, (:) represents a physical association between two sequences, wherein a portion of a first sequence is in close proximity to a second sequence such that the first sequence shields or blocks a portion of the second sequence

from interacting with another moiety, and further that this physical association is maintained without allowing the second sequence to interact with other moieties. The orientation of the polypeptide formulas herein is listed from N-terminus (left) to C-terminus (right). For example, formula (b1) V-L1-X-L2-FVIII means formula NH<sub>2</sub>-V-L1-X-L2-FVIII-COOH. In one embodiment, the formulas described herein can comprise any additional sequences between the two moieties. In another embodiment, the hyphen (-) indicates a peptide bond.

[0111] Another aspect of the present invention is to provide a FVIII chimeric protein having reduced or no interactions with a FVIII half-life limiting factor, *e.g.*, endogenous VWF, and at the same time maximizing the half-life of the FVIII protein using an XTEN sequence (a first half-life extender) in combination with a second half-life extender or a moiety providing a covalent bond between the FVIII protein and the VWF fragment, *e.g.*, an Ig constant region or a portion thereof. In one embodiment, a chimeric protein of the invention comprises (i) a VWF fragment comprising a D' domain and a D3 domain of VWF, (ii) an XTEN sequence, (iii) a FVIII protein, and (iv) an Ig constant region or a portion thereof (also referred to herein as F), wherein (1) the VWF fragment is linked to the XTEN sequence by an optional linker, *e.g.*, a cleavable linker, (2) the VWF fragment is associated with or linked to the FVIII protein by an additional optional linker, *e.g.*, a cleavable linker, and (3) the Ig constant region or a portion thereof is linked to the VWF fragment, the XTEN sequence, or the FVIII protein. In another embodiment, a chimeric protein of the invention comprises (i) a VWF fragment comprising a D' domain and a D3 domain of VWF, (ii) an XTEN sequence, (iii) a FVIII protein, (iv) an Ig constant region or a portion thereof (F1 or a first Ig constant region or a portion thereof), and (v) an additional Ig constant region or a portion thereof (F2 or a second Ig constant region or a portion thereof), wherein (1) the VWF fragment is linked to the XTEN sequence by an optional linker, *e.g.*, a cleavable linker, (2) the XTEN sequence or the VWF fragment is linked to the Ig constant region or a portion thereof, (3) the FVIII is linked to the additional Ig constant region or a portion thereof, and (4) the Ig constant region or a portion thereof is associated with or linked to the additional Ig constant region or a portion thereof. In one embodiment, the association or linkage between the two Ig constant regions or a portion thereof is a covalent bond, *e.g.*, a disulfide bond. In

another embodiment, the association or linkage between the two Ig constant regions or a portion thereof is a processable linker, wherein the processable linker is intracellularly processed by a protease. For example, the chimeric protein comprises a formula comprising:

- (g) V-L2-X-L1-F1: FVIII-L3-F2;
- (h) V-L2-X-L1-F1:F2-L3-FVIII;
- (i) F-L1-X-L2-V: FVIII-L3-F2;
- (j) F-L1-X-L2-V:F2-L3-FVIII;
- (k) V-L2-X-L1-F1-L4-FVIII-L3-F2;
- (l) F2-L3-FVIII-L4-F1-L1-X-L2-V;
- (m) FVIII-L2-F2-L4-V-L2-X-L1-F1; or
- (n) F1-L1-X-L2-V-L4-F2-L2-FVIII,

wherein V comprises a VWF fragment,  
 each of L1 and L3 comprises an optional linker,  
 L2 comprises an optional linker, *e.g.*, a cleavable linker,  
 L4 is an optional linker, *e.g.*, a processable linker,  
 FVIII comprises a FVIII protein,  
 X comprises one or more XTEN sequences,  
 F1 comprises an optional Ig constant region or a portion thereof,  
 F2 comprises an optional additional Ig constant region or a portion thereof;  
 (-) is a peptide bond or one or more amino acids; and  
 (:) is a chemical association or a physical association.

**[0112]** In some embodiments, the FVIII protein in any constructs or formulas disclosed herein can further comprises at least one, at least two, at least three, at least four, at least five, or at least six XTEN sequences, each of the XTEN sequences inserted immediately downstream of one or more amino acids in the FVIII protein or linked to the N-terminus or the C-terminus of the FVIII protein. Non-limiting examples of the XTEN insertion sites are disclosed elsewhere herein.

**[0113]** In one embodiment, (:) represents a chemical association, *e.g.*, at least one non-peptide bond. In another embodiment, the chemical association, *i.e.*, (:) is a covalent bond. In other embodiments, the chemical association, *i.e.*, (:) is a non-covalent interaction, *e.g.*, an ionic interaction, a hydrophobic interaction, a

hydrophilic interaction, a Van der Waals interaction, or a hydrogen bond. In other embodiments, (:) is a non-peptide covalent bond. In still other embodiments, (:) is a peptide bond. In yet other embodiments, (:) represents a physical association between two sequences, wherein a portion of a first sequence is in close proximity to a second sequence such that the first sequence shields or blocks a portion of the second sequence from interacting with another moiety, and further that this physical association is maintained without allowing the second sequence to interact with other moieties. The orientation of the polypeptide formulas herein is listed from N-terminus (left) to C-terminus (right). For example, formula (n) F1-L1-X-L2-V-L4-F2-L2-FVIII means formula NH<sub>2</sub>-F1-L1-X-L2-V-L4-F2-L2-FVIII -COOH. In one embodiment, the formulas described herein can comprise any additional sequences between the two moieties. In another embodiment, the hyphen (-) indicates a peptide bond.

**[0114]** In one embodiment, either or both of the Ig constant region or a portion thereof (sometimes indicated herein by "F" or "F1") and the additional Ig constant region or a portion thereof (sometimes indicated herein by "F2") linked to the VWF fragment or the FVIII protein can extend the half-life of the VWF fragment, the FVIII protein, or both. In another embodiment, a pair of the Ig constant region or a portion thereof (sometimes indicated herein by "F" or "F1") and the additional Ig constant region or a portion thereof (sometimes indicated herein by "F2"), each of which are linked to the VWF fragment and the FVIII protein, provides a bond stronger than the non-covalent bond between the FVIII protein and the VWF fragment, *i.e.*, a covalent bond, *e.g.*, a disulfide bond, thereby preventing endogenous VWF from replacing the VWF fragment *in vivo*. F1 or F2 can comprise an Fc region or an FcRn binding partner. In other embodiments, either or both of F1 and F2 linked to the VWF fragment and/or the FVIII protein form a covalent bond (*e.g.*, a disulfide bond) between F1 and F2, thereby placing the VWF fragment and the FVIII protein in close proximity to prevent interaction of the FVIII protein with the VWF fragment. In some embodiments, F1 and F2 are identical or different. Non-limiting examples of F1 and F2 can be selected from the group consisting of a CH1 domain, a CH2 domain, a CH3 domain, a CH4 domain, a hinge domain, any functional fragments, derivatives, or analogs thereof, and two or more combinations thereof. In one embodiment, F1, F2, or both



comprise at least one CH1 domain, at least one CH2 domain, at least one CH3 domain, at least one CH4 domain, or the functional fragments, derivatives, or analogs thereof. In another embodiment, F1, F2, or both comprise at least one hinge domain or portion thereof and at least one CH2 domain or portion thereof (*e.g.*, in the hinge-CH2 orientation). In other embodiments, F1, F2, or both comprise at least one CH2 domain or portion thereof and at least one CH3 domain or portion thereof (*e.g.*, in the CH2-CH3 orientation.) Examples of the combination include, but are not limited to, a CH2 domain, a CH3 domain, and a hinge domain, which are also known as an Fc region (or Fc domain), *e.g.*, a first Fc region or a first FcRn binding partner for F1 and a second Fc region or a second FcRn binding partner for F2. In other embodiments, F1 is linked to the VWF fragment by a linker, and/or F2 is linked to the FVIII protein by a linker. In some embodiments, F1 and/or F2 comprises, consisting essentially of, or consisting of a hinge region. Additional non-limiting examples of the Fc regions or the FcRn binding partners are described elsewhere herein.

**[0115]** In certain embodiments, a chimeric protein of the invention comprises two polypeptide chains, a first polypeptide chain comprising, consisting essentially of, or consisting of a VWF fragment comprising a D' domain and a D3 domain, an XTEN sequence, a first Ig constant region or a portion thereof (*e.g.*, a first Fc region), and an optional linker between the VWF fragment and the XTEN sequence or the XTEN sequence or the first Ig constant region or a portion thereof and a second polypeptide chain comprising, consisting essentially of, or consisting of a FVIII protein and a second Ig constant region or a portion thereof (*e.g.*, a second Fc region). The linker between the VWF fragment and the first Ig constant region or a portion thereof can be a cleavable linker, *e.g.*, a thrombin cleavable linker, which can be cleaved at the site of coagulation. In some embodiments, the first polypeptide chain and the second polypeptide chain are associated with each other. The association between the first chain and the second chain prevents replacement of the first chain comprising the VWF fragment with endogenous VWF *in vivo*. In one embodiment, the association between the first chain and the second chain can be a covalent bond. In a particular embodiment, the covalent bond is a disulfide bond. In some embodiments, the FVIII protein in the second chain further comprises one or more XTEN sequences linked to the C-terminus or

N-terminus of the FVIII protein or inserted immediately downstream of one or more amino acids (*e.g.*, at least one insertion site disclosed herein) in the FVIII protein. Non-limiting examples of the insertion sites are described elsewhere herein.

**[0116]** In other embodiments, a chimeric protein of the invention comprises three polypeptide chains, wherein a first polypeptide chain comprises, consists essentially of, or consists of a heavy chain of a FVIII protein, a second polypeptide chain comprises, consists essentially of, or consists of a light chain of a FVIII protein fused to a first Ig constant region or a portion thereof (*e.g.*, a first Fc region), and a third polypeptide chain comprises, consists essentially of, or consists of a VWF fragment comprising a D' domain and a D3 domain, an XTEN sequence, a second Ig constant region or a portion thereof (*e.g.*, a second Fc region), and an optional linker between the XTEN sequence and the second Ig constant region or a portion thereof or the VWF fragment and the XTEN sequence. The linker in the third chain can be a cleavable linker, which is cleaved at the site of coagulation, *e.g.*, a thrombin cleavage site. In some embodiments, the heavy chain FVIII or the light chain FVIII is linked to one or more XTEN sequences, which can be linked to the N-terminus, the C-terminus, or inserted within one or more insertion sites within the FVIII sequence. Non-limiting examples of the insertion sites are disclosed elsewhere herein.

**[0117]** In yet other embodiments, a chimeric protein of the invention comprises two polypeptide chains, a first polypeptide chain comprising, consisting essentially of, or consisting of a heavy chain of a FVIII protein and a second polypeptide chain comprising, consisting essentially of, or consisting of a light chain of a FVIII protein, a first Ig constant region or a portion thereof (*e.g.*, a first Fc region), a first linker (*e.g.*, a processable linker, which contains one or more protease cleavage sites comprising one or more intracellular processing sites), a VWF fragment, a second linker (*e.g.*, a thrombin cleavable linker), an XTEN sequence, and a second Ig constant region or a portion thereof (*e.g.*, a second Fc region), wherein the light chain of the FVIII protein is linked to the first Ig constant region or a portion thereof (*e.g.*, the first Fc region), which is further linked to the VWF fragment by the first linker, and wherein the VWF fragment is linked to the XTEN sequence, which is further linked to the second Ig constant

region or a portion thereof by the second linker. In certain embodiments, the first linker is a processable linker, and the second linker is a cleavable linker. Upon expression, the chimeric protein can be processed by an intracellular processing enzyme, which cleaves the processable linker, and thus the chimeric protein can comprise, consists essentially of, or consists of three polypeptide chains. In addition, the VWF fragment can be cleaved off at the site of coagulation due to the cleavable linker.

[0118] In certain embodiments, a chimeric protein of the invention comprises one polypeptide chain, which comprises a single chain FVIII protein, a first Ig constant region or a portion thereof (*e.g.*, a first Fc region), a first linker (*e.g.*, a processable linker), a VWF fragment, an XTEN sequence, a second linker (*e.g.*, a thrombin cleavable linker), and a second Ig constant region or a portion thereof (*e.g.*, a second Fc region), wherein the single chain FVIII protein is linked to the first Ig constant region or a portion thereof, which is also linked to the VWF fragment by the first linker, and the VWF fragment is linked to the XTEN sequence, which is further linked to the second Ig constant region or a portion thereof. In one embodiment, the VWF fragment and the XTEN sequence are linked by the second linker. In another embodiment, the XTEN sequence and the second Ig constant region or a portion thereof are linked by the second linker. In other embodiments, the second chain further comprises a third linker. The single polypeptide chain can thus comprise the VWF fragment linked to the XTEN sequence by the second linker and the XTEN linked to the second Ig constant region or a portion thereof by the third linker. The second linker and the third linker can be identical or different. In one embodiment, the first linker is a processable linker. In another embodiment, the second linker or the third linker is a cleavable linker comprising one or two cleavable sites. In a specific embodiment, the second linker is a thrombin cleavable linker. The linkers useful in the invention are described elsewhere herein.

## **(2) FVIII, XTEN, and Fc**

[0119] A chimeric protein of the invention also comprises (i) a FVIII protein, (ii) an XTEN sequence (a first half-life extender), and (iii) an Ig constant region or a portion thereof (a second half-life extender), in which the XTEN sequence is linked to the FVIII protein by an optional linker and the Ig constant region or a

portion thereof by an additional optional linker. The XTEN sequence and the Ig constant region or a portion thereof can be used together to extend half-life of the FVIII protein. In one embodiment, the chimeric protein is a monomer. In another embodiment, the chimeric protein is a dimer (a homodimer or a heterodimer).

**[0120]** The present invention is also directed to a chimeric protein comprising (i) a FVIII protein, (ii) an XTEN sequence, (iii) an Ig constant region or a portion thereof (*i.e.*, a first Ig constant region or a portion thereof, "F," or "F1"), and (iv) an additional Ig constant region or a portion thereof (*i.e.*, a second Ig constant region or a portion thereof or "F2"). In one embodiment, the XTEN sequence is linked to the FVIII protein at the C-terminus or the N-terminus or inserted immediately downstream of one or more amino acids in the FVIII protein (*e.g.*, one or more XTEN insertion sites), the FVIII protein is linked to the first Ig constant region or a portion thereof, and the first Ig constant region or a portion thereof and the second Ig constant region or a portion thereof are associated with or linked to each other by an optional linker. In certain aspects, the chimeric protein is a monomer-dimer hybrid, which comprises a first polypeptide chain and a second polypeptide chain, wherein the first polypeptide chain comprises a FVIII protein, an XTEN sequence, and a first Ig constant region or a portion thereof, and the second polypeptide chain comprises, consists essentially of, or consists of a second Ig constant region or a portion thereof without the FVIII protein and wherein the first chain and the second chain are associated with each other. The association between the Ig constant region or a portion thereof (*e.g.*, the first Fc region) and the additional Ig constant region or a portion thereof (*e.g.*, a second Fc region) is a chemical association or a physical association. In certain embodiments, the chemical association is a covalent bond. In other embodiments, the chemical association is a non-covalent interaction, *e.g.*, an ionic interaction, a hydrophobic interaction, a hydrophilic interaction, a Van der Waals interaction, or a hydrogen bond. In other embodiments, the association is a non-peptide covalent bond. In still other embodiments, the association is a peptide bond.

**[0121]** In other aspects, the chimeric protein is a single polypeptide chain comprising a FVIII protein, an XTEN sequence, a first Ig constant region or a portion thereof, a linker, *e.g.*, a processable linker, and a second Ig constant region

or a portion thereof, wherein the single polypeptide chain is processed after expression by an intracellular enzyme and becomes two polypeptide chains.

[0122] In one embodiment, the Ig constant region or a portion thereof (sometimes indicated herein by "F" or "F1") linked to the FVIII protein can extend the half-life of the FVIII protein together with the XTEN sequence. In another embodiment, the Ig constant region or a portion thereof ("F" or "F1") is an Fc region or an FcRn binding partner described elsewhere herein.

[0123] In other embodiments, the additional Ig constant region or a portion thereof (sometimes indicated herein by "F2" or a second Ig constant region or a portion thereof) associated with or linked to the first Ig constant region or a portion thereof can also extend the half-life of the FVIII protein. In other embodiments, the second Ig constant region or a portion thereof ("F2") together with the first Ig constant region or a portion thereof and the XTEN sequence can extend the half-life of the FVIII protein. The additional Ig constant region or a portion thereof can be an Fc region or an FcRn binding partner described elsewhere herein.

[0124] In certain embodiments, the second Ig constant region or a portion thereof associated with the first Ig constant region or a portion thereof is further linked to a VWF fragment described elsewhere herein and an optional XTEN sequence.

[0125] In some embodiments, either or both of the Ig constant region or a portion thereof ("F" or "F1" or a first Ig constant region or a portion thereof) and an additional Ig constant region or a portion thereof (*i.e.*, a second Ig constant region or a portion thereof or "F2") (indicated in this paragraph as "the Ig constant regions or portion thereof") can include, but not limited to, a CH1 domain, a CH2 domain, a CH3 domain, a CH4 domain, a hinge domain, any functional fragments, derivatives, or analogs thereof or two or more combinations thereof. In one embodiment, the Ig constant region or a portion thereof comprises at least one CH1 domain, at least one CH2 domain, at least one CH3 domain, at least one CH4 domain, or the functional fragments, derivatives, or analogues thereof. In another embodiment, the Ig constant region or a portion thereof comprises at least one hinge domain or portion thereof and at least one CH2 domain or portion thereof (*e.g.*, in the hinge-CH2 orientation). In other embodiments, the Ig constant domain or portion thereof comprises at least one CH2 domain or portion thereof

and at least one CH3 domain or portion thereof (*e.g.*, in the CF2-CH3 orientation). Examples of the combination include, but are not limited to, a CH2 domain, a CH3 domain, and a hinge domain, which are also known as an Fc region (or Fc domain), *e.g.*, first Fc region. Additional examples of the Ig constant regions or portion thereof are described elsewhere herein.

[0126] The chimeric protein of the invention can have an extended half-life of the FVIII protein compared to wild-type FVIII. In one embodiment, the half-life of the FVIII protein is extended at least about 1.5 times, at least about 2 times, at least about 2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than the half-life of wild type FVIII. In another embodiment, the half-life of the FVIII protein is at least about 10 hours, at least about 11 hours, at least about 12 hours, at least about 13 hours, at least about 14 hours, at least about 15 hours, at least about 16 hours, at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours.

### **(3) FVIII, XTEN, and VWF**

[0127] In one aspect, a chimeric protein of the present invention comprises (i) a FVIII protein, (ii) an XTEN sequence, and (iii) a VWF fragment comprising a D' domain and a D3 domain of VWF, wherein the FVIII protein is linked to the XTEN sequence and wherein the FVIII protein is associated with or linked to the VWF fragment. In one embodiment, the VWF fragment of the chimeric protein described herein is not capable of binding to a VWF clearance receptor. In another embodiment, the VWF fragment is capable of protecting the FVIII protein from one or more protease cleavages, protecting the FVIII protein from activation, stabilizing the heavy chain and/or the light chain of the FVIII protein, or preventing clearance of the FVIII protein by one or more scavenger receptors. In other embodiments, the VWF fragment prevents or inhibits binding of endogenous VWF to the VWF binding site in the FVIII protein. The VWF binding site can be located in the A3 domain or the C2 domain of the FVIII protein or both the A3

domain and the C2 domain. In a specific embodiment, the VWF binding site comprises the amino acid sequence corresponding to amino acids 1669 to 1689 and/or amino acids 2303 to 2332 of SEQ ID NO: 2.

**[0128]** In another aspect, a chimeric protein comprises (i) a FVIII protein, (ii) an XTEN sequence, (iii) a VWF fragment, which comprises a D' domain and a D3 domain of VWF, and (iv) an Ig constant region or a portion thereof, wherein the XTEN sequence is linked to the FVIII protein at the C-terminus or the N-terminus or inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites disclosed herein) in the FVIII protein, the VWF fragment is linked to or associated with the FVIII protein or the XTEN sequence, and the Ig constant region or a portion thereof is linked to the FVIII protein, the XTEN sequence, the VWF fragment, or any combinations thereof. The Ig constant region or a portion thereof useful for chimeric proteins of the invention is described elsewhere herein. In one embodiment, the Ig constant region or a portion thereof is capable of extending the half-life of a FVIII protein. In another embodiment, the Ig constant region or a portion thereof comprises a first Fc region or a first FcRn binding partner. In yet other embodiments, the Ig constant region or a portion thereof is linked to the FVIII protein by an optional linker. In still other embodiments, the linker comprises a cleavable linker. The chimeric protein can be a single polypeptide chain, *i.e.*, a monomer (*i.e.*, a single chain), containing (i), (ii), (iii), and (iv) or two chains containing a first chain comprising (i) and (ii) and a second chain comprising (iii) and (iv). In other aspects, the chimeric protein is a dimer (*e.g.*, a homodimer or a heterodimer). In one embodiment, the chimeric protein comprises two chains, each comprising (i), (ii), (iii), and (iv).

**[0129]** In certain embodiments, a chimeric protein comprises (i) a FVIII protein, (ii) an XTEN sequence, (iii) a VWF fragment, which comprises a D' domain and a D3 domain of VWF, (iv) an Ig constant region or a portion thereof (sometimes also indicated as "F," "a first Ig constant region or a portion thereof", or "F2"), and (v) an additional Ig constant region or a portion thereof (sometimes also indicated as "F2" or "a second Ig constant region or a portion thereof"), wherein (1) the FVIII protein is linked to the XTEN sequence at the C-terminus or N-terminus of the FVIII protein or inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites disclosed herein) in the FVIII protein, (2)

either the XTEN sequence or the FVIII protein is linked to the Ig constant region or a portion thereof, (3) the VWF fragment is linked to the second Ig constant region or a portion thereof, and (4) the Ig constant region or a portion thereof is associated with the second Ig constant region or a portion thereof. In one embodiment, the Ig constant region or a portion thereof linked to the FVII protein or the XTEN sequence is further linked to the VWF fragment by a linker, *e.g.*, a processable linker. In another embodiment, the additional Ig constant region or a portion thereof useful for chimeric proteins of the invention can further be linked to the FVIII protein or the Ig constant region or a portion thereof by an optional linker, *e.g.*, a processable linker. In some embodiments, a pair of the Ig constant region or a portion thereof and the additional Ig constant region or a portion thereof, each of which are linked to the VWF fragment and the FVIII protein, provides a bond stronger than the non-covalent bond between the FVIII protein and the VWF fragment, *i.e.*, a covalent bond, *e.g.*, a disulfide bond, thereby preventing endogenous VWF from replacing the VWF fragment *in vivo*. In other embodiments, either or both of the Ig constant region or a portion thereof and the additional Ig constant region or a portion thereof are capable of extending a half-life of the FVIII protein or the VWF fragment. In other embodiments, the additional Ig constant region or a portion thereof comprises a second Fc region or an FcRn binding partner. The Ig constant region or a portion thereof and the additional Ig constant region or a portion thereof in the chimeric proteins are identical or different.

**[0130]** In certain embodiments, the Ig constant region or a portion thereof and the additional Ig constant region or a portion thereof are associated by a chemical association or a physical association. In one embodiment, the chemical association, *i.e.*, (:), is at least one non-peptide bond. In certain embodiments, the chemical association, *i.e.*, (:), is a covalent bond. In other embodiments, the chemical association, *i.e.*, (:), is a non-covalent interaction, *e.g.*, an ionic interaction, a hydrophobic interaction, a hydrophilic interaction, a Van der Waals interaction, or a hydrogen bond. In other embodiments, (:) is a non-peptide covalent bond. In still other embodiments, (:) is a peptide bond. In yet other embodiments, (:) represents a physical association between two sequences, wherein a portion of a first sequence is in close proximity to a second sequence



such that the first sequence shields or blocks a portion of the second sequence from interacting with another moiety. In some embodiments, the association between the Ig constant region or a portion thereof and the additional Ig constant region or a portion thereof can be a covalent bond, *e.g.*, a disulfide bond, which prevents replacement the VWF fragment or the polypeptide containing the VWF fragment with endogenous VWF. Therefore, preventing interaction between the FVIII protein and endogenous VWF reduces or eliminates this half-life limiting factor for the FVIII protein, and thus the half-life of the FVIII protein is extended compared to a FVIII protein without the VWF protein or wild-type FVIII.

**[0131]** In other aspects, a chimeric protein comprises a formula comprising:

- (1) FVIII(X1)-L1-F1:V-L2-X2-L3-F2;
- (2) FVIII(X1)-L1-F1:F2-L3-X2-L2-V;
- (3) F1-L1-FVIII(X1):V-L2-X2-L3-F2;
- (4) F1-L1-FVIII(X1):F2-L3-X2-L2-V;
- (5) FVIII(X1)-L1-F1-L4-V-L2-X2-L3-F2;
- (6) FVIII(X1)-L1-F1-L4-F2-L3-X2-L2-V;
- (7) F1-L1-FVIII(X1)-L4-V-L2-X2-L3-F2, or
- (8) F1-L1-FVIII(X1)-L4- F2-L3-X2-L2-V,

wherein FVIII(X1) comprises a FVIII protein and one or more XTEN sequences, wherein the one or more XTEN sequence are linked to the N-terminus or C-terminus of the FVIII protein or inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites disclosed herein) in the FVIII protein;

each of L1, L2, or L3 comprises an optional linker, *e.g.*, a cleavable linker;

L4 is a linker, *e.g.*, a processable linker;

X2 comprises one or more optional XTEN sequences;

F1 comprises an Ig constant region or a portion thereof;

F2 comprises an optional additional Ig constant region or a portion thereof, and

V comprises a VWF fragment;

(-) is a peptide bond or one or more amino acids; and

(:) comprises a chemical association or a physical association. In one embodiment, (:) represents a chemical association, *e.g.*, at least one non-peptide bond. In another embodiment, the chemical association, *i.e.*, (:) is a covalent bond. In other embodiments, the chemical association, *i.e.*, (:) is a non-covalent interaction, *e.g.*, an ionic interaction, a hydrophobic interaction, a hydrophilic interaction, a Van der Waals interaction, or a hydrogen bond. In other embodiments, (:) is a non-peptide covalent bond. In still other embodiments, (:) is a peptide bond. In yet other embodiments, (:) represents a physical association between two sequences, wherein a portion of a first sequence is in close proximity to a second sequence such that the first sequence shields or blocks a portion of the second sequence from interacting with another moiety, and further that this physical association is maintained without allowing the second sequence to interact with other moieties. The orientation of the polypeptide formulas herein is listed from N-terminus (left) to C-terminus (right). For example, formula V-X-FVIII means formula NH<sub>2</sub>-V-X-FVIII-COOH. In one embodiment, the formulas described herein can comprise any additional sequences between the two moieties. For example, formula V-X-FVIII can further comprise any sequences at the N-terminus of V between V and X, between X and FVIII, or at the C-terminus of FVIII unless otherwise specified. In another embodiment, the hyphen (-) indicates a peptide bond.

**[0132]** In one aspect, the chimeric protein comprises two polypeptide chains, (A) a first chain comprising (i) a single chain FVIII protein (ii) an XTEN sequence, and (iii) a first Ig constant region or a portion thereof, *e.g.*, a first Fc region or FcRn binding partner, wherein the XTEN sequence is linked to the FVIII protein at the N-terminus or C-terminus or inserted immediately downstream of one or more amino acids of the FVIII protein (*e.g.*, one or more XTEN insertion sites disclosed herein) and the first Ig constant region or a portion thereof is linked to the XTEN sequence when the XTEN sequence is linked to the FVIII protein at the N-terminus or the C-terminus or the FVIII protein when the XTEN sequence is inserted within the FVIII protein, and (B) a second chain comprising (iv) a VWF fragment comprising a D' domain and a D3 domain, (v) a linker, and (vi) a second Ig constant region or a portion thereof, *e.g.*, a second Fc region or a second FcRn binding partner, wherein the VWF fragment is linked to the linker, *e.g.*, a

cleavable linker, which is further linked to the second Ig constant region or a portion thereof, and wherein the first polypeptide chain and the second polypeptide chain are associated with each other, *e.g.*, a covalent bond, *e.g.*, a disulfide bond. In one embodiment, the linker is a cleavable linker described elsewhere herein, *e.g.*, a thrombin cleavable linker. In some embodiments, the second chain comprises one or more XTEN sequences between (iv) and (v) or (v) and (vi).

[0133] In other aspects, the chimeric protein comprises one polypeptide chain comprising (i) a single chain FVIII protein (ii) an XTEN sequence, (iii) a first Ig constant region or a portion thereof, *e.g.*, a first Fc region or a first FcRn binding partner, (iv) a first linker, (v) a VWF fragment comprising a D' domain and a D3 domain, (vi) a second linker, and (vii) a second Ig constant region or a portion thereof, *e.g.*, a second Fc region or a second FcRn binding partner, wherein (i) to (vii) are linked in the order or in any orders. In one embodiment, the first linker is a processable linker, which can be intracellularly processed or cleaved after expression and makes the single polypeptide chain into two polypeptide chains. In another embodiment, the second linker is a cleavable linker described herein, *e.g.*, a thrombin cleavable linker. The XTEN sequence used herein can be linked to the FVIII protein by an optional linker at the N-terminus or the C terminus of the FVIII protein or inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites) in the FVIII protein.

[0134] In certain aspects, a chimeric protein comprises three polypeptide chains, (A) a first polypeptide chain comprising (i) a heavy chain of a FVIII protein and (ii) an XTEN sequence, which are linked to each other and (B) a second polypeptide chain comprising (iii) a light chain of the FVIII protein and (iv) a first Ig constant region or a portion thereof, *e.g.*, a first Fc region or a first FcRn binding partner, which are linked to each other, and (C) a third polypeptide chain comprising (v) a VWF fragment comprising a D' domain and a D3 domain, (vi) a linker, and (vii) a second Ig constant region or a portion thereof, *e.g.*, a second Fc region or a second FcRn binding partner, wherein the second chain is associated with the first chain and the third chain. In one embodiment, the association between the first chain and the second chain is a chemical association or a physical association. For example, the association between the first chain and the

second chain can be a metal bond. In another embodiment, the association between the second chain and the third chain is also a chemical association or a physical association, *e.g.*, a covalent bond or a non-covalent bond. In certain embodiments, the association between the second chain and the third chain is through the two Ig constant regions or a portion thereof and is a disulfide bond. The bonding between the second chain and the third chain prevents or inhibits binding of the FVIII protein with endogenous VWF, thus preventing the FVIII protein being cleared by the VWF clearance pathway. In some embodiments, the linker is a processable linker, which is intracellularly cleaved after expression in a host cell. The XTEN sequence used herein is linked to the FVIII protein by an optional linker at the N-terminus or C terminus of the FVIII protein or inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites) in the FVIII protein.

**[0135]** In certain embodiments, the VWF fragment is directly linked to the FVIII protein, which comprises one or more XTENs, by a peptide bond or a linker. As one way of linking the VWF fragment and the FVIII protein, in which one or more XTENs are inserted or linked, through a direct link (*e.g.* a peptide bond) or a linker, an enzymatic ligation (*e.g.*, sortase) can be employed. For example, sortase refers to a group of prokaryotic enzymes that modify surface proteins by recognizing and cleaving a carboxyl-terminal sorting signal. For most substrates of sortase enzymes, the recognition signal consists of the motif LPXTG (Leu-Pro-any-Thr-Gly (SEQ ID NO: 51), then a highly hydrophobic transmembrane sequence, then a cluster of basic residues such as arginine. Cleavage occurs between the Thr and Gly, with transient attachment through the Thr residue to the active site Cys residue of a ligation partner, followed by transpeptidation that attaches the protein covalently to the cell wall. In some embodiments, the ligation partner contains Gly(n). In other embodiments, the chimeric protein further comprises a sortase recognition motif. In some embodiments, the VWF fragment is attached to FVIII comprising one or more XTENs inserted within or linked to using sortase mediated *in vitro* protein ligation.

**[0136]** In one embodiment, a VWF fragment linked to a sortase recognition motif by an optional linker can be fused to a FVIII protein linked to Gly(n) by a sortase, wherein n can be any integer and wherein one or more XTENs are inserted within

or linked to the FVIII protein. A ligation construct comprises the VWF fragment (N-terminal portion of the construct) and the FVIII protein, in which one or more XTENs are inserted or linked (C-terminal portion of the construct), wherein the sortase recognition motif is inserted in between. Another ligation construct comprises the VWF fragment (N-terminal portion of the construct, the linker, the sortase recognition motif, and the FVIII protein, in which one or more XTENs are inserted or linked (C-terminal portion of the construct). In another embodiment, a FVIII protein linked to a sortase recognition motif by an optional linker can be fused to a VWF fragment linked to Gly(n) by a sortase, wherein n is any integer. A resulting ligation construct comprises the FVIII protein (N-terminal portion of the construct), in which one or more XTENs are inserted or linked, and the VWF fragment (C-terminal portion of the construct), wherein the sortase recognition motif is inserted in between. Another resulting ligation construct comprises the FVIII protein (N-terminal portion of the construct), in which one or more XTENs are inserted or linked, the linker, the sortase recognition motif, and the VWF fragment (C-terminal portion of the construct). In other embodiments, a VWF fragment linked to a sortase recognition motif by a first optional linker can be fused to a heterologous moiety, *e.g.*, an immunoglobulin constant region or a portion thereof, *e.g.*, an Fc region, linked to a thrombin cleavage site by a second optional linker. A resulting construct can comprise the VWF fragment (N-terminal portion), the first linker, the sortase recognition motif, the protease cleavage site, the second optional linker, and the heterologous moiety.

[0137] In some embodiments, the VWF fragment is associated with the FVIII protein. The association between the VWF fragment and the FVIII protein can be a chemical association or a physical association. The chemical association can be a non-covalent interaction, *e.g.*, an ionic interaction, a hydrophobic interaction, a hydrophilic interaction, a Van der Waals interaction, or a hydrogen bond. In yet other embodiments, the association between the FVIII protein and the VWF fragment is a physical association between two sequences, *e.g.*, due to an additional association between the sequence having the FVIII protein and the sequence having the VWF fragment, wherein a portion of a first sequence is in close proximity to a second sequence such that the first sequence shields or blocks a portion of the second sequence from interacting with another moiety.

**[0138]** As a result of preventing or inhibiting endogenous VWF interaction with the FVIII protein by the VWF fragment, the chimeric protein described herein have an extended half-life compared to wild-type FVIII or the corresponding chimeric protein without the VWF fragment. In one embodiment, the half-life of the FVIII protein is extended at least about 1.5 times, at least about 2 times, at least about 2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than a FVIII protein without the VWF fragment. In another embodiment, the half-life of the FVIII protein is at least about 10 hours, at least about 11 hours, at least about 12 hours, at least about 13 hours, at least about 14 hours, at least about 15 hours, at least about 16 hours, at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours. In a particular embodiment, the half-life of the FVIII protein is extended at least 10 hours, at least about 11 hours, at least about 12 hours, at least about 13 hours, at least about 14 hours, at least about 15 hours, at least about 16 hours, at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, or at least about 27 hours in HemA mice.

#### **A) Von Willebrand Factor (VWF) Fragments**

**[0139]** VWF (also known as F8VWF) is a large multimeric glycoprotein present in blood plasma and produced constitutively in endothelium (in the Weibel-Palade bodies), megakaryocytes ( $\alpha$ -granules of platelets), and subendothelial connective tissue. The basic VWF monomer is a 2813 amino acid protein. Every monomer contains a number of specific domains with a specific function, the D'/D3 domain (which binds to Factor VIII), the A1 domain (which binds to platelet GPIb-receptor, heparin, and/or possibly collagen), the A3 domain (which binds to collagen), the C1 domain (in which the RGD domain binds to platelet integrin  $\alpha$ IIb $\beta$ 3 when this is activated), and the "cysteine knot" domain at the C-terminal

end of the protein (which VWF shares with platelet-derived growth factor (PDGF), transforming growth factor- $\beta$  (TGF $\beta$ ) and  $\beta$ -human chorionic gonadotropin ( $\beta$ HCG)).

[0140] The term "a VWF fragment" as used herein includes, but is not limited to, functional VWF fragments comprising a D' domain and a D3 domain, which are capable of inhibiting binding of endogenous VWF to FVIII. In one embodiment, the VWF fragment binds to the FVIII protein. In another embodiment, the VWF fragment blocks the VWF binding site on the FVIII protein, thereby inhibiting interaction of the FVIII protein with endogenous VWF. The VWF fragments include derivatives, variants, mutants, or analogues that retain these activities of VWF.

[0141] The 2813 monomer amino acid sequence for human VWF is reported as Accession Number \_NP\_000543.2\_ in Genbank. The nucleotide sequence encoding the human VWF is reported as Accession Number \_\_NM\_000552.3\_ in Genbank. The nucleotide sequence of human VWF is designated as SEQ ID NO: 1. SEQ ID NO: 2 is the amino acid sequence encoded by SEQ ID NO: 1. Each domain of VWF is listed in Table 1.

**TABLE 1. VWF Sequences**

| VWF domains                                                    | Amino acid Sequence |                                 |                              |
|----------------------------------------------------------------|---------------------|---------------------------------|------------------------------|
| VWF Signal Peptide<br>(Amino acids 1 to 22<br>of SEQ ID NO: 2) | 1                   | <u>MIPARFAGVL LALALILPGT LC</u> |                              |
|                                                                | 22                  |                                 |                              |
| VWF D1D2 region<br>(Amino acids 23 to<br>763 of SEQ ID NO: 2)  | 23                  | <b>AEGTRGRS</b>                 |                              |
|                                                                |                     | <b>STARCSLFGS</b>               | <b>DFVNTFDGSM</b>            |
|                                                                | 51                  | <b>YSFAGYCSYL</b>               | <b>LAGGCQKRSF SIIGDFQNGK</b> |
|                                                                |                     | <b>RVSLSVYLGE</b>               | <b>FFDIHLFVNG</b>            |
|                                                                | 101                 | <b>TVTQGDQRV</b>                | <b>MPYASKGLYL ETEAGYYKLS</b> |
|                                                                |                     | <b>GEAYGFVARI</b>               | <b>DGSGNFQVLL</b>            |
|                                                                | 151                 | <b>SDRYFNKTCG</b>               | <b>LCGNFNIFAE DDFMTQEGTL</b> |
|                                                                |                     | <b>TSDPYDFANS</b>               | <b>WALSSGEQWC</b>            |
|                                                                | 201                 | <b>ERASPPSSSC</b>               | <b>NISSGEMQKG LWEQCQLLKS</b> |
|                                                                |                     | <b>TSVFARCHPL</b>               | <b>VDPEPFVALC</b>            |
|                                                                | 251                 | <b>EKTLCECAGG</b>               | <b>LECACPALLE YARTCAQEGM</b> |
|                                                                |                     | <b>VLYGWTDHSA</b>               | <b>CSPVCPAGME</b>            |
|                                                                | 301                 | <b>YRQCVSPCAR</b>               | <b>TCQSLHINEM CQERCVDGCS</b> |
|                                                                |                     | <b>CPEGQLLDEG</b>               | <b>LCVESTECPC</b>            |
|                                                                | 351                 | <b>VHSGKRYPPG</b>               | <b>TSLSRDCNTC ICRNSQWICS</b> |
|                                                                |                     | <b>NEECPGECLV</b>               | <b>TGQSHFKSFD</b>            |
|                                                                | 401                 | <b>NRYFTFSGIC</b>               | <b>QYLLARDCQD HSFSIVIETV</b> |

|               |      |                                                                                   |               |
|---------------|------|-----------------------------------------------------------------------------------|---------------|
|               | 451  | QCADDRDAVC TRSVTVRLPG<br>LHNSLVKLKH GAGVAMDQD IQLPLLKGDL<br>RIQHTVTASV RLSYGEDLQM |               |
|               | 501  | DWDGRGRLV KLSPVYAGKT CGLCGNYNGN<br>QGDDFLTPSG LAEPRVEDFG                          |               |
|               | 551  | NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS<br>EEACAVLTSP TFEACHRAVS                        |               |
|               | 601  | PLPYLRNCRY DVCSCSDGRE CLCGALASYA<br>AACAGRGVRV AWREPGRCEL                         |               |
|               | 651  | NCPKGQVYLQ CGTPCNLTCT SLSYPDEECN<br>EACLEGCFCP PGLYMDERGD                         |               |
|               | 701  | CVPKAQCPCY YDGEIFQPED IFSDHHTMCY<br>CEDGFMHCTM SGVPGSLLPD                         |               |
|               | 751  | AVLSSPLSHR                                                                        | SKR           |
|               | 763  |                                                                                   |               |
| VWF D' Domain | 764  | <u>SLSCRPP MVKLVC PADN</u><br><u>LRAEGLECTK</u><br><u>TCQNYDLECM</u>              |               |
|               | 801  | <u>SMGCVSGCLC PPGMVRHENR CVALERCPCF</u><br><u>HQKEYAPGE TVKIGCNTCV</u>            |               |
|               | 851  | <u>CRDRKWNCTD</u>                                                                 | <u>HVCDAT</u> |
|               | 866  |                                                                                   |               |
| VWF D3 Domain | 867  | <u>CSTI_GMAHYLTFDG</u><br><u>LKYLFPGECQ YVLVQDYCGS</u>                            |               |
|               | 901  | <u>NPGTFRILVG NKGCSHPSVK CKKRVITLVE</u><br><u>GGEIELFDGE VNVKRPMKDE</u>           |               |
|               | 951  | <u>THFEVVESGR YIILLGKAL SVVWDRHLSI</u><br><u>SVVLKQTYQE KVCGLCGNFD</u>            |               |
|               | 1001 | <u>GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ</u><br><u>CADTRKVPLD SSPATCHNNI</u>           |               |
|               | 1051 | <u>MKQTMVDSSC RILTSDVFQD CNKLVDPEPY</u><br><u>LDVCIYDTCS CESIGDCACF</u>           |               |
|               | 1101 | <u>CDTIAAYAHV CAQHGKVVTW RTATLCPQSC</u><br><u>EERNLRENGY ECEWRYNSCA</u>           |               |
|               | 1151 | <u>PACQVTCQHP EPLACPVQCV EGCHAHCPPG</u><br><u>KILDELLQTC VDPEDCPVCE</u>           |               |
|               | 1201 | <u>VAGRRFASGK KVTLNPSDPE HCQICHCDVV</u><br><u>NLTCEACQEP</u>                      |               |
|               | 1240 |                                                                                   |               |
| VWF A1 Domain | 1241 | GGLVVPPTDA                                                                        |               |
|               | 1251 | PVSPPTLYVE DISEPPLHDF YCSRLLDLVE<br>LLDGSSRLSE AEFEVLKAFV                         |               |
|               | 1301 | VDMMERLRIS QKWVRVAVVE YHDGSHAYIG<br>LKDRKRPSEL RRIASQVKYA                         |               |
|               | 1351 | GSQVASTSEV LKYTLFQIFS KIDRPEASRI<br>ALLLMASQEP QRMSRNFVRY                         |               |
|               | 1401 | VQGLKKKKVI VIPVGIGPHA NLKQIRLIEK                                                  |               |



|  |      |                                  |                                       |
|--|------|----------------------------------|---------------------------------------|
|  | 1451 | QAPENKAFVL<br>DEIVSYLCDL<br>1479 | SSVDELEQQR<br>APEAPPPTLP<br>PDMAQVTVG |
|  | 1480 |                                  | P                                     |
|  |      | GLLGVSTLGP                       | KRNSMVLDVA                            |
|  | 1501 | FVLEGSDKIG<br>GQDSIHVTVL         | EADFNRSKEF<br>QYSYMTVEY               |
|  | 1551 | PFSEAQSKGD<br>ALRYLSDHSF<br>1600 | ILQRVREIRY<br>LVSQGDREQA              |
|  | 1601 | PNLVYMTGN                        | PASDEIKRLP                            |
|  |      | GPANANVQELE                      | RIGWPNAPIL                            |
|  | 1651 | IQDFETLPRE<br>SPAPDCSQPL         | APDLVLQRCC<br>DVILLLDGSS              |
|  | 1701 | SFPASYFDEM                       | KSFAKAFISK                            |
|  |      | SVLQYGSITT                       | IDVPWNVPE                             |
|  | 1751 | KAHLLSLVDV<br>LTSEMHGARP         | MQREGGPSQI<br>GASKAVVILV              |
|  | 1801 | TDVSVDSVDA                       | AADAARSNRV                            |
|  |      | YDAAQLRILA                       | GPAGDSNVVK                            |
|  | 1851 | LQRIEDLPTM                       | VTLGNSFLHK                            |
|  |      | DEDGNEKRPG                       | DVWTLPDQCH                            |
|  | 1901 | TVTCQPDGQT                       | LLKSHRVNCD                            |
|  |      | QSPVKVEETC                       | GCRWTCPCVC                            |
|  | 1951 | TGSSTRHIVT                       | FDGQNFKLTG                            |
|  |      | EQDLEVILHN                       | GACSPGARQG                            |
|  | 2001 | CMKSIEVKHS                       | ALSVEXHSDM                            |
|  |      | VPYVGGNMEV                       | NVYGAIMHEV                            |
|  | 2051 | RFNHLGHIFT                       | FTPQNNFQQL                            |
|  |      | TYGLCGICDE                       | NGANDFMLRD                            |
|  | 2101 | GTVTTDWCTL                       | VQEWTVQRPQ                            |
|  |      | CLVPDSSHQ                        | VLLLPLFAEC                            |
|  | 2151 | HKVLAPATFY                       | AICQQDSCHQ                            |
|  |      | AHLCRTNGVC                       | VDWRTPDFCA                            |
|  | 2201 | MSCPPSLVYN                       | HCEHGCPRHC                            |
|  |      | PSEGCFPCPD                       | KVMLEGSCVP                            |
|  | 2251 | EEACTQCIGE                       | DGVQHQFLEA                            |
|  |      | CTCLSGRKVN                       | CTTQPCPTAK                            |
|  | 2301 | APTCGLCEVA                       | RLRQNAQDCC                            |
|  |      | SCDLPPVPHC                       | ERGLQPTLTN                            |
|  | 2351 | PGECPNFTC                        | ACRKEECKRV                            |
|  |      | PTLRKTQCCD                       | EYECACNCVN                            |
|  | 2401 | STVSCPLGYL                       | ASTATNDCGC                            |
|  |      | CVHRSTIYPV                       | GQFWEEGCDV                            |
|  | 2451 | CTCTDMEDAV                       | MGLRVAQCSQ                            |
|  |      | FTYVLHEGEC                       | CGRCLPSACE                            |
|  | 2501 | VVTGSPRGDS                       | QSSWKSQGSQ                            |
|  |      | NECVRVKEEV                       | FIQQRNVSCP                            |
|  | 2551 | QLEVPVCPSP                       | FQLSCKTSAC                            |
|  |      | ACMLNGTVIG                       | PGKTVMIDVC                            |

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                 | 2601 TTCRCMVQVG VISGFKLECR KTTNCPCLG<br>YKEENNTGEC CGRCLPTACT<br>2651 IQLRGGQIMT LKRDETLQDG CDTHFCKVNE<br>RGEYFWEKRV TGCPPFDEHK<br>2701 CLAEKKIMK IPGTCCDTCE EPECNDITAR<br>LQYVKVGSCK SEVEVDIHYC<br>2751 QGKCASKAMY SIDINDVQDQ CSCCSPTRTE<br>PMQVALHCTN GSVVYHEVLN<br>2801 AMECKCSPRK CSK                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|                 | <b>Nucleotide Sequence (SEQ ID NO: 1)</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Full-length VWF | 1 ATGATTCCTG CCAGATTTCG<br>CGGGGTGCTG CTTGCTCTGG CCCTCATTTT<br>51 GCCAGGGACC CTTTGTGCAG<br>AAGGAACCTG CGGCAGGTCA TCCACGGCCC<br>101 GATGCAGCCT TTTCGGAAGT<br>GACTTCGTCA ACACCTTTGA TGGGAGCATG<br>151 TACAGCTTTG CGGGATACTG<br>CAGTTACCTC CTGGCAGGGG GCTGCCAGAA<br>201 ACGCTCCTTC TCGATTATTG<br>GGGACTTCCA GAATGGCAAG AGAGTGAGCC<br>251 TCTCCGTGTA TCTTGGGGAA<br>TTTTTTGACA TCCATTTGTT TGTCAATGGT<br>301 ACCGTGACAC AGGGGGACCA<br>AAGAGTCTCC ATGCCCTATG CCTCCAAAGG<br>351 GCTGTATCTA GAAACTGAGG<br>CTGGGTACTA CAAGCTGTCC GGTGAGGCCT<br>401 ATGGCTTTGT GGCCAGGATC<br>GATGGCAGCG GCAACTTTCA AGTCCTGCTG<br>451 TCAGACAGAT ACTTCAACAA<br>GACCTGCGGG CTGTGTGGCA ACTTTAACAT<br>501 CTTTGCTGAA GATGACTTTA<br>TGACCCAAGA AGGGACCTTG ACCTCGGACC<br>551 CTTATGACTT TGCCAACTCA<br>TGGGCTCTGA GCAGTGGAGA ACAGTGGTGT<br>601 GAACGGGCAT CTCCTCCAG<br>CAGCTCATGC AACATCTCCT CTGGGGAAAT<br>651 GCAGAAGGGC CTGTGGGAGC<br>AGTGCCAGCT TCTGAAGAGC ACCTCGGTGT<br>701 TTGCCCCTG CCACCTCTG<br>GTGGACCCCG AGCCTTTTGT GGCCCTGTGT<br>751 GAGAAGACTT TGTGTGAGTG<br>TGCTGGGGGG CTGGAGTGCG CCTGCCCTGC<br>801 CCTCCTGGAG TACGCCCGGA<br>CCTGTGCCCCA GGAGGGAATG GTGCTGTACG<br>851 GCTGGACCGA CCACAGCGCG<br>TGCAGCCCAG TGTGCCCTGC TGGTATGGAG<br>901 TATAGGCAGT GTGTGTCCCC<br>TTGCGCCAGG ACCTGCCAGA GCCTGCACAT<br>951 CAATGAAATG TGTCAGGAGC |

|      |                                  |
|------|----------------------------------|
|      | GATGCGTGGA TGGCTGCAGC TGCCCTGAGG |
| 1001 | GACAGCTCCT GGATGAAGGC            |
|      | CTCTGCGTGG AGAGCACCGA GTGTCCCTGC |
| 1051 | GTGCATTCCG GAAAGCGCTA            |
|      | CCCTCCCGGC ACCTCCCTCT CTCGAGACTG |
| 1101 | CAACACCTGC ATTTGCCGAA            |
|      | ACAGCCAGTG GATCTGCAGC AATGAAGAAT |
| 1151 | GTCCAGGGGA GTGCCTTGTC            |
|      | ACTGGTCAAT CCCACTTCAA GAGCTTTGAC |
| 1201 | AACAGATACT TCACCTTCAG            |
|      | TGGGATCTGC CAGTACCTGC TGGCCCGGGA |
| 1251 | TTGCCAGGAC CACTCCTTCT            |
|      | CCATTGTCAT TGAGACTGTC CAGTGTGCTG |
| 1301 | ATGACCGCGA CGCTGTGTGC            |
|      | ACCCGCTCCG TCACCGTCCG GCTGCCTGGC |
| 1351 | CTGCACAACA GCCTTGTA              |
|      | ACTGAAGCAT GGGGCAGGAG TTGCCATGGA |
| 1401 | TGGCCAGGAC ATCCAGCTCC            |
|      | CCCTCCTGAA AGGTGACCTC CGCATCCAGC |
| 1451 | ATACAGTGAC GGCCTCCGTG            |
|      | CGCCTCAGCT ACGGGGAGGA CCTGCAGATG |
| 1501 | GACTGGGATG GCCGCGGGAG            |
|      | GCTGCTGGTG AAGCTGTCCC CCGTCTATGC |
| 1551 | CGGGAAGACC TGCGGCCTGT            |
|      | GTGGGAATTA CAATGGCAAC CAGGGCGACG |
| 1601 | ACTTCCTTAC CCCCTCTGGG            |
|      | CTGGCRGAGC CCCGGGTGGA GGACTTCGGG |
| 1651 | AACGCCTGGA AGCTGCACGG            |
|      | GGACTGCCAG GACCTGCAGA AGCAGCACAG |
| 1701 | CGATCCCTGC GCCCTCAACC            |
|      | CGCGCATGAC CAGGTTCTCC GAGGAGGCGT |
| 1751 | GCGCGGTCCT GACGTCCCCC            |
|      | ACATTCGAGG CCTGCCATCG TGCCGTCAGC |
| 1801 | CCGCTGCCCT ACCTGCGGAA            |
|      | CTGCCGCTAC GACGTGTGCT CCTGCTCGGA |
| 1851 | CGGCCGCGAG TGCCTGTGCG            |
|      | GCGCCCTGGC CAGCTATGCC GCGGCCTGCG |
| 1901 | CGGGGAGAGG CGTGCGCGTC            |
|      | GCGTGGCGCG AGCCAGGCCG CTGTGAGCTG |
| 1951 | AACTGCCCCA AAGGCCAGGT            |
|      | GTACCTGCAG TCGGGGACCC CCTGCAACCT |
| 2001 | GACCTGCCGC TCTCTCTCTT            |
|      | ACCCGGATGA GGAATGCAAT GAGGCCTGCC |
| 2051 | TGGAGGGCTG CTTCTGCCCC            |
|      | CCAGGGCTCT ACATGGATGA GAGGGGGGAC |
| 2101 | TGCGTGCCCA AGGCCAGTG             |
|      | CCCCTGTTAC TATGACGGTG AGATCTTCCA |
| 2151 | GCCAGAAGAC ATCTTCTCAG            |
|      | ACCATCACAC CATGTGCTAC TGTGAGGATG |
| 2201 | GCTTCATGCA CTGTACCATG            |
|      | AGTGGAGTCC CCGGAAGCTT GCTGCCTGAC |

|      |                                   |
|------|-----------------------------------|
| 2251 | GCTGTCCTCA GCAGTCCCCCT            |
|      | GTCTCATCGC AGCAAAAGGA GCCTATCCTG  |
| 2301 | TCGGCCCCCC ATGGTCAAGC             |
|      | TGGTGTGTCC CGCTGACAAC CTGCGGGCTG  |
| 2351 | AAGGGCTCGA GTGTACCAAA             |
|      | ACGTGCCAGA ACTATGACCT GGAGTGCATG  |
| 2401 | AGCATGGGCT GTGTCTCTGG             |
|      | CTGCCTCTGC CCCCCGGGCA TGGTCCGGCA  |
| 2451 | TGAGAACAGA TGTGTGGCCC             |
|      | TGGAAAGGTG TCCCTGCTTC CATCAGGGCA  |
| 2501 | AGGAGTATGC CCCTGGAGAA             |
|      | ACAGTGAAGA TTGGCTGCAA CACTTGTGTC  |
| 2551 | TGTCGGGACC GGAAGTGGAA             |
|      | CTGCACAGAC CATGTGTGTG ATGCCACGTG  |
| 2601 | CTCCACGATC GGCATGGCCC             |
|      | ACTACCTCAC CTTCGACGGG CTCAAATACC  |
| 2651 | TGTTCCCCCG GGAGTGCCAG             |
|      | TACGTTCTGG TGCAGGATTA CTGCGGCAGT  |
| 2701 | AACCCTGGGA CCTTTCGGAT             |
|      | CCTAGTGGGG AATAAGGGAT GCAGCCACCC  |
| 2751 | CTCAGTGAAA TGCAAGAAAC             |
|      | GGGTCACCAT CCTGGTGGAG GGAGGAGAGA  |
| 2801 | TTGAGCTGTT TGACGGGGAG             |
|      | GTGAATGTGA AGAGGCCCAT GAAGGATGAG  |
| 2851 | ACTCACTTTG AGGTGGTGGAA            |
|      | GTCTGGCCGG TACATCATTC TGCTGCTGGG  |
| 2901 | CAAAGCCCTC TCCGTGGTCT             |
|      | GGGACCGCCA CCTGAGCATC TCCGTGGTCC  |
| 2951 | TGAAGCAGAC ATACCAGGAG             |
|      | AAAGTGTGTG GCCTGTGTGG GAATTTTGAT  |
| 3001 | GGCATCCAGA ACAATGACCT             |
|      | CACCAGCAGC AACCTCCAAG TGGAGGAAGA  |
| 3051 | CCCTGTGGAC TTTGGGAACT             |
|      | CCTGGAAAGT GAGCTCGCAG TGTGCTGACA  |
| 3101 | CCAGAAAAGT GCCTCTGGAC             |
|      | TCATCCCCCTG CCACCTGCCA TAACAACATC |
| 3151 | ATGAAGCAGA CGATGGTGGAA            |
|      | TTCTCCTGT AGAATCCTTA CCAGTGACGT   |
| 3201 | CTTCCAGGAC TGCAACAAGC             |
|      | TGGTGGACCC CGAGCCATAT CTGGATGTCT  |
| 3251 | GCATTTACGA CACCTGCTCC             |
|      | TGTGAGTCCA TTGGGGACTG CGCCTGCTTC  |
| 3301 | TGCGACACCA TTGCTGCCTA             |
|      | TGCCCACGTG TGTGCCCAGC ATGGCAAGGT  |
| 3351 | GGTGACCTGG AGGACGGCCA             |
|      | CATTGTGCCC CCAGAGCTGC GAGGAGAGGA  |
| 3401 | ATCTCCGGGA GAACGGGTAT             |
|      | GAGTGTGAGT GCGCTATAA CAGCTGTGCA   |
| 3451 | CCTGCCTGTC AAGTCACGTG             |
|      | TCAGCACCTT GAGCCACTGG CCTGCCCTGT  |
| 3501 | GCAGTGTGTG GAGGGCTGCC             |

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|  | ATGCCCACTG CCCTCCAGGG AAAATCCTGG<br>3551 ATGAGCTTTT GCAGACCTGC<br>GTTGACCCTG AAGACTGTCC AGTGTGTGAG<br>3601 GTGGCTGGCC GGCCTTTTGC<br>CTCAGGAAAG AAAGTCACCT TGAATCCCAG<br>3651 TGACCCTGAG CACTGCCAGA<br>TTTGCCACTG TGATGTTGTC AACCTCACCT<br>3701 GTGAAGCCTG CCAGGAGCCG<br>GGAGGCCTGG TGGTGCCTCC CACAGATGCC<br>3751 CCGGTGAGCC CCACCACTCT<br>GTATGTGGAG GACATCTCGG AACCGCCGTT<br>3801 GCACGATTTT TACTGCAGCA<br>GGCTACTGGA CCTGGTCTTC CTGCTGGATG<br>3851 GCTCCTCCAG GCTGTCCGAG<br>GCTGAGTTTG AAGTGCTGAA GGCCTTTGTG<br>3901 GTGGACATGA TGGAGCGGCT<br>GCGCATCTCC CAGAAGTGGG TCCGCGTGGC<br>3951 CGTGGTGGAG TACCACGACG<br>GCTCCACGC CTACATCGGG CTCAAGGACC<br>4001 GGAAGCGACC GTCAGAGCTG<br>CGGCGCATTG CCAGCCAGGT GAAGTATGCG<br>4051 GGCAGCCAGG TGGCCTCCAC<br>CAGCGAGGTC TTGAAATACA CACTGTTCCA<br>4101 AATCTTCAGC AAGATCGACC<br>GCCCTGAAGC CTCCCGCATC GCCCTGCTCC<br>4151 TGATGGCCAG CCAGGAGCCC<br>CAACGGATGT CCCGGAAGTT TGTCCGCTAC<br>4201 GTCCAGGGCC TGAAGAAGAA<br>GAAGGTCATT GTGATCCCGG TGGGCATTGG<br>4251 GCCCCATGCC AACCTCAAGC<br>AGATCCGCCT CATCGAGAAG CAGGCCCCCTG<br>4301 AGAACAAGGC CTTCGTGCTG<br>AGCAGTGTGG ATGAGCTGGA GCAGCAAAGG<br>4351 GACGAGATCG TTAGCTACCT<br>CTGTGACCTT GCCCCTGAAG CCCCTCCTCC<br>4401 TACTCTGCCC CCCGACATGG<br>CACAAGTCAC TGTGGGCCCC GGGCTCTTGG<br>4451 GGGTTTCGAC CCTGGGGCCC<br>AAGAGGAACT CCATGGTTCT GGATGTGGCG<br>4501 TTCGTCCTGG AAGGATCGGA<br>CAAAATTGGT GAAGCCGACT TCAACAGGAG<br>4551 CAAGGAGTTC ATGGAGGAGG<br>TGATTCAGCG GATGGATGTG GGCCAGGACA<br>4601 GCATCCACGT CACGGTGTG<br>CAGTACTCCT ACATGGTGAC CGTGGAGTAC<br>4651 CCCTTCAGCG AGGCACAGTC<br>CAAAGGGGAC ATCCTGCAGC GGGTGCAGAG<br>4701 GATCCGCTAC CAGGGCGGCA<br>ACAGGACCAA CACTGGGCTG GCCCTGCGGT<br>4751 ACCTCTCTGA CCACAGCTTC<br>TTGGTCAGCC AGGGTGACCG GGAGCAGGCG |
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| 4801 | CCCAACCTGG TCTACATGGT             |
|      | CACCGGAAAT CCTGCCTCTG ATGAGATCAA  |
| 4851 | GAGGCTGCCT GGAGACATCC             |
|      | AGGTGGTGCC CATTGGAGTG GGCCCTAATG  |
| 4901 | CCAACGTGCA GGAGCTGGAG             |
|      | AGGATTGGCT GGCCCAATGC CCCTATCCTC  |
| 4951 | ATCCAGGACT TTGAGACGCT             |
|      | CCCCCGAGAG GCTCCTGACC TGGTGCTGCA  |
| 5001 | GAGGTGCTGC TCCGGAGAGG             |
|      | GGCTGCAGAT CCCACCCCTC TCCCCTGCAC  |
| 5051 | CTGACTGCAG CCAGCCCCTG             |
|      | GACGTGATCC TTCTCCTGGA TGGCTCCTCC  |
| 5101 | AGTTTCCCAG CTTCTTATTT             |
|      | TGATGAAATG AAGAGTTTCG CCAAGGCTTT  |
| 5151 | CATTTCAAAA GCCAATATAG             |
|      | GGCCTCGTCT CACTCAGGTG TCAGTGCTGC  |
| 5201 | AGTATGGAAG CATCACCACC             |
|      | ATTGACGTGC CATGGAACGT GGTCCCGGAG  |
| 5251 | AAAGCCCATT TGCTGAGCCT             |
|      | TGTGGACGTC ATGCAGCGGG AGGGAGGCCC  |
| 5301 | CAGCCAAATC GGGGATGCCT             |
|      | TGGGCTTTGC TGTGCGATAC TTGACTTCAG  |
| 5351 | AAATGCATGG TGCCAGGCCG             |
|      | GGAGCCTCAA AGGCGGTGGT CATCCTGGTC  |
| 5401 | ACGGACGTCT CTGTGGATTC             |
|      | AGTGGATGCA GCAGCTGATG CCGCCAGGTC  |
| 5451 | CAACAGAGTG ACAGTGTTCC             |
|      | CTATTGGAAT TGGAGATCGC TACGATGCAG  |
| 5501 | CCCAGCTACG GATCTTGGA              |
|      | GGCCCAGCAG GCGACTCCAA CGTGGTGAAG  |
| 5551 | CTCCAGCGAA TCGAAGACCT             |
|      | CCCTACCATG GTCACCTTGG GCAATTCCTT  |
| 5601 | CCTCCACAAA CTGTGCTCTG             |
|      | GATTTGTTAG GATTTGCATG GATGAGGATG  |
| 5651 | GGAATGAGAA GAGGCCCGGG             |
|      | GACGTCTGGA CCTTGCCAGA CCAGTGCCAC  |
| 5701 | ACCGTGACTT GCCAGCCAGA             |
|      | TGGCCAGACC TTGCTGAAGA GTCATCGGGT  |
| 5751 | CAACTGTGAC CGGGGGCTGA             |
|      | GGCCTTCGTG CCCTAACAGC CAGTCCCCTG  |
| 5801 | TTAAAGTGGA AGAGACCTGT             |
|      | GGCTGCCGCT GGACCTGCCC CTGYGTGTGC  |
| 5851 | ACAGGCAGCT CCACTCGGCA             |
|      | CATCGTGACC TTTGATGGGC AGAATTTCAA  |
| 5901 | GCTGACTGGC AGCTGTTCTT             |
|      | ATGTCCTATT TCAAAACAAG GAGCAGGACC  |
| 5951 | TGGAGGTGAT TCTCCATAAT             |
|      | GGTGCCCTGCA GCCCTGGAGC AAGGCAGGGC |
| 6001 | TGCATGAAAT CCATCGAGGT             |
|      | GAAGCACAGT GCCCTCTCCG TCGAGSTGCA  |
| 6051 | CAGTGACATG GAGGTGACGG             |

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|  | TGAATGGGAG ACTGGTCTCT GTTCCTTACG<br>6101 TGGGTGGGAA CATGGAAGTC<br>AACGTTTATG GTGCCATCAT GCATGAGGTC<br>6151 AGATTCAATC ACCTTGGTCA<br>CATCTTCACA TTCACTCCAC AAAACAATGA<br>6201 GTTCCAAGT CAGCTCAGCC<br>CCAAGACTTT TGCTTCAAAG ACGTATGGTC<br>6251 TGTGTGGGAT CTGTGATGAG<br>AACGGAGCCA ATGACTTCAT GCTGAGGGAT<br>6301 GGCACAGTCA CCACAGACTG<br>GAAAACACTT GTTCAGGAAT GGAAGTGCA<br>6351 GCGGCCAGGG CAGACGTGCC<br>AGCCCATCCT GGAGGAGCAG TGTCTTGTCC<br>6401 CCGACAGCTC CCACTGCCAG<br>GTCCTCCTCT TACCACTGTT TGCTGAATGC<br>6451 CACAAGGTCC TGGCTCCAGC<br>CACATTCTAT GCCATCTGCC AGCAGGACAG<br>6501 TTGCCACCAG GAGCAAGTGT<br>GTGAGGTGAT CGCCTCTTAT GCCCACCTCT<br>6551 GTCGGACCAA CGGGGTCTGC<br>GTTGAAGTGA GGACACCTGA TTTCTGTGCT<br>6601 ATGTCATGCC CACCATCTCT<br>GGTCTACAAC CACTGTGAGC ATGGCTGTCC<br>6651 CCGGCACTGT GATGGCAACG<br>TGAGCTCCTG TGGGGACCAT CCCTCCGAAG<br>6701 GCTGTTTCTG CCCTCCAGAT<br>AAAGTCATGT TGGAAGGCAG CTGTGTCCCT<br>6751 GAAGAGGCCT GCACTCAGTG<br>CATTGGTGAG GATGGAGTCC AGCACCAGTT<br>6801 CCTGGAAGCC TGGGTCCCGG<br>ACCACCAGCC CTGTCAGATC TGCACATGCC<br>6851 TCAGCGGGCG GAAGGTCAAC<br>TGCACAACGC AGCCCTGCCC CACGGCCAAA<br>6901 GCTCCCACGT GTGGCCTGTG<br>TGAAGTAGCC CGCCTCCGCC AGAATGCAGA<br>6951 CCAGTGCTGC CCCGAGTATG<br>AGTGTGTGTG TGACCCAGTG AGCTGTGACC<br>7001 TGCCCCCAGT GCCTCACTGT<br>GAACGTGGCC TCCAGCCCAC ACTGACCAAC<br>7051 CCTGGCGAGT GCAGACCCAA<br>CTTACCTGC GCCTGCAGGA AGGAGGAGTG<br>7101 CAAAAGAGTG TCCCCACCCT<br>CCTGCCCCC GCACCGTTTG CCCACCCTTC<br>7151 GGAAGACCCA GTGCTGTGAT<br>GAGTATGAGT GTGCCTGCAA CTGTGTCAAC<br>7201 TCCACAGTGA GCTGTCCCCT<br>TGGGTACTTG GCCTCAACCG CCACCAATGA<br>7251 CTGTGGCTGT ACCACAACCA<br>CCTGCCTTCC CGACAAGGTG TGTGTCCACC<br>7301 GAAGCACCAT CTACCCTGTG<br>GGCCAGTTCT GGGAGGAGGG CTGCGATGTG |
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|  | 7351 TGCACCTGCA CCGACATGGA<br>GGATGCCGTG ATGGGCCTCC GCGTGGCCCA<br>7401 GTGCTCCCAG AAGCCCTGTG<br>AGGACAGCTG TCGGTCGGGC TTCACTTACG<br>7451 TTCTGCATGA AGGCGAGTGC<br>TGTGGAAGGT GCCTGCCATC TGCCTGTGAG<br>7501 GTGGTGA CTG GCTCACC GCG<br>GGGGGACTCC CAGTCTTCCT GGAAGAGTGT<br>7551 CGGCTCCCAG TGGGCCTCCC<br>CGGAGAACCC CTGCCTCATC AATGAGTGTG<br>7601 TCCGAGTGAA GGAGGAGGTC<br>TTTATACAAC AAAGGAACGT CTCCTGCCCC<br>7651 CAGCTGGAGG TCCCTGTCTG<br>CCCCTCGGGC TTTCAGCTGA GCTGTAAGAC<br>7701 CTCAGCGTGC TGCCCAAGCT<br>GTCGCTGTGA GCGCATGGAG GCCTGCATGC<br>7751 TCAATGGCAC TGTCATTGGG<br>CCCGGGAAGA CTGTGATGAT CGATGTGTGC<br>7801 ACGACCTGCC GCTGCATGGT<br>GCAGGTGGGG GTCATCTCTG GATTCAAGCT<br>7851 GGAGTGCAGG AAGACCACCT<br>GCAACCCCTG CCCCCTGGGT TACAAGGAAG<br>7901 AAAATAACAC AGGTGAATGT<br>TGTGGGAGAT GTTTGCCTAC GGCTTGCACC<br>7951 ATTCAGCTAA GAGGAGGACA<br>GATCATGACA CTGAAGCGTG ATGAGACGCT<br>8001 CCAGGATGGC TGTGATACTC<br>ACTTCTGCAA GGTCAATGAG AGAGGAGAGT<br>8051 ACTTCTGGGA GAAGAGGGTC<br>ACAGGCTGCC CACCCTTTGA TGAACACAAG<br>8101 TGTCTTGCTG AGGGAGGTAA<br>AATTATGAAA ATTCCAGGCA CCTGCTGTGA<br>8151 CACATGTGAG GAGCCTGAGT<br>GCAACGACAT CACTGCCAGG CTGCAGTATG<br>8201 TCAAGGTGGG AAGCTGTAA<br>TCTGAAGTAG AGGTGGATAT CCACTACTGC<br>8251 CAGGGCAAAT GTGCCAGCAA<br>AGCCATGTAC TCCATTGACA TCAACGATGT<br>8301 GCAGGACCAG TGCTCCTGCT<br>GCTCTCCGAC ACGGACGGAG CCCATGCAGG<br>8351 TGGCCCTGCA CTGCACCAAT<br>GGCTCTGTTG TGTACCATGA GGTTCTCAAT<br>8401 GCCATGGAGT GCAAATGCTC<br>CCCCAGGAAG TGCAGCAAGT GA |
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[0142] The VWF fragment as used herein can be a VWF fragment comprising a D' domain and a D3 domain of VWF, wherein the VWF fragment binds to Factor



VIII (FVIII) and inhibits binding of endogenous VWF (full-length VWF) to FVIII. The VWF fragment comprising the D' domain and the D3 domain can further comprise a VWF domain selected from the group consisting of an A1 domain, an A2 domain, an A3 domain, a D1 domain, a D2 domain, a D4 domain, a B1 domain, a B2 domain, a B3 domain, a C1 domain, a C2 domain, a CK domain, one or more fragments thereof, and any combinations thereof. In one embodiment, a VWF fragment comprises, consists essentially of, or consists of: (1) the D' and D3 domains of VWF or fragments thereof; (2) the D1, D', and D3 domains of VWF or fragments thereof; (3) the D2, D', and D3 domains of VWF or fragments thereof; (4) the D1, D2, D', and D3 domains of VWF or fragments thereof; or (5) the D1, D2, D', D3, and A1 domains of VWF or fragments thereof. The VWF fragment described herein does not contain a site binding to a VWF clearance receptor. In another embodiment, the VWF fragment described herein is not amino acids 764 to 1274 of SEQ ID NO: 2. The VWF fragment of the present invention can comprise any other sequences linked to or fused to the VWF fragment. For example, a VWF fragment described herein can further comprise a signal peptide.

**[0143]** In one embodiment, the VWF fragment binds to or is associated with a FVIII protein. By binding to or associating with a FVIII protein, a VWF fragment of the invention protects FVIII from protease cleavage and FVIII activation, stabilizes the heavy chain and light chain of FVIII, and prevents clearance of FVIII by scavenger receptors. In another embodiment, the VWF fragment binds to or associates with a FVIII protein and blocks or prevents binding of the FVIII protein to phospholipid and activated Protein C. By preventing or inhibiting binding of the FVIII protein with endogenous, full-length VWF, the VWF fragment of the invention reduces the clearance of FVIII by VWF clearance receptors and thus extends half-life of the FVIII protein. In one embodiment, the half-life extension of a FVIII protein is thus due to the binding of or associating with the VWF fragment lacking a VWF clearance receptor binding site to the FVIII protein and shielding or protecting of the FVIII protein by the VWF fragment from endogenous VWF which contains the VWF clearance receptor binding site. The FVIII protein bound to or protected by the VWF fragment can also allow recycling of a FVIII protein. By eliminating the VWF clearance

pathway receptor binding sites contained in the full length VWF molecule, the FVIII/VWF heterodimers of the invention are shielded from the VWF clearance pathway, further extending FVIII half-life.

**[0144]** In one embodiment, a VWF fragment of the present invention comprises the D' domain and the D3 domain of VWF, wherein the D' domain is at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 866 of SEQ ID NO: 2, wherein the VWF fragment prevents binding of endogenous VWF to FVIII. In another embodiment, a VWF fragment comprises the D' domain and the D3 domain of VWF, wherein the D3 domain is at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 867 to 1240 of SEQ ID NO: 2, wherein the VWF fragment prevents binding of endogenous VWF to FVIII. In some embodiments, a VWF fragment described herein comprises, consists essentially of, or consists of the D' domain and D3 domain of VWF, which are at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 1240 of SEQ ID NO: 2, wherein the VWF fragment prevents binding of endogenous VWF to FVIII. In other embodiments, a VWF fragment comprises, consists essentially of, or consists of the D1, D2, D', and D3 domains at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23 to 1240 of SEQ ID NO: 2, wherein the VWF fragment prevents binding of endogenous VWF to FVIII. In still other embodiments, the VWF fragment further comprises a signal peptide operably linked thereto.

**[0145]** In some embodiments, a VWF fragment of the invention consists essentially of or consists of (1) the D'D3 domain, the D1D'D3 domain, D2D'D3 domain, or D1D2D'D3 domain and (2) an additional VWF sequence up to about 10 amino acids (*e.g.*, any sequences from amino acids 764 to 1240 of SEQ ID NO: 2 to amino acids 764 to 1250 of SEQ ID NO: 2), up to about 15 amino acids (*e.g.*, any sequences from amino acids 764 to 1240 of SEQ ID NO: 2 to amino acids 764 to 1255 of SEQ ID NO: 2), up to about 20 amino acids (*e.g.*, any sequences from amino acids 764 to 1240 of SEQ ID NO: 2 to amino acids 764 to 1260 of SEQ ID NO: 2), up to about 25 amino acids (*e.g.*, any sequences from amino acids 764 to 1240 of SEQ ID NO: 2 to amino acids 764 to 1265 of SEQ ID NO: 2), or up to about 30 amino acids (*e.g.*, any sequences from amino acids 764 to 1240 of SEQ

ID NO: 2 to amino acids 764 to 1260 of SEQ ID NO: 2). In a particular embodiment, the VWF fragment comprising or consisting essentially of the D' domain and the D3 domain is neither amino acids 764 to 1274 of SEQ ID NO: 2 nor the full-length mature VWF. In some embodiments, the D1D2 domain is expressed in trans with the D'D3 domain. In some embodiments, the D1D2 domain is expressed in cis with the D'D3 domain.

[0146] In other embodiments, the VWF fragment comprising the D'D3 domains linked to the D1D2 domains further comprises an intracellular cleavage site, *e.g.*, (a cleavage site by PACE (furin) or PC5), allowing cleavage of the D1D2 domains from the D'D3 domains upon expression. Non-limiting examples of the intracellular cleavage site are disclosed elsewhere herein.

[0147] In yet other embodiments, a VWF fragment comprises the D' domain and the D3 domain, but does not comprise an amino acid sequence selected from the group consisting of (1) amino acids 1241 to 2813 of SEQ ID NO: 2, (2) amino acids 1270 to amino acids 2813 of SEQ ID NO: 2, (3) amino acids 1271 to amino acids 2813 of SEQ ID NO: 2, (4) amino acids 1272 to amino acids 2813 of SEQ ID NO: 2, (5) amino acids 1273 to amino acids 2813 of SEQ ID NO: 2, (6) amino acids 1274 to amino acids 2813 of SEQ ID NO: 2, and any combinations thereof.

[0148] In still other embodiments, a VWF fragment of the present invention comprises, consists essentially of, or consists of an amino acid sequence corresponding to the D' domain, D3 domain, and A1 domain, wherein the amino acid sequence is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acid 764 to 1479 of SEQ ID NO: 2, wherein the VWF fragment prevents binding of endogenous VWF to FVIII. In a particular embodiment, the VWF fragment is not amino acids 764 to 1274 of SEQ ID NO: 2.

[0149] In some embodiments, a VWF fragment of the invention comprises the D' domain and the D3 domain, but does not comprise at least one VWF domain selected from the group consisting of (1) an A1 domain, (2) an A2 domain, (3) an A3 domain, (4) a D4 domain, (5) a B1 domain, (6) a B2 domain, (7) a B3 domain, (8) a C1 domain, (9) a C2 domain, (10) a CK domain, (11) a CK domain and C2 domain, (12) a CK domain, a C2 domain, and a C1 domain, (13) a CK domain, a C2 domain, a C1 domain, a B3 domain, (14) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, (15) a CK domain, a C2 domain, a C1

domain, a B3 domain, a B2 domain, and a B1 domain, (16) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, and a D4 domain, (17) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, and an A3 domain, (18) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, an A3 domain, and an A2 domain, (19) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, an A3 domain, an A2 domain, and an A1 domain, and (20) any combinations thereof.

**[0150]** In yet other embodiments, the VWF fragment comprises the D'D3 domains and one or more domains or modules. Examples of such domains or modules include, but are not limited to, the domains and modules disclosed in Zhour et al., Blood published online April 6, 2012: DOI 10.1182/blood-2012-01-405134. For example, the VWF fragment can comprise the D'D3 domain and one or more domains or modules selected from the group consisting of A1 domain, A2 domain, A3 domain, D4N module, VWD4 module, C8-4 module, TIL-4 module, C1 module, C2 module, C3 module, C4 module, C5 module, C5 module, C6 module, and any combinations thereof.

**[0151]** In still other embodiments, the VWF fragment is linked to a heterologous moiety, wherein the heterologous moiety is linked to the N-terminus or the C-terminus of the VWF fragment or inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites) in the FVIII protein in the VWF fragment. For example, the insertion sites for the heterologous moiety in the VWF fragment can be in the D' domain, the D3 domain, or both. The heterologous moiety can be a half-life extender.

**[0152]** In certain embodiments, a VWF fragment of the invention forms a multimer, *e.g.*, dimer, trimer, tetramer, pentamer, hexamer, heptamer, or the higher order multimers. In other embodiments, the VWF fragment is a monomer having only one VWF fragment. In some embodiments, the VWF fragment of the present invention can have one or more amino acid substitutions, deletions, additions, or modifications. In one embodiment, the VWF fragment can include amino acid substitutions, deletions, additions, or modifications such that the VWF fragment is not capable of forming a disulfide bond or forming a dimer or a multimer. In another embodiment, the amino acid substitution is within the D'

domain and the D3 domain. In a particular embodiment, a VWF fragment of the invention contains at least one amino acid substitution at a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 of SEQ ID NO: 2. The at least one amino acid substitution can be any amino acids that are not occurring naturally in the wild type VWF. For example, the amino acid substitution can be any amino acids other than cysteine, *e.g.*, Isoleucine, Alanine, Leucine, Asparagine, Lysine, Aspartic acid, Methionine, Phenylalanine, Glutamic acid, Threonine, Glutamine, Tryptophan, Glycine, Valine, Proline, Serine, Tyrosine, Arginine, or Histidine. In another example, the amino acid substitution has one or more amino acids that prevent or inhibit the VWF fragments from forming multimers.

**[0153]** In certain embodiments, the VWF fragment useful herein can be further modified to improve its interaction with FVIII, *e.g.*, to improve binding affinity to FVIII. As a non-limiting example, the VWF fragment comprises a serine residue at the residue corresponding to amino acid 764 of SEQ ID NO: 2 and a lysine residue at the residue corresponding to amino acid 773 of SEQ ID NO: 2. Residues 764 and/or 773 can contribute to the binding affinity of the VWF fragments to FVIII. In other embodiments, the VWF fragments useful for the invention can have other modifications, *e.g.*, the protein can be pegylated, glycosylated, hesylated, or polysialylated.

## **B) XTEN Sequences**

**[0154]** As used here "XTEN sequence" refers to extended length polypeptides with non-naturally occurring, substantially non-repetitive sequences that are composed mainly of small hydrophilic amino acids, with the sequence having a low degree or no secondary or tertiary structure under physiologic conditions. As a chimeric protein partner, XTENs can serve as a carrier, conferring certain desirable pharmacokinetic, physicochemical and pharmaceutical properties when linked to a VWF fragment or a FVIII sequence of the invention to create a chimeric protein. Such desirable properties include but are not limited to enhanced pharmacokinetic parameters and solubility characteristics. As used herein, "XTEN" specifically excludes antibodies or antibody fragments such as single-chain antibodies or Fc fragments of a light chain or a heavy chain.

[0155] In some embodiments, the XTEN sequence of the invention is a peptide or a polypeptide having greater than about 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1200, 1400, 1600, 1800, or 2000 amino acid residues. In certain embodiments, XTEN is a peptide or a polypeptide having greater than about 20 to about 3000 amino acid residues, greater than 30 to about 2500 residues, greater than 40 to about 2000 residues, greater than 50 to about 1500 residues, greater than 60 to about 1000 residues, greater than 70 to about 900 residues, greater than 80 to about 800 residues, greater than 90 to about 700 residues, greater than 100 to about 600 residues, greater than 110 to about 500 residues, or greater than 120 to about 400 residues.

[0156] The XTEN sequence of the invention can comprise one or more sequence motif of 9 to 14 amino acid residues or an amino acid sequence at least 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the sequence motif, wherein the motif comprises, consists essentially of, or consists of 4 to 6 types of amino acids selected from the group consisting of glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P). *See* US 2010-0239554 A1.

[0157] In some embodiments, the XTEN comprises non-overlapping sequence motifs in which about 80%, or at least about 85%, or at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% or about 100% of the sequence consists of multiple units of non-overlapping sequences selected from a single motif family selected from Table 2A, resulting in a family sequence. As used herein, "family" means that the XTEN has motifs selected only from a single motif category from Table 2A; *i.e.*, AD, AE, AF, AG, AM, AQ, BC, or BD XTEN, and that any other amino acids in the XTEN not from a family motif are selected to achieve a needed property, such as to permit incorporation of a restriction site by the encoding nucleotides, incorporation of a cleavage sequence, or to achieve a better linkage to FVIII or VWF. In some embodiments of XTEN families, an XTEN sequence comprises multiple units of non-overlapping sequence motifs of the AD motif family, or of the AE motif family, or of the AF motif family, or of the AG motif family, or of the AM motif family, or of the AQ motif family, or of the BC family,

or of the BD family, with the resulting XTEN exhibiting the range of homology described above. In other embodiments, the XTEN comprises multiple units of motif sequences from two or more of the motif families of Table 2A. These sequences can be selected to achieve desired physical/chemical characteristics, including such properties as net charge, hydrophilicity, lack of secondary structure, or lack of repetitiveness that are conferred by the amino acid composition of the motifs, described more fully below. In the embodiments hereinabove described in this paragraph, the motifs incorporated into the XTEN can be selected and assembled using the methods described herein to achieve an XTEN of about 36 to about 3000 amino acid residues.

**Table 2A. XTEN Sequence Motifs of 12 Amino Acids and Motif Families**

| <b>Motif Family*</b> | <b>MOTIF SEQUENCE</b> | <b>SEQ ID NO:</b> |
|----------------------|-----------------------|-------------------|
| AD                   | GESPGGSSGSES          | 141               |
| AD                   | GSEGSSGPGESS          | 142               |
| AD                   | GSESSEGSSEGGP         | 143               |
| AD                   | GSGGEPSESGSS          | 144               |
| AE, AM               | GSPAGSPTSTEE          | 145               |
| AE, AM, AQ           | GSEPATSGSETP          | 146               |
| AE, AM, AQ           | GTSESATPESGP          | 147               |
| AE, AM, AQ           | GTSTEPSEGSAP          | 148               |
| AF, AM               | GSTSESPSGTAP          | 149               |
| AF, AM               | GTSTPESGSASP          | 150               |
| AF, AM               | GTSPSGESSTAP          | 151               |
| AF, AM               | GSTSSTAESPGP          | 152               |
| AG, AM               | GTPGSGTASSSP          | 153               |
| AG, AM               | GSSTPSGATGSP          | 154               |
| AG, AM               | GSSPSASTGTGP          | 155               |
| AG, AM               | GASPGTSSTGSP          | 156               |
| AQ                   | GEPAGSPTSTSE          | 157               |
| AQ                   | GTGEPSTPASE           | 158               |
| AQ                   | GSGPSTESAPTE          | 159               |
| AQ                   | GSETPSGPSETA          | 160               |
| AQ                   | GPSETSTSEPGA          | 161               |
| AQ                   | GSPSEPTEGTSA          | 162               |
| BC                   | GSGASEPTSTEP          | 163               |
| BC                   | GSEPATSGTEPS          | 164               |
| BC                   | GTSEPSTSEPGA          | 165               |
| BC                   | GTSTEPSEPGSA          | 166               |
| BD                   | GSTAGSETSTEA          | 167               |
| BD                   | GSETATSGSETA          | 168               |
| BD                   | GTSESATSESGA          | 169               |
| BD                   | GTSTEASEGSAS          | 170               |

- Denotes individual motif sequences that, when used together in various permutations, results in a "family sequence"

**[0158]** XTEN can have varying lengths for insertion into or linkage to FVIII or VWF. In one embodiment, the length of the XTEN sequence(s) is chosen based on the property or function to be achieved in the fusion protein. Depending on the intended property or function, XTEN can be short or intermediate length sequence or longer sequence that can serve as carriers. In certain embodiments, the XTEN include short segments of about 6 to about 99 amino acid residues, intermediate lengths of about 100 to about 399 amino acid residues, and longer lengths of about 400 to about 1000 and up to about 3000 amino acid residues. Thus, the XTEN inserted into or linked to FVIII or VWF can have lengths of about 6, about 12, about 36, about 40, about 42, about 72, about 96, about 144, about 288, about 400, about 500, about 576, about 600, about 700, about 800, about 864, about 900, about 1000, about 1500, about 2000, about 2500, or up to about 3000 amino acid residues in length. In other embodiments, the XTEN sequences is about 6 to about 50, about 50 to about 100, about 100 to 150, about 150 to 250, about 250 to 400, about 400 to about 500, about 500 to about 900, about 900 to 1500, about 1500 to 2000, or about 2000 to about 3000 amino acid residues in length. The precise length of an XTEN inserted into or linked to FVIII or VWF can vary without adversely affecting the activity of the FVIII or VWF. In one embodiment, one or more of the XTEN used herein has 36 amino acids, 42 amino acids, 72 amino acids, 144 amino acids, 288 amino acids, 576 amino acids, or 864 amino acids in length and can be selected from one or more of the XTEN family sequences; *i.e.*, AD, AE, AF, AG, AM, AQ, BC or BD.

**[0159]** In some embodiments, the XTEN sequence used in the invention is at least 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of AE42, AG42, AE48, AM48, AE72, AG72, AE108, AG108, AE144, AF144, AG144, AE180, AG180, AE216, AG216, AE252, AG252, AE288, AG288, AE324, AG324, AE360, AG360, AE396, AG396, AE432, AG432, AE468, AG468, AE504, AG504, AF504, AE540, AG540, AF540, AD576, AE576, AF576, AG576, AE612, AG612, AE624, AE648, AG648, AG684, AE720, AG720, AE756, AG756, AE792, AG792, AE828, AG828, AD836, AE864, AF864,



AG864, AM875, AE912, AM923, AM1318, BC864, BD864, AE948, AE1044, AE1140, AE1236, AE1332, AE1428, AE1524, AE1620, AE1716, AE1812, AE1908, AE2004A, AG948, AG1044, AG1140, AG1236, AG1332, AG1428, AG1524, AG1620, AG1716, AG1812, AG1908, and AG2004. *See* US 2010-0239554 A1.

**[0160]** In one embodiment, the XTEN sequence is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of AE42 (SEQ ID NO: 36), AE72 (SEQ ID NO: 127), AE144\_2A (SEQ IDNO: 128), AE144\_3B (SEQ ID NO: 129), AE144\_4A (SEQ ID NO: 130), AE144\_5A (SEQ IDNO: 131), AE144\_6B (SEQ IDNO: 132), AG144\_A (SEQ ID NO: 133), AG144\_B (SEQ IDNO: 134), AG144\_C (SEQ ID NO: 135), AG144\_F (SEQ IDNO: 136), AE864 (SEQ ID NO: 43), AE576 (SEQ ID NO: 41), AE288 (SEQ IDNO: 39), AE288\_2 (SEQ ID NO: 137), AE144 (SEQ ID NO: 37), AG864 (SEQ ID NO: 44), AG576 (SEQ ID NO: 42), AG288 (SEQ ID NO: 40), AG144 (SEQ ID NO: 38), and any combinations thereof.

**[0161]** In some embodiments, less than 100% of amino acids of an XTEN are selected from glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P), or less than 100% of the sequence consists of the sequence motifs from Table 2A or the XTEN sequences of Table 2B. In such embodiments, the remaining amino acid residues of the XTEN are selected from any of the other 14 natural L-amino acids, but may be preferentially selected from hydrophilic amino acids such that the XTEN sequence contains at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% hydrophilic amino acids. The content of hydrophobic amino acids in the XTEN utilized in the conjugation constructs may be less than 5%, or less than 2%, or less than 1% hydrophobic amino acid content. Hydrophobic residues that are less favored in construction of XTEN include tryptophan, phenylalanine, tyrosine, leucine, isoleucine, valine, and methionine. Additionally, XTEN sequences may contain less than 5% or less than 4% or less than 3% or less than 2% or less than 1% or none of the following amino acids: methionine (for example, to avoid oxidation), or asparagine and glutamine (to avoid desamidation).

**[0162]** In another embodiment, the XTEN sequence is selected from the group consisting of AE42 (SEQ ID NO: 36), AE72 (SEQ ID NO: 127), AE144\_2A

(SEQ IDNO: 128), AE144\_3B (SEQ ID NO: 129), AE144\_4A (SEQ ID NO: 130), AE144\_5A (SEQ IDNO: 131), AE144\_6B (SEQ IDNO: 132), AG144\_A (SEQ ID NO: 133), AG144\_B (SEQ IDNO: 134), AG144\_C (SEQ ID NO: 135), AG144\_F (SEQ IDNO: 136), AE864 (SEQ ID NO: 43), AE576 (SEQ ID NO: 41), AE288 (SEQ IDNO: 39), AE288\_2 (SEQ ID NO: 137), AE144 (SEQ ID NO: 37), AG864 (SEQ ID NO: 44), AG576 (SEQ ID NO: 42), AG288 (SEQ ID NO: 40), AG144 (SEQ ID NO: 38), and any combinations thereof. In a specific embodiment, the XTEN sequence is AE288. The amino acid sequences for certain XTEN sequences of the invention are shown in Table 2B.

**TABLE 2B. XTEN Sequences**

| XTEN                            | Amino Acid Sequence                                                                                                                                             |
|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| AE42<br>SEQ ID NO:<br>36        | GAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPASS                                                                                                                      |
| AE72<br>SEQ ID NO:<br>127       | GAPTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPA<br>TSGSETPGTSESATPESGPGTSTEPSEGSAPGASS                                                                              |
| AE144<br>SEQ ID<br>NO:37        | GSEPATSGSETPGTSESATPESGPGSEPATSGSETPGSPAGSPTSTE<br>EGTSTEPSEG<br>SAPGSEPATSGSETPGSEPATSGSETPGSEPATSGSETPGTSTEPSE<br>GSAPGTSESA<br>PESGPGSEPATSGSETPGTSTEPSEGSAP |
| AE144_2A<br>(SEQ ID NO:<br>128) | TSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAP<br>GTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSEPATSGSET<br>PGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESATPES<br>GPG    |
| AE144_3B<br>(SEQ ID NO:<br>129) | SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGP<br>GTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGS<br>PGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGS<br>APG     |
| AE144_4A<br>(SEQ ID NO:<br>130) | TSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETP<br>GTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTE<br>EGSPAGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGS<br>APG    |
| AE144_5A<br>(SEQ ID NO:         | TSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETP<br>GTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESG<br>PGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTST           |

|                                 |                                                                                                                                                                                                                                                                                                                                       |
|---------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 131)                            | EEG                                                                                                                                                                                                                                                                                                                                   |
| AE144_6B<br>(SEQ ID NO:<br>132) | TSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGP<br>GSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGS<br>PGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEGS<br>APG                                                                                                                                                                           |
| AG144<br>SEQ ID<br>NO:38        | GTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTG<br>PGASPGTSST<br>GSPGASPGTSSTGSPGSSTPSGATGSPGSSPSASTGTGPGASPGTSS<br>TGSPGSSPSA<br>STGTGPGTPGSGTASSSPGSSTPSGATGSP                                                                                                                                                                      |
| AG144_A<br>(SEQ ID NO:<br>133)  | GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSS<br>PGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASS<br>SPGSSTPSGATGSPGTPGSGTASSSPGASPGTSSTGSPGASPGTSST<br>GSP                                                                                                                                                                          |
| AG144_B<br>(SEQ ID NO:<br>134)  | GTPGSGTASSSPGSSTPSGATGSPGASPGTSSTGSPGTPGSGTASSS<br>PGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSTPSGATG<br>SPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSST<br>GSP                                                                                                                                                                          |
| AG144_C<br>(SEQ ID NO:<br>135)  | GTPGSGTASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGS<br>PGSSPSASTGTGPGTPGSGTASSSPGASPGTSSTGSPGASPGTSSTG<br>SPGASPGTSSTGSPGSSTPSGATGSPGSSTPSGATGSPGASPGTSST<br>GSP                                                                                                                                                                          |
| AG144_F<br>(SEQ ID NO:<br>136)  | GSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGS<br>PGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSASTGT<br>GPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGSPGASPGTSST<br>GSP                                                                                                                                                                          |
| AE288<br>SEQ ID<br>NO:39        | GTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSET<br>PGTSESATPESG<br>PGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSE<br>TPGTSESATPES<br>GPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPE<br>SGPGTSESATPE<br>SGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPT<br>STEEGTSTEPSE<br>GSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSE<br>EGSAP |
| AE288_2<br>(SEQ ID NO:<br>137)  | GSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESG<br>PGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGS<br>APGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEG<br>SAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSG<br>SETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSP<br>TSTEEGSPAGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEP<br>SEGSAP              |
| AG288                           | PGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATG                                                                                                                                                                                                                                                                                       |

|                       |    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|-----------------------|----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ<br>NO:40          | ID | SPGTPGSGTASS<br>SPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGAT<br>GSPGSSPSASTG<br>TGPSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGA<br>TGSPGSSPSAST<br>GTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGSSTPSG<br>ATGSPGSSPSAS<br>TGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASSSPGSSTPS<br>GATGS                                                                                                                                                                                                                                                                                                                                                                                         |
| AE576<br>SEQ<br>NO:41 | ID | GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTE<br>EGTSTEPSEGA<br>PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSE<br>TPGSPAGSPTST<br>EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTS<br>TEEGTSTEPSEG<br>SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATP<br>ESGPGSEPATSG<br>SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESAT<br>PESGPGSPAGSP<br>TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEP<br>SEGSAPGTSTEP<br>SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTE<br>PSEGSAPGSPAG<br>SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSE<br>SATPESGPGSEP<br>ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSP<br>AGSPTSTEEGSP<br>AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAP  |
| AG576<br>SEQ<br>NO:42 | ID | PGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGT<br>GPGSSTPSGATG<br>SPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSST<br>GSPGTPGSGTAS<br>SSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSAST<br>GTGPGTPGSGTA<br>SSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSG<br>ATGSPGSSTPSG<br>ATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPS<br>GATGSPGSSTPS<br>GATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTPGS<br>GTASSSPGASPG<br>TSSTGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPG<br>SGTASSSPGSST<br>PSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSS<br>TPSGATGSPGSS<br>TPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGT<br>PGSGTASSSPGS<br>STPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGS |
| AE864                 |    | GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTE<br>EGTSTEPSEGA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

|                                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|--------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>SEQ</b><br><b>NO:43</b>                 | <b>ID</b><br>PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSE<br>TPGSPAGSPTST<br>EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTS<br>TEEGTSTEPSEG<br>SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATP<br>ESGPGSEPATSG<br>SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESAT<br>PESGPGSPAGSP<br>TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEP<br>SEGSAPGTSTEP<br>SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTE<br>PSEGSAPGSPAG<br>SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSE<br>SATPESGPGSEP<br>ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSP<br>AGSPTSTEEGSP<br>AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGT<br>SESATPESGPGS<br>EPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPG<br>TSTEPSEGSAPG<br>SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGP<br>GSPAGSPTSTEE<br>GSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESG<br>PGTSESATPESG<br>PGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGS<br>APGTSTEPSEGS<br>APGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP |
| <b>AG864</b><br><b>SEQ</b><br><b>NO:44</b> | <b>ID</b><br>GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSS<br>PGSSTPSGATGS<br>PGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATG<br>SPGTPGSGTASS<br>SPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGAT<br>GSPGASPGTSST<br>GSPGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSAST<br>GTGPGSSTPSGA<br>TGSPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTS<br>STGSPGTPGSGT<br>ASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSA<br>STGTGPGTPGSG<br>TASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTP<br>SGATGSPGSSTP<br>SGATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSST<br>PSGATGSPGSST<br>PSGATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTP<br>GSGTASSSPGAS<br>PGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGT<br>PGSGTASSSPGS<br>STPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPG<br>SSTPSGATGSPG<br>SSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSP<br>GTPGSGTASSSP                                                                                                              |

|  |                                                                                                                                                                              |
|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | GSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGS<br>PGASPGTSSTGS<br>PGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSASTGT<br>GPGTPGSGTASS<br>SPGSSTPSGATGSPGSSTPSGATGSPGASPGTSSTGSP |
|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**[0163]** In further embodiments, the XTEN sequence used in the invention affects the physical or chemical property, *e.g.*, pharmacokinetics, of the chimeric protein of the present invention. The XTEN sequence used in the present invention can exhibit one or more of the following advantageous properties: conformational flexibility, enhanced aqueous solubility, high degree of protease resistance, low immunogenicity, low binding to mammalian receptors, or increased hydrodynamic (or Stokes) radii. In a specific embodiment, the XTEN sequence linked to a FVIII protein in this invention increases pharmacokinetic properties such as longer terminal half-life or increased area under the curve (AUC), so that the chimeric protein described herein stays *in vivo* for an increased period of time compared to wild type FVIII. In further embodiments, the XTEN sequence used in this invention increases pharmacokinetic properties such as longer terminal half-life or increased area under the curve (AUC), so that FVIII protein stays *in vivo* for an increased period of time compared to wild type FVIII.

**[0164]** A variety of methods and assays can be employed to determine the physical/chemical properties of proteins comprising the XTEN sequence. Such methods include, but are not limited to analytical centrifugation, EPR, HPLC-ion exchange, HPLC-size exclusion, HPLC-reverse phase, light scattering, capillary electrophoresis, circular dichroism, differential scanning calorimetry, fluorescence, HPLC-ion exchange, HPLC-size exclusion, IR, NMR, Raman spectroscopy, refractometry, and UV/Visible spectroscopy. Additional methods are disclosed in Amau *et al.*, *Prot Expr and Purif* 48, 1-13 (2006).

**[0165]** Additional examples of XTEN sequences that can be used according to the present invention and are disclosed in US Patent Publication Nos. 2010/0239554 A1, 2010/0323956 A1, 2011/0046060 A1, 2011/0046061 A1, 2011/0077199 A1, or 2011/0172146 A1, or International Patent Publication Nos. WO 2010091122 A1, WO 2010144502 A2, WO 2010144508 A1, WO 2011028228 A1, WO 2011028229 A1, or WO 2011028344 A2.

### **C) Factor VIII (FVIII) Protein**

[0166] "A FVIII protein" as used herein means a functional FVIII polypeptide in its normal role in coagulation, unless otherwise specified. The term a FVIII protein includes a functional fragment, variant, analog, or derivative thereof that retains the function of full-length wild-type Factor VIII in the coagulation pathway. "A FVIII protein" is used interchangeably with FVIII polypeptide (or protein) or FVIII. Examples of the FVIII functions include, but not limited to, an ability to activate coagulation, an ability to act as a cofactor for factor IX, or an ability to form a tenase complex with factor IX in the presence of  $\text{Ca}^{2+}$  and phospholipids, which then converts Factor X to the activated form Xa. The FVIII protein can be the human, porcine, canine, rat, or murine FVIII protein. In addition, comparisons between FVIII from humans and other species have identified conserved residues that are likely to be required for function (Cameron *et al.*, *Thromb. Haemost.* 79:317-22 (1998); US 6,251,632).

[0167] A number of tests are available to assess the function of the coagulation system: activated partial thromboplastin time (aPTT) test, chromogenic assay, ROTEM assay, prothrombin time (PT) test (also used to determine INR), fibrinogen testing (often by the Clauss method), platelet count, platelet function testing (often by PFA-100), TCT, bleeding time, mixing test (whether an abnormality corrects if the patient's plasma is mixed with normal plasma), coagulation factor assays, antiphospholipid antibodies, D-dimer, genetic tests (*e.g.*, factor V Leiden, prothrombin mutation G20210A), dilute Russell's viper venom time (dRVVT), miscellaneous platelet function tests, thromboelastography (TEG or Sonoclot), thromboelastometry (TEM<sup>®</sup>, *e.g.*, ROTEM<sup>®</sup>), or euglobulin lysis time (ELT).

[0168] The aPTT test is a performance indicator measuring the efficacy of both the "intrinsic" (also referred to the contact activation pathway) and the common coagulation pathways. This test is commonly used to measure clotting activity of commercially available recombinant clotting factors, *e.g.*, FVIII or FIX. It is used in conjunction with prothrombin time (PT), which measures the extrinsic pathway.

[0169] ROTEM analysis provides information on the whole kinetics of haemostasis: clotting time, clot formation, clot stability and lysis. The different parameters in thromboelastometry are dependent on the activity of the plasmatic coagulation system, platelet function, fibrinolysis, or many factors which

influence these interactions. This assay can provide a complete view of secondary haemostasis.

[0170] The FVIII polypeptide and polynucleotide sequences are known, as are many functional fragments, mutants and modified versions. Examples of human FVIII sequences (full-length) are shown below.

**TABLE 3. Amino Acid Sequence of Full-length Factor VIII**

(Full-length FVIII (FVIII signal peptide underlined; FVIII heavy chain is double underlined; B domain is italicized; and FVIII light chain is in plain text)

Signal Peptide: (SEQ ID NO: 3)

MQIELSTCFFLCLLRFCFS

Mature Factor VIII (SEQ ID NO: 4)\*

ATRRYYLGAVELSWDYMOSDLGELPVDARFPFPRVPKSFPEFNTSVVYKKTLEFVEFT  
DHLFENIAKPRPPWMGLLGPTIOAEVYDVTVVITLKNMASHPVSLHAVGVSYWKASE  
GAEYDDQTSOREKEDDKVFPGGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLV  
KDLNSGLIGALLVCREGSLAKEKTQTLHKFILLFAVFDEGKSWHSETKNSLMQDR  
DAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGMGTTEPVHSIFLEGHT  
FLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVKVDSCP  
EEPQLRMKNNEEAEDYDDDLTDSEMDVVRFDDDDNSPSFIQIRSVAKKHPKTTWVHY  
IAAEEDWDYAPLV LAPDDRSYKSOYLNNGPQRIGRKYKKVRFMAYTDETFKTR  
AIQHESGILGPLLYGEVGDILLIFKNQASRPYNIYPHGITDVRPLYSRRLPKGV  
KHLKDFPILPGEIFKYKWTVTVEDGPTKSDPRCLTRYSSFVNMERDLASGLIGP  
LLICYKESVDORGNOIMSDKRNVLFSVFDENRSWYLTENIQRFLLPNPAGVQLED  
PEFQASNIMHSINGYVFDLSQLSVCLHEVAYWYILSIGAQTDFLSVFFSGYTFKH  
KMVEYEDTLTLFPFSGETVFMSENPGLWILGCHNSDFRNRGMTALLKVSSCDKNT  
GDYYEDSYEDISAYLLSKNNAIEPRSFSONSRHPSTROKQFNATTIPENDIEKTD  
PWFAHRTMPMPKIQNVSSDLLMLLRQSPTPHGLSLSDLQEAKEYETFSDDPSPGA  
DSNNLSSEMTHFRPQLHHS GDMVFTPESEGLQLRLNEKLGTTAATELKKLDFKVSS  
TSNNLISTIPSDNLAAGTDNTSSLGPPSMPVHYDSQLDITLFGKKSSPLTESGGP  
LSLSEENND SKLLESGLMNSQESSWGKNVSSSTESGRLFKGKRAHGPAALLTKDNAL  
FKVSI SLLKTNKTSNNSATNRKTHIDGPSLLIENS PSVWQNILES DTEFKKVTPL  
IHDRMLMDKNATALRLNHMSNKTTS SKNMEMVQKKEGP I PPDAQNPDM SFFKML  
FLPESARWIQORTHGKNSLNSGQGPS PKQLVSLGPEKSVEGQNF LSEKNKVVGKG  
EFTKDVGLKEMVFPSSRNLF LTNLDNLHENNTHNQEKKIQEEIEKKETLIQENVV  
LPQIHVTVTGKNFMKNL FLLSTRQNVESYDGAYAPVLQDFRSLNDSTNR TKKHT  
AHFSKKGEEENLEGLGNQTKQIVEKYACTTRISPNTSQQNFVTQRSKRALKQFRL  
PLEETELEKRIIVDDTSTQWSKNMKHLTPSTLTQIDYNEKEKGAITQSPLSDCLT  
RSHSIPQANRSPLPIAKVSSFP SIRPIYLTRVLFQDNSSHLPAASYRKKDSGVQE  
SSHFLQGA KKNLSLA ILTLEMTGDQREVGS LGTSATNSV TYKKVENTVLPK PDL  
PKTSGKV ELLPKVHIYQKDLFPTETSN GSPGHLDLVEGSLLQGT EGAIKWNEANR  
PGKVPFLRVATESSAKTPSKLLDPLAWDNHYGTQIPKEEWSQEKSP EKTAFKKK  
DTILSLNACESNHAI AAINEGQNKPEIEVTWAKQGRTERLCSQNP PVLKRHQREI  
TRTTLQSDQEEIDYDDTISVEMKKEDFDIYDE DENQSPRSFQKKTRHYFIAAVER  
LWDYGMSSSPHVLNRNAQSGSVPQFKKVVFQEF TDG SFTQPLYRGELNEHLGLLG



PYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGAEPKKNFVKPNETKTY  
 FWKVQHMAPTKDEFDCKAWAYFSDVDLEKDVHSGLIGPLLCHTNTLNPAHGRQ  
 VTVQEFALFFTIFDETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYI  
 MDTLPGLVMAQDQIRIRWYLLSMGSENENIHSIHFSGHVFTVRKKEEYKMALYNLYP  
 GVFETVEMLPSKAGIWRVECLIGEHLHAGMSTLFLVYSNKCQTPLGMASGHIRDF  
 QITASGQYGQWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMI IHGIKTQGAR  
 QKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNI FNPPII  
 ARYIRLHPHTHYSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMF  
 ATWSPSKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVTQGVKSLLTSM  
 YVKEFLISSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHP  
 QSWVHQIALRMEVLGCEAQDLY

**TABLE 4. Nucleotide Sequence Encoding Full-Length FVIII (SEQ ID NO: 5)\***

|      |                   |                   |                   |
|------|-------------------|-------------------|-------------------|
| 661  |                   |                   | <u>ATG</u>        |
|      | <u>CAAATAGAGC</u> | <u>TCTCCACCTG</u> |                   |
| 721  | <u>CTTCTTTCTG</u> | <u>TGCCTTTTGC</u> | <u>GATTCTGCTT</u> |
|      | <u>AGAAGATACT</u> | <u>ACCTGGGTGC</u> |                   |
| 781  | AGTGGAAGCTG       | TCATGGGACT        | ATATGCAAAG        |
|      | GAGCTGCCTG        | TGGACGCAAG        |                   |
| 841  | ATTCCTCCT         | AGAGTGCCAA        | AATCTTTTCC        |
|      | TCAGTCGTGT        | ACAAAAAGAC        | ATTCAACACC        |
| 901  | TCTGTTTGT         | GAATTCACGG        | ATCACCTTTT        |
|      | AAGCCAAGGC        | CACCCTGGAT        | CAACATCGCT        |
| 961  | GGGTCTGCTA        | GGTCCTACCA        | TCCAGGCTGA        |
|      | ACAGTGGTCA        | TTACACTTAA        | GGTTTATGAT        |
| 1021 | GAACATGGCT        | TCCCATCCTG        | TCAGTCTTCA        |
|      | GTATCCTACT        | GGAAAGCTTC        | TGCTGTTGGT        |
| 1081 | TGAGGGAGCT        | GAATATGATG        | ATCAGACCAG        |
|      | AAAGAAGATG        | ATAAAGTCTT        | TCAAAGGGAG        |
| 1141 | CCCTGGTGGA        | AGCCATACAT        | ATGTCTGGCA        |
|      | GAGAATGGTC        | CAATGGCCTC        | GGTCCTGAAA        |
| 1201 | TGACCCACTG        | TGCCTTACCT        | ACTCATATCT        |
|      | GACCTGGTAA        | AAGACTTGAA        | TTCTCATGTG        |
| 1261 | TTCAGGCCTC        | ATTGGAGCCC        | TACTAGTATG        |
|      | AGTCTGGCCA        | AGGAAAAGAC        | TAGAGAAGGG        |
| 1321 | ACAGACCTTG        | CACAAATTTA        | TACTACTTTT        |
|      | GATGAAGGGA        | AAAGTTGGCA        | TGCTGTATTT        |
| 1381 | CTCAGAAACA        | AAGAACTCCT        | TGATGCAGGA        |
|      | GCATCTGCTC        | GGGCCTGGCC        | TAGGGATGCT        |
| 1441 | TAAAATGCAC        | ACAGTCAATG        | GTTATGTAAA        |
|      | CCAGGTCTGA        | TTGGATGCCA        | CAGGTCTCTG        |
| 1501 | CAGGAAATCA        | GTCTATTGGC        | ATGTGATTGG        |
|      | ACTCCTGAAG        | TGCACTCAAT        | AATGGGCACC        |

1561 ATTCCTCGAA GGTACACAT TTCTTGTGAG GAACCATCGC  
 CAGGCGTCCT TGGAAATCTC  
 1621 GCCAATAACT TTCCTTACTG CTCAAACACT CTTGATGGAC  
 CTTGGACAGT TTCTACTGTT  
 1681 TTGTCATATC TCTTCCCACC AACATGATGG CATGGAAGCT  
 TATGTCAAAG TAGACAGCTG  
 1741 TCCAGAGGAA CCCCAACTAC GAATGAAAAA TAATGAAGAA  
 GCGGAAGACT ATGATGATGA  
 1801 TCTTACTGAT TCTGAAATGG ATGTGGTCAG GTTTGATGAT  
 GACAACTCTC CTTCCTTTAT  
 1861 CCAAATTCGC TCAGTTGCCA AGAAGCATCC TAAAACTTGG  
 GTACATTACA TTGCTGCTGA  
 1921 AGAGGAGGAC TGGGACTATG CTCCCTTAGT CCTCGCCCCC  
 GATGACAGAA GTTATAAAAG  
 1981 TCAATATTTG AACAATGGCC CTCAGCGGAT TGGTAGGAAG  
 TACAAAAAAG TCCGATTTAT  
 2041 GGCATACACA GATGAAACCT TTAAGACTCG TGAAGCTATT  
 CAGCATGAAT CAGGAATCTT  
 2101 GGGACCTTTA CTTTATGGGG AAGTTGGAGA CACACTGTTG  
 ATTATATTTA AGAATCAAGC  
 2161 AAGCAGACCA TATAACATCT ACCCTCACGG AATCACTGAT  
 GTCCGTCCTT TGTATTCAAG  
 2221 GAGATTACCA AAAGGTGTAA AACATTTGAA GGATTTTCCA  
 ATTCTGCCAG GAGAAATATT  
 2281 CAAATATAAA TGGACAGTGA CTGTAGAAGA TGGGCCAACT  
 AAATCAGATC CTCGGTGCCT  
 2341 GACCCGCTAT TACTCTAGTT TCGTTAATAT GGAGAGAGAT  
 CTAGCTTCAG GACTCATTGG  
 2401 CCCTCTCCTC ATCTGCTACA AAGAATCTGT AGATCAAAGA  
 GGAAACCAGA TAATGTCAGA  
 2461 CAAGAGGAAT GTCATCCTGT TTTCTGTATT TGATGAGAAC  
 CGAAGCTGGT ACCTCACAGA  
 2521 GAATATACAA CGCTTTCTCC CCAATCCAGC TGGAGTGCAG  
 CTTGAGGATC CAGAGTTCCA  
 2581 AGCCTCCAAC ATCATGCACA GCATCAATGG CTATGTTTTT  
 GATAGTTTGC AGTTGTCAGT  
 2641 TTGTTTGCAT GAGGTGGCAT ACTGGTACAT TCTAAGCATT  
 GGAGCACAGA CTGACTTCCT  
 2701 TTCTGTCTTC TTCTCTGGAT ATACCTTCAA ACACAAAATG  
 GTCTATGAAG ACACACTCAC  
 2761 CCTATTCCCA TTCTCAGGAG AACTGTCTT CATGTCGATG  
 GAAAACCCAG GTCTATGGAT  
 2821 TCTGGGGTGC CACAACCTCAG ACTTTCGGAA CAGAGGCATG  
 ACCGCCTTAC TGAAGGTTTC  
 2881 TAGTTGTGAC AAGAACACTG GTGATTATTA CGAGGACAGT  
 TATGAAGATA TTTCAGCATA  
 2941 CTTGCTGAGT AAAAACAATG CCATTGAACC AAGAAGCTTC  
 TCCCAGAATT CAAGACACCC  
 3001 TAGCACTAGG CAAAAGCAAT TTAATGCCAC CACAATTCCA  
 GAAAATGACA TAGAGAAGAC

3061 TGACCCTTGG TTTGCACACA GAACACCTAT GCCTAAAATA  
 CAAAATGTCT CCTCTAGTGA  
 3121 TTTGTTGATG CTCTTGCGAC AGAGTCCTAC TCCACATGGG  
 CTATCCTTAT CTGATCTCCA  
 3181 AGAAGCCAAA TATGAGACTT TTTCTGATGA TCCATCACCT  
 GGAGCAATAG ACAGTAATAA  
 3241 CAGCCTGTCT GAAATGACAC ACTTCAGGCC ACAGCTCCAT  
 CACAGTGGGG ACATGGTATT  
 3301 TACCCCTGAG TCAGGCCTCC AATTAAGATT AAATGAGAAA  
 CTGGGGACAA CTGCAGCAAC  
 3361 AGAGTTGAAG AAACCTTGATT TCAAAGTTTC TAGTACATCA  
 AATAATCTGA TTTCAACAAT  
 3421 TCCATCAGAC AATTTGGCAG CAGGTACTGA TAATACAAGT  
 TCCTTAGGAC CCCCAAGTAT  
 3481 GCCAGTTCAT TATGATAGTC AATTAGATAC CACTCTATTT  
 GGCAAAAAGT CATCTCCCCT  
 3541 TACTGAGTCT GGTGGACCTC TGAGCTTGAG TGAAGAAAAT  
 AATGATTCAA AGTTGTTAGA  
 3601 ATCAGGTTTA ATGAATAGCC AAGAAAGTTC ATGGGGAAAA  
 AATGTATCGT CAACAGAGAG  
 3661 TGGTAGGTTA TTAAAGGGA AAAGAGCTCA TGGACCTGCT  
 TTGTTGACTA AAGATAATGC  
 3721 CTTATTCAAA GTTAGCATCT CTTTGTTAAA GACAAACAAA  
 ACTTCCAATA ATTCAGCAAC  
 3781 TAATAGAAAG ACTCACATTG ATGGCCCATC ATTATTAATT  
 GAGAATAGTC CATCAGTCTG  
 3841 GCAAAATATA TTAGAAAGTG ACACTGAGTT TAAAAAAGTG  
 ACACCTTTGA TTCATGACAG  
 3901 AATGCTTATG GACAAAAATG CTACAGCTTT GAGGCTAAAT  
 CATATGTCAA ATAAACTAC  
 3961 TTCATCAAAA AACATGGAAA TGGTCCAACA GAAAAAAGAG  
 GGCCCCATTC CACCAGATGC  
 4021 ACAAATCCA GATATGTCGT TCTTTAAGAT GCTATTCTTG  
 CCAGAATCAG CAAGGTGGAT  
 4081 ACAAAGGACT CATGGAAAGA ACTCTCTGAA CTCTGGGCAA  
 GGCCCCAGTC CAAAGCAATT  
 4141 AGTATCCTTA GGACCAGAAA AATCTGTGGA AGGTCAGAAT  
 TTCTTGTCTG AGAAAAACAA  
 4201 AGTGGTAGTA GGAAAGGGTG AATTTACAAA GGACGTAGGA  
 CTCAAAGAGA TGGTTTTTCC  
 4261 AAGCAGCAGA AACCTATTTT TACTAACTT GGATAATTTA  
 CATGAAAATA ATACACACAA  
 4321 TCAAGAAAAA AAAATTCAGG AAGAAATAGA AAAGAAGGAA  
 ACATTAATCC AAGAGAATGT  
 4381 AGTTTTGCCT CAGATACATA CAGTGA CTGACTGG CACTAAGAAT  
 TTCATGAAGA ACCTTTTCTT  
 4441 ACTGAGCACT AGGCAAAATG TAGAAGGTTC ATATGACGGG  
 GCATATGCTC CAGTACTTCA  
 4501 AGATTTTAGG TCATTAAATG ATTCAACAAA TAGAACAAAG  
 AAACACACAG CTCATTTCTC

4561 AAAAAAAGGG GAGGAAGAAA ACTTGAAGG CTTGGGAAAT  
 CAAACCAAGC AAATTGTAGA  
 4621 GAAATATGCA TGCACCACAA GGATATCTCC TAATACAAGC  
 CAGCAGAATT TTGTCACGCA  
 4681 ACGTAGTAAG AGAGCTTTGA AACAAATTCAG ACTCCCCTA  
 GAAGAAACAG AACTTGAAAA  
 4741 AAGGATAATT GTGGATGACA CCTCAACCCA GTGGTCCAAA  
 AACATGAAAC ATTTGACCCC  
 4801 GAGCACCTC ACACAGATAG ACTACAATGA GAAGGAGAAA  
 GGGGCCATTA CTCAGTCTCC  
 4861 CTTATCAGAT TGCCTTACGA GGAGTCATAG CATCCCTCAA  
 GCAAATAGAT CTCCATTACC  
 4921 CATTGCAAAG GTATCATCAT TTCCATCTAT TAGACCTATA  
 TATCTGACCA GGGTCCTATT  
 4981 CCAAGACAAC TCTTCTCATC TTCCAGCAGC ATCTTATAGA  
 AAGAAAGATT CTGGGGTCCA  
 5041 AGAAAGCAGT CATTTCTTAC AAGGAGCCAA AAAAAATAAC  
 CTTTCTTTAG CCATTCTAAC  
 5101 CTTGGAGATG ACTGGTGATC AAAGAGAGGT TGGCTCCCTG  
 GGGACAAGTG CCACAAATTC  
 5161 AGTCACATAC AAGAAAGTTG AGAACACTGT TCTCCCGAAA  
 CCAGACTTGC CCAAACATC  
 5221 TGGCAAAGTT GAATTGCTTC CAAAAGTTCA CATTTATCAG  
 AAGGACCTAT TCCCTACGGA  
 5281 AACTAGCAAT GGGTCTCCTG GCCATCTGGA TCTCGTGGA  
 GGGAGCCTTC TTCAGGGAAC  
 5341 AGAGGGAGCG ATTAAGTGGA ATGAAGCAAA CAGACCTGGA  
 AAAGTTCCCT TTCTGAGAGT  
 5401 AGCAACAGAA AGCTCTGCAA AGACTCCCTC CAAGCTATTG  
 GATCCTCTTG CTTGGGATAA  
 5461 CCACTATGGT ACTCAGATAC CAAAAGAAGA GTGGAAATCC  
 CAAGAGAAGT CACCAGAAAA  
 5521 AACAGCTTTT AAGAAAAAGG ATACCATTTT GTCCCTGAAC  
 GCTTGTGAAA GCAATCATGC  
 5581 AATAGCAGCA ATAAATGAGG GACAAAATAA GCCCGAAATA  
 GAAGTCACCT GGGCAAAGCA  
 5641 AGGTAGGACT GAAAGGCTGT GCTCTCAAAA CCCACCAGTC  
 TTGAAACGCC ATCAACGGGA  
 5701 AATAACTCGT ACTACTCTTC AGTCAGATCA AGAGGAAATT  
 GACTATGATG ATACCATATC  
 5761 AGTTGAAATG AAGAAGGAAG ATTTTGACAT TTATGATGAG  
 GATGAAAATC AGAGCCCCCG  
 5821 CAGCTTTCAA AAGAAAACAC GACACTATTT TATTGCTGCA  
 GTGGAGAGGC TCTGGGATTA  
 5881 TGGGATGAGT AGCTCCCCAC ATGTTCTAAG AAACAGGGCT  
 CAGAGTGGCA GTGTCCCTCA  
 5941 GTTCAAGAAA GTTGTTTTCC AGGAATTTAC TGATGGCTCC  
 TTTACTCAGC CCTTATACCG  
 6001 TGGAGAACTA AATGAACATT TGGGACTCCT GGGGCCATAT  
 ATAAGAGCAG AAGTTGAAGA

6061 TAATATCATG GTAAC TTTCA GAAATCAGGC CTCTCGTCCC  
 TATTCCTTCT ATTCTAGCCT  
 6121 TATTTCTTAT GAGGAAGATC AGAGGCAAGG AGCAGAACCT  
 AGAAAAAACT TTGTCAAGCC  
 6181 TAATGAAACC AAAACTTACT TTTGGAAAGT GCAACATCAT  
 ATGGCACCCA CTAAAGATGA  
 6241 GTTTGACTGC AAAGCCTGGG CTTATTTCTC TGATGTTGAC  
 CTGGAAAAAG ATGTGCACTC  
 6301 AGGCCTGATT GGACCCCTTC TGGTCTGCCA CACTAACACA  
 CTGAACCCCTG CTCATGGGAG  
 6361 ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTCAACATC  
 TTTGATGAGA CCAAAGCTG  
 6421 GTACTTCACT GAAAATATGG AAAGAAACTG CAGGGCTCCC  
 TGCAATATCC AGATGGAAGA  
 6481 TCCCACTTTT AAAGAGAATT ATCGCTTCCA TGCAATCAAT  
 GGCTACATAA TGGATACACT  
 6541 ACCTGGCTTA GTAATGGCTC AGGATCAAAG GATTCGATGG  
 TATCTGCTCA GCATGGGCAG  
 6601 CAATGAAAAC ATCCATTCTA TTCATTTTCA TGGACATGTG  
 TTTCACTGTAC GAAAAAAGA  
 6661 GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT  
 TTTGAGACAG TGGAATGTT  
 6721 ACCATCCAAA GCTGGAATTT GGCGGGTGGA ATGCCTTATT  
 GGCGAGCATC TACATGCTGG  
 6781 GATGAGCACA CTTTTTCTGG TGTACAGCAA TAAGTGTCAG  
 ACTCCCCTGG GAATGGCTTC  
 6841 TGGACACATT AGAGATTTTC AGATTACAGC TTCAGGACAA  
 TATGGACAGT GGGCCCCAAA  
 6901 GCTGGCCAGA CTTCAATTATT CCGGATCAAT CAATGCCTGG  
 AGCACCAAGG AGCCCTTTTC  
 6961 TTGGATCAAG GTGGATCTGT TGGCACCAAT GATTATTCAC  
 GGCATCAAGA CCCAGGGTGC  
 7021 CCGTCAGAAG TTCTCCAGCC TCTACATCTC TCAGTTTATC  
 ATCATGTATA GTCTTGATGG  
 7081 GAAGAAGTGG CAGACTTATC GAGGAAATTC CACTGGAACC  
 TTAATGGTCT TCTTTGGCAA  
 7141 TGTGGATTCA TCTGGGATAA AACACAATAT TTTTAACCCT  
 CCAATTATTG CTCGATACAT  
 7201 CCGTTTGCAC CCAACTCATT ATAGCATTCG CAGCACTCTT  
 CGCATGGAGT TGATGGGCTG  
 7261 TGATTTAAAT AGTTGCAGCA TGCCATTGGG AATGGAGAGT  
 AAAGCAATAT CAGATGCACA  
 7321 GATTACTGCT TCATCCTACT TTACCAATAT GTTTGCCACC  
 TGGTCTCCTT CAAAAGCTCG  
 7381 ACTTCACCTC CAAGGGAGGA GTAATGCCTG GAGACCTCAG  
 GTGAATAATC CAAAAGAGTG  
 7441 GCTGCAAGTG GACTTCCAGA AGACAATGAA AGTCACAGGA  
 GTAAC TACTC AGGGAGTAAA  
 7501 ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC  
 TCCAGCAGTC AAGATGGCCA

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7561 TCAGTGGACT CTCTTTTTC AGAATGGCAA AGTAAAGGTT
      TTTCAGGGAA ATCAAGACTC
7621 CTTACACCT GTGGTGAAC CTCTAGACCC ACCGTTACTG
      ACTCGCTACC TTCGAATTCA
7681 CCCCCAGAGT TGGGTGCACC AGATTGCCCT GAGGATGGAG
      GTTCTGGGCT GCGAGGCACA
7741 GGACCTCTAC

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\*The underlined nucleic acids encode a signal peptide.

**[0171]** FVIII polypeptides include full-length FVIII, full-length FVIII minus Met at the N-terminus, mature FVIII (minus the signal sequence), mature FVIII with an additional Met at the N-terminus, and/or FVIII with a full or partial deletion of the B domain. In certain embodiments, FVIII variants include B domain deletions, whether partial or full deletions.

**[0172]** The sequence of native mature human FVIII is presented as SEQ ID NO: 4. A native FVIII protein has the following formula: A1-a1-A2-a2-B-a3-A3-C1-C2, where A1, A2, and A3 are the structurally-related "A domains," B is the "B domain," C1 and C2 are the structurally-related "C domains," and a1, a2 and a3 are acidic spacer regions. Referring to the primary amino acid sequence position in SEQ ID NO:4, the A1 domain of human FVIII extends from Ala1 to about Arg336, the a1 spacer region extends from about Met337 to about Val374, the A2 domain extends from about Ala375 to about Tyr719, the a2 spacer region extends from about Glu720 to about Arg740, the B domain extends from about Ser741 to about Arg 1648, the a3 spacer region extends from about Glu1649 to about Arg1689, the A3 domain extends from about Ser1690 to about Leu2025, the C1 domain extends from about Gly2026 to about Asn2072, and the C2 domain extends from about Ser2073 to Tyr2332. Other than specific proteolytic cleavage sites, designation of the locations of the boundaries between the domains and regions of FVIII can vary in different literature references. The boundaries noted herein are therefore designated as approximate by use of the term "about."

**[0173]** The human FVIII gene was isolated and expressed in mammalian cells (Toole, J. J., *et al.*, *Nature* 312:342-347 (1984); Gitschier, J., *et al.*, *Nature* 312:326-330 (1984); Wood, W. I., *et al.*, *Nature* 312:330-337 (1984); Vehar, G. A., *et al.*, *Nature* 312:337-342 (1984); WO 87/04187; WO 88/08035; WO 88/03558; and U.S. Pat. No. 4,757,006). The FVIII amino acid sequence was

deduced from cDNA as shown in U.S. Pat. No. 4,965,199. In addition, partially or fully B-domain deleted FVIII is shown in U.S. Pat. Nos. 4,994,371 and 4,868,112. In some embodiments, the human FVIII B-domain is replaced with the human Factor V B-domain as shown in U.S. Pat. No. 5,004,803. The cDNA sequence encoding human Factor VIII and amino acid sequence are shown in SEQ ID NOs: 4 and 5, respectively, of US Application Publ. No. 2005/0100990.

**[0174]** The porcine FVIII sequence is published in Toole, J. J., *et al.*, *Proc. Natl. Acad. Sci. USA* 83:5939-5942 (1986). Further, the complete porcine cDNA sequence obtained from PCR amplification of FVIII sequences from a pig spleen cDNA library has been reported in Healey, J. F., *et al.*, *Blood* 88:4209-4214 (1996). Hybrid human/porcine FVIII having substitutions of all domains, all subunits, and specific amino acid sequences were disclosed in U.S. Pat. No. 5,364,771 by Lollar and Runge, and in WO 93/20093. More recently, the nucleotide and corresponding amino acid sequences of the A1 and A2 domains of porcine FVIII and a chimeric FVIII with porcine A1 and/or A2 domains substituted for the corresponding human domains were reported in WO 94/11503. U.S. Pat. No. 5,859,204, Lollar, J. S., also discloses the porcine cDNA and deduced amino acid sequences. U.S. Pat. No. 6,458,563 discloses a B-domain-deleted porcine FVIII.

**[0175]** U.S. Pat. No. 5,859,204 to Lollar, J. S. reports functional mutants of FVIII having reduced antigenicity and reduced immunoreactivity. U.S. Pat. No. 6,376,463 to Lollar, J. S. also reports mutants of FVIII having reduced immunoreactivity. US Appl. Publ. No. 2005/0100990 to Saenko *et al.* reports functional mutations in the A2 domain of FVIII.

**[0176]** In one embodiment, the FVIII (or FVIII portion of a chimeric protein) may be at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a FVIII amino acid sequence of amino acids 1 to 1438 of SEQ ID NO: 6 or amino acids 1 to 2332 of SEQ ID NO: 4 (without a signal sequence) or a FVIII amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 3 and 1 to 1438 of SEQ ID NO: 6 or amino acids 1 to 19 of SEQ ID NO: 3 and amino acids 1 to 2332 of SEQ ID NO: 4 (with a signal sequence), wherein the FVIII has a clotting activity, *e.g.*, activates Factor IX as a cofactor to convert Factor X to activated Factor X. The FVIII (or FVIII portion of a chimeric protein) may be identical to a

FVIII amino acid sequence of amino acids 1 to 1438 of SEQ ID NO: 6 or amino acids 1 to 2332 of SEQ ID NO: 4 (without a signal sequence). The FVIII may further comprise a signal sequence.

[0177] The "B-domain" of FVIII, as used herein, is the same as the B-domain known in the art that is defined by internal amino acid sequence identity and sites of proteolytic cleavage, *e.g.*, residues Ser741-Arg1648 of full-length human FVIII. The other human FVIII domains are defined by the following amino acid residues: A1, residues Ala1-Arg372; A2, residues Ser373-Arg740; A3, residues Ser1690-Asn2019; C1, residues Lys2020-Asn2172; C2, residues Ser2173-Tyr2332. The A3-C1-C2 sequence includes residues Ser1690-Tyr2332. The remaining sequence, residues Glu1649-Arg1689, is usually referred to as the a3 acidic region. The locations of the boundaries for all of the domains, including the B-domains, for porcine, mouse and canine FVIII are also known in the art. In one embodiment, the B domain of FVIII is deleted ("B-domain-deleted factor VIII" or "BDD FVIII"). An example of a BDD FVIII is REFACTO<sup>®</sup> (recombinant BDD FVIII), which has the same sequence as the Factor VIII portion of the sequence in Table 5. (BDD FVIII heavy chain is double underlined; B domain is italicized; and BDD FVIII light chain is in plain text). A nucleotide sequence encoding the amino acid sequence set forth in Table 5 (SEQ ID NO: 7) is shown in Table 6.

**TABLE 5. Amino Acid Sequence of B-domain Deleted Factor VIII (BDD FVIII)**

**BDD FVIII (SEQ ID NO: 6)**

ATRRYYLGAVELSWDYMOSDLGELPVDARFPFPRVPKSFPFNTSVVYKKTLEVEFT  
DHLFNIAKPRPPWMGLLGPTTQAEVYDVTVVITLKNMASHPVSLHAVGVSYWKASE  
GAEYDDQTSQREKEDDKVFPGGSHTYVWQVLKENGPMASDPLCLTYSYLSHVDLV  
KDLNSGLIGALLVCREGLAKEKTQTLHKFILLFAVFDEGKSWHSETKNSLMQDR  
DAASARAWPKMHTVNGYVNRSLPGLIGCHRKSIVYWHVIGMGTTPEVHSIFLEGHT  
FLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHDGMEAYVKVDSCP  
EFPQLRMKNNEEAEDYDDDLTDSEMDVVRFDNNSPSFIQIRSVAKKHPKTTWVHY  
IAAEEEDWDYAPLV LAPDDR SYKSOYLNNGPQRIGRKYKKVRFMAYTDETFKTR  
AIQHESGILGPLYGEVGDTLIIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGV  
KHLKDFPILPGEIFKYKWTVTVEDGPTKSDPRCLTRYSSFVNMERDLASGLIGP  
LLICYKESVDQRGNOIMSDKRNVLFSVFDENRSWYLTENIQRF LNPAGVQLED  
PEFQASNIMHSINGYVFDLSQLSVCLHEVAYWYILSIGAQTDFLSVFFSGYTFKH  
KMVEYEDTLTLFPFSGETVFMSENPGLWILGCHNSDFRNRGMTALLKVSSCDKNT  
GDYYEDSYEDISAYLLSKNNAIEPRSFSONPPVLKRHQREITRTTLQSDQEEIDY  
DDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYGMSSSPHVL



NRAQSGSVPOFKKVVFQEFDTGDSFTQPLYRGELNEHLGLLGPYIRAEVEDNIMVT  
 FRNQASRPYSFYSSLISYEEDQRQGAEPKKNFVKPNETKTYFWKVQHMAPTKDE  
 FDCKAWAYFSDVDLEKDVHSGLLGPLLVCHTNLTNPAGHRQVTVQEFALFFTIFD  
 ETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQR  
 IRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPSKAG  
 IWRVECLIGEHLHAGMSTLFLVYSNKCQTPGLMASGHIRDFQITASGQYQWAPK  
 LARLHYSGSINAWSTKEPFSWIKVDLLAPMI IHGIKTQGARQKFSSLYISQFIIM  
 YSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNIFNPPIIARYIRLHPHTHSIR  
 STLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQG  
 RSNAWRPQVNNPKEWLQVDFQKTMKVTGVTTQGVKSLTSMYVKEFLISSSQDGH  
 QWTLFFQNGKVVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVL  
 GCEAQDLY

**TABLE 6. Nucleotide Sequence Encoding BDD FVIII (SEQ ID NO: 7)\***

|      |                              |                  |            |
|------|------------------------------|------------------|------------|
| 661  | <u>A TGCAAATAGA</u>          |                  |            |
|      | <u>GCTCTCCACC TGCTTCTTTC</u> |                  |            |
| 721  | <u>TGTGCCTTTT GCGATTCTGC</u> | <u>TTAGTGCCA</u> | CCAGAAGATA |
|      | CTACCTGGGT GCAGTGAAC         |                  |            |
| 781  | TGTCATGGGA CTATATGCAA        | AGTGATCTCG       | GTGAGCTGCC |
|      | TGTGGACGCA AGATTTCCTC        |                  |            |
| 841  | CTAGAGTGCC AAAATCTTTT        | CCATTCAACA       | CCTCAGTCGT |
|      | GTACAAAAAG ACTCTGTTTG        |                  |            |
| 901  | TAGAATTCAC GGATCACCTT        | TTCAACATCG       | CTAAGCCAAG |
|      | GCCACCCTGG ATGGGTCTGC        |                  |            |
| 961  | TAGGTCCTAC CATCCAGGCT        | GAGGTTTATG       | ATACAGTGGT |
|      | CATTACACTT AAGAACATGG        |                  |            |
| 1021 | CTTCCCATCC TGTCAGTCTT        | CATGCTGTTG       | GTGTATCCTA |
|      | CTGGAAAGCT TCTGAGGGAG        |                  |            |
| 1081 | CTGAATATGA TGATCAGACC        | AGTCAAAGGG       | AGAAAGAAGA |
|      | TGATAAAGTC TTCCCTGGTG        |                  |            |
| 1141 | GAAGCCATAC ATATGTCTGG        | CAGGTCCTGA       | AAGAGAATGG |
|      | TCCAATGGCC TCTGACCCAC        |                  |            |
| 1201 | TGTGCCTTAC CTA CTACTATAT     | CTTTCTCATG       | TGGACCTGGT |
|      | AAAAGACTTG AATTCAGGCC        |                  |            |
| 1261 | TCATTGGAGC CTA CTAGTA        | TGTAGAGAAG       | GGAGTCTGGC |
|      | CAAGGAAAAG ACACAGACCT        |                  |            |
| 1321 | TGCACAAATT TATACTACTT        | TTTGCTGTAT       | TTGATGAAGG |
|      | GAAAAGTTGG CACTCAGAAA        |                  |            |
| 1381 | CAAAGAACTC CTGATGCAG         | GATAGGGATG       | CTGCATCTGC |
|      | TCGGGCCTGG CCTAAAATGC        |                  |            |
| 1441 | ACACAGTCAA TGGTTATGTA        | AACAGGTCTC       | TGCCAGGTCT |
|      | GATTGGATGC CACAGGAAAT        |                  |            |
| 1501 | CAGTCTATTG GCATGTGATT        | GGAATGGGCA       | CCACTCCTGA |
|      | AGTGCACTCA ATATTCCTCG        |                  |            |
| 1561 | AAGGTCACAC ATTTCTTGTG        | AGGAACCATC       | GCCAGGCGTC |
|      | CTTGGAATC TCGCCAATAA         |                  |            |
| 1621 | CTTTCCTTAC TGCTCAAACA        | CTCTTGATGG       | ACCTTGACAA |
|      | GTTTCTACTG TTTTGTGATA        |                  |            |

1681 TCTCTTCCCA CCAACATGAT GGCATGGAAG CTTATGTCAA  
 AGTAGACAGC TGTCCAGAGG  
 1741 AACCCCAACT ACGAATGAAA AATAATGAAG AAGCGGAAGA  
 CTATGATGAT GATCTTACTG  
 1801 ATTCTGAAAT GGATGTGGTC AGGTTTGATG ATGACAACTC  
 TCCTTCCTTT ATCCAAATTC  
 1861 GCTCAGTTGC CAAGAAGCAT CCTAAAACCTT GGGTACATTA  
 CATTGCTGCT GAAGAGGAGG  
 1921 ACTGGGACTA TGCTCCCTTA GTCCTCGCCC CCGATGACAG  
 AAGTTATAAA AGTCAATATT  
 1981 TGAACAATGG CCCTCAGCGG ATTGGTAGGA AGTACAAAAA  
 AGTCCGATTT ATGGCATAACA  
 2041 CAGATGAAAC CTTTAAGACT CGTGAAGCTA TTCAGCATGA  
 ATCAGGAATC TTGGGACCTT  
 2101 TACTTTATGG GGAAGTTGGA GACACACTGT TGATTATATT  
 TAAGAATCAA GCAAGCAGAC  
 2161 CATATAACAT CTACCCTCAC GGAATCACTG ATGTCCGTCC  
 TTTGTATTCA AGGAGATTAC  
 2221 CAAAAGGTGT AAAACATTTG AAGGATTTTC CAATTCTGCC  
 AGGAGAAATA TTCAAATATA  
 2281 AATGGACAGT GACTGTAGAA GATGGGCCAA CTAAATCAGA  
 TCCTCGGTGC CTGACCCGCT  
 2341 ATTACTCTAG TTTCGTTAAT ATGGAGAGAG ATCTAGCTTC  
 AGGACTCATT GGCCCTCTCC  
 2401 TCATCTGCTA CAAAGAATCT GTAGATCAAA GAGGAAACCA  
 GATAATGTCA GACAAGAGGA  
 2461 ATGTCATCCT GTTTTCTGTA TTTGATGAGA ACCGAAGCTG  
 GTACCTCACA GAGAATATAC  
 2521 AACGCTTTCT CCCCAATCCA GCTGGAGTGC AGCTTGAGGA  
 TCCAGAGTTC CAAGCCTCCA  
 2581 ACATCATGCA CAGCATCAAT GGCTATGTTT TTGATAGTTT  
 GCAGTTGTCA GTTTGTTTGC  
 2641 ATGAGGTGGC ATACTGGTAC ATTCTAAGCA TTGGAGCACA  
 GACTGACTTC CTTTCTGTCT  
 2701 TCTTCTCTGG ATATACCTTC AAACACAAAA TGGTCTATGA  
 AGACACACTC ACCCTATTCC  
 2761 CATTCTCAGG AGAACTGTC TTCATGTCGA TGGAACCC  
 AGGTCTATGG ATTCTGGGGT  
 2821 GCCACAACCTC AGACTTTCGG AACAGAGGCA TGACCGCCTT  
 ACTGAAGGTT TCTAGTTGTG  
 2881 ACAAGAACAC TGGTGATTAT TACGAGGACA GTTATGAAGA  
 TATTTTCAGCA TACTTGCTGA  
 2941 GTAAAAACAA TGCCATTGAA CCAAGAAGCT TCTCTCAAAA  
 CCCACCAGTC TTGAAACGCC  
 3001 ATCAACGGGA AATAACTCGT ACTACTCTTC AGTCAGATCA  
 AGAGGAAATT GACTATGATG  
 3061 ATACCATATC AGTTGAAATG AAGAAGGAAG ATTTTGACAT  
 TTATGATGAG GATGAAAATC  
 3121 AGAGCCCCCG CAGCTTTCAA AAGAAAACAC GACACTATTT  
 TATTGCTGCA GTGGAGAGGC

3181 TCTGGGATTA TGGGATGAGT AGCTCCCCAC ATGTTCTAAG  
AAACAGGGCT CAGAGTGGCA  
3241 GTGTCCCTCA GTTCAAGAAA GTTGTTTTCC AGGAATTTAC  
TGATGGCTCC TTTACTCAGC  
3301 CCTTATACCG TGGAGAACTA AATGAACATT TGGGACTCCT  
GGGGCCATAT ATAAGAGCAG  
3361 AAGTTGAAGA TAATATCATG GTAACCTTCA GAAATCAGGC  
CTCTCGTCCC TATTCCTTCT  
3421 ATTCTAGCCT TATTTCTTAT GAGGAAGATC AGAGGCAAGG  
AGCAGAACCT AGAAAAAACT  
3481 TTGTCAAGCC TAATGAAACC AAAACTTACT TTTGGAAAGT  
GCAACATCAT ATGGCACCCA  
3541 CTAAAGATGA GTTTGACTGC AAAGCCTGGG CTTATTTCTC  
TGATGTTGAC CTGGAAAAAG  
3601 ATGTGCACTC AGGCCTGATT GGACCCCTTC TGGTCTGCCA  
CACTAACACA CTGAACCCTG  
3661 CTCATGGGAG ACAAGTGACA GTACAGGAAT TTGCTCTGTT  
TTTCACCATC TTTGATGAGA  
3721 CCAAAGCTG GTACTTCACT GAAAATATGG AAAGAACTG  
CAGGGCTCCC TGCAATATCC  
3781 AGATGGAAGA TCCCACCTTTT AAAGAGAATT ATCGCTTCCA  
TGCAATCAAT GGCTACATAA  
3841 TGGATACACT ACCTGGCTTA GTAATGGCTC AGGATCAAAG  
GATTCGATGG TATCTGCTCA  
3901 GCATGGGCAG CAATGAAAAC ATCCATTCTA TTCATTTTCA  
TGGACATGTG TTTACTGTAC  
3961 GAAAAAAGA GGAGTATAAA ATGGCACTGT ACAATCTCTA  
TCCAGGTGTT TTTGAGACAG  
4021 TGGAAATGTT ACCATCCAAA GCTGGAATTT GGCGGGTGGA  
ATGCCTTATT GGCGAGCATC  
4081 TACATGCTGG GATGAGCACA CTTTTTCTGG TGTACAGCAA  
TAAGTGTGAG ACTCCCCTGG  
4141 GAATGGCTTC TGGACACATT AGAGATTTTC AGATTACAGC  
TTCAGGACAA TATGGACAGT  
4201 GGGCCCCAAA GCTGGCCAGA CTTTATTATT CCGGATCAAT  
CAATGCCTGG AGCACCAAGG  
4261 AGCCCTTTTC TTGGATCAAG GTGGATCTGT TGGCACCAAT  
GATTATTAC GGCATCAAGA  
4321 CCCAGGGTGC CCGTCAGAAG TTCTCCAGCC TCTACATCTC  
TCAGTTTATC ATCATGTATA  
4381 GTCTTGATGG GAAGAAGTGG CAGACTTATC GAGGAAATTC  
CACTGGAACC TTAATGGTCT  
4441 TCTTTGGCAA TGTGGATTCA TCTGGGATAA AACACAATAT  
TTTTAACCCT CCAATTATTG  
4501 CTCGATACAT CCGTTTGCAC CCAACTCATT ATAGCATTCG  
CAGCACTCTT CGCATGGAGT  
4561 TGATGGGCTG TGATTTAAAT AGTTGCAGCA TGCCATTGGG  
AATGGAGAGT AAAGCAATAT  
4621 CAGATGCACA GATTACTGCT TCATCCTACT TTACCAATAT  
GTTTGCCACC TGGTCTCCTT

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4681  CAAAAGCTCG ACTTCACCTC CAAGGGAGGA GTAATGCCTG
      GAGACCTCAG GTGAATAATC
4741  CAAAAGAGTG GCTGCAAGTG GACTTCCAGA AGACAATGAA
      AGTCACAGGA GTAAC TACTC
4801  AGGGAGTAAA ATCTCTGCTT ACCAGCATGT ATGTGAAGGA
      GTTCCTCATC TCCAGCAGTC
4861  AAGATGGCCA TCAGTGGACT CTCTTTTTTC AGAATGGCAA
      AGTAAAGGTT TTTCAGGGAA
4921  ATCAAGACTC CTTCACACCT GTGGTGAAC TCTAGACCC
      ACCGTTACTG ACTCGCTACC
4981  TTGGAATTCA CCCCAGAGT TGGGTGCACC AGATTGCCCT
      GAGGATGGAG GTTCTGGGCT
5041  GCGAGGCACA GGACCTCTAC

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\*The underlined nucleic acids encode a signal peptide.

**[0178]** A "B-domain-deleted FVIII" may have the full or partial deletions disclosed in U.S. Pat. Nos. 6,316,226, 6,346,513, 7,041,635, 5,789,203, 6,060,447, 5,595,886, 6,228,620, 5,972,885, 6,048,720, 5,543,502, 5,610,278, 5,171,844, 5,112,950, 4,868,112, and 6,458,563. In some embodiments, a B-domain-deleted FVIII sequence of the present invention comprises any one of the deletions disclosed at col. 4, line 4 to col. 5, line 28 and Examples 1-5 of U.S. Pat. No. 6,316,226 (also in US 6,346,513). In another embodiment, a B-domain deleted Factor VIII is the S743/Q1638 B-domain deleted Factor VIII (SQ BDD FVIII) (*e.g.*, Factor VIII having a deletion from amino acid 744 to amino acid 1637, *e.g.*, Factor VIII having amino acids 1-743 and amino acids 1638-2332 of SEQ ID NO: 4, *i.e.*, SEQ ID NO: 6). In some embodiments, a B-domain-deleted FVIII of the present invention has a deletion disclosed at col. 2, lines 26-51 and examples 5-8 of U.S. Patent No. 5,789,203 (also US 6,060,447, US 5,595,886, and US 6,228,620). In some embodiments, a B-domain-deleted Factor VIII has a deletion described in col. 1, lines 25 to col. 2, line 40 of US Patent No. 5,972,885; col. 6, lines 1-22 and example 1 of U.S. Patent no. 6,048,720; col. 2, lines 17-46 of U.S. Patent No. 5,543,502; col. 4, line 22 to col. 5, line 36 of U.S. Patent no. 5,171,844; col. 2, lines 55-68, figure 2, and example 1 of U.S. Patent No. 5,112,950; col. 2, line 2 to col. 19, line 21 and table 2 of U.S. Patent No. 4,868,112; col. 2, line 1 to col. 3, line 19, col. 3, line 40 to col. 4, line 67, col. 7, line 43 to col. 8, line 26, and col. 11, line 5 to col. 13, line 39 of U.S. Patent no. 7,041,635; or col. 4, lines 25-53, of U.S. Patent No. 6,458,563. In some embodiments, a B-domain-deleted FVIII has a deletion of most of the B domain, but still contains amino-terminal

sequences of the B domain that are essential for *in vivo* proteolytic processing of the primary translation product into two polypeptide chain, as disclosed in WO 91/09122. In some embodiments, a B-domain-deleted FVIII is constructed with a deletion of amino acids 747-1638, *i.e.*, virtually a complete deletion of the B domain. Hoeben R.C., *et al. J. Biol. Chem.* 265 (13): 7318-7323 (1990). A B-domain-deleted Factor VIII may also contain a deletion of amino acids 771-1666 or amino acids 868-1562 of FVIII. Meulien P., *et al. Protein Eng.* 2(4): 301-6 (1988). Additional B domain deletions that are part of the invention include: deletion of amino acids 982 through 1562 or 760 through 1639 (Toole *et al., Proc. Natl. Acad. Sci. U.S.A.* (1986) 83, 5939-5942)), 797 through 1562 (Eaton, *et al. Biochemistry* (1986) 25:8343-8347)), 741 through 1646 (Kaufman (PCT published application No. WO 87/04187)), 747-1560 (Sarver, *et al., DNA* (1987) 6:553-564)), 741 through 1648 (Pasek (PCT application No.88/00831)), or 816 through 1598 or 741 through 1648 (Lagner (Behring Inst. Mitt. (1988) No 82:16-25, EP 295597)). In other embodiments, BDD FVIII includes a FVIII polypeptide containing fragments of the B-domain that retain one or more N-linked glycosylation sites, *e.g.*, residues 757, 784, 828, 900, 963, or optionally 943, which correspond to the amino acid sequence of the full-length FVIII sequence. Examples of the B-domain fragments include 226 amino acids or 163 amino acids of the B-domain as disclosed in Miao, H.Z., *et al., Blood* 103(a): 3412-3419 (2004), Kasuda, A, *et al., J. Thromb. Haemost.* 6: 1352-1359 (2008), and Pipe, S.W., *et al., J. Thromb. Haemost.* 9: 2235-2242 (2011) (*i.e.*, the first 226 amino acids or 163 amino acids of the B domain are retained). In still other embodiments, BDD FVIII further comprises a point mutation at residue 309 (from Phe to Ser) to improve expression of the BDD FVIII protein. *See* Miao, H.Z., *et al., Blood* 103(a): 3412-3419 (2004). In still other embodiments, the BDD FVIII includes a FVIII polypeptide containing a portion of the B-domain, but not containing one or more furin cleavage sites (*e.g.*, Arg1313 and Arg 1648). *See* Pipe, S.W., *et al., J. Thromb. Haemost.* 9: 2235-2242 (2011). Each of the foregoing deletions may be made in any FVIII sequence.

**[0179]** In some embodiments, the FVIII has a partial B-domain. In some embodiments, the FVIII protein with a partial B-domain is FVIII198 (SEQ ID NO: 89). FVIII198 is a partial B-domain containing single chain FVIII<sub>FC</sub>

molecule-226N6. 226 represents the N-terminus 226 amino acid of the FVIII B-domain, and N6 represents six N-glycosylation sites in the B-domain.

**[0180]** In one embodiment, FVIII is cleaved right after arginine at amino acid 1648 (in full-length Factor VIII or SEQ ID NO: 4), amino acid 754 (in the S743/Q1638 B-domain deleted Factor VIII or SEQ ID NO: 6), or the corresponding arginine residue (in other variants), thereby resulting in a heavy chain and a light chain. In another embodiment, FVIII comprises a heavy chain and a light chain, which are linked or associated by a metal ion-mediated non-covalent bond.

**[0181]** In other embodiments, FVIII is a single chain FVIII that has not been cleaved right after Arginine at amino acid 1648 (in full-length FVIII or SEQ ID NO: 4), amino acid 754 (in the S743/Q1638 B-domain-deleted FVIII or SEQ ID NO: 6), or the corresponding Arginine residue (in other variants). A single chain FVIII may comprise one or more amino acid substitutions. In one embodiment, the amino acid substitution is at a residue corresponding to residue 1648, residue 1645, or both of full-length mature Factor VIII polypeptide (SEQ ID NO: 4) or residue 754, residue 751, or both of SQ BDD Factor VIII (SEQ ID NO: 6). The amino acid substitution can be any amino acids other than arginine, *e.g.*, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, selenocysteine, serine, tyrosine, histidine, ornithine, pyrrolysine, or taurine.

**[0182]** FVIII can further be cleaved by thrombin and then activated as FVIIIa, serving as a cofactor for activated Factor IX (FIXa). And the activated FIX together with activated FVIII forms a Xase complex and converts Factor X to activated Factor X (FXa). For activation, FVIII is cleaved by thrombin after three Arginine residues, at amino acids 372, 740, and 1689 (corresponding to amino acids 372, 740, and 795 in the B-domain deleted FVIII sequence), the cleavage generating FVIIIa having the 50kDa A1, 43kDa A2, and 73kDa A3-C1-C2 chains. In one embodiment, the FVIII protein useful for the present invention is non-active FVIII. In another embodiment, the FVIII protein is an activated FVIII.

**[0183]** The protein having FVIII polypeptide linked to or associated with the VWF fragment can comprise a sequence at least 50%, 60%, 70%, 80%, 90%,

95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 4 or 6, wherein the sequence has the FVIII clotting activity, *e.g.*, activating Factor IX as a cofactor to convert Factor X to activated Factor X (FXa).

**[0184]** "Hybrid" or "chimeric" polypeptides and proteins, as used herein, includes a combination of a first polypeptide chain, *e.g.*, the VWF fragment, optionally fused to a first Ig constant region or a portion thereof, with a second polypeptide chain, *e.g.*, a FVIII protein linked to an XTEN sequence, optionally fused to a second Ig constant region or a portion thereof, thereby forming a heterodimer. In one embodiment, the first polypeptide and the second polypeptide in a hybrid are associated with each other via protein-protein interactions, such as charge-charge or hydrophobic interactions. In another embodiment, the first polypeptide and the second polypeptide in a hybrid are associated with each other via disulfide or other covalent bond(s). Hybrids are described, for example, in US 2004/101740 and US 2006/074199. The second polypeptide may be an identical copy of the first polypeptide or a non-identical polypeptide. In one embodiment, the first polypeptide is a FVIII protein(X)-Fc fusion protein, and the second polypeptide is a polypeptide comprising, consisting essentially of, or consisting of an Fc region, wherein the first polypeptide and the second polypeptide are associated with each other. In another embodiment, the first polypeptide comprises a VWF fragment-XTEN-Fc fusion protein, and the second polypeptide comprises FVIII-Fc fusion protein, making the hybrid a heterodimer. In other embodiments, the first polypeptide comprises a VWF fragment-Fc fusion protein, and the second polypeptide comprises FVIII(X)-Fc fusion protein, making the hybrid a heterodimer. In yet other embodiments, the first polypeptide comprises a VWF fragment-XTEN-Fc fusion protein, and the second polypeptide comprises FVIII(X)-Fc fusion protein. The first polypeptide and the second polypeptide can be associated through a covalent bond, *e.g.*, a disulfide bond, between the first Fc region and the second Fc region. The first polypeptide and the second polypeptide can further be associated with each other by binding between the VWF fragment and the FVIII protein.

**[0185]** A FVIII protein useful in the present invention can include FVIII having one or more additional XTEN sequences, which do not affect the FVIII coagulation activity. Such XTEN sequences can be fused to the C-terminus or N-

terminus of the FVIII protein or inserted between one or more of the two amino acid residues in the FVIII protein wherein the insertions do not affect the FVIII coagulation activity or FVIII function. In one embodiment, the insertions improve pharmacokinetic properties of the FVIII protein (*e.g.*, half-life). In another embodiment, the insertions can be multiple insertions, *e.g.*, more than two, three, four, five, six, seven, eight, nine, or ten insertions. Examples of the insertion sites include, but are not limited to, the sites listed in Tables 7, 8, 9, 10, 11, 12, 13, 14, 15 or any combinations thereof.

**[0186]** The FVIII protein linked to one or more XTEN sequences can be represented as FVIII(X), FVIII(X1), FVIII<sub>(a→b)</sub>-X-FVIII<sub>(c→d)</sub>, wherein FVIII<sub>(a→b)</sub> comprises, consists essentially of, or consists of a first portion of a FVIII protein from amino acid residue "a" to amino acid residue "b"; X or X1 comprises, consists essentially of, or consists of one or more XTEN sequences, FVIII<sub>(c→d)</sub> comprises, consists essentially of, or consists of a second portion of a FVIII protein from amino acid residue "c" to amino acid residue "d"; a is the N-terminal amino acid residue of the first portion of the FVIII protein, b is the C-terminal amino acid residue of the first portion of the FVIII protein but is also the N-terminal amino acid residue of the two amino acids of an insertion site in which the XTEN sequence is inserted, c is the N-terminal amino acid residue of the second portion of the FVIII protein but is also the C-terminal amino acid residue of the two amino acids of an insertion site in which the XTEN sequence is inserted, and d is the C-terminal amino acid residue of the FVIII protein, and wherein the first portion of the FVIII protein and the second portion of the FVIII protein are not identical to each other and are of sufficient length together such that the FVIII protein has a FVIII coagulation activity.

**[0187]** In one embodiment, the first portion of the FVIII protein and the second portion of the FVIII protein are fragments of SEQ ID NO: 4 [full length mature FVIII sequence] or SEQ ID NO: 6 [B-domain deleted FVIII], *e.g.*, N-terminal portion and C-terminal portion, respectively. In certain embodiments, the first portion of the FVIII protein comprises the A1 domain and the A2 domain of the FVIII protein. The second portion of the FVIII protein comprises the A3 domain, the C1 domain, and optionally the C2 domain. In yet other embodiments, the first



portion of the FVIII protein comprises the A1 domain and A2 domain, and the second portion of the FVIII protein comprises a portion of the B domain, the A3 domain, the C1 domain, and optionally the C2 domain. In still other embodiments, the first portion of the FVIII protein comprises the A1 domain, A2 domain, and a portion of the B domain of the FVIII protein, and the second portion of the FVIII protein comprises the A3 domain, the C1 domain, and optionally the C2 domain. In still other embodiments, the first portion of the FVIII protein comprises the A1 domain, A2 domain, and a first portion of the B domain of the FVIII protein. The second portion of the FVIII protein comprises a second portion of the B domain, the A3 domain, the C1 domain, and optionally the C2 domain. In some embodiments, the two amino acids ("b" and "c") can be any one or more of the amino acid residues insertion sites shown in Tables 7, 8, 9, 10, 11, 12, 13, 14, and 15. For example, "b" can be the amino acid residue immediately upstream of the site in which one or more XTEN sequences are inserted or linked, and "c" can be the amino acid residue immediately downstream of the site in which the one or more XTEN sequences are inserted or linked. In some embodiments, "a" is the first mature amino acid sequence of a FVIII protein, and "d" is the last amino acid sequence of a FVIII protein. For example, FVIII<sub>(a→b)</sub> can be an amino acid sequence at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 1 to 745 of SEQ ID NO: 6 [B domain deleted FVIII amino acid sequence] or SEQ ID NO: 4 [full length FVIII] and FVIII<sub>(c→d)</sub> can be amino acids 746 to 1438 of SEQ ID NO: 6 or amino acids 1641 to 2332 of SEQ ID NO: 4, respectively.

**[0188]** In some aspects, the insertion site in the FVIII protein is located in one or more domains of the FVIII protein, which is the N-terminus, the A1 domain, the A2 domain, the A3 domain, the B domain, the C1 domain, the C2 domain, the C-terminus, or two or more combinations thereof or between two domains of the FVIII protein, which are the A1 domain and a1 acidic region, and the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, and the A3 domain and C1 domain, the C1 domain and C2 domain, or any combinations thereof. For example, the insertion sites in which the XTEN sequence can be inserted are selected from the group consisting of the N-terminus and A1 domain, the N-terminus and A2

domain, the N-terminus and A3 domain, the N-terminus and B domain, the N-terminus and C1 domain, the N-terminus and C2 domain, the N-terminus and the C-terminus, the A1 and A2 domains, the A1 and A3 domains, the A1 and B domains, the A1 and C1 domains, the A1 and C2 domains, the A1 domain and the C-terminus, the A2 and A3 domains, the A2 and B domains, the A2 and C1 domains, the A2 and C2 domains, the A2 domain and the C-terminus, the A3 and B domains, the A3 and C1 domains, the A3 and C2 domains, the A3 domain and the C-terminus, the B and C1 domains, the B and C2 domains, the B domain and the C-terminus, the C1 and C2 domains, the C1 and the C-terminus, the C2 domain, and the C-terminus, and two or more combinations thereof. Non-limiting examples of the insertion sites are listed in Tables 7, 8, 9, 10, 11, 12, 13, 14, and 15.

**[0189]** The FVIII protein, in which the XTEN sequence is inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites) in the FVIII protein or linked at the C-terminus or the N-terminus, retains the FVIII activity after linkage to or insertion by the XTEN sequence. The XTEN sequence can be inserted in the FVIII protein once or more than once, twice, three times, four times, five times, or six times such that the insertions do not affect the FVIII activity (*i.e.*, the FVIII protein still retains the coagulation property).

**[0190]** The FVIII protein useful in the present invention can be linked to one or more XTEN polypeptides at the N-terminus or C-terminus of the FVIII protein by an optional linker or inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites) in the FVIII protein by one or more optional linkers. In one embodiment, the two amino acid residues in which the XTEN sequence is inserted or the amino acid residue to which the XTEN sequence is linked correspond to the two or one amino acid residues of SEQ ID NO: 4 [full length mature FVIII] selected from the group consisting of the residues in Table 7, Table 8, Table 9, and Table 10 and any combinations thereof.

**[0191]** In other embodiments, at least one XTEN sequence is inserted in any one or more XTEN insertion sites disclosed herein or any combinations thereof. In one aspect, at least one XTEN sequence is inserted in one or more XTEN insertion sites disclosed in one or more amino acids disclosed in Table 7.

**TABLE 7: Exemplary XTEN Insertion Sites**

| <b>No.</b> | <b>XTEN<br/>Insertion<br/>Point*</b> | <b>Insertion<br/>Residue</b> | <b>FVIII BDD<br/>Downstream<br/>Sequence</b> | <b>FVIII Domain</b> |
|------------|--------------------------------------|------------------------------|----------------------------------------------|---------------------|
| 1          | 0                                    | (N-terminus)                 | ATR                                          | A1                  |
| 2          | 3                                    | R                            | RYY                                          | A1                  |
| 3          | 17                                   | M                            | QSD                                          | A1                  |
| 4          | 18                                   | Q                            | SDL                                          | A1                  |
| 5          | 22                                   | G                            | ELP                                          | A1                  |
| 6          | 24                                   | L                            | PVD                                          | A1                  |
| 7          | 26                                   | V                            | DAR                                          | A1                  |
| 8          | 28                                   | A                            | RFP                                          | A1                  |
| 9          | 32                                   | P                            | RVP                                          | A1                  |
| 10         | 38                                   | F                            | PFN                                          | A1                  |
| 11         | 40                                   | F                            | NTS                                          | A1                  |
| 12         | 41                                   | N                            | TSV                                          | A1                  |
| 13         | 60                                   | N                            | IAK                                          | A1                  |
| 14         | 61                                   | I                            | AKP                                          | A1                  |
| 15         | 65                                   | R                            | PPW                                          | A1                  |
| 16         | 81                                   | Y                            | DTV                                          | A1                  |
| 17         | 111                                  | G                            | AEY                                          | A1                  |
| 18         | 116                                  | D                            | QTS                                          | A1                  |
| 19         | 119                                  | S                            | QRE                                          | A1                  |
| 20         | 120                                  | Q                            | REK                                          | A1                  |
| 21         | 128                                  | V                            | FPG                                          | A1                  |
| 22         | 129                                  | F                            | PGG                                          | A1                  |
| 23         | 130                                  | P                            | GGG                                          | A1                  |
| 24         | 182                                  | G                            | SLA                                          | A1                  |
| 25         | 185                                  | A                            | KEK                                          | A1                  |
| 26         | 188                                  | K                            | TQT                                          | A1                  |
| 27         | 205                                  | G                            | KSW                                          | A1                  |
| 28         | 210                                  | S                            | ETK                                          | A1                  |
| 29         | 211                                  | E                            | TKN                                          | A1                  |
| 30         | 216                                  | L                            | MQD                                          | A1                  |
| 31         | 220                                  | R                            | DAA                                          | A1                  |
| 32         | 222                                  | A                            | ASA                                          | A1                  |
| 33         | 223                                  | A                            | SAR                                          | A1                  |
| 34         | 224                                  | S                            | ARA                                          | A1                  |
| 35         | 230                                  | K                            | MHT                                          | A1                  |
| 36         | 243                                  | P                            | GLI                                          | A1                  |
| 37         | 244                                  | G                            | LIG                                          | A1                  |
| 38         | 250                                  | R                            | KSV                                          | A1                  |
| 39         | 318                                  | D                            | GME                                          | A1                  |
| 40         | 333                                  | P                            | QLR                                          | A1                  |
| 42         | 334                                  | Q                            | LRM                                          | A1                  |
| 43         | 336                                  | R                            | MKN                                          | a1                  |

| No. | XTEN<br>Insertion<br>Point* | Insertion<br>Residue | FVIII BDD<br>Downstream<br>Sequence | FVIII Domain |
|-----|-----------------------------|----------------------|-------------------------------------|--------------|
| 44  | 339                         | N                    | NEE                                 | a1           |
| 45  | 345                         | D                    | YDD                                 | a1           |
| 46  | 357                         | V                    | VRF                                 | a1           |
| 47  | 367                         | S                    | FIQ                                 | a1           |
| 48  | 370                         | S                    | RPY                                 | a1           |
| 49  | 375                         | A                    | KKH                                 | A2           |
| 50  | 376                         | K                    | KHP                                 | A2           |
| 51  | 378                         | H                    | PKT                                 | A2           |
| 52  | 399                         | V                    | LAP                                 | A2           |
| 53  | 403                         | D                    | DRS                                 | A2           |
| 54  | 405                         | R                    | SYK                                 | A2           |
| 55  | 409                         | S                    | QYL                                 | A2           |
| 56  | 416                         | P                    | QRI                                 | A2           |
| 57  | 434                         | E                    | TFK                                 | A2           |
| 58  | 438                         | T                    | REA                                 | A2           |
| 59  | 441                         | A                    | IQH                                 | A2           |
| 60  | 442                         | I                    | QHE                                 | A2           |
| 61  | 463                         | I                    | IFK                                 | A2           |
| 62  | 487                         | Y                    | SRR                                 | A2           |
| 63  | 490                         | R                    | LPK                                 | A2           |
| 64  | 492                         | P                    | KGV                                 | A2           |
| 65  | 493                         | K                    | GVK                                 | A2           |
| 66  | 494                         | G                    | VKH                                 | A2           |
| 67  | 500                         | D                    | FPI                                 | A2           |
| 68  | 506                         | G                    | EIF                                 | A2           |
| 69  | 518                         | E                    | DGP                                 | A2           |
| 70  | 556                         | K                    | ESV                                 | A2           |
| 71  | 565                         | Q                    | IMS                                 | A2           |
| 72  | 566                         | I                    | MSD                                 | A2           |
| 73  | 598                         | P                    | AGV                                 | A2           |
| 74  | 599                         | A                    | GVQ                                 | A2           |
| 75  | 603                         | L                    | EDP                                 | A2           |
| 76  | 616                         | S                    | ING                                 | A2           |
| 77  | 686                         | G                    | LWI                                 | A2           |
| 78  | 713                         | K                    | NTG                                 | A2           |
| 79  | 719                         | Y                    | EDS                                 | A2           |
| 80  | 730                         | L                    | LSK                                 | A2           |
| 81  | 733                         | K                    | NNA                                 | A2           |
| 82  | 745                         | N                    | PPV**                               | B            |
| 83  | 1640                        | P                    | PVL                                 | B            |
| 84  | 1652                        | R                    | TTL                                 | B            |
| 85  | 1656                        | Q                    | SDQ                                 | A3           |
| 86  | 1685                        | N                    | QSP                                 | A3           |
| 87  | 1711                        | M                    | SSS                                 | A3           |
| 88  | 1713                        | S                    | SPH                                 | A3           |

| No. | XTEN<br>Insertion<br>Point* | Insertion<br>Residue | FVIII BDD<br>Downstream<br>Sequence | FVIII Domain |
|-----|-----------------------------|----------------------|-------------------------------------|--------------|
| 89  | 1720                        | N                    | RAQ                                 | A3           |
| 90  | 1724                        | S                    | GSV                                 | A3           |
| 91  | 1725                        | G                    | SVP                                 | A3           |
| 92  | 1726                        | S                    | VPQ                                 | A3           |
| 93  | 1741                        | G                    | SFT                                 | A3           |
| 94  | 1744                        | T                    | QPL                                 | A3           |
| 95  | 1749                        | R                    | GEL                                 | A3           |
| 96  | 1773                        | V                    | TFR                                 | A3           |
| 97  | 1792                        | Y                    | EED                                 | A3           |
| 98  | 1793                        | E                    | EDQ                                 | A3           |
| 99  | 1796                        | Q                    | RQG                                 | A3           |
| 100 | 1798                        | Q                    | GAE                                 | A3           |
| 101 | 1799                        | G                    | AEP                                 | A3           |
| 102 | 1802                        | P                    | RKN                                 | A3           |
| 103 | 1803                        | R                    | KNF                                 | A3           |
| 104 | 1807                        | V                    | KPN                                 | A3           |
| 105 | 1808                        | K                    | PNE                                 | A3           |
| 106 | 1827                        | K                    | DEF                                 | A3           |
| 107 | 1844                        | E                    | KDV                                 | A3           |
| 108 | 1861                        | N                    | TLN                                 | A3           |
| 109 | 1863                        | L                    | NPA                                 | A3           |
| 110 | 1896                        | E                    | RNC                                 | A3           |
| 111 | 1900                        | R                    | APC                                 | A3           |
| 112 | 1904                        | N                    | IQM                                 | A3           |
| 113 | 1905                        | I                    | QME                                 | A3           |
| 114 | 1910                        | P                    | TFK                                 | A3           |
| 115 | 1920                        | A                    | ING                                 | A3           |
| 116 | 1937                        | D                    | QRI                                 | A3           |
| 117 | 1981                        | G                    | VFE                                 | A3           |
| 118 | 2019                        | N                    | KCQ                                 | A3           |
| 119 | 2020                        | K                    | CQT                                 | C1           |
| 120 | 2044                        | G                    | QWA                                 | C1           |
| 121 | 2068                        | F                    | SWI                                 | C1           |
| 122 | 2073                        | V                    | DLL                                 | C1           |
| 123 | 2090                        | R                    | QKF                                 | C1           |
| 124 | 2092                        | K                    | FSS                                 | C1           |
| 125 | 2093                        | F                    | SSL                                 | C1           |
| 126 | 2111                        | K                    | WQT                                 | C1           |
| 127 | 2115                        | Y                    | RGN                                 | C1           |
| 128 | 2120                        | T                    | GTL                                 | C1           |
| 129 | 2125                        | V                    | FFG                                 | C1           |
| 130 | 2171                        | L                    | NSC                                 | C1           |
| 131 | 2173                        | S                    | CSM                                 | C2           |
| 132 | 2188                        | A                    | QIT                                 | C2           |
| 133 | 2223                        | V                    | NNP                                 | C2           |

| No. | XTEN Insertion Point* | Insertion Residue | FVIII BDD Downstream Sequence | FVIII Domain |
|-----|-----------------------|-------------------|-------------------------------|--------------|
| 134 | 2224                  | N                 | NPK                           | C2           |
| 135 | 2227                  | K                 | EWL                           | C2           |
| 136 | 2268                  | G                 | HQW                           | C2           |
| 137 | 2277                  | N                 | GKV                           | C2           |
| 138 | 2278                  | G                 | KVK                           | C2           |
| 139 | 2290                  | F                 | TPV                           | C2           |
| 140 | 2332                  | Y                 | C terminus of FVIII           | CT           |

\* Indicates an insertion point for XTEN based on the amino acid number of mature full-length human FVIII, wherein the insertion could be either on the N- or C-terminal side of the indicated amino acid.

[0192] In some embodiments, one or more XTEN sequences are inserted within about six amino acids up or down from amino acids 32, 220, 224, 336, 339, 399, 416, 603, 1656, 1711, 1725, 1905, or 1910, corresponding to SEQ ID NO: 4 or any combinations thereof.

**TABLE 8. Exemplary XTEN Insertion Ranges**

| No. | XTEN Insertion Point | Insertion Residue | FVIII BDD Downstream Sequence | FVIII Domain | Distance from insertion residue* |
|-----|----------------------|-------------------|-------------------------------|--------------|----------------------------------|
| 9   | 32                   | P                 | RVP                           | A1           | -3, +6                           |
| 31  | 220                  | R                 | DAA                           | A1           | -                                |
| 34  | 224                  | S                 | ARA                           | A1           | +5                               |
| 43  | 336                  | R                 | MKN                           | a1           | -1, +6                           |
| 44  | 339                  | N                 | NEE                           | a1           | -4, +5                           |
| 52  | 399                  | V                 | LAP                           | A2           | -6, +3                           |
| 56  | 416                  | P                 | QRI                           | A2           | +6                               |
| 75  | 603                  | L                 | EDP                           | A2           | -6, +6                           |
| 85  | 1656                 | Q                 | SDQ                           | B            | -3, +6                           |
| 87  | 1711                 | M                 | SSS                           | A3           | -6, +1                           |
| 91  | 1725                 | G                 | SVP                           | A3           | +6                               |
| 113 | 1905                 | I                 | QME                           | A3           | +6                               |
| 114 | 1910                 | P                 | TFK                           | A3           | -5, +6                           |

\*Distance from insertion residue refers to the relative number of amino acids away from the N-terminus (negative numbers) or C-terminus (positive numbers) of the designated insertion residue (residue "0") where an insertion may be made. The designation "-x" refers to an insertion site which is x amino acids away on the N-terminal side of the designated insertion residue. Similarly, the designation "+x"

refers to an insertion site which is x amino acids away on the C-terminal side of the designated insertion residue.

For example, "-1, +2" indicates that the insertion is made at the N-terminus or C-terminus of amino acid residues denoted -1, 0, +1 or +2.

**[0193]** In other embodiments, one or more XTEN sequences are inserted immediately down stream of one or more amino acids corresponding to the full-length mature human FVIII selected from the group consisting of one or more insertion sites in Table 9.

**TABLE 9. Exemplary XTEN Insertion Sites or Ranges**

| No. | XTEN Insertion Point Range* | First Insertion Residue | FVIII Domain |
|-----|-----------------------------|-------------------------|--------------|
| 3   | 18-32                       | Q                       | A1           |
| 8   | 40                          | F                       | A1           |
| 18  | 211-224                     | E                       | A1           |
| 27  | 336-403                     | R                       | A1, A2       |
| 43  | 599                         | A                       | A2           |
| 47  | 745-1640                    | N                       | B            |
| 50  | 1656-1728                   | Q                       | B, a3, A3    |
| 57  | 1796-1804                   | R                       | A3           |
| 65  | 1900-1912                   | R                       | A3           |
| 81  | 2171-2332                   | L                       | C1, C2       |

\* indicates range of insertion sites numbered relative to the amino acid number of mature human FVIII

**[0194]** In yet other embodiments, one or more XTENs are inserted in the B domain of FVIII. In one example, an XTEN is inserted between amino acids 740 and 1640 corresponding to SEQ ID NO: 4, wherein the FVIII sequence between amino acids 740 and 1640 is optionally not present. In another example, an XTEN is inserted between amino acids 741 and 1690 corresponding to SEQ ID NO: 4, wherein the FVIII sequence between amino acids 740 and 1690 is optionally not present. In other examples, an XTEN is inserted between amino acids 741 and 1648 corresponding to SEQ ID NO: 4, wherein the FVIII sequence between amino acids 741 and 1648 is optionally not present. In yet other examples, an XTEN is inserted between amino acids 743 and 1638 corresponding to SEQ ID NO: 4, wherein the FVIII sequence between amino acids 743 and 1638 is optionally not present. In still other examples, an XTEN is inserted between

amino acids 745 and 1656 corresponding to SEQ ID NO: 4, wherein the FVIII sequence between amino acids 745 and 1656 is optionally not present. In some examples, an XTEN is inserted between amino acids 745 and 1657 corresponding to SEQ ID NO: 4, wherein the FVIII sequence between amino acids 745 and 1657 is optionally not present. In certain examples, an XTEN is inserted between amino acids 745 and 1667 corresponding to SEQ ID NO: 4, wherein the FVIII sequence between amino acids 745 and 1667 is optionally not present. In still other examples, an XTEN is inserted between amino acids 745 and 1686 corresponding to SEQ ID NO: 4, wherein the FVIII sequence between amino acids 745 and 1686 is optionally not present. In some other examples, an XTEN is inserted between amino acids 747 and 1642 corresponding to SEQ ID NO: 4, wherein the FVIII sequence between amino acids 747 and 1642 is optionally not present. In still other examples, an XTEN is inserted between amino acids 751 and 1667 corresponding to SEQ ID NO: 4, wherein the FVIII sequence between amino acids 751 and 1667 is optionally not present.

**[0195]** In some embodiments, one or more XTENs are inserted in one or more amino acids immediately downstream of an amino acid of an insertion site selected from the group consisting of the amino acid residues in Table 10.



**TABLE 10: FVIII XTEN insertion sites and construct designations**

| Construct Number | Domain | Upstream Residue No.* | Downstream Residue No.* | Upstream Sequence | Downstream Sequence |
|------------------|--------|-----------------------|-------------------------|-------------------|---------------------|
| F8X-1            | A1     | 3                     | 4                       | ATR               | RYY                 |
| F8X-2            | A1     | 18                    | 19                      | YMQ               | SDL                 |
| F8X-3            | A1     | 22                    | 23                      | DLG               | ELP                 |
| F8X-4            | A1     | 26                    | 27                      | LPV               | DAR                 |
| F8X-5            | A1     | 40                    | 41                      | FPF               | NTS                 |
| F8X-6            | A1     | 60                    | 61                      | LFN               | IAK                 |
| F8X-7            | A1     | 116                   | 117                     | YDD               | QTS                 |
| F8X-8            | A1     | 130                   | 131                     | VFP               | GGG                 |
| F8X-9            | A1     | 188                   | 189                     | KEK               | TQT                 |
| F8X-10           | A1     | 216                   | 217                     | NSL               | MQD                 |
| F8X-11           | A1     | 230                   | 231                     | WPK               | MHT                 |
| F8X-12           | A1     | 333                   | 334                     | EEP               | QLR                 |
| F8X-13           | A2     | 375                   | 376                     | SVA               | KKH                 |
| F8X-14           | A2     | 403                   | 404                     | APD               | DRS                 |
| F8X-15           | A2     | 442                   | 443                     | EAI               | QHE                 |
| F8X-16           | A2     | 490                   | 491                     | RRL               | PKG                 |
| F8X-17           | A2     | 518                   | 519                     | TVE               | DGP                 |
| F8X-18           | A2     | 599                   | 600                     | NPA               | GVQ                 |
| F8X-19           | A2     | 713                   | 714                     | CDK               | NTG                 |
| F8X-20           | BD     | 745                   | 746                     | SQN               | PPV                 |
| F8X-21           | BD     | 745                   | 746                     | SQN               | PPV                 |
| F8X-22           | BD**   | 745                   | 746                     | SQN               | PPV                 |
| F8X-23           | A3     | 1720                  | 1721                    | APT               | KDE                 |
| F8X-24           | A3     | 1796                  | 1797                    | EDQ               | RQG                 |
| F8X-25           | A3     | 1802                  | 1803                    | AEP               | RKN                 |
| F8X-26           | A3     | 1827                  | 1828                    | PTK               | DEF                 |
| F8X-27           | A3     | 1861                  | 1862                    | HTN               | TLN                 |
| F8X-28           | A3     | 1896                  | 1897                    | NME               | RNC                 |
| F8X-29           | A3     | 1900                  | 1901                    | NCR               | APC                 |
| F8X-30           | A3     | 1904                  | 1905                    | PCN               | IQM                 |
| F8X-31           | A3     | 1937                  | 1938                    | AQD               | QRI                 |
| F8X-32           | C1     | 2019                  | 2020                    | YSN               | KCQ                 |
| F8X-33           | C1     | 2068                  | 2069                    | EPF               | SWI                 |
| F8X-34           | C1     | 2111                  | 2112                    | GKK               | WQT                 |
| F8X-35           | C1     | 2120                  | 2121                    | NST               | GTL                 |
| F8X-36           | C2     | 2171                  | 2172                    | CDL               | NSC                 |
| F8X-37           | C2     | 2188                  | 2189                    | SDA               | QIT                 |
| F8X-38           | C2     | 2227                  | 2228                    | NPK               | EWL                 |
| F8X-39           | C2     | 2277                  | 2278                    | FQN               | GKV                 |
| F8X-40           | CT     | 2332                  | NA                      | DLY               | NA                  |
| F8X-41           | CT     | 2332                  | NA                      | DLY               | NA                  |
| F8X-42           | A1     | 3                     | 4                       | ATR               | ATR                 |
| pSD0001          | A2     | 403                   | 404                     |                   |                     |
| pSD0002          | A2     | 599                   | 600                     |                   |                     |

|         |        |      |      |  |  |
|---------|--------|------|------|--|--|
| pSD0021 | N-term | 0    | 1    |  |  |
| pSD0022 | A1     | 32   | 33   |  |  |
| pSD0023 | A1     | 65   | 66   |  |  |
| pSD0024 | A1     | 81   | 82   |  |  |
| pSD0025 | A1     | 119  | 120  |  |  |
| pSD0026 | A1     | 211  | 212  |  |  |
| pSD0027 | A1     | 220  | 221  |  |  |
| pSD0028 | A1     | 224  | 225  |  |  |
| pSD0029 | A1     | 336  | 337  |  |  |
| pSD0030 | A1     | 339  | 340  |  |  |
| pSD0031 | A2     | 378  | 379  |  |  |
| pSD0032 | A2     | 399  | 400  |  |  |
| pSD0033 | A2     | 409  | 410  |  |  |
| pSD0034 | A2     | 416  | 417  |  |  |
| pSD0035 | A2     | 487  | 488  |  |  |
| pSD0036 | A2     | 494  | 495  |  |  |
| pSD0037 | A2     | 500  | 501  |  |  |
| pSD0038 | A2     | 603  | 604  |  |  |
| pSD0039 | A3     | 1656 | 1657 |  |  |
| pSD0040 | A3     | 1711 | 1712 |  |  |
| pSD0041 | A3     | 1725 | 1726 |  |  |
| pSD0042 | A3     | 1749 | 1750 |  |  |
| pSD0043 | A3     | 1905 | 1906 |  |  |
| pSD0044 | A3     | 1910 | 1911 |  |  |
| pDS0062 | A3     | 1900 | 1901 |  |  |

\* Indicates the amino acid number of the mature FVIII protein

[0196] In one embodiment, the one or more XTEN insertion sites are located within one or more surface-exposed, flexible loop structure of the FVIII protein (*e.g.*, a permissive loop). For example, at least one XTEN sequence can be inserted in each FVIII "A" domain comprising at least two "permissive loops" into which at least one XTEN polypeptide can be inserted without eliminating procoagulant activity of the recombinant protein, or the ability of the recombinant proteins to be expressed *in vivo* or *in vitro* in a host cell. The permissive loops are regions that allow insertion of at least one XTEN sequence with, among other attributes, high surface or solvent exposure and high conformational flexibility. The A1 domain comprises a permissive loop-1 (A1-1) region and a permissive loop-2 (A1-2) region, the A2 domain comprises a permissive loop-1 (A2-1) region and a permissive loop-2 (A2-2) region, the A3 domain comprises a permissive loop-1 (A3-1) region and a permissive loop-2 (A3-2) region.

[0197] In one aspect, a first permissive loop in the FVIII A1 domain (A1-1) is located between beta strand 1 and beta strand 2, and a second permissive loop in the FVIII A2 domain (A1-2) is located between beta strand 11 and beta strand 12. A first permissive loop in the FVIII A2 domain (A2-1) is located between beta strand 22 and beta strand 23, and a second permissive loop in the FVIII A2 domain (A2-2) is located between beta strand 32 and beta strand 33. A first permissive loop in the FVIII A3 domain (A3-1) is located between beta strand 38 and beta strand 39, and a second permissive loop in the FVIII A3 (A3-2) is located between beta strand 45 and beta strand 46. In certain aspects, the surface-exposed, flexible loop structure comprising A1-1 corresponds to a region in native mature human FVIII from about amino acid 15 to about amino acid 45 of SEQ ID NO: 4, *e.g.*, from about amino acid 18 to about amino acid 41 of SEQ ID NO: 4. In other aspects, the surface-exposed, flexible loop structure comprising A1-2 corresponds to a region in native mature human FVIII from about amino acid 201 to about amino acid 232 of SEQ ID NO: 4, *e.g.*, from about amino acid 218 to about amino acid 229 of SEQ ID NO: 4. In yet other aspects, the surface-exposed, flexible loop structure comprising A2-1 corresponds to a region in native mature human FVIII from about amino acid 395 to about amino acid 421 of SEQ ID NO: 4, *e.g.*, from about amino acid 397 to about amino acid 418 of SEQ ID NO: 4. In still other embodiments, the surface-exposed, flexible loop structure comprising A2-2 corresponds to a region in native mature human FVIII from about amino acid 577 to about amino acid 635 of SEQ ID NO: 4, *e.g.*, from about amino acid 595 to about amino acid 607 of SEQ ID NO: 4. In certain aspects the surface-exposed, flexible loop structure comprising A3-1 corresponds to a region in native mature human FVIII from about amino acid 1705 to about amino acid 1732 of SEQ ID NO: 4, *e.g.*, from about amino acid 1711 to about amino acid 1725 of SEQ ID NO: 4. In yet other aspects, the surface-exposed, flexible loop structure comprising A3-2 corresponds to a region in native mature human FVIII from about amino acid 1884 to about amino acid 1917 of SEQ ID NO: 4, *e.g.*, from about amino acid 1899 to about amino acid 1911 of SEQ ID NO: 4.

[0198] In another embodiment, the one or more amino acids in which at least one XTEN sequence is inserted is located within a3 domain, *e.g.*, amino acids 1649 to 1689, corresponding to full-length mature FVIII polypeptide. In a particular

embodiment, an XTEN sequence is inserted between amino acids 1656 and 1657 of SEQ ID NO: 4 (full-length mature FVIII). In a specific embodiment, a FVIII protein comprising an XTEN sequence inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 4 further comprises a deletion from amino acid 745 to amino acid 1656 corresponding to SEQ ID NO: 4.

**[0199]** In some embodiments, the one or more insertion sites for one or more XTEN insertions are immediately downstream of one or more amino acids selected from the group consisting of:

- |                       |                       |                      |
|-----------------------|-----------------------|----------------------|
| (1) amino acid 3,     | (2) amino acid 18,    | (3) amino acid 22,   |
| (4) amino acid 26,    | (5) amino acid 32,    | (6) amino acid 40,   |
| (7) amino acid 60,    | (8) amino acid 65,    | (9) amino acid 81,   |
| (10) amino acid 116,  | (11) amino acid 119,  | (12) amino acid 130, |
| (13) amino acid 188,  | (14) amino acid 211,  | (15) amino acid 216, |
| (16) amino acid 220,  | (17) amino acid 224,  | (18) amino acid 230, |
| (19) amino acid 333,  | (20) amino acid 336,  | (21) amino acid 339, |
| (22) amino acid 375,  | (23) amino acid 399,  | (24) amino acid 403, |
| (25) amino acid 409,  | (26) amino acid 416,  | (26) amino acid 442, |
| (28) amino acid 487,  | (29) amino acid 490,  | (30) amino acid 494, |
| (31) amino acid 500,  | (32) amino acid 518,  | (33) amino acid 599, |
| (34) amino acid 603,  | (35) amino acid 713,  | (36) amino acid 745, |
| (37) amino acid 1656, | (38) amino acid 1711, | (39)                 |
| amino acid 1720,      |                       |                      |
| (40) amino acid 1725, | (41) amino acid 1749, | (42)                 |
| amino acid 1796,      |                       |                      |
| (43) amino acid 1802, | (44) amino acid 1827, | (45)                 |
| amino acid 1861,      |                       |                      |
| (46) amino acid 1896, | (47) amino acid 1900, | (48)                 |
| amino acid 1904,      |                       |                      |
| (49) amino acid 1905, | (50) amino acid 1910, | (51)                 |
| amino acid 1937,      |                       |                      |
| (52) amino acid 2019, | (53) amino acid 2068, | (54)                 |
| amino acid 2111,      |                       |                      |

(55) amino acid 2120, (56) amino acid 2171, (57) amino acid 2188,  
 (58) amino acid 2227, (59) amino acid 2277, and  
 (60) two or more combinations thereof.

**[0200]** In one embodiment, a FVIII protein useful for the invention comprises two XTEN sequences, a first XTEN sequence inserted into a first XTEN insertion site and a second XTEN inserted into a second XTEN insertion site. Non-limiting examples of the first XTEN insertion site and the second XTEN insertion site are listed in Table 11.

**TABLE 11. Exemplary Insertion Sites for Two XTENs**

| Insertion 1    |        | Insertion 2    |        |
|----------------|--------|----------------|--------|
| Insertion Site | Domain | Insertion Site | Domain |
| 745            | B      | 2332           | CT     |
| 26             | A1     | 403            | A2     |
| 40             | A1     | 403            | A2     |
| 18             | A1     | 403            | A2     |
| 26             | A1     | 599            | A2     |
| 40             | A1     | 599            | A2     |
| 18             | A1     | 599            | A2     |
| 1720           | A3     | 1900           | A3     |
| 1725           | A3     | 1900           | A3     |
| 1711           | A3     | 1905           | A3     |
| 1720           | A3     | 1905           | A3     |
| 1725           | A3     | 1905           | A3     |
| 1656           | A3     | 26             | A1     |
| 1656           | A3     | 18             | A1     |
| 1656           | A3     | 40             | A1     |
| 1656           | A3     | 399            | A2     |
| 1656           | A3     | 403            | A2     |
| 1656           | A3     | 1725           | A3     |
| 1656           | A3     | 1720           | A3     |
| 1900           | A3     | 18             | A1     |
| 1900           | A3     | 26             | A1     |
| 1900           | A3     | 40             | A1     |
| 1905           | A3     | 18             | A1     |
| 1905           | A3     | 40             | A1     |
| 1905           | A3     | 26             | A1     |
| 1910           | A3     | 26             | A1     |
| 18             | A1     | 399            | A2     |
| 26             | A1     | 399            | A2     |
| 40             | A1     | 399            | A2     |
| 18             | A1     | 403            | A2     |

| Insertion 1    |        | Insertion 2    |        |
|----------------|--------|----------------|--------|
| Insertion Site | Domain | Insertion Site | Domain |
| 1656           | A3     | 1900           | A3     |
| 1656           | A3     | 1905           | A3     |
| 1711           | A3     | 40             | A1     |
| 1711           | A3     | 26             | A1     |
| 1720           | A3     | 26             | A1     |
| 1720           | A3     | 40             | A1     |
| 1720           | A3     | 18             | A1     |
| 1725           | A3     | 26             | A1     |
| 1725           | A3     | 40             | A1     |
| 1725           | A3     | 18             | A1     |
| 1720           | A3     | 403            | A2     |
| 1720           | A3     | 399            | A2     |
| 1711           | A3     | 403            | A2     |
| 1720           | A3     | 403            | A2     |
| 1725           | A3     | 403            | A2     |
| 1725           | A3     | 399            | A2     |
| 1711           | A3     | 403            | A2     |
| 1900           | A3     | 399            | A2     |
| 1900           | A3     | 403            | A2     |
| 1905           | A3     | 403            | A2     |
| 1905           | A3     | 399            | A2     |
| 1910           | A3     | 403            | A2     |

**[0201]** The two XTENS inserted or linked to the FVIII protein can be identical or different. In some embodiments, a FVIII protein useful for the invention comprises two XTEN sequences inserted in the FVIII protein, a first XTEN sequence inserted immediately downstream of amino acid 745 corresponding to SEQ ID NO: 4, and a second XTEN sequence inserted immediately downstream of amino acid 2332 corresponding to SEQ ID NO: 4 (the C-terminus). In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, 40, 1656, or 1720 corresponding to SEQ ID NO: 4, and a second XTEN sequence inserted immediately downstream of amino acid 403 corresponding to SEQ ID NO: 4. In yet other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 4, and a second XTEN sequence inserted immediately downstream of amino acid 599 corresponding to SEQ ID NO: 4. In still other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 4, and a second XTEN sequence inserted immediately downstream of amino acid 18, 26, 40, 399,

403, 1725, 1720, 1900, 1905, or 2332 corresponding to SEQ ID NO: 4. In certain embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1900 corresponding to SEQ ID NO: 4, and a second XTEN sequence inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 4. In some embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 4, and a second XTEN sequence inserted immediately downstream of amino acid 399 corresponding to SEQ ID NO: 4. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 4, and a second XTEN sequence inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 4. In still other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 4, and a second XTEN sequence inserted immediately downstream of amino acid 18 corresponding to SEQ ID NO: 4. In a particular embodiment, the FVIII protein comprising two XTEN sequences, a first XTEN sequence inserted immediately downstream of amino acid 745 corresponding to SEQ ID NO: 4 and a second XTEN sequence inserted immediately downstream of amino acid 2332 corresponding to SEQ ID NO: 4, wherein the FVIII protein further has a deletion from amino acid 745 corresponding to SEQ ID NO: 4 to amino acid 1685 corresponding to SEQ ID NO: 4, a mutation or substitution at amino acid 1680 corresponding to SEQ ID NO: 4, *e.g.*, Y1680F, a mutation or substitution at amino acid 1648 corresponding to SEQ ID NO: 4, *e.g.*, R1648A, or at least two mutations or substitutions at amino acid 1648 corresponding to SEQ ID NO: 4, *e.g.*, R1648A, and amino acid 1680 corresponding to SEQ ID NO: 4, *e.g.*, Y1680F. In a specific embodiment, the FVIII protein comprises two XTEN sequences, a first XTEN inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 4 and a second XTEN sequence inserted immediately downstream of amino acid 2332 of SEQ ID NO: 4, wherein the FVIII protein further has a deletion from amino acid 745 to amino acid 1656 corresponding to SEQ ID NO: 4.

**[0202]** In certain embodiments, a FVIII protein comprises three XTEN sequences, a first XTEN sequence inserted into a first XTEN insertion site, a second XTEN sequence inserted into a second XTEN sequence, and a third XTEN sequence

inserted into a third XTEN insertion site. The first, second, or third XTEN sequences can be identical or different. The first, second, and third insertion sites can be selected from the group of any one of the insertion sites disclosed herein. In some embodiments, the FVIII protein comprising three XTEN sequences can further comprise a mutation or substitution, *e.g.*, amino acid 1648 corresponding to SEQ ID NO: 4, *e.g.*, R1648A. For example, non-limiting examples of the first, second, and third XTEN insertion sites are listed in Table 12.

**TABLE 12. Exemplary Insertion Sites for Three XTENs**

| Insertion 1    |        | Insertion 2    |        | Insertion 3    |        |
|----------------|--------|----------------|--------|----------------|--------|
| Insertion Site | Domain | Insertion Site | Domain | Insertion Site | Domain |
| 26             | A1     | 403            | A2     | 1656           | A3     |
| 26             | A1     | 403            | A2     | 1720           | A3     |
| 26             | A1     | 403            | A2     | 1900           | A3     |
| 26             | A1     | 1656           | A3     | 1720           | A3     |
| 26             | A1     | 1656           | A3     | 1900           | A3     |
| 26             | A1     | 1720           | A3     | 1900           | A3     |
| 403            | A2     | 1656           | A3     | 1720           | A3     |
| 403            | A2     | 1656           | A3     | 1900           | A3     |
| 403            | A2     | 1720           | A3     | 1900           | A3     |
| 1656           | A3     | 1720           | A3     | 1900           | A3     |
| 745            | B      | 1900           |        | 2332           | CT     |
| 18             | A1     | 745            | B      | 2332           | CT     |
| 26             | A1     | 745            | B      | 2332           | CT     |
| 40             | A1     | 745            | B      | 2332           | CT     |
| 18             | A1     | 745            | B      | 2332           | CT     |
| 40             | A1     | 745            | B      | 2332           | CT     |
| 403            | A2     | 745            | B      | 2332           | CT     |
| 399            | A2     | 745            | B      | 2332           | CT     |
| 1725           | A3     | 745            | B      | 2332           | CT     |
| 1720           | A3     | 745            | B      | 2332           | CT     |
| 1711           | A3     | 745            | B      | 2332           | CT     |
| 1900           | A3     | 745            | B      | 2332           | CT     |
| 1905           | A3     | 745            | B      | 2332           | CT     |
| 1910           | A3     | 745            | B      | 2332           | CT     |

[0203] In some embodiments, a FVIII protein comprises three XTEN sequences, a first XTEN sequence inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 4, a second XTEN sequence inserted downstream of amino acid 403 corresponding to SEQ ID NO: 4, and a third XTEN sequence inserted downstream of amino acid 1656, 1720, or 1900 corresponding to SEQ ID



NO: 4. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 4, a second XTEN sequence is inserted downstream of amino acid 1656 corresponding to SEQ ID NO: 4, and a third XTEN sequence is inserted downstream of amino acid 1720 or 1900 corresponding to SEQ ID NO: 4. In yet other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 4, a second XTEN sequence is inserted downstream of amino acid 1720 corresponding to SEQ ID NO: 4, and a third XTEN sequence is inserted downstream of amino acid 1900 corresponding to SEQ ID NO: 4. In still other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 403 corresponding to SEQ ID NO: 4, a second XTEN sequence is inserted downstream of amino acid 1656 corresponding to SEQ ID NO: 4, and a third XTEN sequence is inserted downstream of amino acid 1720 or 1900 corresponding to SEQ ID NO: 4. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 403 or 1656 corresponding to SEQ ID NO: 4, a second XTEN sequence is inserted downstream of amino acid 1720 corresponding to SEQ ID NO: 4, and a third XTEN sequence is inserted downstream of amino acid 1900 corresponding to SEQ ID NO: 4. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, 40, 399, 403, 1711, 1720, 1725, 1900, 1905, or 1910 corresponding to SEQ ID NO: 4, a second XTEN sequence is inserted downstream of amino acid 745 corresponding to SEQ ID NO: 4, and a third XTEN sequence is inserted downstream of amino acid 2332 corresponding to SEQ ID NO: 4.

**[0204]** In other embodiments, a FVIII protein in the invention comprises four XTEN sequences, a first XTEN sequence inserted into a first insertion site, a second XTEN sequence inserted into a second insertion site, a third XTEN sequence inserted into a third insertion site, and a fourth XTEN sequence inserted into a fourth insertion site. The first, second, third, and fourth XTEN sequences can be identical, different, or combinations thereof. In some embodiments, the FVIII protein comprising four XTEN sequences can further comprise a mutation or substitution, *e.g.*, amino acid 1648 corresponding to SEQ ID NO: 4, *e.g.*,

R1648A. Non-limiting examples of the first, second, third, and fourth XTEN insertion sites are listed in Table 13.

**TABLE 13. Exemplary Insertion Sites for Four XTENs**

| Insertion 1    |        | Insertion 2    |        | Insertion 3    |        | Insertion 4    |        |
|----------------|--------|----------------|--------|----------------|--------|----------------|--------|
| Insertion Site | Domain | Insertion Site | Domain | Insertion Site | Domain | Insertion Site | Domain |
| 26             | A1     | 403            | A2     | 1656           | a3     | 1720           | A3     |
| 26             | A1     | 403            | A2     | 1656           | a3     | 1900           | A3     |
| 26             | A1     | 403            | A2     | 1720           | A3     | 1900           | A3     |
| 26             | A1     | 1656           | a3     | 1720           | A3     | 1900           | A3     |
| 403            | A2     | 1656           | a3     | 1720           | A3     | 1900           | A3     |
| 0040           | A1     | 0403           | A2     | 745            | B      | 2332           | CT     |
| 0040           | A1     | 0403           | A2     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 0409           | A2     | 745            | B      | 2332           | CT     |
| 0040           | A1     | 0409           | A2     | 745            | B      | 2332           | CT     |
| 0040           | A1     | 0409           | A2     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 0409           | A2     | 745            | B      | 2332           | CT     |
| 0040           | A1     | 1720           | A3     | 745            | B      | 2332           | CT     |
| 0026           | A1     | 1720           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1720           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1720           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1720           | A3     | 745            | B      | 2332           | CT     |
| 0026           | A1     | 1720           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1720           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1900           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1900           | A3     | 745            | B      | 2332           | CT     |
| 0026           | A1     | 1900           | A3     | 745            | B      | 2332           | CT     |
| 0040           | A1     | 1900           | A3     | 745            | B      | 2332           | CT     |
| 0040           | A1     | 1905           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1905           | A3     | 745            | B      | 2332           | CT     |
| 0040           | A1     | 1905           | A3     | 745            | B      | 2332           | CT     |
| 0026           | A1     | 1905           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1905           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1905           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1910           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1910           | A3     | 745            | B      | 2332           | CT     |
| 0040           | A1     | 1910           | A3     | 745            | B      | 2332           | CT     |
| 0026           | A1     | 1910           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1910           | A3     | 745            | B      | 2332           | CT     |
| 0026           | A1     | 1910           | A3     | 745            | B      | 2332           | CT     |
| 0040           | A1     | 1910           | A3     | 745            | B      | 2332           | CT     |
| 0018           | A1     | 1910           | A3     | 745            | B      | 2332           | CT     |
| 0409           | A2     | 1720           | A3     | 745            | B      | 2332           | CT     |
| 0403           | A2     | 1720           | A3     | 745            | B      | 2332           | CT     |
| 0409           | A2     | 1720           | A3     | 745            | B      | 2332           | CT     |
| 0403           | A2     | 1720           | A3     | 745            | B      | 2332           | CT     |

|      |    |      |    |      |    |      |    |
|------|----|------|----|------|----|------|----|
| 0403 | A2 | 1720 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1900 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1900 | A3 | 745  | B  | 2332 | CT |
| 0409 | A2 | 1900 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1900 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1900 | A3 | 745  | B  | 2332 | CT |
| 0409 | A2 | 1900 | A3 | 745  | B  | 2332 | CT |
| 0409 | A2 | 1905 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1905 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1905 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1905 | A3 | 745  | B  | 2332 | CT |
| 0409 | A2 | 1905 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1905 | A3 | 745  | B  | 2332 | CT |
| 0409 | A2 | 1910 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1910 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1910 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1910 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1910 | A3 | 745  | B  | 2332 | CT |
| 1720 | A3 | 1900 | A3 | 745  | B  | 2332 | CT |
| 1720 | A3 | 1905 | A3 | 745  | B  | 2332 | CT |
| 1720 | A3 | 1910 | A3 | 745  | B  | 2332 | CT |
| 1720 | A3 | 1910 | A3 | 745  | B  | 2332 | CT |
| 0403 | A2 | 1656 | a3 | 1720 | A3 | 2332 | CT |
| 0403 | A2 | 1656 | a3 | 1900 | A3 | 2332 | CT |
| 0403 | A2 | 1720 | A3 | 1900 | A3 | 2332 | CT |
| 1656 | a3 | 1720 | A3 | 1900 | A3 | 2332 | CT |
| 0018 | A1 | 0403 | A2 | 1656 | a3 | 2332 | CT |
| 0018 | A1 | 0403 | A2 | 1720 | A3 | 2332 | CT |
| 0018 | A1 | 0403 | A2 | 1900 | A3 | 2332 | CT |
| 0018 | A1 | 1656 | a3 | 1720 | A3 | 2332 | CT |
| 0018 | A1 | 1656 | a3 | 1900 | A3 | 2332 | CT |
| 0018 | A1 | 1720 | A3 | 1900 | A3 | 2332 | CT |
| 0018 | A1 | 0403 | A2 | 0745 | B  | 2332 | CT |
| 0018 | A1 | 0745 | B  | 1720 | A3 | 2332 | CT |
| 0018 | A1 | 0745 | B  | 1900 | A3 | 2332 | CT |
| 0403 | A2 | 0745 | B  | 1720 | A3 | 2332 | CT |
| 0403 | A2 | 0745 | B  | 1900 | A3 | 2332 | CT |
| 0745 | B  | 1720 | A3 | 1900 | A3 | 2332 | CT |
| 0188 | A1 | 1900 | A3 | 0745 | B  | 2332 | CT |
| 0599 |    | 1900 | A3 | 0745 | B  | 2332 | CT |
| 2068 |    | 1900 | A3 | 0745 | B  | 2332 | CT |
| 2171 |    | 1900 | A3 | 0745 | B  | 2332 | CT |
| 2227 |    | 1900 | A3 | 0745 | B  | 2332 | CT |
| 2277 |    | 1900 | A3 | 0745 | B  | 2332 | CT |

[0205] In some embodiments, a FVIII protein comprises five XTEN sequences, a first XTEN sequence inserted into a first insertion site, a second XTEN sequence

inserted into a second insertion site, a third XTEN sequence inserted into a third XTEN insertion site, a fourth XTEN sequence inserted into a fourth XTEN insertion site, and a fifth XTEN sequence inserted into a fifth XTEN insertion site. The first, second, third, fourth, or fifth XTEN sequences can be identical, different, or combinations thereof. Non-limiting examples of the first, second, third, fourth, and fifth insertion sites are listed in Table 14.

**TABLE 14. Exemplary Insertion Sites for Five XTENs**

| XTEN Insertion 1 | XTEN insertion 2 | XTEN Insertion 3 | XTEN Insertion 4 | XTEN Insertion 5 |
|------------------|------------------|------------------|------------------|------------------|
| 0403             | 1656             | 1720             | 1900             | 2332             |
| 0018             | 0403             | 1656             | 1720             | 2332             |
| 0018             | 0403             | 1656             | 1900             | 2332             |
| 0018             | 0403             | 1720             | 1900             | 2332             |
| 0018             | 1656             | 1720             | 1900             | 2332             |
| 0018             | 0403             | 0745             | 1720             | 2332             |
| 0018             | 0403             | 0745             | 1900             | 2332             |
| 0018             | 0745             | 1720             | 1900             | 2332             |
| 0403             | 0745             | 1720             | 1900             | 2332             |

[0206] In certain embodiments, a FVIII protein comprises six XTEN sequences, a first XTEN sequence inserted into a first XTEN insertion site, a second XTEN sequence inserted into a second XTEN insertion site, a third XTEN sequence inserted into a third XTEN insertion site, a fourth XTEN sequence inserted into a fourth XTEN insertion site, a fifth XTEN sequence inserted into a fifth XTEN insertion site, and a sixth XTEN sequence inserted into a sixth XTEN insertion site. The first, second, third, fourth, fifth, or sixth XTEN sequences can be identical, different, or combinations thereof. Examples of the six XTEN insertion sites include, but are not limited to the insertion sites listed in Table 15.

**TABLE 15. Exemplary XTEN Insertion Sites for Six XTENs**

| XTEN Insertion 1 | XTEN insertion 2 | XTEN Insertion 3 | XTEN Insertion 4 | XTEN Insertion 5 | XTEN Insertion 6 |
|------------------|------------------|------------------|------------------|------------------|------------------|
| 0018             | 0403             | 1656             | 1720             | 1900             | 2332             |
| 0018             | 0403             | 0745             | 1720             | 1900             | 2332             |

[0207] In a particular example, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 4, and a second XTEN is inserted between

amino acids 1720 and 1721 corresponding to SEQ ID NO: 4 (full-length mature FVIII). In another example, a first XTEN is inserted between amino acids 403 and 404 corresponding to SEQ ID NO: 4, and a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 4. In some examples, a first XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 4, and a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 4. In other examples, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 4, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 4, and a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 4. In yet other embodiments, a first XTEN is inserted between amino acids 403 and 404 corresponding to SEQ ID NO: 4, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 4, and a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 4. In still other embodiments, a first XTEN is inserted between amino acids 403 and 404 corresponding to SEQ ID NO: 4, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 4, and a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 4. In certain embodiments, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 4, a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 4, and a third XTEN is inserted between amino acids 1900 and 1901 corresponding to SEQ ID NO: 4. In some embodiments, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 4, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 4, a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 4, and a fourth XTEN is inserted between 1900 and 1901 corresponding to SEQ ID NO: 4.

**[0208]** In a particular embodiment, an XTEN sequence is inserted between amino acids 745 and 746 of a full-length Factor VIII or the corresponding insertion site of the B-domain deleted Factor VIII.

**[0209]** In some embodiments, a chimeric protein of the invention comprises two polypeptide sequences, a first polypeptide sequence comprising an amino acid

sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from FVIII-161 (SEQ ID NO: 101), FVIII-169 (SEQ ID NO: 103), FVIII-170 (SEQ ID NO: 102), FVIII-173 (SEQ ID NO: 104); FVIII-195 (SEQ ID NO: 105); FVIII-196 (SEQ ID NO: 106), FVIII-199 (SEQ ID NO: 107), FVIII-201 (SEQ ID NO: 108); FVIII-203 (SEQ ID NO: 109), FVIII-204 (SEQ ID NO: 110), FVIII-205 (SEQ ID NO: 111), FVIII-266 (SEQ ID NO: 112), FVIII-267 (SEQ ID NO: 113), FVIII-268 (SEQ ID NO: 114), FVIII-269 (SEQ ID NO: 115), FVIII-271 (SEQ ID NO: 116), or FVIII-272 (SEQ ID NO: 117) and a second polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from VWF031 (SEQ ID NO: 118), VWF034 (SEQ ID NO: 119), or VWF-036 (SEQ ID NO: 120).

**D) Ig Constant Region or a portion thereof**

[0210] The VWF fragment or the FVIII protein linked to an XTEN sequence in the present invention can further comprise an Ig constant region or a portion thereof. The Ig constant region or a portion thereof can improve pharmacokinetic or pharmacodynamic properties of the VWF fragment or the FVIII protein in combination with the XTEN sequence. In certain embodiments, the Ig constant region or a portion thereof extends a half-life of a molecule fused to the Ig constant region or a portion thereof.

[0211] An Ig constant region is comprised of domains denoted CH (constant heavy) domains (CH1, CH2, etc.). Depending on the isotype, (*i.e.* IgG, IgM, IgA, IgD, or IgE), the constant region can be comprised of three or four CH domains. Some isotypes (*e.g.* IgG) constant regions also contain a hinge region. *See* Janeway *et al.* 2001, *Immunobiology*, Garland Publishing, N.Y., N.Y.

[0212] An Ig constant region or a portion thereof for producing the chimeric protein of the present invention may be obtained from a number of different sources. In some embodiments, an Ig constant region or a portion thereof is derived from a human Ig. It is understood, however, that the Ig constant region or a portion thereof may be derived from an Ig of another mammalian species, including for example, a rodent (*e.g.* a mouse, rat, rabbit, guinea pig) or non-human primate (*e.g.* chimpanzee, macaque) species. Moreover, the Ig constant region or a portion thereof may be derived from any Ig class, including IgM, IgG,

IgD, IgA, and IgE, and any Ig isotype, including IgG1, IgG2, IgG3, and IgG4. In one embodiment, the human isotype IgG1 is used.

[0213] A variety of the Ig constant region gene sequences (*e.g.*, human constant region gene sequences) are available in the form of publicly accessible deposits. Constant region domains sequence can be selected having a particular effector function (or lacking a particular effector function) or with a particular modification to reduce immunogenicity. Many sequences of antibodies and antibody-encoding genes have been published and suitable Ig constant region sequences (*e.g.*, hinge, CH2, and/or CH3 sequences, or portions thereof) can be derived from these sequences using art recognized techniques. The genetic material obtained using any of the foregoing methods may then be altered or synthesized to obtain polypeptides of the present invention. It will further be appreciated that the scope of this invention encompasses alleles, variants and mutations of constant region DNA sequences.

[0214] The sequences of the Ig constant region or a portion thereof can be cloned, *e.g.*, using the polymerase chain reaction and primers which are selected to amplify the domain of interest. To clone a sequence of the Ig constant region or a portion thereof from an antibody, mRNA can be isolated from hybridoma, spleen, or lymph cells, reverse transcribed into DNA, and antibody genes amplified by PCR. PCR amplification methods are described in detail in U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; 4,965,188; and in, *e.g.*, "PCR Protocols: A Guide to Methods and Applications" Innis et al. eds., Academic Press, San Diego, CA (1990); Ho et al. 1989. *Gene* 77:51; Horton et al. 1993. *Methods Enzymol.* 217:270). PCR may be initiated by consensus constant region primers or by more specific primers based on the published heavy and light chain DNA and amino acid sequences. As discussed above, PCR also may be used to isolate DNA clones encoding the antibody light and heavy chains. In this case the libraries may be screened by consensus primers or larger homologous probes, such as mouse constant region probes. Numerous primer sets suitable for amplification of antibody genes are known in the art (*e.g.*, 5' primers based on the N-terminal sequence of purified antibodies (Benhar and Pastan. 1994. *Protein Engineering* 7:1509); rapid amplification of cDNA ends (Ruberti, F. et al. 1994. *J. Immunol. Methods* 173:33); antibody leader sequences (Larrick et al. 1989 *Biochem.*

Biophys. Res. Commun. 160:1250). The cloning of antibody sequences is further described in Newman et al., U.S. Pat. No. 5,658,570, filed January 25, 1995, which is incorporated by reference herein.

[0215] An Ig constant region used herein can include all domains and the hinge region or portions thereof. In one embodiment, the Ig constant region or a portion thereof comprises CH2 domain, CH3 domain, and a hinge region, *i.e.*, an Fc region or an FcRn binding partner.

[0216] As used herein, the term "Fc region" is defined as the portion of a polypeptide which corresponds to the Fc region of native Ig, *i.e.*, as formed by the dimeric association of the respective Fc domains of its two heavy chains. A native Fc region forms a homodimer with another Fc region. In contrast, the term "genetically-fused Fc region" or "single-chain Fc region" (scFc region), as used herein, refers to a synthetic dimeric Fc region comprised of Fc domains genetically linked within a single polypeptide chain (*i.e.*, encoded in a single contiguous genetic sequence).

[0217] In one embodiment, the "Fc region" refers to the portion of a single Ig heavy chain beginning in the hinge region just upstream of the papain cleavage site (*i.e.* residue 216 in IgG, taking the first residue of heavy chain constant region to be 114) and ending at the C-terminus of the antibody. Accordingly, a complete Fc domain comprises at least a hinge domain, a CH2 domain, and a CH3 domain.

[0218] The Fc region of an Ig constant region, depending on the Ig isotype can include the CH2, CH3, and CH4 domains, as well as the hinge region. Chimeric proteins comprising an Fc region of an Ig bestow several desirable properties on a chimeric protein including increased stability, increased serum half-life (see Capon *et al.*, 1989, *Nature* 337:525) as well as binding to Fc receptors such as the neonatal Fc receptor (FcRn) (U.S. Pat. Nos. 6,086,875, 6,485,726, 6,030,613; WO 03/077834; US2003-0235536A1), which are incorporated herein by reference in their entirety.

[0219] An Ig constant region or a portion thereof can be an FcRn binding partner. FcRn is active in adult epithelial tissues and expressed in the lumen of the intestines, pulmonary airways, nasal surfaces, vaginal surfaces, colon and rectal surfaces (U.S. Pat. No. 6,485,726). An FcRn binding partner is a portion of an Ig that binds to FcRn.



[0220] The FcRn receptor has been isolated from several mammalian species including humans. The sequences of the human FcRn, monkey FcRn, rat FcRn, and mouse FcRn are known (Story et al. 1994, J. Exp. Med. 180:2377). The FcRn receptor binds IgG (but not other Ig classes such as IgA, IgM, IgD, and IgE) at relatively low pH, actively transports the IgG transcellularly in a luminal to serosal direction, and then releases the IgG at relatively higher pH found in the interstitial fluids. It is expressed in adult epithelial tissue (U.S. Pat. Nos. 6,485,726, 6,030,613, 6,086,875; WO 03/077834; US2003-0235536A1) including lung and intestinal epithelium (Israel et al. 1997, Immunology 92:69) renal proximal tubular epithelium (Kobayashi et al. 2002, Am. J. Physiol. Renal Physiol. 282:F358) as well as nasal epithelium, vaginal surfaces, and biliary tree surfaces.

[0221] FcRn binding partners useful in the present invention encompass molecules that can be specifically bound by the FcRn receptor including whole IgG, the Fc fragment of IgG, and other fragments that include the complete binding region of the FcRn receptor. The region of the Fc portion of IgG that binds to the FcRn receptor has been described based on X-ray crystallography (Burmeister et al. 1994, Nature 372:379). The major contact area of the Fc with the FcRn is near the junction of the CH2 and CH3 domains. Fc-FcRn contacts are all within a single Ig heavy chain. The FcRn binding partners include whole IgG, the Fc fragment of IgG, and other fragments of IgG that include the complete binding region of FcRn. The major contact sites include amino acid residues 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. References made to amino acid numbering of Igs or Ig fragments, or regions, are all based on Kabat et al. 1991, Sequences of Proteins of Immunological Interest, U.S. Department of Public Health, Bethesda, Md.

[0222] Fc regions or FcRn binding partners bound to FcRn can be effectively shuttled across epithelial barriers by FcRn, thus providing a non-invasive means to systemically administer a desired therapeutic molecule. Additionally, fusion proteins comprising an Fc region or an FcRn binding partner are endocytosed by cells expressing the FcRn. But instead of being marked for degradation, these fusion proteins are recycled out into circulation again, thus increasing the *in vivo*

half-life of these proteins. In certain embodiments, the portions of Ig constant regions are an Fc region or an FcRn binding partner that typically associates, via disulfide bonds and other non-specific interactions, with another Fc region or another FcRn binding partner to form dimers and higher order multimers.

[0223] Two FcRn receptors can bind a single Fc molecule. Crystallographic data suggest that each FcRn molecule binds a single polypeptide of the Fc homodimer. In one embodiment, linking the FcRn binding partner, *e.g.*, an Fc fragment of an IgG, to a biologically active molecule provides a means of delivering the biologically active molecule orally, buccally, sublingually, rectally, vaginally, as an aerosol administered nasally or via a pulmonary route, or via an ocular route. In another embodiment, the chimeric protein can be administered invasively, *e.g.*, subcutaneously, intravenously.

[0224] An FcRn binding partner region is a molecule or a portion thereof that can be specifically bound by the FcRn receptor with consequent active transport by the FcRn receptor of the Fc region. Specifically bound refers to two molecules forming a complex that is relatively stable under physiologic conditions. Specific binding is characterized by a high affinity and a low to moderate capacity as distinguished from nonspecific binding which usually has a low affinity with a moderate to high capacity. Typically, binding is considered specific when the affinity constant  $KA$  is higher than  $10^6 \text{ M}^{-1}$ , or higher than  $10^8 \text{ M}^{-1}$ . If necessary, non-specific binding can be reduced without substantially affecting specific binding by varying the binding conditions. The appropriate binding conditions such as concentration of the molecules, ionic strength of the solution, temperature, time allowed for binding, concentration of a blocking agent (*e.g.* serum albumin, milk casein), etc., may be optimized by a skilled artisan using routine techniques.

[0225] In certain embodiments, a chimeric protein of the invention comprises one or more truncated Fc regions that are nonetheless sufficient to confer Fc receptor (FcR) binding properties to the Fc region. For example, the portion of an Fc region that binds to FcRn (*i.e.*, the FcRn binding portion) comprises from about amino acids 282-438 of IgG1, EU numbering (with the primary contact sites being amino acids 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. Thus, an Fc region of the invention may comprise or consist of an FcRn binding

portion. FcRn binding portions may be derived from heavy chains of any isotype, including IgG1, IgG2, IgG3 and IgG4. In one embodiment, an FcRn binding portion from an antibody of the human isotype IgG1 is used. In another embodiment, an FcRn binding portion from an antibody of the human isotype IgG4 is used.

[0226] In another embodiment, the "Fc region" includes an amino acid sequence of an Fc domain or derived from an Fc domain. In certain embodiments, an Fc region comprises at least one of: a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain (about amino acids 216-230 of an antibody Fc region according to EU numbering), a CH2 domain (about amino acids 231-340 of an antibody Fc region according to EU numbering), a CH3 domain (about amino acids 341-438 of an antibody Fc region according to EU numbering), a CH4 domain, or a variant, portion, or fragment thereof. In other embodiments, an Fc region comprises a complete Fc domain (*i.e.*, a hinge domain, a CH2 domain, and a CH3 domain). In some embodiments, an Fc region comprises, consists essentially of, or consists of a hinge domain (or a portion thereof) fused to a CH3 domain (or a portion thereof), a hinge domain (or a portion thereof) fused to a CH2 domain (or a portion thereof), a CH2 domain (or a portion thereof) fused to a CH3 domain (or a portion thereof), a CH2 domain (or a portion thereof) fused to both a hinge domain (or a portion thereof) and a CH3 domain (or a portion thereof). In still other embodiments, an Fc region lacks at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). In a particular embodiment, an Fc region comprises or consists of amino acids corresponding to EU numbers 221 to 447.

[0227] The Fc regions denoted as F, F1, or F2 herein may be obtained from a number of different sources. In one embodiment, an Fc region of the polypeptide is derived from a human Ig. It is understood, however, that an Fc region may be derived from an Ig of another mammalian species, including for example, a rodent (*e.g.* a mouse, rat, rabbit, or guinea pig) or non-human primate (*e.g.* chimpanzee, macaque) species. Moreover, the polypeptide of the Fc domains or portions thereof may be derived from any Ig class, including IgM, IgG, IgD, IgA and IgE, and any Ig isotype, including IgG1, IgG2, IgG3 and IgG4. In another embodiment, the human isotype IgG1 is used.

[0228] In certain embodiments, the Fc variant confers a change in at least one effector function imparted by an Fc region comprising said wild-type Fc domain (*e.g.*, an improvement or reduction in the ability of the Fc region to bind to Fc receptors (*e.g.* FcγRI, FcγRII, or FcγRIII) or complement proteins (*e.g.* C1q), or to trigger antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cytotoxicity (CDCC)). In other embodiments, the Fc variant provides an engineered cysteine residue.

[0229] The Fc regions of the invention may employ art-recognized Fc variants which are known to impart a change (*e.g.*, an enhancement or reduction) in effector function and/or FcR or FcRn binding. Specifically, a binding molecule of the invention may include, for example, a change (*e.g.*, a substitution) at one or more of the amino acid positions disclosed in International PCT Publications WO88/07089A1, WO96/14339A1, WO98/05787A1, WO98/23289A1, WO99/51642A1, WO99/58572A1, WO00/09560A2, WO00/32767A1, WO00/42072A2, WO02/44215A2, WO02/060919A2, WO03/074569A2, WO04/016750A2, WO04/029207A2, WO04/035752A2, WO04/063351A2, WO04/074455A2, WO04/099249A2, WO05/040217A2, WO04/044859, WO05/070963A1, WO05/077981A2, WO05/092925A2, WO05/123780A2, WO06/019447A1, WO06/047350A2, and WO06/085967A2; US Patent Publication Nos. US2007/0231329, US2007/0231329, US2007/0237765, US2007/0237766, US2007/0237767, US2007/0243188, US20070248603, US20070286859, US20080057056 ; or US Patents 5,648,260; 5,739,277; 5,834,250; 5,869,046; 6,096,871; 6,121,022; 6,194,551; 6,242,195; 6,277,375; 6,528,624; 6,538,124; 6,737,056; 6,821,505; 6,998,253; 7,083,784; 7,404,956, and 7,317,091, each of which is incorporated by reference herein. In one embodiment, the specific change (*e.g.*, the specific substitution of one or more amino acids disclosed in the art) may be made at one or more of the disclosed amino acid positions. In another embodiment, a different change at one or more of the disclosed amino acid positions (*e.g.*, the different substitution of one or more amino acid position disclosed in the art) may be made.

[0230] The Fc region or FcRn binding partner of IgG can be modified according to well recognized procedures such as site directed mutagenesis and the like to yield modified IgG or Fc fragments or portions thereof that will be bound by

FcRn. Such modifications include modifications remote from the FcRn contact sites as well as modifications within the contact sites that preserve or even enhance binding to the FcRn. For example, the following single amino acid residues in human IgG1 Fc (Fc  $\gamma$ 1) can be substituted without significant loss of Fc binding affinity for FcRn: P238A, S239A, K246A, K248A, D249A, M252A, T256A, E258A, T260A, D265A, S267A, H268A, E269A, D270A, E272A, L274A, N276A, Y278A, D280A, V282A, E283A, H285A, N286A, T289A, K290A, R292A, E293A, E294A, Q295A, Y296F, N297A, S298A, Y300F, R301A, V303A, V305A, T307A, L309A, Q311A, D312A, N315A, K317A, E318A, K320A, K322A, S324A, K326A, A327Q, P329A, A330Q, P331A, E333A, K334A, T335A, S337A, K338A, K340A, Q342A, R344A, E345A, Q347A, R355A, E356A, M358A, T359A, K360A, N361A, Q362A, Y373A, S375A, D376A, A378Q, E380A, E382A, S383A, N384A, Q386A, E388A, N389A, N390A, Y391F, K392A, L398A, S400A, D401A, D413A, K414A, R416A, Q418A, Q419A, N421A, V422A, S424A, E430A, N434A, T437A, Q438A, K439A, S440A, S444A, and K447A, where for example P238A represents wild type proline substituted by alanine at position number 238. As an example, a specific embodiment incorporates the N297A mutation, removing a highly conserved N-glycosylation site. In addition to alanine other amino acids may be substituted for the wild type amino acids at the positions specified above. Mutations may be introduced singly into Fc giving rise to more than one hundred Fc regions distinct from the native Fc. Additionally, combinations of two, three, or more of these individual mutations may be introduced together, giving rise to hundreds more Fc regions. Moreover, one of the Fc region of a construct of the invention may be mutated and the other Fc region of the construct not mutated at all, or they both may be mutated but with different mutations.

**[0231]** Certain of the above mutations may confer new functionality upon the Fc region or FcRn binding partner. For example, one embodiment incorporates N297A, removing a highly conserved N-glycosylation site. The effect of this mutation is to reduce immunogenicity, thereby enhancing circulating half-life of the Fc region, and to render the Fc region incapable of binding to Fc $\gamma$ RI, Fc $\gamma$ RIIA, Fc $\gamma$ RIIB, and Fc $\gamma$ RIIIA, without compromising affinity for FcRn (Routledge et al. 1995, Transplantation 60:847; Friend et al. 1999, Transplantation 68:1632; Shields

et al. 1995, J. Biol. Chem. 276:6591). As a further example of new functionality arising from mutations described above affinity for FcRn may be increased beyond that of wild type in some instances. This increased affinity may reflect an increased "on" rate, a decreased "off" rate or both an increased "on" rate and a decreased "off" rate. Examples of mutations believed to impart an increased affinity for FcRn include, but not limited to, T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591).

[0232] Additionally, at least three human Fc gamma receptors appear to recognize a binding site on IgG within the lower hinge region, generally amino acids 234-237. Therefore, another example of new functionality and potential decreased immunogenicity may arise from mutations of this region, as for example by replacing amino acids 233-236 of human IgG1 "ELLG" to the corresponding sequence from IgG2 "PVA" (with one amino acid deletion). It has been shown that FcγRI, FcγRII, and FcγRIII, which mediate various effector functions will not bind to IgG1 when such mutations have been introduced. Ward and Ghetie 1995, *Therapeutic Immunology* 2:77 and Armour et al. 1999, *Eur. J. Immunol.* 29:2613.

[0233] In one embodiment, the Ig constant region or a portion thereof, e.g., an Fc region, is a polypeptide including the sequence PKNSSMISNTP (SEQ ID NO: 52) and optionally further including a sequence selected from HQSLGTQ (SEQ ID NO: 53), HQNLSDGK (SEQ ID NO: 54), HQNISDGK (SEQ ID NO: 55), or VISSHLGQ (SEQ ID NO: 56) (U.S. Pat. No. 5,739,277).

[0234] In another embodiment, the immunoglobulin constant region or a portion thereof comprises an amino acid sequence in the hinge region or a portion thereof that forms one or more disulfide bonds with another immunoglobulin constant region or a portion thereof. The disulfide bond by the immunoglobulin constant region or a portion thereof places the first polypeptide comprising FVIII and the second polypeptide comprising the VWF fragment together so that endogenous VWF does not replace the VWF fragment and does not bind to the FVIII. Therefore, the disulfide bond between the first immunoglobulin constant region or a portion thereof and a second immunoglobulin constant region or a portion thereof prevents interaction between endogenous VWF and the FVIII protein. This inhibition of interaction between the VWF and the FVIII protein allows the half-life of the FVIII protein to go beyond the two fold limit. The hinge region or

a portion thereof can further be linked to one or more domains of CH1, CH2, CH3, a fragment thereof, and any combinations thereof. In a particular embodiment, the immunoglobulin constant region or a portion thereof is a hinge region and CH2.

[0235] In certain embodiments, the Ig constant region or a portion thereof is hemi-glycosylated. For example, the chimeric protein comprising two Fc regions or FcRn binding partners may contain a first, glycosylated, Fc region (*e.g.*, a glycosylated CH2 region) or FcRn binding partner and a second, aglycosylated, Fc region (*e.g.*, an aglycosylated CH2 region) or FcRn binding partner. In one embodiment, a linker may be interposed between the glycosylated and aglycosylated Fc regions. In another embodiment, the Fc region or FcRn binding partner is fully glycosylated, *i.e.*, all of the Fc regions are glycosylated. In other embodiments, the Fc region may be aglycosylated, *i.e.*, none of the Fc moieties are glycosylated.

[0236] In certain embodiments, a chimeric protein of the invention comprises an amino acid substitution to an Ig constant region or a portion thereof (*e.g.*, Fc variants), which alters the antigen-independent effector functions of the Ig constant region, in particular the circulating half-life of the protein.

[0237] Such proteins exhibit either increased or decreased binding to FcRn when compared to proteins lacking these substitutions and, therefore, have an increased or decreased half-life in serum, respectively. Fc variants with improved affinity for FcRn are anticipated to have longer serum half-lives, and such molecules have useful applications in methods of treating mammals where long half-life of the administered polypeptide is desired, *e.g.*, to treat a chronic disease or disorder (*see, e.g.*, US Patents 7,348,004, 7,404,956, and 7,862,820). In contrast, Fc variants with decreased FcRn binding affinity are expected to have shorter half-lives, and such molecules are also useful, for example, for administration to a mammal where a shortened circulation time may be advantageous, *e.g.* for in vivo diagnostic imaging or in situations where the starting polypeptide has toxic side effects when present in the circulation for prolonged periods. Fc variants with decreased FcRn binding affinity are also less likely to cross the placenta and, thus, are also useful in the treatment of diseases or disorders in pregnant women. In addition, other applications in which reduced FcRn binding affinity may be

desired include those applications in which localization the brain, kidney, and/or liver is desired. In one exemplary embodiment, the chimeric protein of the invention exhibit reduced transport across the epithelium of kidney glomeruli from the vasculature. In another embodiment, the chimeric protein of the invention exhibit reduced transport across the blood brain barrier (BBB) from the brain, into the vascular space. In one embodiment, a protein with altered FcRn binding comprises at least one Fc region or FcRn binding partner (e.g, one or two Fc regions or FcRn binding partners) having one or more amino acid substitutions within the "FcRn binding loop" of an Ig constant region. The FcRn binding loop is comprised of amino acid residues 280-299 (according to EU numbering) of a wild-type, full-length, Fc region. In other embodiments, an Ig constant region or a portion thereof in a chimeric protein of the invention having altered FcRn binding affinity comprises at least one Fc region or FcRn binding partner having one or more amino acid substitutions within the 15 Å FcRn "contact zone." As used herein, the term 15 Å FcRn "contact zone" includes residues at the following positions of a wild-type, full-length Fc moiety: 243-261, 275-280, 282-293, 302-319, 336-348, 367, 369, 372-389, 391, 393, 408, 424, 425-440 (EU numbering). In other embodiments, a Ig constant region or a portion thereof of the invention having altered FcRn binding affinity comprises at least one Fc region or FcRn binding partner having one or more amino acid substitutions at an amino acid position corresponding to any one of the following EU positions: 256, 277-281, 283-288, 303-309, 313, 338, 342, 376, 381, 384, 385, 387, 434 (e.g., N434A or N434K), and 438. Exemplary amino acid substitutions which altered FcRn binding activity are disclosed in International PCT Publication No. WO05/047327 which is incorporated by reference herein.

[0238] An Fc region or FcRn binding partner used in the invention may also comprise an art recognized amino acid substitution which alters the glycosylation of the chimeric protein. For example, the Fc region or FcRn binding partner of the chimeric protein linked to a VWF fragment or a FVIII protein may comprise an Fc region having a mutation leading to reduced glycosylation (e.g., N- or O-linked glycosylation) or may comprise an altered glycoform of the wild-type Fc moiety (e.g., a low fucose or fucose-free glycan).



[0239] In one embodiment, an unprocessed chimeric protein of the invention may comprise a genetically fused Fc region (*i.e.*, scFc region) having two or more of its constituent Ig constant region or a portion thereof independently selected from the Ig constant region or a portion thereof described herein. In one embodiment, the Fc regions of a dimeric Fc region are the same. In another embodiment, at least two of the Fc regions are different. For example, the Fc regions or FcRn binding partners of the proteins of the invention comprise the same number of amino acid residues or they may differ in length by one or more amino acid residues (*e.g.*, by about 5 amino acid residues (*e.g.*, 1, 2, 3, 4, or 5 amino acid residues), about 10 residues, about 15 residues, about 20 residues, about 30 residues, about 40 residues, or about 50 residues). In yet other embodiments, the Fc regions or FcRn binding partners of the protein of the invention may differ in sequence at one or more amino acid positions. For example, at least two of the Fc regions or FcRn binding partners may differ at about 5 amino acid positions (*e.g.*, 1, 2, 3, 4, or 5 amino acid positions), about 10 positions, about 15 positions, about 20 positions, about 30 positions, about 40 positions, or about 50 positions).

#### **E) Linkers**

[0240] The chimeric protein of the present invention further comprises one or more linkers. One type of the linkers is a cleavable linker, which can be cleaved by various proteases when administered to a subject *in vivo*, *e.g.*, at a site of coagulation. In one embodiment, the cleavable linker allows cleavage of moiety, *e.g.*, a VWF fragment, from the chimeric protein at the site of the coagulation cascade, thus allowing activated FVIII (FVIIIa) to have its FVIIIa activity. Another type of the linkers is a processable linker, which contains an intracellular cleavage site and thus can be cleaved by an intracellular processing enzyme in a host cell, allowing convenient expression of a polypeptide and formation of a chimeric protein.

[0241] One or more linkers can be present between any two proteins in the chimeric protein. In one embodiment, a chimeric protein comprises (i) a VWF fragment, (ii) an XTEN sequence, and (iii) a FVIII protein, wherein the VWF fragment is linked to the XTEN sequence by a linker, *e.g.*, a cleavable linker, and the XTEN sequence is further linked to the FVIII protein (*i.e.*, V-L-X-FVIII). In another embodiment, a chimeric protein comprises (i) a VWF fragment, (ii) an

XTEN sequence, and (iii) a FVIII protein, wherein the VWF fragment is linked to the XTEN sequence, and the XTEN sequence is linked to the FVIII protein by a linker, *e.g.*, a cleavable linker (*i.e.*, V-X-L-FVIII).

[0242] In certain embodiments, a chimeric protein comprises (i) a VWF fragment, (ii) an XTEN sequence, (iii) a first Ig constant region or a portion thereof (*e.g.*, a first Fc region), (iv) a FVIII protein, and (v) a second Ig constant region or a portion thereof (*e.g.*, a second Fc region), wherein the VWF fragment is linked to the XTEN sequence by an optional linker, *e.g.*, a cleavable linker. The XTEN sequence can be further linked to the first Ig constant region or a portion thereof by a linker, *e.g.*, a cleavable linker. The FVIII protein (with or without an XTEN sequence) can also be linked to the second Ig constant region or a portion thereof by an optional linker, *e.g.* a cleavable linker. In certain embodiments, the chimeric protein further comprises one or more linkers, *e.g.*, processable linkers, between the first Ig constant region or a portion thereof (*e.g.*, first Fc region) and the second Ig constant region or a portion thereof (*e.g.*, second Fc region), between the VWF fragment and the second Ig constant region or a portion thereof, or between the FVIII protein and the first Ig constant region or a portion thereof (*e.g.*, first Fc region).

[0243] In some embodiments, the present invention includes a chimeric protein comprising (i) a FVIII protein, (ii) an XTEN sequence, (iii) a first Ig constant region or a portion thereof, and (iv) a second Ig constant region or a portion thereof, wherein the first Ig constant region or a portion thereof and the second Ig constant region or a portion thereof are linked by a processable linker.

[0244] The linker useful in the present invention can comprise any organic molecule. In one embodiment, the linker comprises a polymer, *e.g.*, polyethylene glycol (PEG) or hydroxyethyl starch (HES). In another embodiment, the linker comprises an amino acids sequence. The linker can comprise at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 amino acids. The linker can comprise 1-5 amino acids, 1-10 amino acids, 1-20 amino acids, 10-50 amino acids, 50-100 amino acids, 100-200 amino acids, 200-300 amino acids, 300-400 amino acids, 400-500 amino acids, 500-600 amino acids, 600-700 amino acids, 700-800 amino acids, 800-900 amino acids, or 900-1000

amino acids. In one embodiment, the linker comprises an XTEN sequence.

Additional examples of XTEN can be used according to the present invention and are disclosed in US Patent Publication Nos. 2010/0239554 A1, 2010/0323956 A1, 2011/0046060 A1, 2011/0046061 A1, 2011/0077199 A1, or 2011/0172146 A1, or International Patent Publication Nos. WO 2010091122 A1, WO 2010144502 A2, WO 2010144508 A1, WO 2011028228 A1, WO 2011028229 A1, or WO 2011028344 A2. In another embodiment, the linker is a PAS sequence.

[0245] The linker useful in the present invention can comprise any organic molecule. In one embodiment, the linker is a polymer, *e.g.*, polyethylene glycol (PEG) or hydroxyethyl starch (HES). In another embodiment, the linker is an amino acid sequence. The linker can comprise at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 amino acids. The linker can comprise 1-5 amino acids, 1-10 amino acids, 1-20 amino acids, 10-50 amino acids, 50-100 amino acids, 100-200 amino acids, 200-300 amino acids, 300-400 amino acids, 400-500 amino acids, 500-600 amino acids, 600-700 amino acids, 700-800 amino acids, 800-900 amino acids, or 900-1000 amino acids.

[0246] Examples of linkers are well known in the art. In one embodiment, the linker comprises the sequence  $G_n$ . The linker can comprise the sequence  $(GA)_n$ . The linker can comprise the sequence  $(GGS)_n$ . In other embodiments, the linker comprises  $(GGGS)_n$  (SEQ ID NO: 57). In still other embodiments, the linker comprises the sequence  $(GGS)_n(GGGGS)_n$  (SEQ ID NO: 58). In these instances,  $n$  may be an integer from 1-100. In other instances,  $n$  may be an integer from 1-20, *i.e.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. Examples of linkers include, but are not limited to, GGG, SGGSGGS (SEQ ID NO: 59), GGSGGSGGS (SEQ ID NO: 60), GGSGGSGGGSGGGGS (SEQ ID NO: 61), GGSGGSGGS (SEQ ID NO: 62), or GGGSGGGSGGGGS (SEQ ID NO: 63). The linker does not eliminate or diminish the VWF fragment activity or the clotting activity of Factor VIII. Optionally, the linker enhances the VWF fragment activity or the clotting activity of Factor VIII protein, *e.g.*, by further diminishing the effects of steric hindrance and making the VWF fragment or Factor VIII portion more accessible to its target binding site.

[0247] In one embodiment, the linker useful for the chimeric protein is 15-25 amino acids long. In another embodiment, the linker useful for the chimeric protein is 15-20 amino acids long. In some embodiments, the linker for the chimeric protein is 10-25 amino acids long. In other embodiments, the linker for the chimeric protein is 15 amino acids long. In still other embodiments, the linker for the chimeric protein is (GGGS)<sub>n</sub> (SEQ ID NO: 64) where G represents glycine, S represents serine and n is an integer from 1-20.

#### **F) Cleavage Sites**

[0248] The linker may also incorporate a moiety capable of being cleaved either chemically (*e.g.*, hydrolysis of an ester bond), enzymatically (*i.e.*, incorporation of a protease cleavage sequence), or photolytically (*e.g.*, a chromophore such as 3-amino-3-(2-nitrophenyl) propionic acid (ANP)) in order to release one molecule from another.

[0249] In one embodiment, the linker is a cleavable linker. The cleavable linkers can comprise one or more cleavage sites at the N-terminus or C-terminus or both. In another embodiment, the cleavable linker consists essentially of or consists of one or more cleavable sites. In other embodiments, the cleavable linker comprises heterologous amino acid linker sequences described herein or polymers and one or more cleavable sites.

[0250] In certain embodiments, a cleavable linker comprises one or more cleavage sites that can be cleaved in a host cell (*i.e.*, intracellular processing sites). Non limiting examples of the cleavage site include RRRR (SEQ ID NO: 9), RKRRKR (SEQ ID NO: 10), and RRRRS (SEQ ID NO: 11).

[0251] In other embodiments, a cleavable linker comprises one or more cleavage sites that are cleaved by a protease after a chimeric protein comprising the cleavable linker is administered to a subject. In one embodiment, the cleavage site is cleaved by a protease selected from the group consisting of factor XIa, factor XIIa, kallikrein, factor VIIa, factor IXa, factor Xa, factor IIa (thrombin), Elastase-2, MMP-12, MMP-13, MMP-17, and MMP-20. In another embodiment, the cleavage site is selected from the group consisting of a FXIa cleavage site (*e.g.*, KLTR↓AET (SEQ ID NO: 65)), a FXIa cleavage site (*e.g.*, DFTR↓VVG (SEQ ID NO: 66)), a FXIIa cleavage site (*e.g.*, TMTR↓IVGG (SEQ ID NO: 67)), a Kallikrein cleavage site (*e.g.*, SPFR↓STGG (SEQ ID NO: 68)), a FVIIa cleavage

site (*e.g.*, LQVR↓IVGG (SEQ ID NO: 69)), a FIXa cleavage site (*e.g.*, PLGR↓IVGG (SEQ ID NO: 70)), a FXa cleavage site (*e.g.*, IEGR↓TVGG (SEQ ID NO: 71)), a FIIa (thrombin) cleavage site (*e.g.*, LTPR↓SLLV (SEQ ID NO: 72)), a Elastase-2 cleavage site (*e.g.*, LGPV↓SGVP (SEQ ID NO: 73)), a Granzyme-B cleavage site (*e.g.*, VAGD↓SLEE (SEQ ID NO: 74)), a MMP-12 cleavage site (*e.g.*, GPAG↓LGGA (SEQ ID NO: 75)), a MMP-13 cleavage site (*e.g.*, GPAG↓LRGA (SEQ ID NO: 76)), a MMP-17 cleavage site (*e.g.*, APLG↓LRLR (SEQ ID NO: 77)), a MMP-20 cleavage site (*e.g.*, PALP↓LVAQ (SEQ ID NO: 78)), a TEV cleavage site (*e.g.*, ENLYFQ↓G (SEQ ID NO: 79)), a Enterokinase cleavage site (*e.g.*, DDDK↓IVGG (SEQ ID NO: 80)), a Protease 3C (PRESCISSION™) cleavage site (*e.g.*, LEVLFQ↓GP (SEQ ID NO: 81)), and a Sortase A cleavage site (*e.g.*, LPKT↓GSES) (SEQ ID NO: 82). In certain embodiments, the FXIa cleavage sites include, but are not limited to, *e.g.*, TQSFNDFTR (SEQ ID NO: 83) and SVSQTSKLTR (SEQ ID NO: 84). Non-limiting exemplary thrombin cleavage sites include, *e.g.*, DFLAEGGGVR (SEQ ID NO: 85), TTKIKPR (SEQ ID NO: 86), or LVPRG (SEQ ID NO: 87), and a sequence comprising, consisting essentially of, or consisting of ALRPR (SEQ ID NO: 17) (*e.g.*, ALRPRVVGGA (SEQ ID NO: 88)).

[0252] In a specific embodiment, the cleavage site is TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 8).

#### **POLYNUCLEOTIDES, VECTORS, AND HOST CELLS**

[0253] Also provided in the invention is a polynucleotide encoding (a) a VWF fragment linked to an XTEN sequence and a FVIII protein, (b) a FVIII protein linked to an XTEN sequence and Fc, or (c) a FVIII protein linked to an XTEN sequence and a VWF fragment described herein. When a chimeric protein is a single polypeptide chain (*e.g.*, F2-L2-X-V-L1-F1-FVIII, wherein FVIII comprises a FVIII protein, F1 comprises a first Ig constant region or a portion thereof, *e.g.*, a first Fc region, L1 comprises a first linker, V comprises a VWF fragment, X comprises an XTEN sequence, L2 comprises a second linker, and F2 comprises a second Ig constant region or a portion thereof, *e.g.*, a second Fc region), the invention is drawn to a single polynucleotide chain encoding the single polypeptide chain. When the chimeric protein comprises a first and a second polypeptide chains (F2-L2-X-V:FVIII-F1), the first polypeptide chain comprising

a VWF fragment linked to a XTEN sequence, which is further linked to a first Ig constant region or a portion thereof (*e.g.*, a first Fc region) by a cleavable linker (*e.g.*, F2-L2-X-V) and the second polypeptide chain comprising a FVIII protein and a second Ig constant region or a portion thereof (*e.g.*, a second Fc region) (*e.g.*, FVIII-F1), wherein the first polypeptide chain and the second polypeptide chain are associated with each other, a polynucleotide can comprise the first nucleotide sequence and the second nucleotide sequence. In one embodiment, the first polypeptide chain and the second polypeptide chain can be encoded by a single polynucleotide chain. In another embodiment, the first polypeptide chain and the second polypeptide chain are encoded by two different polynucleotides, *i.e.*, a first nucleotide sequence and a second nucleotide sequence. In another embodiment, the first nucleotide sequence and the second nucleotide sequence are on two different polynucleotides (*e.g.*, different vectors). In certain embodiments, the present invention is directed to a set of polynucleotides comprising a first nucleotide chain and a second nucleotide chain, wherein the first nucleotide chain encodes the VWF fragment of the chimeric protein and the second nucleotide chain encodes the FVIII protein. In some embodiments, a chimeric protein comprising two polypeptide chains or three polypeptide chains can be encoded by a single polynucleotide chain, and then processed into two or three (or more) polypeptide chains. In yet other embodiments, a chimeric protein comprising these polypeptide chains can be encoded by two or three polynucleotide chains.

[0254] In other embodiments, the set of the polynucleotides further comprises an additional nucleotide chain (*e.g.*, a second nucleotide chain when the chimeric polypeptide is encoded by a single polynucleotide chain or a third nucleotide chain when the chimeric protein is encoded by two polynucleotide chains) which encodes a protein convertase. The protein convertase can be selected from the group consisting of proprotein convertase subtilisin/kexin type 5 (PCSK5 or PC5), proprotein convertase subtilisin/kexin type 7 (PCSK7 or PC7), a yeast Kex 2, proprotein convertase subtilisin/kexin type 3 (PACE or PCSK3), and two or more combinations thereof. In some embodiments, the protein convertase is PACE, PC5, or PC7. In a specific embodiment, the protein convertase is PC5 or PC7. See International Application no. PCT/US2011/043568.

[0255] As used herein, an expression vector refers to any nucleic acid construct which contains the necessary elements for the transcription and translation of an inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation, when introduced into an appropriate host cell. Expression vectors can include plasmids, phagemids, viruses, and derivatives thereof.

[0256] Expression vectors of the invention will include polynucleotides encoding the chimeric protein described herein. In one embodiment, one or more of the coding sequences for the VWF fragment and XTEN, the FVIII protein and XTEN, or both are operably linked to an expression control sequence. As used herein, two nucleic acid sequences are operably linked when they are covalently linked in such a way as to permit each component nucleic acid sequence to retain its functionality. A coding sequence and a gene expression control sequence are said to be operably linked when they are covalently linked in such a way as to place the expression or transcription and/or translation of the coding sequence under the influence or control of the gene expression control sequence. Two DNA sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to a coding nucleic acid sequence if the gene expression sequence were capable of effecting transcription of that coding nucleic acid sequence such that the resulting transcript is translated into the desired protein or polypeptide.

[0257] A gene expression control sequence as used herein is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the coding nucleic acid to which it is operably linked. The gene expression control sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine

phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, beta-actin promoter, and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example, promoters from the cytomegalovirus (CMV), simian virus (*e.g.*, SV40), papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, cytomegalovirus, the long terminal repeats (LTR) of Moloney leukemia virus, and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

[0258] In general, the gene expression control sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined coding nucleic acid. The gene expression sequences optionally include enhancer sequences or upstream activator sequences as desired.

[0259] Viral vectors include, but are not limited to, nucleic acid sequences from the following viruses: retrovirus, such as Moloney murine leukemia virus, Harvey murine sarcoma virus, murine mammary tumor virus, and Rous sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyomaviruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors well-known in the art. Certain viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (*i.e.*, capable of



directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high efficiency transduction of genes *in vivo*. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M., Gene Transfer and Expression, A Laboratory Manual, W.H. Freeman Co., New York (1990) and Murry, E. J., Methods in Molecular Biology, Vol. 7, Humana Press, Inc., Clifton, N.J. (1991).

**[0260]** In one embodiment, the virus is an adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus can be engineered to be replication-deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hematopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

**[0261]** Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well-known to those of skill in the art. See, *e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been found to be particularly advantageous for delivering genes to cells *in vivo* because of their inability to replicate within and integrate into a host genome. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operably encoded within the plasmid. Some

commonly used plasmids available from commercial suppliers include pBR322, pUC18, pUC19, various pcDNA plasmids, pRC/CMV, various pCMV plasmids, pSV40, and pBlueScript. Additional examples of specific plasmids include pcDNA3.1, catalog number V79020; pcDNA3.1/hygro, catalog number V87020; pcDNA4/myc-His, catalog number V86320; and pBudCE4.1, catalog number V53220, all from Invitrogen (Carlsbad, CA.). Other plasmids are well-known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using standard molecular biology techniques to remove and/or add specific fragments of DNA.

[0262] In one insect expression system that may be used to produce the proteins of the invention, *Autographa californica* nuclear polyhydrosis virus (AcNPV) is used as a vector to express the foreign genes. The virus grows in *Spodoptera frugiperda* cells. A coding sequence may be cloned into non-essential regions (for example, the polyhedron gene) of the virus and placed under control of an ACNPV promoter (for example, the polyhedron promoter). Successful insertion of a coding sequence will result in inactivation of the polyhedron gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedron gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (see, *e.g.*, Smith *et al.* (1983) *J Virol* 46:584; U.S. Pat. No. 4,215,051). Further examples of this expression system may be found in Ausubel *et al.*, eds. (1989) *Current Protocols in Molecular Biology*, Vol. 2, Greene Publish. Assoc. & Wiley Interscience.

[0263] Another system which can be used to express the proteins of the invention is the glutamine synthetase gene expression system, also referred to as the "GS expression system" (Lonza Biologics PLC, Berkshire UK). This expression system is described in detail in U.S. Pat. No. 5,981,216.

[0264] In mammalian host cells, a number of viral based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region

E1 or E3) will result in a recombinant virus that is viable and capable of expressing peptide in infected hosts. See, *e.g.*, Logan & Shenk (1984) *Proc Natl Acad Sci USA* 81:3655). Alternatively, the vaccinia 7.5 K promoter may be used. See, *e.g.*, Mackett *et al.* (1982) *Proc Natl Acad Sci USA* 79:7415; Mackett *et al.* (1984) *J Virol* 49:857; Panicali *et al.* (1982) *Proc Natl Acad Sci USA* 79:4927.

[0265] To increase efficiency of production, the polynucleotides can be designed to encode multiple units of the protein of the invention separated by enzymatic cleavage sites. The resulting polypeptide can be cleaved (*e.g.*, by treatment with the appropriate enzyme) in order to recover the polypeptide units. This can increase the yield of polypeptides driven by a single promoter. When used in appropriate viral expression systems, the translation of each polypeptide encoded by the mRNA is directed internally in the transcript; *e.g.*, by an internal ribosome entry site, IRES. Thus, the polycistronic construct directs the transcription of a single, large polycistronic mRNA which, in turn, directs the translation of multiple, individual polypeptides. This approach eliminates the production and enzymatic processing of polyproteins and may significantly increase the yield of polypeptides driven by a single promoter.

[0266] Vectors used in transformation will usually contain a selectable marker used to identify transformants. In bacterial systems, this can include an antibiotic resistance gene such as ampicillin or kanamycin. Selectable markers for use in cultured mammalian cells include genes that confer resistance to drugs, such as neomycin, hygromycin, and methotrexate. The selectable marker may be an amplifiable selectable marker. One amplifiable selectable marker is the dihydrofolate reductase (DHFR) gene. Simonsen C C *et al.* (1983) *Proc Natl Acad Sci USA* 80:2495-9. Selectable markers are reviewed by Thilly (1986) *Mammalian Cell Technology*, Butterworth Publishers, Stoneham, Mass., and the choice of selectable markers is well within the level of ordinary skill in the art.

[0267] Selectable markers may be introduced into the cell on a separate plasmid at the same time as the gene of interest, or they may be introduced on the same plasmid. If on the same plasmid, the selectable marker and the gene of interest may be under the control of different promoters or the same promoter, the latter arrangement producing a dicistronic message. Constructs of this type are known in the art (for example, U.S. Pat. No. 4,713,339).

[0268] The expression vectors can encode for tags that permit easy purification of the recombinantly produced protein. Examples include, but are not limited to, vector pUR278 (Ruther *et al.* (1983) *EMBO J* 2:1791), in which coding sequences for the protein to be expressed may be ligated into the vector in frame with the lac z coding region so that a tagged fusion protein is produced; pGEX vectors may be used to express proteins of the invention with a glutathione S-transferase (GST) tag. These proteins are usually soluble and can easily be purified from cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The vectors include cleavage sites (thrombin or Factor Xa protease or PRESCISSION PROTEASE<sup>TM</sup> (Pharmacia, Peapack, N.J.)) for easy removal of the tag after purification.

[0269] The expression vector or vectors are then transfected or co-transfected into a suitable target cell, which will express the polypeptides. Transfection techniques known in the art include, but are not limited to, calcium phosphate precipitation (Wigler *et al.* (1978) *Cell* 14:725), electroporation (Neumann *et al.* (1982) *EMBO J* 1:841), and liposome-based reagents. A variety of host-expression vector systems may be utilized to express the proteins described herein including both prokaryotic and eukaryotic cells. These include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli*) transformed with recombinant bacteriophage DNA or plasmid DNA expression vectors containing an appropriate coding sequence; yeast or filamentous fungi transformed with recombinant yeast or fungi expression vectors containing an appropriate coding sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing an appropriate coding sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus or tobacco mosaic virus) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing an appropriate coding sequence; or animal cell systems, including mammalian cells (*e.g.*, HEK 293, CHO, Cos, HeLa, HKB11, and BHK cells).

[0270] In one embodiment, the host cell is a eukaryotic cell. As used herein, a eukaryotic cell refers to any animal or plant cell having a definitive nucleus. Eukaryotic cells of animals include cells of vertebrates, *e.g.*, mammals, and cells of invertebrates, *e.g.*, insects. Eukaryotic cells of plants specifically can include,

without limitation, yeast cells. A eukaryotic cell is distinct from a prokaryotic cell, *e.g.*, bacteria.

[0271] In certain embodiments, the eukaryotic cell is a mammalian cell. A mammalian cell is any cell derived from a mammal. Mammalian cells specifically include, but are not limited to, mammalian cell lines. In one embodiment, the mammalian cell is a human cell. In another embodiment, the mammalian cell is a HEK 293 cell, which is a human embryonic kidney cell line. HEK 293 cells are available as CRL-1533 from American Type Culture Collection, Manassas, VA, and as 293-H cells, Catalog No. 11631-017 or 293-F cells, Catalog No. 11625-019 from Invitrogen (Carlsbad, Calif.). In some embodiments, the mammalian cell is a PER.C6<sup>®</sup> cell, which is a human cell line derived from retina. PER.C6<sup>®</sup> cells are available from Crucell (Leiden, The Netherlands). In other embodiments, the mammalian cell is a Chinese hamster ovary (CHO) cell. CHO cells are available from American Type Culture Collection, Manassas, VA. (*e.g.*, CHO-K1; CCL-61). In still other embodiments, the mammalian cell is a baby hamster kidney (BHK) cell. BHK cells are available from American Type Culture Collection, Manassas, Va. (*e.g.*, CRL-1632). In some embodiments, the mammalian cell is a HKB11 cell, which is a hybrid cell line of a HEK293 cell and a human B cell line. Mei *et al.*, *Mol. Biotechnol.* 34(2): 165-78 (2006).

[0272] In one embodiment, a plasmid including a FVIII(X)-Fc fusion coding sequence, a VWF fragment-L-Fc fusion coding sequence, or both and a selectable marker, *e.g.*, zeocin resistance, are transfected into HEK 293 cells, for production of a chimeric protein.

[0273] In another embodiment, a plasmid including a FVIII-Fc fusion coding sequence, a VWF fragment-XTEN-L-Fc fusion coding sequence, or both and a selectable marker, *e.g.*, zeocin resistance, are transfected into HEK 293 cells, for production of a chimeric protein.

[0274] In other embodiments, a plasmid including a FVIII(X)-Fc fusion coding sequence, a Fc coding sequence, or both and a selectable marker, *e.g.*, zeocin resistance, are transfected into HEK 293 cells, for production of a chimeric protein.

[0275] In some embodiments, a first plasmid including a FVIII(X)-Fc fusion coding sequence and a first selectable marker, *e.g.*, a zeocin resistance gene, and a

second plasmid including an Fc coding sequence or a VWF fragment-L-Fc coding sequence and a second selectable marker, *e.g.*, a neomycin resistance gene, and a third plasmid including a protein convertase coding sequence and a third selectable marker, *e.g.*, a hygromycin resistance gene, are cotransfected into HEK 293 cells, for production of the chimeric protein. The first and second plasmids can be introduced in equal amounts (*i.e.*, 1:1 molar ratio), or they can be introduced in unequal amounts.

[0276] In still other embodiments, a first plasmid including a FVIII-Fc fusion coding sequence and a first selectable marker, *e.g.*, a zeocin resistance gene, and a second plasmid including a VWF fragment-XTEN-L-Fc coding sequence and a second selectable marker, *e.g.*, a neomycin resistance gene, and a third plasmid including a protein convertase coding sequence and a third selectable marker, *e.g.*, a hygromycin resistance gene, are cotransfected into HEK 293 cells, for production of the chimeric protein. The first and second plasmids can be introduced in equal amounts (*i.e.*, 1:1 molar ratio), or they can be introduced in unequal amounts.

[0277] In yet other embodiments, a first plasmid including a FVIII(X)-Fc fusion coding sequence and a first selectable marker, *e.g.*, a zeocin resistance gene, and a second plasmid including a VWF fragment-XTEN-L-Fc coding sequence and a second selectable marker, *e.g.*, a neomycin resistance gene, and a third plasmid including a protein convertase coding sequence and a third selectable marker, *e.g.*, a hygromycin resistance gene, are cotransfected into HEK 293 cells, for production of the chimeric protein. The first and second plasmids can be introduced in equal amounts (*i.e.*, 1:1 molar ratio), or they can be introduced in unequal amounts.

[0278] In certain embodiments, a first plasmid, including a chimeric protein encoding FVIII (with or without XTEN)-F1-L1-V-XTEN-L2-F2 coding sequence and a first selectable marker, *e.g.*, a zeocin resistance gene, and a second plasmid including a protein convertase coding sequence and a second selectable marker, *e.g.*, a hygromycin resistance gene, are cotransfected into HEK 293 cells, for production of the chimeric protein. The promoters for the FVIII(X)-Fc coding sequence and the VWF-XTEN-Fc coding sequence can be different or they can be the same.

- [0279] In still other embodiments, transfected cells are stably transfected. These cells can be selected and maintained as a stable cell line, using conventional techniques known to those of skill in the art.
- [0280] Host cells containing DNA constructs of the protein are grown in an appropriate growth medium. As used herein, the term "appropriate growth medium" means a medium containing nutrients required for the growth of cells. Nutrients required for cell growth may include a carbon source, a nitrogen source, essential amino acids, vitamins, minerals, and growth factors. Optionally, the media can contain one or more selection factors. Optionally the media can contain bovine calf serum or fetal calf serum (FCS). In one embodiment, the media contains substantially no IgG. The growth medium will generally select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker on the DNA construct or co-transfected with the DNA construct. Cultured mammalian cells are generally grown in commercially available serum-containing or serum-free media (*e.g.*, MEM, DMEM, DMEM/F12). In one embodiment, the medium is CD293 (Invitrogen, Carlsbad, CA.). In another embodiment, the medium is CD17 (Invitrogen, Carlsbad, CA.). Selection of a medium appropriate for the particular cell line used is within the level of those ordinary skilled in the art.
- [0281] In order to co-express the two polypeptide chains of the chimeric protein, the host cells are cultured under conditions that allow expression of both chains. As used herein, culturing refers to maintaining living cells *in vitro* for at least a definite time. Maintaining can, but need not include, an increase in population of living cells. For example, cells maintained in culture can be static in population, but still viable and capable of producing a desired product, *e.g.*, a recombinant protein or recombinant fusion protein. Suitable conditions for culturing eukaryotic cells are well known in the art and include appropriate selection of culture media, media supplements, temperature, pH, oxygen saturation, and the like. For commercial purposes, culturing can include the use of any of various types of scale-up systems including shaker flasks, roller bottles, hollow fiber bioreactors, stirred-tank bioreactors, airlift bioreactors, Wave bioreactors, and others.

[0282] The cell culture conditions are also selected to allow association of the VWF fragment with the FVIII protein. Conditions that allow expression of the VWF fragment and/or the FVIII protein may include the presence of a source of vitamin K. For example, in one embodiment, stably transfected HEK 293 cells are cultured in CD293 media (Invitrogen, Carlsbad, CA) or OptiCHO media (Invitrogen, Carlsbad, CA) supplemented with 4 mM glutamine.

[0283] In one aspect, the present invention is directed to a method of expressing, making, or producing the chimeric protein of the invention comprising a) transfecting a host cell comprising a polynucleotide encoding the chimeric protein and b) culturing the host cell in a culture medium under a condition suitable for expressing the chimeric protein, wherein the chimeric protein is expressed.

[0284] In further embodiments, the protein product containing the VWF fragment linked to an XTEN sequence or the FVIII protein linked to an XTEN sequence is secreted into the media. Media is separated from the cells, concentrated, filtered, and then passed over two or three affinity columns, *e.g.*, a protein A column and one or two anion exchange columns.

[0285] In certain aspects, the present invention relates to the chimeric protein produced by the methods described herein.

[0286] *In vitro* production allows scale-up to give large amounts of the desired altered polypeptides of the invention. Techniques for mammalian cell cultivation under tissue culture conditions are known in the art and include homogeneous suspension culture, *e.g.* in an airlift reactor or in a continuous stirrer reactor, or immobilized or entrapped cell culture, *e.g.* in hollow fibers, microcapsules, on agarose microbeads or ceramic cartridges. If necessary and/or desired, the solutions of polypeptides can be purified by the customary chromatography methods, for example gel filtration, ion-exchange chromatography, hydrophobic interaction chromatography (HIC, chromatography over DEAE-cellulose or affinity chromatography.

#### PHARMACEUTICAL COMPOSITION

[0287] Compositions containing the chimeric protein of the present invention may contain a suitable pharmaceutically acceptable carrier. For example, they may contain excipients and/or auxiliaries that facilitate processing of the active compounds into preparations designed for delivery to the site of action.



[0288] The pharmaceutical composition can be formulated for parenteral administration (*i.e.* intravenous, subcutaneous, or intramuscular) by bolus injection. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampoules or in multidose containers with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, *e.g.*, pyrogen free water.

[0289] Suitable formulations for parenteral administration also include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, including, for example, sodium carboxymethyl cellulose, sorbitol and dextran. Optionally, the suspension may also contain stabilizers. Liposomes also can be used to encapsulate the molecules of the invention for delivery into cells or interstitial spaces. Exemplary pharmaceutically acceptable carriers are physiologically compatible solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like. In some embodiments, the composition comprises isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride. In other embodiments, the compositions comprise pharmaceutically acceptable substances such as wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the active ingredients.

[0290] Compositions of the invention may be in a variety of forms, including, for example, liquid (*e.g.*, injectable and infusible solutions), dispersions, suspensions, semi-solid and solid dosage forms. The preferred form depends on the mode of administration and therapeutic application.

[0291] The composition can be formulated as a solution, micro emulsion, dispersion, liposome, or other ordered structure suitable to high drug

concentration. Sterile injectable solutions can be prepared by incorporating the active ingredient in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active ingredient into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0292] The active ingredient can be formulated with a controlled-release formulation or device. Examples of such formulations and devices include implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, for example, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for the preparation of such formulations and devices are known in the art. See *e.g.*, Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0293] Injectable depot formulations can be made by forming microencapsulated matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the polymer employed, the rate of drug release can be controlled. Other exemplary biodegradable polymers are polyorthoesters and polyanhydrides. Depot injectable formulations also can be prepared by entrapping the drug in liposomes or microemulsions.

[0294] Supplementary active compounds can be incorporated into the compositions. In one embodiment, the chimeric protein of the invention is formulated with another clotting factor, or a variant, fragment, analogue, or derivative thereof. For example, the clotting factor includes, but is not limited to,

factor V, factor VII, factor VIII, factor IX, factor X, factor XI, factor XII, factor XIII, prothrombin, fibrinogen, von Willebrand factor or recombinant soluble tissue factor (rsTF) or activated forms of any of the preceding. The clotting factor of hemostatic agent can also include anti-fibrinolytic drugs, *e.g.*, epsilon-amino-caproic acid, tranexamic acid.

[0295] Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. See, *e.g.*, Remington's Pharmaceutical Sciences (Mack Pub. Co., Easton, Pa. 1980).

[0296] In addition to the active compound, the liquid dosage form may contain inert ingredients such as water, ethyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols, and fatty acid esters of sorbitan.

[0297] Non-limiting examples of suitable pharmaceutical carriers are also described in Remington's Pharmaceutical Sciences by E. W. Martin. Some examples of excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. The composition can also contain pH buffering reagents, and wetting or emulsifying agents.

[0298] For oral administration, the pharmaceutical composition can take the form of tablets or capsules prepared by conventional means. The composition can also be prepared as a liquid for example a syrup or a suspension. The liquid can include suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats), emulsifying agents (lecithin or acacia), non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils), and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations can also include flavoring, coloring and sweetening agents.

Alternatively, the composition can be presented as a dry product for constitution with water or another suitable vehicle.

[0299] For buccal administration, the composition may take the form of tablets or lozenges according to conventional protocols.

[0300] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of a nebulized aerosol with or without excipients or in the form of an aerosol spray from a pressurized pack or nebulizer, with optionally a propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoromethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0301] The pharmaceutical composition can also be formulated for rectal administration as a suppository or retention enema, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[0302] In one embodiment, a pharmaceutical composition comprises a chimeric protein, the polynucleotide encoding the chimeric protein, the vector comprising the polynucleotide, or the host cell comprising the vector, and a pharmaceutically acceptable carrier. The FVIII protein in a chimeric protein has extended half-life compared to wild type FVIII protein or the corresponding FVIII protein without the VWF fragment. In one embodiment, wherein the half-life of the FVIII protein is extended at least about 1.5 times, at least about 2 times, at least about 2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than wild type FVIII. In another embodiment, the half-life of Factor VIII is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at

least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours.

[0303] In some embodiments, the composition is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration. The parenteral administration can be intravenous or subcutaneous administration.

[0304] In other embodiments, the composition is used to treat a bleeding disease or condition in a subject in need thereof. The bleeding disease or condition is selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the iliopsoas sheath and any combinations thereof. In still other embodiments, the subject is scheduled to undergo a surgery. In yet other embodiments, the treatment is prophylactic or on-demand.

#### **GENE THERAPY**

[0305] A chimeric protein thereof of the invention can be produced in vivo in a mammal, *e.g.*, a human patient, using a gene therapy approach to treatment of a bleeding disease or disorder selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, and bleeding in the iliopsoas sheath would be therapeutically beneficial. In one embodiment, the bleeding disease or disorder is hemophilia. In another embodiment, the bleeding disease or disorder is hemophilia A. This involves administration of a suitable chimeric protein-encoding nucleic acid operably linked to suitable expression control sequences. In certain embodiment, these sequences are incorporated into a viral vector. Suitable viral vectors for such gene

therapy include adenoviral vectors, lentiviral vectors, baculoviral vectors, Epstein Barr viral vectors, papovaviral vectors, vaccinia viral vectors, herpes simplex viral vectors, and adeno associated virus (AAV) vectors. The viral vector can be a replication-defective viral vector. In other embodiments, an adenoviral vector has a deletion in its E1 gene or E3 gene. When an adenoviral vector is used, the mammal may not be exposed to a nucleic acid encoding a selectable marker gene. In other embodiments, the sequences are incorporated into a non-viral vector known to those skilled in the art.

#### **METHODS OF USING CHIMERIC PROTEIN**

- [0306] The present invention is directed to a method of using a chimeric protein described herein to prevent or inhibit endogenous VWF binding to a FVIII protein. The present invention is also directed to a method of using a chimeric protein having a FVIII protein linked to XTEN and an Ig constant region or a portion thereof.
- [0307] One aspect of the present invention is directed to preventing or inhibiting FVIII interaction with endogenous VWF by blocking or shielding the VWF binding site on the FVIII from endogenous VWF and at the same time extending half-life of the FVIII protein using an XTEN sequence in combination with an Ig constant region or a portion thereof, which can also be a half-life extender. In one embodiment, the invention is directed to a method of constructing a FVIII protein having half-life longer than wild-type FVIII. In one embodiment, an XTEN sequence inhibits or prevents interaction of a FVIII protein in a chimeric protein with endogenous VWF. In another embodiment, an Ig constant region or a portion thereof inhibits or prevents interaction of the FVIII protein with endogenous VWF. The chimeric protein useful in the method includes any one or more chimeric protein described herein.
- [0308] Another aspect of the invention includes a method of administering to a subject in need thereof a chimeric protein comprising a FVIII protein having half-life longer than wild-type FVIII, wherein the method comprises administering the chimeric protein described herein to the subject.
- [0309] In one embodiment, the invention is directed to a method of using an XTEN sequence and an Ig constant region or a portion thereof to extend a half-life of a FVIII protein and a VWF fragment to prevent or inhibit endogenous VWF

interaction with a FVIII protein. A FVIII protein linked to an XTEN sequence (*e.g.*, FVIII(X)) and then bound to or associated with a VWF fragment is shielded or protected from the clearance pathway of VWF and thus has reduced clearance compared to the FVIII protein not bound to the VWF fragment. The shielded FVIII protein thus has maximum extension of a half-life compared to a FVIII protein not bound to or associated with the XTEN sequence and the VWF fragment. In certain embodiments, the FVIII protein associated with or protected by a VWF fragment and linked to an XTEN sequence is not cleared by a VWF clearance receptor. In other embodiments, the FVIII protein associated with or protected by a VWF fragment and linked to an XTEN sequence is cleared from the system slower than the FVIII protein that is not associated with or protected by the VWF fragment and linked to the XTEN sequence.

[0310] In one aspect, the chimeric protein comprising the FVIII protein linked to an XTEN sequence or the FVIII protein bound to or associated with a VWF fragment linked to XTEN has reduced clearance from circulation as the VWF fragment does not contain a VWF clearance receptor binding site. The VWF fragment prevents or inhibits clearance of FVIII bound to or associated with the VWF fragment from the system through the VWF clearance pathway. The VWF fragments useful for the present invention can also provide at least one or more VWF-like FVIII protection properties that are provided by endogenous VWF. In certain embodiments, the VWF fragment or the XTEN sequence can also mask one or more FVIII clearance receptor binding site, thereby preventing clearance of FVIII by its own clearance pathway.

[0311] In some embodiments, the prevention or inhibition of a FVIII protein binding to endogenous VWF by the VWF fragment or the XTEN sequence can be *in vitro* or *in vivo*.

[0312] Also provided is a method of increasing the half-life of a FVIII protein comprising administering the chimeric protein described herein to a subject in need thereof. The half-life of non-activated FVIII bound to or associated with full-length VWF is about 12 to 14 hours in plasma. In VWD type 3, wherein there is almost no VWF in circulation, the half-life of FVIII is only about six hours, leading to symptoms of mild to moderate hemophilia A in such patients due to decreased concentrations of FVIII. The half-life of the FVIII protein linked to or

associated with the VWF fragment or the XTEN sequence of the present invention can increase at least about 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2.0 times, 2.1 times, 2.2 times, 2.3 times, 2.4 times, 2.6 times, 2.7. times, 2.8 times, 2.9 times, 3.0 times, 3.1 times, 3.2 times, 3.3 times, 3.4 times, 3.5 times, 3.6 times, 3.7 times, 3.8 times, 3.9 times, or 4.0 times higher than the half-life of the non-activated FVIII bound to or associated with full-length VWF.

**[0313]** In one embodiment, the half-life of the FVIII protein linked to or associated with the VWF fragment or linked to an Ig constant region or a portion thereof in the chimeric protein comprising an XTEN sequence increases at least about 2 times, 2.5 times, 3.0 times, 3.5 times, 4.0 times, 4.5 times, 5.0 times, 5.5 times, 6.0 times, 7 times, 8 times, 9 times, or 10 times higher than the half-life of the non-activated FVIII bound to or associated with full-length VWF. In another embodiment, the half-life of the FVIII protein linked to or associated with the VWF fragment or an Ig constant region or a portion thereof in the chimeric protein comprising an XTEN sequence increases about 2 to about 5 times, about 3 to about 10 times, about 5 to about 15 times, about 10 to about 20 times, about 15 to about 25 times, about 20 to about 30 times, about 25 to about 35 times, about 30 to about 40 times, about 35 to about 45 times higher than the half-life of the non-activated FVIII bound to or associated with full-length VWF or wild type FVIII. In a specific embodiment, the half-life of the FVIII protein linked to or associated with the VWF fragment or linked to an Ig constant region in the chimeric protein comprising an XTEN sequence increases at least about 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 times higher than the half-life of the wild type FVIII in a FVIII and VWF double knockout mouse.

**[0314]** In some embodiments, the half-life of the chimeric protein comprising the VWF fragment fused to a first Ig constant region or a portion thereof, *e.g.*, a first Fc region and an XTEN sequence, and a FVIII protein linked to an XTEN sequence and a second Ig constant region or a portion thereof, *e.g.*, a second Fc region, is longer than the half-life of a FVIII associated with endogenous VWF. In other embodiments, the half-life of the chimeric protein is at least about 1.5 times, 2 times, 2.5 times, 3.5 times, 3.6 times, 3.7 times, 3.8 times, 3.9 times, 4.0 times, 4.5 times, or 5.0 times the half-life of wild type FVIII or a FVIII protein associated with endogenous VWF.



[0315] In some embodiments, as a result of the invention the half-life of the FVIII protein is extended compared to a FVIII protein without the VWF fragment or wild-type FVIII. The half-life of the chimeric protein of the invention is at least about 1.5 times, at least about 2 times, at least about 2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than the half-life of a FVIII protein without the VWF fragment or wild-type FVIII. In one embodiment, the half-life of FVIII is about 1.5-fold to about 20-fold, about 1.5 fold to about 15 fold, or about 1.5 fold to about 10 fold longer than the half-life of wild-type FVIII. In another embodiment, the half-life of the FVIII is extended about 2-fold to about 10-fold, about 2-fold to about 9-fold, about 2-fold to about 8-fold, about 2-fold to about 7-fold, about 2-fold to about 6-fold, about 2-fold to about 5-fold, about 2-fold to about 4-fold, about 2-fold to about 3-fold, about 2.5-fold to about 10-fold, about 2.5-fold to about 9-fold, about 2.5-fold to about 8-fold, about 2.5-fold to about 7-fold, about 2.5-fold to about 6-fold, about 2.5-fold to about 5-fold, about 2.5-fold to about 4-fold, about 2.5-fold to about 3-fold, about 3-fold to about 10-fold, about 3-fold to about 9-fold, about 3-fold to about 8-fold, about 3-fold to about 7-fold, about 3-fold to about 6-fold, about 3-fold to about 5-fold, about 3-fold to about 4-fold, about 4-fold to about 6 fold, about 5-fold to about 7-fold, or about 6-fold to about 8 fold as compared to wild-type FVIII or a FVIII protein without the VWF fragment. In other embodiments, the half-life of the chimeric protein of the invention is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours. In still other embodiments, the half-life of the chimeric protein of the invention is about 15 hours to about two weeks, about 16 hours to about one week, about 17 hours to about one week, about 18 hours to about one week, about 19 hours to

about one week, about 20 hours to about one week, about 21 hours to about one week, about 22 hours to about one week, about 23 hours to about one week, about 24 hours to about one week, about 36 hours to about one week, about 48 hours to about one week, about 60 hours to about one week, about 24 hours to about six days, about 24 hours to about five days, about 24 hours to about four days, about 24 hours to about three days, or about 24 hours to about two days.

[0316] In some embodiments, the average half-life of the chimeric protein of the invention per subject is about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours (1 day), about 25 hours, about 26 hours, about 27 hours, about 28 hours, about 29 hours, about 30 hours, about 31 hours, about 32 hours, about 33 hours, about 34 hours, about 35 hours, about 36 hours, about 40 hours, about 44 hours, about 48 hours (2 days), about 54 hours, about 60 hours, about 72 hours (3 days), about 84 hours, about 96 hours (4 days), about 108 hours, about 120 hours (5 days), about six days, about seven days (one week), about eight days, about nine days, about 10 days, about 11 days, about 12 days, about 13 days, or about 14 days.

[0317] In addition, the invention provides a method of treating or preventing a bleeding disease or disorder comprising administering an effective amount of a chimeric protein. In one embodiment, the bleeding disease or disorder is selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, and bleeding in the iliopsoas sheath. In a specific embodiment, the bleeding disease or disorder is hemophilia A.

[0318] The chimeric protein comprising an XTEN sequence and an Ig constant region or a portion thereof in combination with a VWF fragment described herein, that prevents or inhibits interaction of the FVIII protein with endogenous VWF prepared by the invention, has many uses as will be recognized by one skilled in the art, including, but not limited to methods of treating a subject having a hemostatic disorder and methods of treating a subject in need of a general

hemostatic agent. In one embodiment, the invention relates to a method of treating a subject having a hemostatic disorder comprising administering a therapeutically effective amount of the chimeric protein.

[0319] The FVIII protein portion in the chimeric protein treats or prevents a hemostatic disorder by serving as a cofactor to Factor IX on a negatively charged phospholipid surface, thereby forming a Xase complex. The binding of activated coagulation factors to a phospholipid surface localizes this process to sites of vascular damage. On a phospholipid surface, Factor VIIIa increases the maximum velocity of Factor X activation by Factor IXa, by approximately 200,000-fold, leading to the large second burst of thrombin generation.

[0320] The chimeric protein of the invention can be used to treat any hemostatic disorder. The hemostatic disorders that may be treated by administration of the chimeric protein of the invention include, but are not limited to, hemophilia A, as well as deficiencies or structural abnormalities relating to Factor VIII. In one embodiment, the hemostatic disorder is hemophilia A.

[0321] The chimeric protein of the invention can be used prophylactically to treat a subject with a hemostatic disorder. The chimeric protein of the invention can be used to treat an acute bleeding episode in a subject with a hemostatic disorder. In another embodiment, the hemostatic disorder can be the result of a defective clotting factor, *e.g.*, von Willebrand's factor. In one embodiment, the hemostatic disorder is an inherited disorder. In another embodiment, the hemostatic disorder is an acquired disorder. The acquired disorder can result from an underlying secondary disease or condition. The unrelated condition can be, as an example, but not as a limitation, cancer, an auto-immune disease, or pregnancy. The acquired disorder can result from old age or from medication to treat an underlying secondary disorder (*e.g.* cancer chemotherapy).

[0322] The invention also relates to methods of treating a subject that does not have a congenital hemostatic disorder, but has a secondary disease or condition resulting in acquisition of a hemostatic disorder, *e.g.*, due to development of an anti-FVIII antibody or a surgery. The invention thus relates to a method of treating a subject in need of a general hemostatic agent comprising administering a therapeutically effective amount of the chimeric protein prepared by the present methods.

- [0323] The present invention is also related to methods of reducing immunogenicity of FVIII or inducing less immunogenicity against FVIII comprising administering an effective amount of the chimeric proteins described herein, or the polynucleotides encoding the same.
- [0324] In one embodiment, the subject in need of a general hemostatic agent is undergoing, or is about to undergo, surgery. The chimeric protein of the invention can be administered prior to, during, or after surgery as a prophylactic regimen. The chimeric protein of the invention can be administered prior to, during, or after surgery to control an acute bleeding episode..
- [0325] The chimeric protein of the invention can be used to treat a subject having an acute bleeding episode who does not have a hemostatic disorder. The acute bleeding episode can result from severe trauma, *e.g.*, surgery, an automobile accident, wound, laceration gun shot, or any other traumatic event resulting in uncontrolled bleeding. Non limiting examples of bleeding episodes include a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the iliopsoas sheath, and any combinations thereof.
- [0326] In prophylactic applications, one or more compositions containing the chimeric protein of the invention or a cocktail thereof are administered to a patient not already in the disease state to enhance the patient's resistance or reduce symptoms associated with a disease or disorder. Such an amount is defined to be a "prophylactic effective dose." In therapeutic applications, a relatively high dosage (*e.g.*, from about 1 to 400 mg/kg of polypeptide per dose, with dosages of from 5 to 25 mg being more commonly used for radioimmuno conjugates and higher doses for cytotoxin-drug modified polypeptides) at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.
- [0327] In some embodiments, a chimeric protein or a composition of the invention is used for on-demand treatment, which includes treatment for a bleeding episode,

hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis (head trauma), gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, or bleeding in the iliopsoas sheath. The subject may be in need of surgical prophylaxis, peri-operative management, or treatment for surgery. Such surgeries include, *e.g.*, minor surgery, major surgery, tooth extraction, tonsillectomy, inguinal herniotomy, synovectomy, total knee replacement, craniotomy, osteosynthesis, trauma surgery, intracranial surgery, intra-abdominal surgery, intrathoracic surgery, or joint replacement surgery.

[0328] In one embodiment, the chimeric protein of the present invention is administered intravenously, subcutaneously, intramuscularly, or via any mucosal surface, *e.g.*, orally, sublingually, buccally, nasally, rectally, vaginally or via pulmonary route. The chimeric protein comprising a VWF fragment and a FVIII protein of the present invention can be implanted within or linked to a biopolymer solid support that allows for the slow release of the chimeric protein to the site of bleeding or implanted into bandage/dressing. The dose of the chimeric protein will vary depending on the subject and upon the particular route of administration used. Dosages can range from 0.1 to 100,000 µg/kg body weight. In one embodiment, the dosing range is 0.1-1,000 µg/kg. In another embodiment, the dosing range is 0.1-500 µg/kg. The protein can be administered continuously or at specific timed intervals. *In vitro* assays may be employed to determine optimal dose ranges and/or schedules for administration. *In vitro* assays that measure clotting factor activity are known in the art, *e.g.*, STA-CLOT VIIa-rTF clotting assay or ROTEM clotting assay. Additionally, effective doses may be extrapolated from dose-response curves obtained from animal models, *e.g.*, a hemophiliac dog (Mount *et al.* 2002, *Blood* 99(8):2670).

[0329] Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention. All patents, publications, and articles referred to herein are expressly and specifically incorporated herein by reference.

## Examples

[0330] Throughout the examples, the following materials and methods were used unless otherwise stated.

## Materials and Methods

[0331] In general, the practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, biophysics, molecular biology, recombinant DNA technology, immunology (especially, *e.g.*, antibody technology), and standard techniques in electrophoresis. See, *e.g.*, Sambrook, Fritsch and Maniatis, *Molecular Cloning*: Cold Spring Harbor Laboratory Press (1989); *Antibody Engineering Protocols* (Methods in Molecular Biology), 510, Paul, S., Humana Pr (1996); *Antibody Engineering: A Practical Approach* (Practical Approach Series, 169), McCafferty, Ed., Irl Pr (1996); *Antibodies: A Laboratory Manual*, Harlow *et al.*, CS.H.L. Press, Pub. (1999); and *Current Protocols in Molecular Biology*, eds. Ausubel *et al.*, John Wiley & Sons (1992).

## Example 1: Cloning different VWF domains (Figure 1)

## (a) Cloning of pSYN-VWF-002

[0332] pSYN-VWF-002 contains nucleotide sequences encoding a VWF fragment, which are amino acids 1-477 of SEQ ID NO: 100. [VWF-D'D3 protein sequence] Amino acid numbering represents the mature VWF sequence without propeptide and corresponds to amino acids 764-1240 of SEQ ID NO: 2. pSYN-VWF-002 construct has the FVIII signal peptide at N-terminus, which allows proper secretion of the synthesized protein and followed by a 6xHis tag at C-terminus, which is used for protein purification. It was synthesized by using following primer combinations:

ESC48- Fwd - VWF-D'D3 with VIII signal and BsiW1 site

TCGCGACGTACGGCCGCCACCATGCAAATAGAGCTCTCCACCTGCTTCTTTCTGT  
GCCTTTTGCGATTCTGCTTTAGCCTATCCTGTCGGCCCCCATG (SEQ ID NO:  
90)

ESC51- Rev- VWF D'D3 (1-477 amino acid) with 6His and Not 1 site

TGACCTCGAGCGGCCGCTCAGTGGTGATGGTGATGATGCGGCTCCTGGCAGGCTT  
CACAGGTGAGGTTGACAAC (SEQ ID NO: 91)

[0333] A 50 µl PCR reaction was carried out with ESC 48/ESC 51 primer combinations and full length VWF plasmid as the template, using the 2 step PCR

amplification cycle: 94 °C 2minutes; 21 cycles of (96 °C 30 seconds, 68 °C 2 minute). The 1460bp band was gel purified with a Gel Extraction kit (Qiagen, Valencia, Calif.) and cloned into the BsiWI and NotI restriction sites of pcDNA 4 to generate pSYN-VWF 002.

(b) Cloning of pSYN-VWF- 010 and 013

[0334] pSYN-VWF-010 was constructed using pSYN-VWF-008 and pSYN-VWF-002. pSYN-VWF-008 contains the full-length VWF sequence in pcDNA 3.1 (amino acids 1-2813 of SEQ ID NO: 2), it includes 763 amino acid propeptide (*i.e.*, D1D2 domains) followed by remaining 2050 amino acids sequence of mature VWF. The FVIII signal peptide in pSYN-VWF-002 was replaced with D1D2 domains from pSYN-VWF-008, the resulting construct is pSYN-VWF-010. pSYN-VWF- 008 has a BamHI site at Arg907 and NotI site at the end of coding region (after stop codon). pSYN- VWF- 008 and 002 were digested with BamHI and NotI restriction enzymes. Inserts from pSYN- VWF-002 (1026 bp) were ligated into bamHI/NotI digested pSYN-VWF- 008 (8242bp) to obtain pSYN-VWF -010 (D1D2D'D3: amino acid 1-1240 of SEQ ID NO: 2), a 6xHis tag was also added at the C-terminus. In transformed cells pSYN-VWF-010 is synthesized with propeptide but due to intracellular processing the secreted products do not contain any propeptide (D1D2). Protein from VWF-010 exists as dimer.

[0335] pSYN-VWF-010 was used to generate pSYN-VWF-013 which has two point mutations at C336A and C379A corresponding to SEQ ID NO: 100 (amino acid numbering represents mature VWF sequence without D1D2 domain-VWF sequence 2). These mutations are predicted to prevent dimerization of VWF D'D3 domain.

(c) Cloning of pSYN-VWF-025 and pSYN-VWF-029

[0336] pSYN-VWF-025 contains wild type D1D2D'D3 sequences of full-length VWF in pLIVE vector, and pSYN-VWF-029 contains D1D2D'D3 sequence with C336A and C379A mutation. For cloning pSYN-VWF-025, the following primer combination was used:

ESC 89-fwd with NheI site= CTCACTATAGGGAGACCCAAGCTGGCTAGCCG  
(SEQ ID NO: 92)

ESC 91-rev with SalI=

CTGGATCCCGGGAGTCGACTCGTCAGTGGTGATGGTGATGATG (SEQ ID NO: 93)

[0337] A 50 µl PCR reaction was carried out with ESC 89/ESC91 primer combinations and either pSYN-VWF 010 ( for pSYN-VWF-025) or pSYN-VWF 013 ( for pSYN-VWF-029) plasmid as the template using the 3 step PCR amplification cycle: 94 °C 2minutes; 21 cycles of (96 °C -30 seconds, 55 °C-30 second, 68 °C-4 minutes). The expected sized band (~3800bp) was gel purified with a Gel Extraction kit (Qiagen, Valencia, Calif.) and cloned into the NheI and SalI restriction sites of pLIVE-Mirus vector (Invitrogen, Carlsbad, Calif.) to generate pSYN-VWF 025 and 029.

(d) Cloning pSYN-VWF-031

[0338] pSYN-VWF-031 is a D1D2D'D3(C336A/C379A) -Fc construct which has a 48 amino acid long thrombin cleavable linker (8x GGGGS (SEQ ID NO 94) + thrombin site) in between the VWF D1D2D'D3(C336A/C379A) and the Fc sequences. To make this construct, VWF-Fc region was amplified from construct pSYN-FVIII-064 (refer FVIII-VWF construct below). pSYN-FVIII-VWF was digested with XbaI and NheI. Resulting insert region of 4165bp, containing the VWF fragment and Fc region was used as a template for amplifying the VWF and Fc region by primer combinations LW 22/LW23.

LW 22-FWD-VWF-D'D3 with FVIII signal sequence and BsiW1 site

GCGCCGGCCGTACGATGCAAATAGAGCTCTCCACCTGCTTCTTTCTGTGCCTTTT  
GCGATTCTGCTTTAGCCTATCCTGTGCGGCCCCCATG (SEQ ID NO: 95)

LW 23-Rev- Fc with stop codon and NotI site

TCATCAATGTATCTTATCATGTCTGAATTCGCGGCCGCTCATTTACC (SEQ ID NO:96)

[0339] The PCR product obtained from LW22/LW23 amplification (~2300bp) was cloned in BsiW1/NotI digested pSYN-VWF-002 to obtain pSYN-VWF-014 intermediate. pSYN-VWF-014 contains FVIII signal peptide-D'D3-20 amino acid thrombin cleavable linker followed by the Fc region.

[0340] To generate the D1D2D'D3-Fc construct, the D1D2D'D3 region was amplified from pSYN-VWF-013 using primer combination LW24/LW27 by standard PCR method.

LW24- Fwd- VWF D1D2D'D3 cloning oligo with BsiW1 site



GCGCCGGCCGTACGATGATTCTGCCAGATTTGCCGGGGTG (SEQ ID NO:97)

LW27-Rev-VWF D'D3 oligo with EcoRV

CCACCGCCAGATATCGGCTCCTGGCAGGCTTCACAGGTGAG (SEQ ID NO:98)

[0341] The PCR product obtained from LW22/LW23 amplification (~3750bp) was cloned in BsiW1/EcoRV digested pSYN-VWF-014 to obtain pSYN-VWF-015 intermediate. The linker length between the VWF fragment and Fc region was changed to obtain pSYN-VWF-031.

VWF-D1D2D'D3 protein sequence 1 (SEQ ID NO: 99)

```

1    MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS
DFVNTFDGSM
51   YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE
FFDIHLFVNG
101  TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI
DGSGNFQVLL
151  SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS
WALSSGEQWC
201  ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL
VDPEPFVALC
251  EKTLCCEAGG LECACPALLE YARTCAQEGM VLYGWDHSA
CSPVCPAGME
301  YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG
LCVESTCPC
351  VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV
TGQSHFKSFD
401  NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC
TRSVTVRLPG
451  LHNSLVKLKH GAGVAMDQD IQLPLLKGD LRIQHTVTASV
RLSYGEDLQM
501  DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG
LAEPRVEDFG
551  NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP
TFEACHRAVS
601  PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV
AWREPGRCEL
651  NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP
PGLYMDERGD
701  CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM
SGVPGSLLPD
751  AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK
TCQNYDLECM
801  SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE
TVKIGCNTCV
851  CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ
YVLVQDYCGS
901  NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE
VNVKRPMKDE

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951  THFEVVESGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE
    KVCGLCGNFD
1001  GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD
    SSPATCHNNI
1051  MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS
    CESIGDCACF
1101  CDTIAAYAHV CAQHKGKVVW RTATLCPQSC EERNLRENGY
    ECEWRYNSCA
1151  PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC
    VDPEDCPVCE
1201  VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP*
    VWF-D'D3 protein sequence 2 (SEQ ID NO: 100)
    1  SLSCRPPMVK LVCPADNLRA EGLECTKTCQ NYDLECMSMG
    CVSGCLCPPG
    51  MVRHENRCVA LERCPCFHQG KEYAPGETVK IGCNTCVCRD
    RKWNCTDHVC
    101  DATCSTIGMA HYLTFDGLKY LFPGECQYVL VQDYCGSNPG
    TFRILVGNGK
    151  CSHPSVKCKK RVTILVEGGE IELFDGEVNV KRPMKDETHF
    EVVESGRYII
    201  LLLGKALSIV WDRHLSISVV LKQTYQEKVC GLCGNFDGIQ
    NNDLTSSNLQ
    251  VEEDPVDFGN SWKVSSQCAD TRKVPLDSSP ATCHNNIMKQ
    TMVDSSCRIL
    301  TSDVVFQDCNK LVDPEPYLDV CIYDTCSCEs IGDCACFCDT
    IAAYAHVCAQ
    351  HGKVVWTRTA TLCPSQCEER NLRENGYECE WRYNSCAPAC
    QVTCQHPEPL
    401  ACPVQCVEGC HAHCPPGKIL DELLQTCVDP EDCPVCEVAG
    RRFASGKKVT
    451  LNPSDPEHCQ ICHCDVVNLT CEACQEP

```

#### Example 2: Effects of D'D3 and XTEN fusion on FVIII half-life extension

**[0342]** To evaluate D'D3 FVIII half-life extension potential on rFVIII-XTEN fusion protein, a VWF D'D3 dimer was introduced into FVIII-VWF DKO mice by hydrodynamic injection of its corresponding DNA construct VWF-025 (Example 1). After D'D3 has reached the steady state expression (day5 post injection), a single dose of rFVIII-XTEN was administered by IV injection at 200 IU/kg dose. Blood samples were collected up to 120hrs post rFVIII-XTEN dosing. Plasma FVIII activity was analyzed by a FVIII chromogenic assay. The D'D3 expression level was measured by VWF ELISA, and rFVIII<sup>h</sup> PK profile was analyzed using WinNonlin program.

**[0343]** The study results were shown in Figure 2, and the PK parameter of rFVIII-XTEN with/without D'D3 in circulation was listed in Table 16. The D'D3 dimer further extended rFVIII-XTEN  $t_{1/2}$  from 3.4hr to 17.8hr, a 5 fold increase. In

addition to half-life, 5 fold of increase on MRT, 3.6 fold increases on AUC, 3.8 fold decreases on clearance were also observed.

[0344] We have observed a synergistic effect of D'D3 fragment and XTEN technology, a serial of FVIII/VWF/XTEN constructs will be evaluated for their FVIII half-life extension potential in Hemophilic animals.

**TABLE 16: rFVIII-XTEN PK parameter with/without D'D3 in blood circulation**

| Treatment          | 5min Recovery (%) | $t_{1/2}$ (hr) | MRT (hr) | Cl (mL/hr/kg) | V <sub>ss</sub> (mL/kg) | AUC <sub>D</sub> (hr*kg*mIU/mL/mIU) |
|--------------------|-------------------|----------------|----------|---------------|-------------------------|-------------------------------------|
| rFVIIIXTEN VWF-025 | 80                | 17.8           | 19.3     | 3.5           | 67.4                    | 0.29                                |
| rFVIIIXTEN         | 74                | 3.4            | 3.8      | 13.1          | 63.68                   | 0.08                                |
| Improvement fold   | 1.1               | 5.2            | 5.1      | 3.8           | 0.9                     | 3.6                                 |

#### Protein purification of FVIII-XTEN

[0345] An AE288 XTEN was inserted at the C-terminus of BDD-FVIII for this study. To purify this protein, a tangential flow filtration (TFF) step was used first to buffer exchange the conditioned media. Products in the filtrate were then captured using a strong anion exchange chromatography, and then further purified using affinity chromatography. Purity of the molecule was acceptable by HPLC-SEC and was further confirmed by western blotting. The specific activity of the molecule was comparable to B-domain deleted FVIII, as measured by aPTT assay and ELISA.

#### FVIII chromogenic assay

[0346] The FVIII activity was measured using the COATEST SP FVIII kit from DiaPharma (lot# N089019) and all incubations were performed on a 37°C plate heater with shaking.

[0347] The range of rFVIII standard was from 100 mIU/mL to 0.78 mIU/mL. A pooled normal human plasma assay control and plasma samples (diluted with 1X Coatest buffer) were added into Immulon 2HB 96-well plates in duplicate (25  $\mu$ L/well). Freshly prepared IXa/FX/Phospholipid mix (50  $\mu$ L), 25  $\mu$ L of 25mM CaCl<sub>2</sub>, and 50  $\mu$ L of FXa substrate were added sequentially into each well with 5

minutes incubation between each addition. After incubating with the substrate, 25  $\mu$ L of 20% Acetic Acid was added to terminate the color reaction, and the absorbance of OD405 was measured with a SpectraMAX plus (Molecular Devices) instrument. Data were analyzed with SoftMax Pro software (version 5.2). The Lowest Level of Quantification (LLOQ) is 7.8 mIU/mL.

VWF ELISA:

- [0348] Goat anti-human VWF antibody (Affinity purified, affinity biological, GAVWF-AP) was used as the capture antibody at 0.5ug/well and VWF-EIA-D (Affinity Biologicals, VWF-EIA-D, 1:100 dilution) was used as the detecting antibody for the VWF ELISA. ELISA assay was performed following the standard ELISA procedure, TMB was used as the HRP substrate, PBST/1.5% BSA/0.5M NaCl buffer was used as blocking and binding buffer. The assay standard range is 100ng to 0.78ng, and assay's lowest limit of quantification (LLOQ) is 7.8ng/mL.

Example 3: Plasmid construction of XTEN containing FVIII/VWF constructs

(a) Cloning of pSYN-FVIII-161 (Figure 3)

- [0349] The FVIII-161 plasmid comprises a single chain Fc (scFc) scaffold with enzyme cleavage sites which are processed during synthesis in a cell. The construct has a FVIII binding domain of full-length VWF ( D'D3).
- [0350] Plasmid (pSYN-FVIII-161) was designed for the expression FVIII-Fc and VWF-Fc heterodimer, where the D'D3 domains to bind FVIII and prevents FVIII interaction with phospholipids and activated protein C. Protein from pSYN-FVIII-161 is expressed in the cell as a single polypeptide where the C-terminus of the FVIII-Fc subunit is linked to the N-terminus of the VWF D'D3-Fc subunit by a 6x (GGGGS) polypeptide linker (SEQ ID NO: 64). In addition, RRRRS (SEQ ID NO: 11) and RKRRKR (SEQ ID NO: 10) sequences were inserted at the 5' and 3' end of the polypeptide linker, respectively, for intracellular cleavage by proprotein convertases following the last Arg at each sequence. Hence, the cells can express a double chain FVIII-Fc/D'D3-Fc heterodimer where the FVIII-Fc chain has a RRRRS sequence (SEQ ID NO: 11) at the C-terminus, but the remainder of the linker sequence has been removed. An AE288 XTEN fragment immediately followed by IS{5X(GGGGS)}LVPRGSGG (SEQ ID NO: 122) polypeptide (contains thrombin cleavage site) is introduced in between the VWF domains and

the Fc region to facilitate release of the VWF fragment from FVIII once the FVIII-VWF hetero-dimeric protein is activated by thrombin allowing interaction of FVIII with other clotting factors.

[0351] pSYN-FVIII-161 (SEQ ID NO: 101).protein sequence (FVIII sequence amino acid position 1-1457; underlined region represents Fc region; curly underline represents cleavable linker in between first Fc and VWF fragment; double underlined region represents VWF fragment; bold region represents cleavable linker in between VWF fragment and Fc.

```

1  MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL
   GELPVDARFP
51  PRVPKSFPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL
   LGPTIQAEVY
101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR
   EKEDDKVFPG
151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG
   LIGALLVCRE
201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD
   AASARAWPKM
251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL
   EGHTFLVRNH
301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME
   AYVKVDSCPE
351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI
   RSVAKKHPKT
401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR
   KYKKVRFMAY
451 TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR
   PYNIYPHGIT
501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP
   TKSDPRCLTR
551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR
   NVILFSVFDE
601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV
   FDSLQLSVCL
651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF
   PFSGETVFMS
701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED
   SYEDISAYLL
751 SKNNAIEPRS FSQNPPVLKR HQREITRTTL QSDQEEIDYD
   DTISVEMKKE
801 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP
   HVLNRNAQSG
851 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA
   EVEDNIMVTF
901 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY
   FWKVQHMAP

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951 TKDEFDCKAW AYFSDVDLEK DVHSGGLIGPL LVCHTNTLNP  
 AHGRQVTVQE  
 1001 FALFFTIFDE TKSIFYFTENM ERNCRAPCNI QMEDPTFKEN  
 YRFHAINGYI  
 1051 MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV  
 RKKEEYKMAL  
 1101 YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL  
 VYSNKCQTPL  
 1151 GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK  
 EPFSWIKVDL  
 1201 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY  
 RGNSTGTLMV  
 1251 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME  
 LMGCDLNSCS  
 1301 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR  
 SNAWRPQVNN  
 1351 PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISS  
 QDGHQWTLFF  
 1401 QNGKVVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH  
 QIALRMEVLG  
 1451 CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI  
SRTPEVTCVV  
 1501 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV  
SVLTVLHQDW  
 1551 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP  
SRDELTKNQV  
 1601 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS  
FFLYSKLTVD  
 1651 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGKRRRRSG  
GGGSGGGGSG  
 1701 GGGSGGGGSG GGGSGGGGSR KRRKRSLSCR PPMVKLVCPA  
DNLRAEGLEC  
 1751 TKTCQNYDLE CMSMGCVSGC LCPPGMVRHE NRCVALERCP  
CFHQGKEYAP  
 1801 GETVKIGCNT CVCRRDRKWC TDHVCDATCS TIGMAHYLTF  
DGLKYLFPGE  
 1851 CQYVLVQDYC GSNPGTFRIL VGNKGCSHPS VKCKKRVTL  
VEGGEIELFD  
 1901 GEVNVKRPMK DETHFEVVES GRYIILLGK ALSVVWDRHL  
SISVVLKQTY  
 1951 QEKVCGLCGN FDGIQNNDLT SSNLQVEEDP VDFGNSWKVS  
SQCADTRKVP  
 2001 LDSSPATCHN NIMKQTMVDS SCRILTSDVF QDCNKLVDPE  
PYLDVCIYDT  
 2051 CSCSIGDCA AFCDTIAAYA HVCAQHGVV TWRTATLCPQ  
SCEERNLREN  
 2101 GYEAEWRYNS CAPACQVTCQ HPEPLACPVO CVEGCHAHCP  
PGKILDELLQ  
 2151 TCVDPEDCPV CEVAGRRFAS GKKVTLNPSD PEHCQICHCD  
VVNLTCEACQ

|                   |                                                    |
|-------------------|----------------------------------------------------|
| 2201              | <u>EPISGTSESA</u> TPESGPGSEP ATSGSETPGT SESATPESGP |
| GSEPATSGSE        |                                                    |
| 2251              | TPGTSESATP ESGPGTSTEP SEGSAPGSPA GSPTSTEEGT        |
| SESATPESGP        |                                                    |
| 2301              | GSEPATSGSE TPGTSESATP ESGPGSPAGS PTSTEEGSPA        |
| GSPTSTEEGT        |                                                    |
| 2351              | STEPSEGSAP GTSESATPES GPGTSESATP ESGPGTSESA        |
| TPESGPGSEP        |                                                    |
| 2401              | ATSGSETPGS EPATSGSETP GSPAGSPTST EEGTSTEPSE        |
| GSAPGTSTEP        |                                                    |
| 2451              | SEGSAPGSEP ATSGSETPGT SESATPESGP GTSTEPSEGS        |
| APDSGGGGSG        |                                                    |
| 2501              | GGGSGGGGSG GGGSGGGGSL VPRGSGGDKT <u>HTCPPCPAPE</u> |
| <u>LLGGPSVFLF</u> |                                                    |
| 2551              | <u>PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE</u> |
| <u>VHNAKTKPRE</u> |                                                    |
| 2601              | <u>EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE</u> |
| <u>KTISKAKGQP</u> |                                                    |
| 2651              | <u>REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES</u> |
| <u>NGQPENNYKT</u> |                                                    |
| 2701              | <u>TPPVLDSGDS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH</u> |
| <u>NHYTQKSLSL</u> |                                                    |
| 2751              | <u>SPGK</u>                                        |

(b) Cloning of pSYN-FVIII-168, 175, 172 and 174 (Figure 4A-4D)

[0352] pSYN-FVIII-168, 172, 174 and 175 are derivatives of pSYN-FVIII-161. R1645A/R1648A mutations were introduced into pSYN-FVIII-161 to form pSYN-FVIII-168, which produces a SC-FVIII isoform, and an AE288 XTEN was directly fused into the C-terminus of FVIII-HC for further half-life extension. To construct pSYN-FVIII-175, the D'D3 codon sequence was removed from pSYN-FVIII-168 for evaluation of the effect of Fc and XTEN technology on FVIII half-life extension.

[0353] To construct pSYN-FVIII-172, the AE288 XTEN fragment was directly fused into the C-terminus of FVIII-HC for further half-life extension, and the D'D3 codon sequence was removed from pSYN-FVIII-172 to form pSYN-FVIII-174 for evaluation of the effect of Fc and XTEN technology on FVIII half-life extension.

(c) Cloning of pSYN-FVIII-170 (Figure 4E)

[0354] pSYN-FVIII-170 was constructed to evaluate the effect of XTEN and D'D3 fragment on FVIII half-life extension. The codon sequence VWF-D1D2D'D3 fragment and BDD-FVIII were introduced into the 5' and 3' end of expression cassette, an AE288 XTEN codon sequence which followed by a 35 aa

thrombin cleavable linker was used to connect the VWF and FVIII molecule. After intra cellular processing, the secreted protein comprises a polypeptide contains the D'D3 fragment of mature VWF molecule which is linked to the N-terminus of mature BDD-FVIII by an AE288 XTEN/35 aa thrombin cleavable linker.

**pSYN-FVIII-170 protein sequence (SEQ ID NO: 102)**

```

1  SLSCRPPMVK LVCPADNLRA EGLECTKTCQ NYDLECMSMG
   CVSGCLCPPG
51  MVRHENRCVA LERCPCFHQG KEYAPGETVK IGCNTCVCRD
   RKWNCTDHVC
101 DATCSTIGMA HYLTFDGLKY LFPGECQYVL VQDYCGSNPG
   TFRILVGNKG
151 CSHPSVKCKK RVTILVEGGE IELFDGEVNV KRPMKDETHF
   EVVESGRYII
201 LLLGKALSVV WDRHLSISVV LKQTYQEKVC GLCGNFDGIQ
   NNDLTSSNLQ
251 VEEDPVDFGN SWKVSSQCAD TRKVPLDSSP ATCHNNIMKQ
   TMVDSSCRIL
301 TSDVFQDCNK LVDPEPYLDV CIYDTCSCES IGDCAAFCDT
   IAAYAHVCAQ
351 HGKVVTWRTA TLCPQSCEER NLRENGYAE WRYNSCAPAC
   QVTCQHPEPL
401 ACPVQCVEGC HAHCPPGKIL DELLQTCVDP EDCPVCEVAG
   RRFASGKKVT
451 LNPSDPEHCQ ICHCDVNLIT CEACQEPISG TSESATPESG
   PGSEPATSGS
501 ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
   TSTEPSEGS
551 PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS
   ESATPESGPG
601 SPAGSPTSTE EGSPAGSPTS TEEGTSTEPS EGSAPGTSES
   ATPESGPGTS
651 ESATPESGPG TSESATPESG PGSEPATSGS ETPGSEPATS
   GSETPGSPAG
701 SPTSTEEGTS TEPSEGSAPG TSTEPSEGS PGSEPATSGS
   ETPGTSESAT
751 PESGPGTSTE PSEGSAPDSG GGSGGGGGSG GGSGGGGGSG
   GGSLVPRGS
801 GGASATRRYY LGAVELSWDY MQSDLGELPV DARFPPrVPK
   SFPFNTSVVY
851 KKTLFVEFTD HLFNIAKPRP PWMGLLGPTI QAEVYDTVVI
   TLKNMASHPV
901 SLHAVGVSYW KASEGAEYDD QTSQREKEDD KVFPGGSHTY
   VWQVLKENG
951 MASDPLCLTY SYLSHVDLVK DLNSGLIGAL LVCREGSLAK
   EKTQTLHKFI

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1001 LLFAVFDEGK SWHSETKNSL MQDRDAASAR AWPKMHTVNG  
YVNRSLPGLI  
1051 GCHRKSVYWH VIGMGTTPEV HSIFLEGHTF LVRNHRQASL  
EISPITFLTA  
1101 QTLLMDLGQF LLFCHISSHQ HDGMEAYVKV DSCPEEPQLR  
MKNNEEAEDY  
1151 DDDLT DSEMD VVRFDDDN SP SFIQIRSVAK KHPKTWVHYI  
AAEEEDWDYA  
1201 PLVLAPDDRS YKSQYLNNGP QRIGRKYKKV RFMAYTDETF  
KTREAIQHE  
1251 GILGPLLYGE VGD TLLIIFK NQASRPYNIY PHGITDVRPL  
YSRRLPKGVK  
1301 HLKDFPILPG EIFKYKWTVT VEDGPTKSDP RCLTRYYSF  
VNMERDLASG  
1351 LIGPLLCYK ESVDQRGNOI MSDKRN VILF SVFDENRSWY  
LTENIQRFLP  
1401 NPAGVQLEDP EFQASNIMHS INGYVFDSLQ LSVCLHEVAY  
WYILSIGAQT  
1451 DFLSVFFSGY TFKHKMVYED TLTLFPFSGE TVFMSMENPG  
LWILGCHNSD  
1501 FRNRGMTALL KVSSCDKNTG DYYEDSYEDI SAYLLSKNNA  
IEPRSFSONP  
1551 PVLKRHQREI TRTTLQSDQE EIDYDDTISV EMKKEDFDIY  
DEDENQSPRS  
1601 FQKKTRHYFI AAVERLWDYG MSSSPHVLRN RAQSGSVPQF  
KKVVFQEFTD  
1651 GSFTQPLYRG ELNEHLGLLG PYIRAEVEDN IMVTFRNQAS  
RPYSFYSSLI  
1701 SYEEDQRQGA EPRKNFVKPN ETKTYFWKVQ HHMAPTKDEF  
DCKAWAYFSD  
1751 VDLEKDVHSG LIGPLLVCHT NTLNPAHGRQ VTVQEFALFF  
TIFDETKSWY  
1801 FTENMERNCR APCNIQMEDP TFKENYRFHA INGYIMDTLP  
GLVMAQDQRI  
1851 RWYLLSMGSN ENIHSIHFG HVFTVRKKEE YKMALYNLYP  
GVFETVEMLP  
1901 SKAGIWRVEC LIGEHLHAGM STLFLVYSNK CQTPLGMASG  
HIRDFQITAS  
1951 GOYGQWAPKL ARLHYSGSIN AWSTKEPFSW IKVDLLAPMI  
IHGIKTQGAR  
2001 QKFSSLYISQ FIIMYSLDGK KWQTYRGNST GTLMVFFGNV  
DSSGIKHNIF  
2051 NPPIIARYIR LHPTHYSIRS TLRMELMGCD LNCSMPLGM  
ESKAISDAQI  
2101 TASSYFTNMF ATWSPSKARL HLQGRSNAWR PQVNNPKEWL  
QVDFQKTMKV  
2151 TGVTTQGVKS LLTSMYVKEF LISSSQDGHQ WTLFFQNGKV  
KVFQGNQDSF  
2201 TPVNSLDPP LLTRYLRIHP QSWVHQIALR MEVLGCEAQD LY

Example 4: Hydrodynamic injection of XTEN containing FVIII/VWF constructs in FVIII and VWF deficient mice

**[0355]** The XTEN containing DNA constructs in Figures 3 and 4 have combined 2-3 half-life extension elements together. To evaluate their FVIII half-life extension potential, a selective group of DNA constructs in figure 3 and figure 4 were introduced into FVIII/VWF double knockout (DKO) mice by Hydrodynamic injection (HDI) at 100ug/mouse dose. Blood samples were then collected by retro orbital blood collection at 24hr post HDI. The post HDI plasma FVIII activity was analyzed by FVIII chromogenic assay, and results were listed in Table 17 and Figure 5. Compared to wild type BDD-FVIII, all XTEN containing DNA constructs yield significantly higher FVIII plasma activity at 24hr post HDI, indicating the corresponding molecules had significant longer circulating protein half-life than BDD-FVIII. The application of the combination of those half-life extending elements was further evaluated in Hemophilic animals.

**TABLE 17: FVIII plasma activity 24hr post HDI in FVIII/VWF DKO mice**

| DNA Construct          | BDD-FVIII | FVIII-161 | FVIII-168 | FVIII-172 | BDD-FVIII | FVIII-170 |
|------------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| DNA Dose (µg/mouse)    | 100       | 100       | 100       | 100       | 50        | 50        |
| FVIII Activity (mU/mL) | 219±72    | 2446±1012 | 2209±609  | 1671±223  | 197±21    | 399±30    |

Hydrodynamic injection:

**[0356]** Hydrodynamic Injection is an efficient and safe non-viral gene delivery method to the liver in small animals, such as mice and rats. It was originally described as a rapid injection of a naked plasmid DNA/saline solution free of endotoxin at a tenth volume of the animal's body weight in about 5-7 seconds. The naked plasmid DNA contains the gene of interest and the liver produced in a tenth volume of the animal's body weight. The targeted protein is produced in the liver from the injected DNA and can be detected within 24 hours post-injection. Plasma samples were then collected to study the therapeutic property of the expressed protein.

**[0357]** For all the hydrodynamic injections that were performed herein, 2 ml of plasmid DNA in 0.9% sterile saline solution was delivered via intravenous tail vein injection within about 4-7 seconds to mice weighing 20-35 grams. The mice

were closely monitored for the first couple of hours until the normal activity resumed. After the blood samples were collected via retro orbital blood collection, plasma samples were then obtained and stored at -80 °C for further analysis.

Example 5: Plasmid construction of co-transfection system for FVIII-Fc-VWF Heterodimer contain XTEN insertions (Figure 6)

[0358] To increase the protein production yield, two co-transfection systems were generated for protein production, which contains three DNA constructs. The first DNA construct encoded a FVIII-Fc fusion protein in which a AE288 XTEN fragment was directly fuse to the C-terminus of the FVIII heavy chain and followed by either a wild type FVIII light chain fragment (pSYN-FVIII-173, Figure 6B) or a FVIII light chain fragment with R1645A/R1648A mutations (pSYN-FVIII-169, Figure 6A), the FVIII light chain was then directly fused to a single Fc fragment. The second DNA construct is pSYN-VWF-031 which encoding a D'D3-Fc fusion protein (Example 1). HEK293F cells were transfected with the two plasmid along with a third plasmid (PC5) at 80:15:5 ratio. The synthesized proteins were secreted as FVIII (XTEN) Fc/D'D3Fc heterodimer and D'D3Fc dimer and the FVIII (XTEN) Fc/D'D3Fc heterodimer was separated from the D'D3Fc dimer by protein purification.

**pSYN-FVIII-169 mature Protein sequence (SEQ ID NO: 103):**

```

1  ATRRYYLGA V ELSWDYMQSD LGELPVDARF PPRVPKSFPF
   NTSVVYKCTL
51  FVEFTDHLFN IAKPRPPWMG LLGPTIQAEV YDTVVITLKN
   MASHPVSLHA
101 VGVSYWKASE GA EYDDQTSQ REKEDDKVFP GGSHTYVWQV
   LKENGPMASD
151 PLCLTYSYLS HVDLVKDLNS GLIGALLVCR EGSLAKEKTQ
   TLHKFILLFA
201 VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR
   SLPGLIGCHR
251 KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP
   ITFLTAQTLL
301 MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKNN
   EEAEDYDDDL
351 TDSEMDVVR F DDDNSPSFIQ IRSVAKKHPK TWVHYIAAEE
   EDWDYAPLVL
401 APDDRSYKSQ YLNNGPQRIG RKYKKVRFMA YTDETFKTRE
   AIQHESGILG
451 PLLYGEVGDT LLIIFKNQAS RPYNIYPHGI TDVRPLYSRR
   LPKGVKHLKD

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501 FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYYSSSFVNME  
RDLASGLIGP  
551 LLICYKESVD QRGNQIMSDK RNVILFSVFD ENRSWYL TEN  
IQRF L PNPAG  
601 VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL  
SIGAQTDFLS  
651 VFFSGYT FKH KMVYEDTLTL FPFSGETVFM SMENPGLWIL  
GCHNSDFRNR  
701 GMTALLKVSS CDKNTGDYIE DSYEDISAYL LSKNNAIEPR  
SFSQNGAPGT  
751 SESATPESGP GSEPATSGSE TPGTSESATP ESGPGSEPAT  
SGSETPGTSE  
801 SATPESGPGT STEPSEGSAP GSPAGSPTST EEGTSESATP  
ESGPGSEPAT  
851 SGSETPGTSE SATPESGPGS PAGSPTSTEE GSPAGSPTST  
EEGTSTEPSE  
901 GSAPGTSESA TPESGPGTSE SATPESGPGT SESATPESGP  
GSEPATSGSE  
951 TPGSEPATSG SETPGSPAGS PTSTEEGTST EPSEGSAPGT  
STEPSEGSAP  
1001 GSEPATSGSE TPGTSESATP ESGPGTSTEP SEGSAPASSP  
PVLKRHQAEI  
1051 TRTTLQSDQE EIDYDDTISV EMKKEDFDIY DEDENQSPRS  
FQKKTRHYFI  
1101 AAVERLWDYG MSSSPHVL RN RAQSGSVPQF KKVVFQEFTD  
GSFTQPLYRG  
1151 ELNEHLG LLG PYIRAEVEDN IMVTFRNQAS RPYSFYSSLI  
SYEEDQRQGA  
1201 EPRKNFVKPN ETKTYFWKVQ HHMAPTKDEF DCKAWAYFSD  
VDLEKDVHSG  
1251 LIGPLL VCHT NTLNPAHGRQ VTVQEFALFF TIFDETKSWY  
FTENMERNCR  
1301 APCNIQMEDP TFKENYRFHA INGYIMDTLP GLVMAQDQRI  
RWYLLSMGSN  
1351 ENIH SIHFSG HVFTVRKKEE YKMALYNLYP GVFETVEMLP  
SKAGIWRVEC  
1401 LIG EHLHAGM STLFLVYSNK CQTPLGMASG HIRDFQITAS  
GOYGQWAPKL  
1451 ARLHYS GSIN AWSTKEPFSW IKVDLLAPMI IHGIKTQGAR  
QKFSSLYISQ  
1501 FIIMYS LDGK KWQTYRGNST GTLMVFFGNV DSSGIKHNIF  
NPPIIARYIR  
1551 LHPTHYSIRS TLRMELMGCD LNSCSMPLGM ESKAISDAQI  
TASSYFTNMF  
1601 ATWSPSKARL HLQGRSNAWR PQVNNPKEWL QVDFQKTMKV  
TGVTTQGVKS  
1651 LLTSMYVKEF LISSSQDGHQ WTLFFQNGKV KVFQGNQDSF  
TPVVNSLDPP  
1701 LLTRYLRIHP QSWVHQIALR MEVLGCEAQD LYDKTHTCPP  
CPAPELLGGP

1751 SVFLFPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY  
 VDGVEVHNAK  
 1801 TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL  
 PAPIEKTISK  
 1851 AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA  
 VEWESNGQPE  
 1901 NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM  
 HEALHNHYTQ  
 1951 KSLSLSPGK

**pSYN-FVIII-173 mature Protein sequencing (SEQ ID NO: 104):**

1 ATRRYYLGA V ELSWDYMQSD LGELPVDARF PPRVPKSFPF  
 NTSVVYKCTL  
 51 FVEFTDHLFN IAKPRPPWMG LLGPTIQAEV YDTVVITLKN  
 MASHPVSLHA  
 101 VGVSYWKASE GAEYDDQTSQ REKEDDKVFP GGSHTYVWQV  
 LKENGPMASD  
 151 PLCLTYSYLS HVDLVKDLNS GLIGALLVCR EGSLAKEKTQ  
 TLHKFILLFA  
 201 VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR  
 SLPGLIGCHR  
 251 KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP  
 ITFLTAQTLL  
 301 MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKNN  
 EEAEDYDDDL  
 351 TDSEMDVVR F DDNSPSFIQ IRSVAKKHPK TWVHYIAAEE  
 EDWDYAPLVL  
 401 APDDRSYKSQ YLNNGPQRIG RKYKKVRFMA YTDETFKTRE  
 AIQHESGILG  
 451 PLYGEVGD T LLIIFKNQAS RPYNIYPHGI TDVRPLYSR  
 LPKGVKHLKD  
 501 FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYSSSFVNME  
 RDLASGLIGP  
 551 LLICYKESVD QRGNQIMSDK RNVILFSVFD ENRSWYL T  
 IQRFLPNPAG  
 601 VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL  
 SIGAQTDFLS  
 651 VFFSGYT FKH KMYIEDTLTL FPFSGETVFM SMENPGLWIL  
 GCHNSDFRNR  
 701 GMTALLKVSS CDKNTGDYEE DSYEDISAYL LSKNNAIEPR  
 SFSQNGAPGT  
 751 SESATPESGP GSEPATSGSE TPGTSESATP ESGPGSEPAT  
 SGSETPGTSE  
 801 SATPESGPGT STEPSEGSAP GSPAGSPTST EEGTSESATP  
 ESGPGSEPAT  
 851 SGSETPGTSE SATPESGPGS PAGSPTSTEE GSPAGSPTST  
 EEGTSTEPSE  
 901 GSAPGTSESA TPESGPGTSE SATPESGPGT SESATPESGP  
 GSEPATSGSE  
 951 TPGSEPATSG SETPGSPAGS PTSTEEGTST EPSEGSAPGT  
 STEPSEGSAP

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1001  GSEPATSGSE TPGTSESATP ESGPGTSTEP SEGSAPASSP
      PVLKRHQREI
1051  TRTTLQSDQE EIDYDDTISV EMKKEDFDIY DEDENQSPRS
      FQKKTRHYFI
1101  AAVERLWDYG MSSSPHVLRN RAQSGSVPQF KKVVFQEFTD
      GSFTQPLYRG
1151  ELNEHLGLLG PYIRAEVEDN IMVTFRNQAS RPYSFYSSLI
      SYEEDQRQGA
1201  EPRKNFVKPN ETKTYFWKVQ HHMAPTKDEF DCKAWAYFSD
      VDLEKDVHSG
1251  LIGPLLVCHT NTLNPAHGRQ VTVQEFALFF TIFDETKSWY
      FTENMERNCR
1301  APCNIQMEDP TFKENYRFHA INGYIMDTLP GLVMAQDQRI
      RWYLLSMGSN
1351  ENIHSIHFSG HVFTVRKKEE YKMALYNLYP GVFETVEMLP
      SKAGIWRVEC
1401  LIGEHLHAGM STLFLVYSNK CQTPLGMASG HIRDFQITAS
      GQYGQWAPKL
1451  ARLHYSGSIN AWSTKEPFSW IKVDLLAPMI IHGIKTQGAR
      QKFSSLYISQ
1501  FIIMYSLDGK KWQTYRGNST GTLMVFFGNV DSSGIKHNIF
      NPPIIARYIR
1551  LHPHYSIRS TLRMELMGCD LNSCSMPLGM ESKAISDAQI
      TASSYFTNMF
1601  ATWSPSKARL HLQGRSNAWR PQVNNPKEWL QVDFQKTMKV
      TGVTTQGVKS
1651  LLTSMYVKEF LISSSQDGHQ WTLFFQNGKV KVFQGNQDSF
      TPVVNSLDP
1701  LLTRYLRIHP QSWVHQIALR MEVLGCEAQD LYDKTHTCPP
      CPAPELLGGP
1751  SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY
      VDGVEVHNAK
1801  TKPREEQYNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL
      PAPIEKTISK
1851  AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA
      VEWESNGQPE
1901  NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
      HEALHNHYTQ
1951  KSLSLSPGK

```

**Example 6. Protein purification for FVIII-169/VWF-031 and FVIII-173/VWF-031**

**[0359]** A tangential flow filtration (TFF) step was used to buffer exchange the clarified conditioned media. The FVIII-169/VWF-031 or FVIII-173/VWF-031 heterodimer was then purified using a two-step chromatography process. A weak anion exchange resin was used, followed by affinity chromatography. The final purified product had acceptable purity by SEC-HPLC. The specific activity was

compatible to B-domain deleted FVIII, as measured by FVIII chromogenic assay and A280 concentration. Purity and the presence of each moiety of this molecule were confirmed by SDS-PAGE and western blotting.

Example 7. Evaluation the VWF binding ability of FVIII-169/VWF-031 by Octet assay

[0360] The VWF binding ability of FVIII-169/VWF-031 was obtained by Bio-Layer Interferometry (BLI) based measurements (Octet assay) at 25 °C with a ForteBio Octet 384 instrument, using Tris binding buffer (50 mM Tris, pH 7.2, 150 mM NaCl, 5 mM CaCl<sub>2</sub>). The Octet assay for determining FVIII binding was based on the hydrophobic immobilization of Human von Willebrand Factor (Haematologic Technologies Catalog No. HCVWF-0191) onto the APS Biosensor, then followed by the binding of 1.0% Bovine Serum Albumin (Jackson ImmunoResearch Catalog No. 001-000-161). Briefly, hvWF (20 µg/mL) was diluted in Tris buffer and loaded across APS Biosensors for 600 sec, yielding approximately 3.0 – 3.5 nm binding on the reaction probes. Control APS probes were loaded with 1.0% BSA in the absence of hvWF for reference subtraction. After loading, all probes were incubated in Tris buffer for 300 sec to establish a new baseline. Subsequently, biosensor probes were incubated in solutions of FVIII-XTEN 169 or FVIII-Fc Drug Substance (0, 0.6, 2, 6, 20, 60, 200, 600 IU/mL) for 5 min at room temperature, followed by a 5 min dissociation step. Using the Octet data analysis software, the binding response (nm) was derived from the subtracted data (Reaction probe minus Reference probe). No binding to immobilized VWF was detected for FVIII-169/VWF-031 (Figure 7), indicating a complete shielding of FVIII from full length VWF molecule by the D'D3 fragment.

Example 8. FVIII-169/VWF-031 PK in HemA and FVIII/VWF DKO mice

[0361] The PK profile of FVIII-169/VWF-031 was tested in HemA and FVIII/VWF DKO mice to evaluate the ability of the D'D3 fragment to shield the FVIII moiety from the endogenous VWF. HemA or FVIII/VWF DKO mice were treated with a single intravenous dose of FVIII-169/VWF-031 at 200 IU/kg, plasma samples were then collected at 5min, 8hr, 24hr, 48hr and 72 hours post dosing. The FVIII activity of plasma sample was tested by FVIII chromogenic

assay, and half-life of FVIII-169/VWF-031 was calculated using WinNonlin program.

[0362] Complete inhibition of the constructs' binding to immobilized VWF was demonstrated by biolayer interferometry (Figure 7) for FVIII-169/VWF-031. This indicates the D'D3 fragment in the molecule had successfully blocked the FVIII binding to native VWF molecules, therefor similar half-life of FVIII-169/VWF-031 was predicted in the two different mouse strains. As shown in Figure 8A and Table 18, as expected, FVIII-169/VWF-031 had similar PK profile in both HemA and FVIII/VWF DKO mice, which has demonstrated that the half-life of FVIII Fc/VWF heterodimer is independent from the half-life of endogenous VWF. The separation of the FVIII Fc/VWF heterodimer half-life from the endogenous VWF half-life, eliminated the FVIII extension ceiling and opened the possibility of further extending FVIII half-life beyond the 2 fold half-life limit imposed by endogenous VWF.

**TABLE 18: FVIII-169/VWF-031 PK in HemA and FVIII/VWF DKO mice**

| Mouse Strain  | Recovery (%) | $t_{1/2}$ (hr) | MRT (hr) | Cl (mL/hr/kg) | Vss (mL/kg) | AUC (hr*kg*mIU/mL/mIU) |
|---------------|--------------|----------------|----------|---------------|-------------|------------------------|
| FVIII/VWF DKO | 69           | 17.94          | 20.1     | 4.06          | 81.69       | 0.2461                 |
| HemA          | 83           | 16.65          | 18.44    | 3.57          | 85.72       | 0.28                   |

[0363] The FVIII protecting ability of the XTEN insertion and D'D3 fragment was evaluated by comparing the half-life of FVIII-169/VWF-031 with FVIII-169/Fc and FVIII Fc in FVIII/VWF DKO mice. After a single IV administration, blood samples were collected at 5min, 8hr, 24hr, 48hr and 72hr for FVIII-169/VWF-031, 5min, 8hr, 24hr, 32hr, 48hr for FVIII-169/Fc and at 5min, 1, 2, 4, 6 and 8hrs for FVIII Fc. The FVIII activity of plasma sample was tested by FVIII chromogenic assay, and half-life of FVIII-155/VWF-031 was calculated using WinNonlin program.

[0364] The study results were summarized in Figure 8B and Table 19, rFVIII Fc has a 1.6hr half-life in DKO mice due to the loss of VWF protection. When an XTEN insertion was introduced into the FVIII Fc molecule, the resulting FVIII-



169/Fc molecule has a 7hr half-life, a 4 fold half-life extension by the XTEN insertion. Finally, when D'D3 fragment was incorporated into the molecule to form FVIII-169/VWF-031, a 17hr half-life was observed, another 2.5 fold further increase by the D'D3 fragment. In addition of the half-life improvement, improved Mean residency time (MRT), Clearance (Cl) and AUC were also observed as shown in Table 19.

[0365] FVIII-169/VWF-031 has achieved 17-18hr  $t_{1/2}$  in both HemA and FVIII/VWF DKO mice, which is the upper limit of the  $t_{1/2}$  extension ceiling that imposed by VWF clearance. More  $t_{1/2}$  extension elements can be further incorporated into this molecule, such as a second XTEN insertion within FVIII. The synergistic effect of D'D3 fragment and XTEN insertions provided the possibility of the complete protection for FVIII from its clearance pathway, a final breakthrough of the 2 fold FVIII  $t_{1/2}$  extension limit might be achieved by the FVIII/Fc/XTEN/VWF variants.

**TABLE 19. FVIII-169/VWF-031 PK in FVIII/VWF DKO mice**

| Mouse Strain  | Treatment          | Recovery (%) | $t_{1/2}$ (hr) | MRT (hr) | Cl (mL/hr/kg) | Vss (mL/kg) | AUC/D (hr*kg*<br>mIU/mL /mIU) |
|---------------|--------------------|--------------|----------------|----------|---------------|-------------|-------------------------------|
| FVIII/VWF DKO | rFVIII/Fc          | 35           | 1.6            | 2.1      | 57.7          | 120.2       | 0.0173                        |
|               | rFVIII-169/Fc      | 77           | 7.0            | 6.2      | 6.4           | 39.2        | 0.1573                        |
|               | rFVIII-169/VWF-031 | 69           | 17.9           | 20.1     | 4.1           | 81.7        | 0.2461                        |

Example 9: FVIII-XTEN variants cell media concentrate PK in D'D3 expressing FVIII/VWF DKO mice

[0366] The ability of D'D3 fragment to extend the  $t_{1/2}$  of FVII-XTEN was evaluated in the D'D3 expressing FVIII/VWF DKO mouse model (described in example 2). In this study, instead of using VWF-025 to introduce the D'D3 dimer into the circulation, VWF-029 construct was used to introduce the D'D3 monomer into the circulation. To prepare FVIII-XTEN variants protein, a small scale (50-100 mL) transient transfection culture media was prepared, at day 4 post transfection, cell culture was harvested and concentrated to reach 10-20 IU/mL of

FVIII activity range which is suitable for PK study. The concentrated cell media were then used for standard PK study in FVIII/VWF DKO mice with or without D'D3 in the circulation.

[0367] Total of 6 FVIII-XTEN variants that contains 1-3 XTEN insertions were tested in the system, their  $t_{1/2}$  were summarized in Table 20 and data from representative variants were plotted in Figure 9A.

[0368] Longer half-life was observed for all the FVIII-XTEN variants with the presents of D'D3 fragment in the circulation (Table 20), which demonstrated the D'D3 protection for FVIII-XTEN from its clearance pathways. Furthermore, when compared to its 14hr half-life in HemA mice, LSD0055.021 has a 20.4hr  $t_{1/2}$  in D'D3 expressing DKO mice (Figure 9B, Table 20), indicates the final breakthrough of the 2 fold half-life extension ceiling for FVIII molecules. By further modify the FVIII(XTEN)/VWF molecule, we could potentially achieve even longer FVIII  $t_{1/2}$ , and provide HemA patients a FVIII protein that only requires once weekly or less frequent dosing regimen.

**TABLE 20. FVIII-XTEN  $t_{1/2}$  in D'D3 expressing FVIII/VWF DKO mice**

| FVIII-XTEN ID | # of XTEN insertions | Insertion sites | XTEN size   | $t_{1/2}$ (hr) DKO mice | $t_{1/2}$ (hr) pLIVE-D'D3/DKO mice | $t_{1/2}$ (hr) HemA mice |
|---------------|----------------------|-----------------|-------------|-------------------------|------------------------------------|--------------------------|
| pSD-0013      | 1                    | CT              | 144         | 3.3                     | 7.9                                |                          |
| LSD0003.009   | 2                    | B*/CT           | 144/288     | 9.7                     | 16.4                               |                          |
| LSD0038.015   | 2                    | 1656/26         | 144/144     | 7.8                     | 17.2                               |                          |
| LSD0049.002   | 3                    | 18/B*/CT        | 144/144/288 | 12.6                    | 17.5                               |                          |
| LSD0051.002   | 3                    | 403/B*/CT       | 144/144/288 | 11.1                    | 19.9                               |                          |
| LSD0055.021   | 3                    | 1900/B*/CT      | 144/144/288 | 16                      | 20.4                               | 14                       |

\*B indicates an XTEN sequence (*e.g.*, 144) is inserted immediately downstream of amino acid residue 745 corresponding to mature FVIII sequence.

Example 10: Stability of VWF- and XTEN-containing FVIII variants in FVIII/VWF double knockout (DKO) plasma

[0369] Plasma stability of rFVIII<sup>h</sup> protein variants was tested in FVIII/VWF double knockout (DKO) mouse plasma. For the stability assay, HEK293 cells were co-transfected with plasmids directing the expression of rFVIII<sup>h</sup> or FVIII-169 (rFVIII<sup>h</sup> with 288 AE XTEN inserted at the B-domain junction) and plasmids directing the expression of either IgG-Fc or VWF-031 (VWF D'D3 region fused to IgG-Fc). At day four post-transfection, cell culture media was

harvested and concentrated to 30 IU/mL based on FVIII chromogenic activity. Concentrated cell culture medium was then added into DKO mouse plasma to yield a FVIII activity of 5 IU/mL and incubated at 37°C. Aliquots were collected at different time points for activity measurement by chromogenic assay. Activity at each time point was measured in duplicate, and the average activity was plotted as a function of time. The activity of FVIII-Fc, a dual chain (dc) FVIII molecule in which heavy and light chains are held together by non-covalent interaction, decreases with time in DKO mouse plasma (Figure 10). The activity of FVIII-169:Fc, which contains a 288 AE XTEN insertion at the B-domain junction, decays at a reduced rate relative to rFVIII-Fc, indicating that enhanced stability is conferred by the XTEN insertion. Given that VWF has been proposed to enhance the stability of FVIII in vivo, we evaluated the plasma stability of FVIII-169:VWF-031. This heterodimeric molecule, in which the FVIII element and the VWF D'D3 element are fused to respective hemi-domains of Fc, exhibited additional plasma stability relative to FVIII-169:Fc, indicating that the VWF D'D3 domain and XTEN have a synergistic effect on the plasma stability of rFVIII-Fc.

Example 11: The effect on FVIII half-life of Fc fusion, XTEN insertion and the D'D3 fragment of VWF.

[0370] To assess the effect of Fc fusion, XTEN insertion and D'D3 fragment of VWF on the half-life of FVIII, the pharmacokinetic properties of B domain deleted recombinant FVIII (rBDD-FVIII), rFVIII-Fc, FVIII-169:Fc and FVIII-169:VWF-031 were evaluated in FVIII/VWF double knockout (DKO) mice.

[0371] DKO mice were treated with a single intra venous administration of 200 IU/kg of FVIII proteins, and plasma samples were collected at designated time points as indicated in Figure 11. FVIII activity of the plasma samples were analyzed by FVIII chromogenic assay and half-life was calculated using the WinNonlin-Phoenix program. The pharmacokinetic parameters of the tested molecules are listed in Table 21. The time regression curve of plasma FVIII activity for each FVIII variants were plotted in Figure 11.

[0372] Unmodified BDD-FVIII had a half-life of 0.23 hr in DKO mice, the FVIII-Fc fusion protein has an extended half-life of 1.66 hr in DKO mice due to the recycling of FVIII-Fc protein through the Fc:FcRn interaction. When a 288 residue of AEXTEN polypeptide was incorporated into the B domain region of

FVIII within the FVIII<sub>Fc</sub> molecule, the half-life of the resulting FVIII169/<sub>Fc</sub> protein was further extended to 7.41 hr in DKO mice. Finally, with the addition of the D'D3 domain of VWF, the half-life of FVIII169/VWF031 heterodimer has reached 17.9 hr in DKO mice (Figure 11, Table 21). In addition of the half-life, all of the other PK parameters also improved proportionally with the addition of each element (Table 21). FVIII can tolerate multiple half-life extension elements, and this synergistic effect of the three elements on FVIII half-life extension, enabled the further improvement of the half-life of FVIII-XTEN VWF heterodimers.

**Table 21: PK parameters of FVIII variants**

| FVIII                   | FVIII Isoform | XTEN Insertions |             | T <sub>1/2</sub> (hr) | MRT (hr) | Cl (mL/hr/kg) | V <sub>ss</sub> (mL/kg) | AUC <sub>D</sub> (kg*hr/mL) |
|-------------------------|---------------|-----------------|-------------|-----------------------|----------|---------------|-------------------------|-----------------------------|
|                         |               | Site            | XTEN Length |                       |          |               |                         |                             |
| BDD-FVIII               | dc            |                 |             | 0.23                  | 0.24     | 407.72        | 97.42                   | 0.0025                      |
| FVIII <sub>Fc</sub>     | dc            |                 |             | 1.66                  | 2.06     | 62.66         | 128.82                  | 0.0161                      |
| FVIII169/ <sub>Fc</sub> | sc            | B*              | AE288       | 7.41                  | 6.67     | 6.24          | 41.61                   | 0.1603                      |
| FVIII169/VWF031         | sc            | B*              | AE288       | 17.94                 | 20.1     | 4.06          | 81.69                   | 0.2463                      |

\*B indicates an XTEN sequence (*e.g.*, 144) is inserted immediately downstream of amino acid residue 745 corresponding to mature FVIII sequence.

Example 12: Pharmacokinetic properties of different FVIII-XTEN\_VWF heterodimers

[0373] To evaluate the combined effect of the VWF-D'D3 fragment and XTEN insertions on the FVIII half-life, the pharmacokinetic properties of FVIII-XTEN-<sub>Fc</sub>:VWF-<sub>Fc</sub> heterodimers were tested in Hema mice and compared to those of the single chain isoform of BDD-FVIII (scBDD-FVIII) and FVIII-169:VWF-031 (example 10). Seven new FVIII-XTEN-<sub>Fc</sub> constructs were generated (protein sequences were listed in Table 24). Schematic diagrams of those constructs are shown in Figure 14A-H. FVIII-195 and FVIII-199, respectively, are the FVIII dual chain and single chain isoforms that each contains two XTEN insertions at positions 1900 and 1656. FVIII-196 and FVIII-201, respectively, are the FVIII dual chain and single chain isoforms that each contains three XTEN insertions at positions 26, 1656 and 1900. FVIII-203, -204 and -205 are sc-FVIII<sub>Fc</sub> molecules with two XTEN insertions at the B domain junction and at positions 1900, 403 or 18, respectively. Each FVIII-XTEN-<sub>Fc</sub> construct was co-expressed with VWF-

031 in HEK293 cells to produce FVIII-XTEN-Fc/VWF heterodimeric proteins. At day four post-transfection, cell culture medium was harvested and either concentrated to 20 IU/mL based on FVIII chromogenic activity (FVIII-195:VWF-031, FVIII-196:VWF-031, FVIII-199:VWF-031, FVIII-203:VWF-031 and FVIII-204:VWF-031) or purified (scBDD-FVIII, FVIII-169:VWF-031, FVIII-201:VWF-031 and FVIII-205:VWF-031). Having demonstrated the complete intra-molecular shielding of FVIII molecule from the endogenous VWF by the D'D3 fragment in the FVIII-XTEN-Fc:VWF-Fc heterodimer (FVIII-169:VWF-031, Example 5), HemA mice was chosen for the PK evaluations. Purified protein or concentrated cell culture medium was administered to 8-12 week-old HemA mice by intravenous administration at a dose of 200 IU/10 mL/kg. Plasma samples were collected at 5 min, 8 hr, 16 hr, 24 hr, 32 hr, 48 hr, 72 hr and 96 hr post-dosing. FVIII activity of the plasma samples were analyzed by FVIII chromogenic assay and half-life was calculated using the WinNonlin-Phoenix program. The pharmacokinetic parameters of the tested molecules are listed in Table 22. The plasma FVIII activities at selected time points for FVIII-XTEN-Fc/VWF-Fc variants were plotted in Figures 12A-C.

[0374] When XTEN was inserted into positions 1900 and 1656 (FVIII-195, FVIII-199), moderate improvement in half-life was observed for the scFVIII isoform (FVIII-199:VWF-031) compared to FVIII-169:VWF-031. However, the dcFVIII isoform exhibited a shorter half-life than did FVIII-169:VWF-031, indicating that the single chain isoform might be significantly more stable than the corresponding dual chain isoform (Table 22 and Figure 12A). When a third XTEN insertion was incorporated into FVIII-199 at position 26, the half-life of the resulting molecule FVIII-201:VWF-031 had reached 24.6 hr, which represents greater than a threefold half-life improvement relative to scBDD-FVIII (Table 22 and Figure 12C). We have also tested the half-life extension effect of the second XTEN insertion at position 403 (A2 domain), 1900 (A3 domain) and 18 (A1 domain) each in combination with the B domain XTEN insertion. While the addition of the A2 or A3 XTEN insertion did not confer an additional half-life benefit (Table 22, Figure 12b), the addition of the A1 insertion further extended the half-life of the FVIII-XTEN-Fc:VWF-Fc heterodimer to 29.4 hr (Table 22, Figure 12C), which is greater than threefold longer than that of scBDD-FVIII.

[0375] When XTENs were incorporated into the FVIII<sub>h</sub>/VWF heterodimer construct, degree of half-life improvement of the resulting molecules was variable, and no obvious correlation was observed between half-lives and either the site or number of XTEN insertion, suggesting that the half-life of the FVIII-XTEN-Fc/VWF heterodimer is determined by the integrity of the whole molecule rather than by the number or placement of XTEN insertions.

[0376] The 24.6 hr and 29.4 hr half-lives observed for FVIII-XTEN-Fc:VWF-Fc heterodimers clearly exceeded the 1.6- to 2-fold limitation on FVIII half-life extension. If this finding translates for HemA patients, it will allow once-weekly or less frequent dosing for FVIII prophylaxis.

**Table 22: PK parameters of FVIII-XTEN-Fc/VWF-Fc heterodimers**

| FVIII           | FVIII Isoform | XTEN Insertions |                   | T <sub>1/2</sub> (hr) | MRT (hr) | Cl (mL/hr/kg) | V <sub>ss</sub> (mL/kg) | AUC <sub>D</sub> (kg*hr/mL) |
|-----------------|---------------|-----------------|-------------------|-----------------------|----------|---------------|-------------------------|-----------------------------|
|                 |               | Site            | XTEN Length       |                       |          |               |                         |                             |
| scBDD-FVIII     | sc            |                 |                   | 7.16                  | 10.16    | 4.38          | 44.44                   | 0.23                        |
| FVIII169/VWF031 | sc            | B*              | AE288             | 16.65                 | 18.44    | 3.57          | 65.79                   | 0.28                        |
| FVIII195/VWF031 | dc            | 1656/1900       | AG144/AE144       | 12.56                 | 13.88    | 9.04          | 125.48                  | 0.11                        |
| FVIII199/VWF031 | sc            | 1656/1900       | AG144/AE144       | 18.57                 | 20.09    | 6.24          | 125.28                  | 0.16                        |
| FVIII201/VWF031 | sc            | 26/1656/1900    | AG144/AG144/AE144 | 24.63                 | 33.67    | 1.9           | 63.97                   | 0.53                        |
| FVIII203/VWF031 | sc            | 403/B*          | AE144/AE288       | 15.52                 | 18       | 3.65          | 65.61                   | 0.27                        |
| FVIII204/VWF031 | sc            | 1900/B*         | AE144/AE288       | 16.3                  | 20.63    | 2.87          | 59.14                   | 0.35                        |
| FVIII205/VWF031 | sc            | 18/B*           | AE144/AE288       | 29.4                  | 37.06    | 1.82          | 67.39                   | 0.55                        |

\*B indicates an XTEN sequence (e.g., 144) is inserted immediately downstream of amino acid residue 745 corresponding to mature FVIII sequence.

[0377] In addition to incorporating XTEN into the FVIII molecule, we also evaluated the potential half-life extension benefit of incorporating XTEN as a linker between the D'D3 and Fc fragment. FVIII-155 (scFVIII<sub>h</sub>Fc) was co-expressed with VWF-034 (VWF-Fc with AE 288 XTEN plus a 35 residue thrombin cleavable linker) in HEK293 cells. At day 4 post-transfection, cell culture medium was harvested and concentrated to 20 IU/mL based on FVIII activity assay. FVIII/VWF DKO mice were dosed with concentrated cell culture

media at 200 IU/10 mL/kg with a single intravenous injection. Plasma samples were collected at 5 min, 8 hr, 24 hr, 48 hr, 72 hr and 96 hr post-dosing. The FVIII activity of plasma samples was analyzed by FVIII chromogenic assay, and the regression curve of plasma FVIII activity as a function of time was plotted (Figure 13). FVIII-155/VWF-034 exhibited the same improvement in half-life as FVIII-169/VWF-031, which has AE 288 XTEN inserted into the B domain junction of FVIII, as illustrated by the over lapping regression curves for the two molecules (Figure 13). The demonstration that XTEN insertion into the VWF-Fc polypeptide confers half-life improvement of a magnitude similar to that conferred by XTEN insertion at the B domain junction of the FVIII polypeptide suggests that further half-life improvement may be possible in a heterodimeric molecule in which intra-molecular XTEN insertion in the FVIII polypeptide is combined with inter-domain XTEN insertion between the VWF and Fc elements of the VWF-Fc polypeptide.

Example 13A: Pharmacokinetic properties of additional FVIII-XTEN\_VWF heterodimers

[0378] In addition to the FVIII-XTEN VWF heterodimers that were listed in Table 22, FVIII-XTEN VWF heterodimers containing different composition of XTEN insertions, single chain and dual chain version of FVIII (Table 23A) are either tested or will be tested in HemA for their pharmacokinetic properties. Various FVIII constructs (Table 23B) and VWF constructs (Table 23C) are also disclosed below. HemA mice will be treated with a single dose of intravenous administration of the heterodimer proteins at 200 IU/10 mL/kg. Plasma samples will then be collected at 5 min, 24, 48, 72, 96 and 120 hrs post-dosing. FVIII activity of the plasma samples will be analyzed by FVIII chromogenic assay and half-life will be calculated using the WinNonlin-Phoenix program. The protein sequences of the listed heterodimers were listed in Table 25.

**Table 23A. Plausible FVIII-XTEN-Fc:VWF-Fc heterodimer combinations for PK and activity improvement.**

|                | pSYN VWF-015                 | pSYN VWF-031                   | pSYN VWF-034 **                | pSYN VWF-036                   |
|----------------|------------------------------|--------------------------------|--------------------------------|--------------------------------|
| pSYN FVIII 010 | -                            | $t_{1/2}$ 8.7 hr<br>DKO mice   | To be tested                   | -                              |
| pSYN FVIII 155 | $t_{1/2}$ 6.3 hr<br>DKO mice | $t_{1/2}$ 10.8 hr<br>HemA mice | $t_{1/2}$ 18.6 hr<br>HemA mice | $t_{1/2}$ 13.3 hr<br>HemA mice |

|                                                                         |   |                                       |                                       |                                       |
|-------------------------------------------------------------------------|---|---------------------------------------|---------------------------------------|---------------------------------------|
| pSYN FVIII<br>169 **                                                    | - | t <sub>1/2</sub> 16.7 hr<br>HemA mice | t <sub>1/2</sub> 31.1 hr<br>HemA mice | -                                     |
| pSYN FVIII<br>173 **                                                    | - | t <sub>1/2</sub> 15.2 hr<br>DKO mice  | t <sub>1/2</sub> 28.9 hr<br>HemA mice | To be tested                          |
| pSYN FVIII<br>205                                                       | - | t <sub>1/2</sub> 29.4 hr<br>HemA mice | t <sub>1/2</sub> 32.4 hr<br>HemA mice | t <sub>1/2</sub> 29.7 hr<br>HemA mice |
| pSYN FVIII<br>266                                                       | - | t <sub>1/2</sub> 24.5 hr<br>HemA mice | t <sub>1/2</sub> 27.4 hr<br>HemA mice | -                                     |
| pSYN FVII<br>267                                                        | - | t <sub>1/2</sub> 23.0 hr<br>HemA mice | t <sub>1/2</sub> 25.7 hr<br>HemA mice |                                       |
| pSYN FVIII<br>268                                                       | - | To be tested                          | To be tested                          | To be tested                          |
| Dual chain<br>isoform of<br>pSYN FVIII<br>268                           |   | To be tested                          | To be tested                          | To be tested                          |
| **Length of XTEN can be changed to 72, 144, 288, 324, 333, 576, or 864. |   |                                       |                                       |                                       |

**Table 23B. FVIII Constructs:**

|                           |                                                                                       |
|---------------------------|---------------------------------------------------------------------------------------|
| <b>pSYN FVIII<br/>010</b> | dual chain FVIII Fc                                                                   |
| <b>pSYN FVIII<br/>169</b> | Single chain FVIII Fc with 288 AE XTEN in B-domain                                    |
| <b>pSYN FVIII<br/>173</b> | dual chain FVIII Fc with 288 AE XTEN in B-domain                                      |
| <b>pSYN FVIII<br/>195</b> | dual chain FVIII Fc with two 144 XTENs at amino acid 1656 and 1900                    |
| <b>pSYN FVIII<br/>196</b> | dual chain FVIII Fc with three 144 XTENs at amino acid 26, 1656 and 1900              |
| <b>pSYN FVIII<br/>199</b> | Single chain FVIII Fc with two 144 XTENs at amino acid 1656 and 1900                  |
| <b>pSYN FVIII<br/>201</b> | Single chain FVIII Fc with three 144 XTENs at amino acid 26, 1656 and 1900            |
| <b>pSYN FVIII<br/>203</b> | Single chain FVIII Fc with 144 AE XTEN at amino acid 1900 and 288 AE XTEN in B-domain |
| <b>pSYN FVIII<br/>204</b> | Single chain FVIII Fc with 144 AE XTEN at amino acid 403 and 288 AE XTEN in B-domain  |
| <b>pSYN FVIII<br/>205</b> | Single chain FVIII Fc with 144 AE XTEN at amino acid 18 and 288 AE XTEN in B-domain   |
| <b>pSYN FVIII<br/>207</b> | Single chain FVIII ( no Fc, no XTEN)                                                  |
| <b>pSYN FVIII<br/>266</b> | Single chain FVIII Fc with 42 AE XTEN at amino acid 18 and 288 AE XTEN in B-domain    |
| <b>pSYN FVIII<br/>267</b> | Single chain FVIII Fc with 72 AE XTEN at amino acid 18 and 288 AE XTEN in B-domain    |
| <b>pSYN FVIII<br/>268</b> | Single chain FVIII Fc with 144 AE XTEN at amino acid 18                               |
| <b>pSYN FVIII</b>         | Single chain FVIII Fc with 72 AE XTEN at amino acid 18                                |



|                       |                                                                                          |
|-----------------------|------------------------------------------------------------------------------------------|
| <b>269</b>            |                                                                                          |
| <b>pSYN FVIII 271</b> | Single chain FVIII <sub>FC</sub> with 42 AE XTEN at amino acid 18                        |
| <b>pSYN FVIII 272</b> | Single chain FVIII with 144 AE XTEN at amino acid 18 and 288 AE XTEN in B-domain (no Fc) |

**Table 23C. VWF Constructs:**

|                    |                                                                                                               |
|--------------------|---------------------------------------------------------------------------------------------------------------|
| <b>pSYN VWF031</b> | VWF-D1D2D'D3- 48aa long thrombin cleavable GS linker-Fc with C1099A/C1142A                                    |
| <b>pSYN VWF034</b> | VWF-D1D2D'D3- 288AE XTEN +35aa long thrombin cleavable GS linker-Fc with C1099A/C1142A                        |
| <b>pSYN VWF035</b> | VWF-D1D2D'D3- 72aa long thrombin cleavable GS linker-Fc with C1099A/C1142A                                    |
| <b>pSYN VWF036</b> | VWF-D1D2D'D3- 98aa long thrombin cleavable GS linker-Fc with C1099A/C1142A                                    |
| <b>pSYN VWF041</b> | VWF-D1D2D'D3 with 288 AE XTEN in D3 and 48aa long thrombin cleavable GS linker after D3-Fc with C1099A/C1142A |

Example 13B: Pharmacokinetic properties of additional FVIII-XTEN\_VWF heterodimers.

[0379] FVIII-XTEN\_VWF heterodimers were tested in HemA mice for their pharmacokinetic properties. The heterodimers tested are FVIII169/VWF034, FVIII205/VWF034, FVIII205/VWF036 and FVIII266/VWF031. HemA mice were administered with a single intravenous dose of various heterodimer proteins at 200 IU/10 mL/kg. Plasma samples were collected at 5 min, 24, 48, 72, 96 and 120 hrs post-dosing. FVIII activity of the plasma samples were analyzed by FVIII chromogenic assay, and half-lives were calculated using the WinNonlin-Phoenix program. The PK results are shown below in Table 24.

**Table 24. Additional FVIII-XTEN\_VWF - PK in HemA Mice**

| <b>Treatment</b> | <b>5min recovery (%)</b> | <b>HL (hr)</b> | <b>MRT (hr)</b> | <b>Cl (mL/hr/kg)</b> | <b>Vss (mL/kg)</b> | <b>AUC_D (hr*kg*mIU/mL/mIU)</b> | <b>Fold of t<sub>1/2</sub> increase vs scBDD-FVIII</b> |
|------------------|--------------------------|----------------|-----------------|----------------------|--------------------|---------------------------------|--------------------------------------------------------|
| ScBDD-FVIII      |                          | 7.16           | 10.16           | 4.83                 | 44.44              | 0.23                            | -                                                      |
| FVIII169/VWF034  | 76                       | <b>31.1</b>    | 34.57           | 1.73                 | 59.77              | 0.58                            | 4.3                                                    |
| FVIII205/VWF034  | 68                       | <b>32.41</b>   | 39.79           | 1.55                 | 61.73              | 0.64                            | 4.6                                                    |
| FVIII205/VWF036  | 74                       | 29.71          | 36.35           | 1.61                 | 58.43              | 0.62                            | 4.1                                                    |
| FVIII266/V       | 66                       | 24.45          | 22.75           | 2.67                 | 60.83              | 0.37                            | 3.4                                                    |

|       |  |  |  |  |  |  |  |
|-------|--|--|--|--|--|--|--|
| WF031 |  |  |  |  |  |  |  |
|-------|--|--|--|--|--|--|--|

**pSYNFVIII 010 nucleotide sequence-(Dual chain FVIII<sub>h</sub>FC) (SEQ ID NO:****125)**

```

1  ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT
   TCGGATTCTG
51  CTTTAGTGCC ACCAGAAGAT ACTACCTGGG TGCAGTGGAA
   CTGTCATGGG
101 ACTATATGCA AAGTGATCTC GGTGAGCTGC CTGTGGACGC
   AAGATTTCTT
151 CCTAGAGTGC CAAAATCTTT TCCATTCAAC ACCTCAGTCG
   TGTACAAAAA
201 GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC
   GCTAAGCCAA
251 GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC
   TGAGGTTTAT
301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC
   CTGTCAGTCT
351 TCATGCTGTT GGTGTATCCT ACTGGAAAGC TTCTGAGGGA
   GCTGAATATG
401 ATGATCAGAC CAGTCAAAGG GAGAAAGAAG ATGATAAAGT
   CTTCCCTGGT
451 GGAAGCCATA CATATGTCTG GCAGGTCCTG AAAGAGAATG
   GTCCAATGGC
501 CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT
   GTGGACCTGG
551 TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT
   ATGTAGAGAA
601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT
   TTATACTACT
651 TTTTGCTGTA TTTGATGAAG GGAAAAGTTG GCACTCAGAA
   ACAAAGAACT
701 CCTTGATGCA GGATAGGGAT GCTGCATCTG CTCGGGCCTG
   GCCTAAAATG
751 CACACAGTCA ATGGTTATGT AAACAGGTCT CTGCCAGGTC
   TGATTGGATG
801 CCACAGGAAA TCAGTCTATT GGCATGTGAT TGAATGGGC
   ACCACTCCTG
851 AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT
   GAGGAACCAT
901 CGCCAGGCGT CCTTGAAAT CTCGCCAATA ACTTTCCTTA
   CTGCTCAAAC
951 ACTCTTGATG GACCTTGGAC AGTTTCTACT GTTTTGTCAT
   ATCTCTTCCC
1001 ACCAACATGA TGGCATGGAA GCTTATGTCA AAGTAGACAG
   CTGTCCAGAG
1051 GAACCCCAAC TACGAATGAA AAATAATGAA GAAGCGGAAG
   ACTATGATGA
1101 TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
   GATGACAACT

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1151 CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA  
 TCCTAAAACT  
 1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT  
 ATGCTCCCTT  
 1251 AGTCCTCGCC CCCGATGACA GAAGTTATAA AAGTCAATAT  
 TTGAACAATG  
 1301 GCCCTCAGCG GATTGGTAGG AAGTACAAAA AAGTCCGATT  
 TATGGCATACT  
 1351 ACAGATGAAA CCTTTAAGAC TCGTGAAGCT ATTCAGCATG  
 AATCAGGAAT  
 1401 CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG  
 TTGATTATAT  
 1451 TTAAGAATCA AGCAAGCAGA CCATATAACA TCTACCCTCA  
 CGGAATCACT  
 1501 GATGTCCGTC CTTTGTATTC AAGGAGATTA CCAAAGGTG  
 TAAAACATTT  
 1551 GAAGGATTTT CCAATTCTGC CAGGAGAAAT ATTCAAATAT  
 AAATGGACAG  
 1601 TGACTGTAGA AGATGGGCCA ACTAAATCAG ATCCTCGGTG  
 CCTGACCCGC  
 1651 TATTACTCTA GTTTCGTAA TATGGAGAGA GATCTAGCTT  
 CAGGACTCAT  
 1701 TGGCCCTCTC CTCATCTGCT ACAAAGAATC TG TAGATCAA  
 AGAGGAAACC  
 1751 AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTCTGT  
 ATTTGATGAG  
 1801 AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC  
 TCCCCAATCC  
 1851 AGCTGGAGTG CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC  
 AACATCATGC  
 1901 ACAGCATCAA TGGCTATGTT TTTGATAGTT TGCAGTTGTC  
 AGTTTGTTTG  
 1951 CATGAGGTGG CATACTGGTA CATTCTAAGC ATTGGAGCAC  
 AGACTGACTT  
 2001 CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA  
 ATGGTCTATG  
 2051 AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT  
 CTTTATGTCG  
 2101 ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACCT  
 CAGACTTTCG  
 2151 GAACAGAGGC ATGACCGCCT TACTGAAGGT TTCTAGTTGT  
 GACAAGAACA  
 2201 CTGGTGATTA TTACGAGGAC AGTTATGAAG ATATTTTCAGC  
 ATACTTGCTG  
 2251 AGTAAAAACA ATGCCATTGA ACCAAGAAGC TTCTCTCAAA  
 ACCCACCAGT  
 2301 CTTGAAACGC CATCAACGGG AAATAACTCG TACTACTCTT  
 CAGTCAGATC  
 2351 AAGAGGAAAT TGAATATGAT GATACCATAT CAGTTGAAAT  
 GAAGAAGGAA

2401 GATTTTGACA TTTATGATGA GGATGAAAAT CAGAGCCCCC  
GCAGCTTTCA  
2451 AAAGAAAACA CGACACTATT TTATTGCTGC AGTGGAGAGG  
CTCTGGGATT  
2501 ATGGGATGAG TAGCTCCCCA CATGTTCTAA GAAACAGGGC  
TCAGAGTGGC  
2551 AGTGTCCCTC AGTTCAAGAA AGTTGTTTTTC CAGGAATTTA  
CTGATGGCTC  
2601 CTTTACTCAG CCCTTATACC GTGGAGAACT AAATGAACAT  
TTGGGACTCC  
2651 TGGGGCCATA TATAAGAGCA GAAGTTGAAG ATAATATCAT  
GGTAACTTTC  
2701 AGAAATCAGG CCTCTCGTCC CTATTCCTTC TATTCTAGCC  
TTATTTCTTA  
2751 TGAGGAAGAT CAGAGGCAAG GAGCAGAACC TAGAAAAAAC  
TTTGTCAAGC  
2801 CTAATGAAAC CAAAACCTAC TTTTGAAAG TGCAACATCA  
TATGGCACCC  
2851 ACTAAAGATG AGTTTGACTG CAAAGCCTGG GCTTATTTCT  
CTGATGTTGA  
2901 CCTGGAAAAA GATGTGCACT CAGGCCTGAT TGGACCCCTT  
CTGGTCTGCC  
2951 AACTAACAC ACTGAACCCT GTCATGGGA GACAAGTGAC  
AGTACAGGAA  
3001 TTTGCTCTGT TTTTCACCAT CTTTGATGAG ACCAAAAGCT  
GGTACTTCAC  
3051 TGAAAATATG GAAAGAACT GCAGGGCTCC CTGCAATATC  
CAGATGGAAG  
3101 ATCCCACCTT TAAAGAGAAT TATCGCTTCC ATGCAATCAA  
TGGCTACATA  
3151 ATGGATACAC TACCTGGCTT AGTAATGGCT CAGGATCAAA  
GGATTGATG  
3201 GTATCTGCTC AGCATGGGCA GCAATGAAAA CATCCATTCT  
ATTCATTTCA  
3251 GTGGACATGT GTTCACTGTA CGAAAAAAG AGGAGTATAA  
AATGGCACTG  
3301 TACAATCTCT ATCCAGGTGT TTTTGAGACA GTGGAAATGT  
TACCATCCAA  
3351 AGCTGGAATT TGGCGGGTGG AATGCCTTAT TGGCGAGCAT  
CTACATGCTG  
3401 GGATGAGCAC ACTTTTTCTG GTGTACAGCA ATAAGTGTCA  
GACTCCCCTG  
3451 GGAATGGCTT CTGGACACAT TAGAGATTTT CAGATTACAG  
CTTCAGGACA  
3501 ATATGGACAG TGGGCCCCAA AGCTGGCCAG ACTTCATTAT  
TCCGGATCAA  
3551 TCAATGCCTG GAGCACCAAG GAGCCCTTTT CTTGGATCAA  
GGTGGATCTG  
3601 TTGGCACCAA TGATTATTCA CGGCATCAAG ACCCAGGGTG  
CCCGTCAGAA

3651 GTTCTCCAGC CTCTACATCT CTCAGTTTAT CATCATGTAT  
 AGTCTTGATG  
 3701 GGAAGAAGTG GCAGACTTAT CGAGGAAATT CCACTGGAAC  
 CTTAATGGTC  
 3751 TTCTTTGGCA ATGTGGATTC ATCTGGGATA AAACACAATA  
 TTTTAAACCC  
 3801 TCCAATTATT GCTCGATACA TCCGTTTGCA CCCAACTCAT  
 TATAGCATTC  
 3851 GCAGCACTCT TCGCATGGAG TTGATGGGCT GTGATTTAAA  
 TAGTTGCAGC  
 3901 ATGCCATTGG GAATGGAGAG TAAAGCAATA TCAGATGCAC  
 AGATTACTGC  
 3951 TTCATCCTAC TTTACCAATA TGTTTGCCAC CTGGTCTCCT  
 TCAAAAGCTC  
 4001 GACTTCACCT CCAAGGGAGG AGTAATGCCT GGAGACCTCA  
 GGTGAATAAT  
 4051 CCAAAAGAGT GGCTGCAAGT GGAATTCCAG AAGACAATGA  
 AAGTCACAGG  
 4101 AGTAACTACT CAGGGAGTAA AATCTCTGCT TACCAGCATG  
 TATGTGAAGG  
 4151 AGTTCCTCAT CTCCAGCAGT CAAGATGGCC ATCAGTGGAC  
 TCTCTTTTTT  
 4201 CAGAATGGCA AAGTAAAGGT TTTTCAGGGA AATCAAGACT  
 CCTTCACACC  
 4251 TGTGGTGAAC TCTCTAGACC CACCGTTACT GACTCGCTAC  
 CTTCGAATTC  
 4301 ACCCCCAGAG TTGGGTGCAC CAGATTGCCC TGAGGATGGA  
 GGTTCCTGGGC  
 4351 TGCGAGGCAC AGGACCTCTA CGACAAAAC CACACATGCC  
 CACCGTGCCC  
 4401 AGCTCCAGAA CTCCTGGGCG GACCGTCAGT CTTCTCTTC  
 CCCCCAAAAC  
 4451 CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC  
 ATGCGTGGTG  
 4501 GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT  
 GGTACGTGGA  
 4551 CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG  
 GAGCAGTACA  
 4601 ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA  
 CCAGGACTGG  
 4651 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG  
 CCCTCCCAGC  
 4701 CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC  
 CGAGAACCAC  
 4751 AGGTGTACAC CCTGCCCCCA TCCCGGGATG AGCTGACCAA  
 GAACCAGGTC  
 4801 AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA  
 TCGCCGTGGA  
 4851 GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC  
 ACGCCTCCCG

4901 TGTGGACTC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT  
CACCGTGGAC  
4951 AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG  
TGATGCATGA  
5001 GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG  
TCTCCGGGTA  
5051 AATGA

**pSYNFVIII 010 protein sequence-(Dual chain FVIII<sub>FC</sub>) (SEQ ID NO: 126)**

1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQSDL  
GELPVDARFP  
51 PRVPKSFPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL  
LGPTIQAEVY  
101 DTVVITLKNM ASHPVSLHAV GVS YWKASEG AEYDDQTSQR  
EKEDDKVFPG  
151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG  
LIGALLVCRE  
201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD  
AASARAWPKM  
251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL  
EGHTFLVRNH  
301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME  
AYVKVDSCPE  
351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI  
RSVAKKHPKT  
401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR  
KYKKVRFMAY  
451 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR  
PYNIPHGIT  
501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP  
TKSDPRCLTR  
551 YYSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR  
NVILFSVFDE  
601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV  
FDSLQLSVCL  
651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF  
PFSGETVFMS  
701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDY YED  
SYEDISAYLL  
751 SKNNAIEPRS FSQNPPVLKR HQREITRTTL QSDQEEIDYD  
DTISVEMKKE  
801 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP  
HVLNRNAQSG  
851 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA  
EVEDNIMVTF  
901 RNQASRPYSF YSSLISYEED QRQGAEP RKN FVKPNETKTY  
FWKVQH HMAP  
951 TKDEFDCKAW AYFSDVDLEK DVHSG LIGPL LVCHTNTLNP  
AHGRQVTVQE  
1001 FALFFTIFDE TKS WYFTENM ERNCRAPCNI QMEDPTFKEN  
YRFHAINGYI

```

1051  MDTLPGLVMA QDQIRRWYLL SMGSNENIHS IHFSGHVFTV
      RKKEEYKMAL
1101  YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL
      VYSNKCQTPL
1151  GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK
      EPFSWIKVDL
1201  LAPMIIHGK  TQGARQKFSS LYISQFIIMY SLDGKKWQTY
      RGNSTGTLMV
1251  FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME
      LMGCDLNSCS
1301  MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR
      SNAWRPQVNN
1351  PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS
      QDGHQWTLFF
1401  QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH
      QIALRMEVLG
1451  CEAQDLYDKT HTPPCPAPE LLGGPSVFLF PPKPKDTLMI
      SRTPEVTCVV
1501  VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
      SVLTVLHQDW
1551  LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
      SRDELTKNQV
1601  SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS
      FFLYSKLTVD
1651  KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

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Example 14: A new class of coagulation factor VIII molecules with greater than three-fold half-life extension in hemophilia A mice

**[0380]** The new class of FVIII molecules was designed to contain two polypeptides; one that consists of a single chain B-domain deleted (BDD) FVIII with XTEN inserted at one or more locations within the FVIII sequence, and one that is composed of the D'D3 region of VWF. Each polypeptide was also recombinantly fused to the Fc region of IgG1 to enable the D'D3 region to be correctly aligned to bind the FVIII moiety. The resulting FVIII variants were expressed in HEK 293 cells by transient transfection, and purified from the conditioned media. FVIII activity was evaluated by FVIII chromogenic assay and the pharmacokinetic properties were assessed in both FVIII knockout (HemA) and FVIII/VWF double knock-out (DKO) mice.

**[0381]** Incorporating XTEN and D'D3 region of VWF into rFVIII led to the uncoupling of the clearance of the fusion proteins from endogenous VWF while extending their circulating half-life. FVIII in this fusion configuration is completely shielded from interacting with VWF, as measured by biolayer

interferometry (Octet) analysis. Consistent with this, their pharmacokinetic profiles in HemA and DKO mice were found to be identical, indicating that their clearance rate in mice was effectively disconnected from VWF. Optimization of XTEN length and the locations for inserting XTEN identified a subset of the proteins that have exceeded the VWF limitation (16 hours), reaching a circulating half-life of up to 30 hours in HemA mice representing a 4-fold improvement over BDD-FVIII. Importantly, these proteins maintained their functionality, as judged by FVIII chromogenic assay.

[0382] The VWF dependency has set a fundamental limitation for half-life of therapeutic FVIII. Uncoupling FVIII from VWF clearance pathways while extending half-life by the fusion of D'D3 region of VWF and XTEN has generated a novel FVIII molecule with a 4-fold half-life extension. This is the first report of an engineered FVIII that has exceeded the half-life limitation observed through industry-wide efforts in development of long-lasting FVIII, representing a potentially significant advancement in prophylactic treatment of hemophilia A.

**Table 25: Protein sequences of FVIII-XTEN-Fc and VWF-Fc constructs**  
**FVIII 195 protein sequence (dual chain FVIII-Fc with two 144 AE XTENs at amino acid 1656 and 1900) (SEQ ID NO: 105)**

```

1      MQIELSTCFE LCLLRFCFSA TRRYYLGAWE LSWDYMQSDL
    GELPVDARFP
51  PRVPKSFPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL
    LGPTIQAEVY
101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR
    EKEDDKVFPG
151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG
    LIGALLVCRE
201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD
    AASARAWPKM
251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL
    EGHTFLVRNH
301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME
    AYVKVDSCPE
351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI
    RSVAKKHPKT
401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR
    KYKKVRFMAY
451 TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR
    PYNIYPHGIT
501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP
    TKSDPRCLTR
551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR
    NVILFSVFDE

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601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV  
 FDSLQLSVCL  
 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MUYEDTLTLF  
 PFSGETVFM  
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYED  
 SYEDISAYLL  
 751 SKNNAIEPRS FSQNPPVLKR HQREITRTTL QGAPGTPGSG  
 TASSSPGASP  
 801 GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST  
 GTGPGTPGSG  
 851 TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG  
 SPGSSTPSGA  
 901 TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD  
 DTISVEMKKE  
 951 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP  
 HVLNRNAQSG  
 1001 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA  
 EVEDNIMVTF  
 1051 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY  
 FWKVQHMAP  
 1101 TKDEFDCKAW AYFSDVDLEK DVHSGGLIGPL LVCHTNTLNP  
 AHGRQVTVQE  
 1151 FALFFTIFDE TKSIFYTENM ERNCRGAPTS ESATPESGPG  
 SEPATSGSET  
 1201 PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS  
 TEPSEGSAPG  
 1251 TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEESGSPAG  
 SPTSTEEGTS  
 1301 ESATPESGPG TSTEPSEGSA PGASSAPCNI QMEDPTFKEN  
 YRFHAINGYI  
 1351 MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV  
 RKKEEYKMAL  
 1401 YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL  
 VYSNKCQTPL  
 1451 GMASGHIRDF QITASGQYQG WAPKLARLHY SGSINAWSTK  
 EPFSWIKVDL  
 1501 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY  
 RGNSTGTLMV  
 1551 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME  
 LMGCDLNSCS  
 1601 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR  
 SNAWRPQVNN  
 1651 PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISS  
 QDGHQWTLFF  
 1701 QNGKVVFQGG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH  
 QIALRMEVLG  
 1751 CEAQDLYDKT HTPPCPAPE LLGGPSVFLF PPKPKDTLMI  
 SRTPEVTCVV  
 1801 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV  
 SVLTVLHQDW

1851 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP  
 SRDELTKNQV  
 1901 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGDS  
 FFLYSKLTVD  
 1951 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK\*

**FVIII 196 protein sequence (dual chain FVIII Fc with three 144 AE XTENS at amino acid 26, 1656 and 1900) (SEQ ID NO: 106)**

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQSDL  
 GELPVGAPGS  
 51 SPSASTGTGP GSSPSASTGT GPGASPGTSS TGSPGASPGT  
 SSTGSPGSST  
 101 PSGATGSPGS SPSASTGTGP GASPGTSSTG SPGSSPSAST  
 GTGPGTPGSG  
 151 TASSSPGSST PSGATGSPGS STPSGATGSP GASPGTSSTG  
 SPASSDARFP  
 201 PRVPKSFPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL  
 LGPTIQAEVY  
 251 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR  
 EKEDDKVFPG  
 301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG  
 LIGALLVCRE  
 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD  
 AASARAWPKM  
 401 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL  
 EGHTFLVRNH  
 451 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME  
 AYVKVDSCPE  
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI  
 RSVAKKHPKT  
 551 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR  
 KYKKVRFMAY  
 601 TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR  
 PYNIPHGIT  
 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP  
 TKSDPRCLTR  
 701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR  
 NVILFSVFDE  
 751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV  
 FDSLQLSVCL  
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFFKHK MVEYEDTLTLF  
 PFSGETVFMS  
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED  
 SYEDISAYLL  
 901 SKNNAIEPRS FSQNPPVLKR HQREITRTTL QGAPGTPGSG  
 TASSSPGASP  
 951 GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST  
 GTGPGTPGSG  
 1001 TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG  
 SPGSSTPSGA

1051 TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD  
 DTISVEMKKE  
 1101 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP  
 HVLRNRAQSG  
 1151 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPIYRA  
 EVEDNIMVTF  
 1201 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY  
 FWKVQHMAP  
 1251 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP  
 AHGRQVTVQE  
 1301 FALFFTIFDE TKSIFYTENM ERNCRGAPTS ESATPESGPG  
 SEPATSGSET  
 1351 PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS  
 TEPSEGSAPG  
 1401 TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEEGSPAG  
 SPTSTEEGTS  
 1451 ESATPESGPG TSTEPSEGSA PGASSAPCNI QMEDPTFKEN  
 YRFHAINGYI  
 1501 MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV  
 RKKEEYKMAL  
 1551 YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL  
 VYSNKCQTPL  
 1601 GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK  
 EPFSWIKVDL  
 1651 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY  
 RGNSTGTLMV  
 1701 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME  
 LMGCDLNSCS  
 1751 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR  
 SNAWRPQVNN  
 1801 PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS  
 QDGHQWTLFF  
 1851 QNGKVKVFGG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH  
 QIALRMEVLG  
 1901 CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI  
 SRTPEVTCVV  
 1951 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV  
 SVLTVLHQDW  
 2001 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP  
 SRDELTKNQV  
 2051 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGGS  
 FFLYSKLTVD  
 2101 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK\*

**FVIII 199 protein sequence (single chain FVIII<sub>FC</sub> with three 144 AE XTENS at amino acid 1656 and 1900) (SEQ ID NO: 107)**

1 MQIELSTCFE LCLLRFCFSA TRRYYLGAWE LSWDYMQSDL  
 GELPVDARFP  
 51 PRVPKSFPEF TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL  
 LGPTIQAEVY

101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR  
 EKEDDKVFPG  
 151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG  
 LIGALLVCRE  
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD  
 AASARAWPKM  
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL  
 EGHTFLVRNH  
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME  
 AYVKVDSCPE  
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI  
 RSVAKKHPKT  
 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR  
 KYKKVRFMAY  
 451 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR  
 PYNIYPHGIT  
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP  
 TKSDPRCLTR  
 551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR  
 NVILFSVFDE  
 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV  
 FDSLQLSVCL  
 651 HEVAYWYILS IGAQTDFLSV FFSGYT FKHK MVEDTLTLF  
 PFSGETVFMS  
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED  
 SYEDISAYLL  
 751 SKNNAIEPRS FSQNPVLKR HQAEITRTTL QGAPGTPGSG  
 TASSSPGASP  
 801 GTSSTGSPGA SPGTSSTGSP GASP GTSSTG SPGSSPSAST  
 GTGPGTPGSG  
 851 TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASP GTSSTG  
 SPGSSTPSGA  
 901 TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD  
 DTISVEMKKE  
 951 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP  
 HVLRNRAQSG  
 1001 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA  
 EVEDNIMVTF  
 1051 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY  
 FWKVQHMAP  
 1101 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP  
 AHGRQVTVQE  
 1151 FALFFTIFDE TKSIFYTENM ERNCRGAPTS ESATPESGPG  
 SEPATSGSET  
 1201 PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS  
 TEPSEGSAPG  
 1251 TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEESGSPAG  
 SPTSTEESGTS  
 1301 ESATPESGPG TSTEPSEGSAPG PGASSAPCNI QMEDPTFKEN  
 YRFHAINGYI

1351 MDTLPGLVMA QDQIRRWYLL SMGSNENIHS IHFSGHVFTV  
 RKKEEYKMAL  
 1401 YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL  
 VYSNKCQTPL  
 1451 GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK  
 EPFSWIKVDL  
 1501 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY  
 RGNSTGTLMV  
 1551 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME  
 LMGCDLNSCS  
 1601 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR  
 SNAWRPQVNN  
 1651 PKEWLQVDFQ KTMKVTGVTT QGVKSLTSM YVKEFLISSS  
 QDGHQWTLFF  
 1701 QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH  
 QIALRMEVLG  
 1751 CEAQDLYDKT HTPPCPAPE LLGGPSVFLF PPKPKDTLMI  
 SRTPEVTCVV  
 1801 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV  
 SVLTVLHQDW  
 1851 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP  
 SRDELTKNQV  
 1901 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDS DGS  
 FFLYSKLTVD  
 1951 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK\*

**FVIII 201 protein sequence (single chain FVIII Fc with three 144 AE XTENs at amino acid 26, 1656 & 1900) (SEQ ID NO: 108)**

1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQSDL  
 GELPVGAPGS  
 51 SPSASTGTGP GSSPSASTGT GPGASPGTSS TGSPGASPGT  
 SSTGSPGSST  
 101 PSGATGSPGS SPSASTGTGP GASPGTSSTG SPGSSPSAST  
 GTGPGTPGSG  
 151 TASSSPGSST PSGATGSPGS STPSGATGSP GASPGTSSTG  
 SPASSDARFP  
 201 PRVPKSFPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL  
 LGPTIQAEVY  
 251 DTVVITLKNM ASHPVSLHAV GVSYWKA SEG AEYDDQTSQR  
 EKEDDKVFPG  
 301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG  
 LIGALLVCRE  
 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD  
 AASARAWPKM  
 401 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL  
 EGHTFLVRNH  
 451 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME  
 AYVKVDSCPE  
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI  
 RSVAKKHPKT

551 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR  
 KYKKVRFMAY  
 601 TDETFKTTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR  
 PYNIPHGIT  
 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP  
 TKSDPRCLTR  
 701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR  
 NVILFSVFDE  
 751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV  
 FDSLQLSVCL  
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF  
 PFSGETVFMS  
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYIED  
 SYEDISAYLL  
 901 SKNNAIEPRS FSQNPPVLKR HQAEITRTTL QGAPGTPGSG  
 TASSSPGASP  
 951 GTSSTGSPGA SPGTSSTGSP GASPGTSSTG SPGSSPSAST  
 GTGPGTPGSG  
 1001 TASSSPGASP GTSSTGSPGA SPGTSSTGSP GASPGTSSTG  
 SPGSSTPSGA  
 1051 TGSPGSSTPS GATGSPGASP GTSSTGSPAS SSDQEEIDYD  
 DTISVEMKKE  
 1101 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP  
 HVLNRNAQSG  
 1151 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA  
 EVEDNIMVTF  
 1201 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY  
 FWKVQHMAP  
 1251 TKDEFDCKAW AYFSDVDLEK DVHSGGLIGPL LVCHTNTLNP  
 AHGRQVTVQE  
 1301 FALFFTIFDE TKSIFYTENM ERNCRGAPTS ESATPESGPG  
 SEPATSGSET  
 1351 PGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGTS  
 TEPSEGSAPG  
 1401 TSESATPESG PGSPAGSPTS TEEGSPAGSP TSTEEGSPAG  
 SPTSTEEGTS  
 1451 ESATPESGPG TSTEPSEGSA PGASSAPCNI QMEDPTFKEN  
 YRFHAINGYI  
 1501 MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV  
 RKKEEYKMAL  
 1551 YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL  
 VYSNKCQTPL  
 1601 GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK  
 EPFSWIKVDL  
 1651 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY  
 RGNSTGTLMV  
 1701 FFGNVDSSGI KHNIFNPPII ARYIRLHPH YSIRSTLRME  
 LMGCDLNSCS  
 1751 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR  
 SNAWRPQVNN

1801 PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS  
 QDGHQWTLFF  
 1851 QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH  
 QIALRMEVLG  
 1901 CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI  
 SRTPEVTCVV  
 1951 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV  
 SVLTVLHQDW  
 2001 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP  
 SRDELTKNQV  
 2051 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGDS  
 FFLYSKLTVD  
 2101 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK\*

**FVIII 203 protein sequence (single chain FVIII<sub>1</sub> with two AE XTENs; one 288AE XTEN**

**in B-domain and one 144 AE XTEN at amino acid 1900) (SEQ ID NO: 109)**

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL  
 GELPVDARFP  
 51 PRVPKSFPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL  
 LGPTIQAEVY  
 101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR  
 EKEDDKVFPG  
 151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG  
 LIGALLVCRE  
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD  
 AASARAWPKM  
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL  
 EGHTFLVRNH  
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME  
 AYVKVDSCPE  
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI  
 RSVAKKHPKT  
 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR  
 KYKKVRFMAY  
 451 TDETFTKTREA IQHESGILGP LLYGEVGDIT LIIFKNQASR  
 PYNIYPHGIT  
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP  
 TKSDPRCLTR  
 551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR  
 NVILFSVFDE  
 601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV  
 FDSLQLSVCL  
 651 HEVAYWYILS IGAQTDFLSV FFSGYTFFKHK MVEYEDTLTLF  
 PFSGETVFMS  
 701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED  
 SYEDISAYLL  
 751 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET  
 PGTSESATPE  
 801 SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG  
 SPAGSPTSTE

851 EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP  
 AGSPTSTEEG  
 901 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES  
 ATPESGPGTS  
 951 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP  
 TSTEEGTSTE  
 1001 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE  
 SGPGTSTEPS  
 1051 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE  
 MKKEDFDIYD  
 1101 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR  
 AQSGSVPQFK  
 1151 KVVFQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI  
 MVTFRNQASR  
 1201 PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH  
 HMAPTKDEFD  
 1251 CKAWAYFSDV DLEKDVHSGI IGPLLVCNTN TLNPAHGRQV  
 TVQEFALFFT  
 1301 IFDETKSWYF TENMERNCRG APTSESATPE SGPGSEPATS  
 GSETPGTSES  
 1351 ATPESGPGSE PATSGSETPG TSESATPESG PGTSTEPSEG  
 SAPGTSESAT  
 1401 PESGPGSPAG SPTSTEEGSP AGSPTSTEEG SPAGSPTSTE  
 EGTSESATPE  
 1451 SGPGTSTEPS EGSAPGASSA PCNIQMEDPT FKENYRFHAI  
 NGYIMDTLPG  
 1501 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY  
 KMALYNLYPG  
 1551 VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC  
 QTPLGMAHGH  
 1601 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI  
 KVDLLAPMII  
 1651 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG  
 TLMVFFGNVD  
 1701 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL  
 NSCSMPLGME  
 1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP  
 QVNNPKEWLQ  
 1801 VDFQKTMKVT GVTQGVKSL LTSMYVKEFL ISSSQDGHQW  
 TLFFQNGKVK  
 1851 VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM  
 EVLGCEAQDL  
 1901 YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV  
 TCVVVDVSHE  
 1951 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL  
 HQDWLNGKEY  
 2001 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT  
 KNQVSLTCLV  
 2051 KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK  
 LTVDKSRWQQ  
 2101 GNVFSCSVMH EALHNHYTQK SLSLSPGK\*



**FVIII 204 protein sequence (single chain FVIII<sub>FC</sub> with two AE XTENs; one 288AE XTEN**

**in B-domain and one 144 AE XTEN at amino acid 403) (SEQ ID NO: 110)**

```

1  MQIELSTCFF LCLLRFCFSA TRYYLGAVE LSWDYMQSDL
   GELPVDARFP
51  PRVPKSFPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL
   LGPTIQAEVY
101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR
   EKEDDKVFPG
151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG
   LIGALLVCRE
201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD
   AASARAWPKM
251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL
   EGHTFLVRNH
301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME
   AYVKVDSCPE
351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI
   RSVAKKHPKT
401 WVHYIAAEEE DWDYAPLVLA PDGAPTSTEP SEGSA PGSPA
   GSPTSTEEGT
451 STEPSEGSA P GTSTEPSEGSA PGTSESATP ESGPGTSTEP
   SEGSA PGTSE
501 SATPESGPGS EPATSGSETP GTSTEPSEGSA PGTSTEPSE
   GSAPGTSESA
551 TPESGPGTSE SATPESGPGA SSDRSYKSQY LNNGPQRIGR
   KYKKVRFMAY
601 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR
   PYNIYPHGIT
651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP
   TKSDPRCLTR
701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR
   NVILFSVFDE
751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV
   FDSLQLSVCL
801 HEVAYWYILS IGAQTDFLSV FFSGYT FKHK MVEYEDTLTLF
   PFSGETVFMS
851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED
   SYEDISAYLL
901 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET
   PGTSESATPE
951 SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSA PG
   SPAGSPTSTE
1001 EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP
   AGSPTSTEEG
1051 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES
   ATPESGPGTS
1101 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP
   TSTEEGTSTE

```

1151 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE  
 SGPGTSTEPS  
 1201 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE  
 MKKEDFDIYD  
 1251 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR  
 AQSGSVPQFK  
 1301 KVVVFQFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI  
 MVTFRNQASR  
 1351 PYSFYSSLIS YEEDQRQGA E PRKNFVKPNE TKTYFWKVQH  
 HMAPTKDEFD  
 1401 CKAWAYFSDV DLEKDVHSGI IGPLLVCNTN TLNPAHGRQV  
 TVQEFALFFT  
 1451 IFDETKSWYF TENMERNCR A PCNIQMEDPT FKENYRFHAI  
 NGYIMDTLPG  
 1501 LVMAQDQRI R WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY  
 KMALYNLYPG  
 1551 VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC  
 QTPLGMASGH  
 1601 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI  
 KVDLLAPMII  
 1651 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG  
 TLMVFFGNVD  
 1701 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL  
 NSCSMPLGME  
 1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP  
 QVNNPKEWLQ  
 1801 VDFQKTMKVT GVTQGVKSL LTSMYVKEFL ISSSQDGHQW  
 TLFFQNGKVK  
 1851 VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM  
 EVLGCEAQDL  
 1901 YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV  
 TCVVVDVSHE  
 1951 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL  
 HQDWLNGKEY  
 2001 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT  
 KNQVSLTCLV  
 2051 KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK  
 LTVDKSRWQQ  
 2101 GNVFSCSVMH EALHNHYTQK SLSLSPGK\*

**FVIII 205 protein sequence (single chain FVIII<sub>1</sub> with two AE XTENs; one 288AE XTEN in B-domain and one 144 AE XTEN at amino acid 18) (SEQ ID NO: 111)**

1 MQIELSTCFE LCLLRFCFSA TRRYYLGA VE LSWDYMQGAP  
 TSESATPESG  
 51 PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS  
 ESATPESGPG  
 101 TSTEPSEGS A PGSPAGSPTS TEEGTSESAT PESGPGSEPA  
 TSGSETPGTS

151 ESATPESGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL  
 GELPVDARFP  
 201 PRVPKSFFPN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL  
 LGPTIQAEVY  
 251 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR  
 EKEDDKVFPG  
 301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG  
 LIGALLVCRE  
 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD  
 AASARAWPKM  
 401 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL  
 EGHTFLVRNH  
 451 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME  
 AYVKVDSCPE  
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI  
 RSVAKKHPKT  
 551 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR  
 KYKKVRFMAY  
 601 TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR  
 PYNIPHGIT  
 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP  
 TKSDPRCLTR  
 701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR  
 NVILFSVFDE  
 751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV  
 FDSLQLSVCL  
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFFKHK MVYEDTLTLF  
 PFSGETVFMS  
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED  
 SYEDISAYLL  
 901 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET  
 PGTSESATPE  
 951 SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG  
 SPAGSPTSTE  
 1001 EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP  
 AGSPTSTEEG  
 1051 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES  
 ATPESGPGTS  
 1101 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP  
 TSTEEGTSTE  
 1151 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE  
 SGPGTSTEPS  
 1201 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE  
 MKKEDFDIYD  
 1251 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR  
 AQSGSVPQFK  
 1301 KVVFEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI  
 MVTFRNQASR  
 1351 PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH  
 HMAPTKDEFD

1401 CKAWAYFSDV DLEKDVHSGL IGPLLVCHTN TLNPAHGRQV  
 TVQEFALFFT  
 1451 IFDETKSWYF TENMERN CRA PCNIQMEDPT FKENYRFHAI  
 NGYIMDTLPG  
 1501 LVMAQDQIR WYLLSMGSNE NIHSIHFS GH VFTVRKKEEY  
 KMALYNLYPG  
 1551 VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC  
 QTPLGMASGH  
 1601 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI  
 KVDLLAPMII  
 1651 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG  
 TLMVFFGNVD  
 1701 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL  
 NSCSMPLGME  
 1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP  
 QVNNPKEWLQ  
 1801 VDFQKTMKVT GVTTQGVKSL LTSMYVKEFL ISSSQDGHQW  
 TLFFQNGKVK  
 1851 VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM  
 EVLGCEAQDL  
 1901 YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV  
 TCVVVDVSHE  
 1951 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL  
 HQDWLNGKEY  
 2001 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT  
 KNQVSLTCLV  
 2051 KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK  
 LTVDKSRWQQ  
 2101 GNVFSCSVMH EALHNHYTQK SLSLSPGK\*

**pSYN FVIII 266 protein sequence (FVIII Fc with 42 AE-XTEN at amino acid 18 and 288 AE XTEN in B-domain) SEQ ID NO: 112**

1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQGAP  
 GSPAGSPTST  
 51 EEGTSESATP ESGPGSEPAT SGSETPASSS DLGELPVDAR  
 FPPRVPKSFP  
 101 FNTSVVYKKT LFVEFTDHLF NIAKPRPPWM GLLGPTIQAE  
 VYDTVVITLK  
 151 NMASHPVSLH AVGVSYWKAS EGA EYDDQTS QREKEDDKVF  
 PGGSHTYVWQ  
 201 VLKENGPMAS DPLCLTYSYL SHVDLVKDLN SGLIGALLVC  
 REGSLAKEKT  
 251 QTLHKFILLF AVFDEGKSWH SETKNSLMQD RDAASARAWP  
 KMHTVNGYVN  
 301 RSLPGLIGCH RKS VYWHVIG MGTTP EVHSI FLEGHTFLVR  
 NHRQASLEIS  
 351 PITFLTAQTL LMDLGQFLLF CHISSHQHDG MEAYVKVDSC  
 PEEPQLRMKN  
 401 NEEAEDYDDD LTDSEMDVVR FDDDNSPSFI QIRSVAKKHP  
 KTWVHYIAAE

451 EEDWDYAPLV LAPDDRSYKS QYLNNGPQRI GRKYKKVRFM  
 AYTDETFKTR  
 501 EAIQHESGIL GPLLYGEVGD TLLIIFKNQA SRPYNIYPHG  
 ITDVRPLYSR  
 551 RLPKGVKHLK DFPILPGEIF KYKWTVTVED GPTKSDPRCL  
 TRYIYSSFVNM  
 601 ERDLASGLIG PLLICYKESV DQRGNQIMSD KRNVIILFSVF  
 DENRSWYLTE  
 651 NIQRFLPNPA GVQLEDPEFQ ASNIMHSING YVFDSLQLSV  
 CLHEVAYWYI  
 701 LSIGAQTDFL SVFFSGYTFK HKMVYEDTLT LFPFSGETVF  
 MSMENPGLWI  
 751 LGCHNSDFRN RGMTALLKVS SCDKNTGDYY EDSYEDISAY  
 LLSKNNAIEP  
 801 RSFSQNGAPG TSESATPESG PGSEPATSGS ETPGTSESAT  
 PESGPGSEPA  
 851 TSGSETPGTS ESATPESGPG TSTEPSEGSA PGSPAGSPTS  
 TEEGTSESAT  
 901 PESGPGSEPA TSGSETPGTS ESATPESGPG SPAGSPTSTE  
 EGSPAGSPTS  
 951 TEEGTSTEPS EGSAPGTSES ATPESGPGTS ESATPESGPG  
 TSESATPESG  
 1001 PGSEPATSGS ETPGSEPATS GSETPGSPAG SPTSTEEGTS  
 TEPSEGSAPG  
 1051 TSTEPSEGSA PGSEPATSGS ETPGTSESAT PESGPGTSTE  
 PSEGSAPASS  
 1101 PPVLKRHQAE ITRTTLQSDQ EEIDYDDTIS VEMKKEDFDI  
 YDEDENQSPR  
 1151 SFQKKTRHYF IAAVERLWDY GMSSSPHVLN NRAQSGSVPO  
 FKKVVFQFT  
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 SRPYSFYSSL  
 1251 ISYEEDQORQG AEPRKNFVKP NETKTYFWKV QHHMAPTKDE  
 FDCKAWAYFS  
 1301 DVDLEKDVHS GLIGPLLCH TNTLNPAHGR QVTVQEFALF  
 FTIFDETKSW  
 1351 YFTENMERNC RAPCNIQMED PTFKENYRFH AINGYIMDTL  
 PGLVMAQDQR  
 1401 IRWYLLSMGS NENIHSIHFS GHVFTVRKKE EYKMALYNLY  
 PGVFETVEML  
 1451 PSKAGIWRVE CLIGEHLHAG MSTLFLVYSN KCQTPLGMAS  
 GHIRDFQITA  
 1501 SGQYGQWAPK LARLHYSISI NAWSTKEPFS WIKVDLLAPM  
 IIHGIKTQGA  
 1551 RQKFSSLYIS QFIIMYSLDG KKWQTYRGNS TGTLMVFFGN  
 VDSSGIKHNI  
 1601 FNPPIIARYI RLHPHTYSIR STLRMELMGC DLNSCSMPLG  
 MESKAISDAQ  
 1651 ITASSYFTNM FATWSPSKAR LHLQGRSNAW RPQVNNPKEW  
 LQVDFQKTMK

1701 VTGVTQTQGVK SLLTSMYVKE FLISSSQDGH QWTLFFQNGK  
 VKVFQGNQDS  
 1751 FTPVVNSLDP PLLTRYLRIH PQSWVHQIAL RMEVLGCEAQ  
 DLYDKTHTCP  
 1801 PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS  
 HEDPEVKFNW  
 1851 YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK  
 EYKCKVSNKA  
 1901 LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC  
 LVKGFYPSDI  
 1951 AVEWESNGQP ENNYKTPPV LDSDGSFFLY SKLTVDKSRW  
 QQGNVFSCSV  
 2001 MHEALHNHYT QKSLSLSPGK \*

**pSYN FVIII 267 protein sequence (FVIII Fc with 72 AE-XTEN at amino acid 18 and 288**

**AE XTEN in B-domain) SEQ ID NO: 113**

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQGAP  
 TSESATPESG  
 51 PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS  
 ESATPESGPG  
 101 TSTEPSEGS A PGASSSDLGE LPVDARFPPR VPKSFPFNTS  
 VVYKKTLEVE  
 151 FTDHLFNI AK PRPPWMGLLG PTIQAEVYDT VVITLKNMAS  
 HPVSLHAVGV  
 201 SYWKASEGAE YDDQTSQREK EDDKVFPFGS HTYVWQVLKE  
 NGPMASDPLC  
 251 LTYSYLSHVD LVKDLNSGLI GALLVCREGS LAKEKTQTLH  
 KFILLFAVFD  
 301 EGKSWHSETK NSLMQDRDAA SARAWPKMHT VNGYVNRSLP  
 GLIGCHRKSV  
 351 YWHVIGMGT PEVHSIFLEG HTFLVRNHRQ ASLEISPITF  
 LTAQTLLMDL  
 401 GQFLLFCHIS SHQHDGMEAY VKVDSCPEEP QLRMKNNEEA  
 EDYDDDLTDS  
 451 EMDVVRFD DD NSPSFIQIRS VAKKHPKTWV HYIAAEEEDW  
 DYAPLV LAPD  
 501 DRSYKSQYLN NGPQRIGRKY KKVRFMAYTD ETFKTREAIQ  
 HESGILGPLL  
 551 YGEVGD TLLI IFKNQASRPY NIYPHGITDV RPLYSRRLPK  
 GVKHLKDFPI  
 601 LPGEIFKYKW TVTVEDGPTK SDPRCLTRY SSFVNMERDL  
 ASGLIGPLLI  
 651 CYKESVDQRG NQIMSDKRN ILFSVFDENR SWYLTENIQR  
 FLPNPAGVQL  
 701 EDPEFQASNI MHSINGYVFD SLQLSVCLHE VAYWYILSIG  
 AQTDFLSVFF  
 751 SGYTFKHKMV YEDTLTLFPF SGETVFMSME NPGLWILGCH  
 NSDFRNRGMT  
 801 ALLKVSSCDK NTGDYYEDSY EDISAYLLSK NNAIEPRSF  
 QNGAPGTSES

851 ATPESGPGSE PATSGSETPG TSESATPESG PGSEPATSGS  
 ETPGTSESAT  
 901 PESGPGTSTE PSEGSA PGSP AGSPTSTEEG TSESATPESG  
 PGSEPATSGS  
 951 ETPGTSESAT PESGPGSPAG SPTSTEEGSP AGSPTSTEEG  
 TSTEPSEGS  
 1001 PGTSESATPE SGPGTSESAT PESGPGTSES ATPESGPGSE  
 PATSGSETPG  
 1051 SEPATSGSET PGSPAGSPTS TEEGTSTEPS EGSAPGTSTE  
 PSEGSA PGSE  
 1101 PATSGSETPG TSESATPESG PGTSTEPSEG SAPASSPPVL  
 KRHQAEITRT  
 1151 TLQSDQEEID YDDTISVEMK KEDFDIYDED ENQSPRSFQK  
 KTRHYFIAAV  
 1201 ERLWDYGMSS SPHVLRNRAQ SGSVPQFKKV VFQEFDTGSGF  
 TQPLYRGELN  
 1251 EHLGLLGPYI RAEVEDNIMV TFRNQASRPY SFYSSLISYE  
 EDQRQGAEP  
 1301 KNFVKPNETK TYFWKVQHMH APTKDEFDCK AWAYFSDVDL  
 EKDVHSGSLIG  
 1351 PLLVCHTNTL NPAHGRQVTV QEFALFFTIF DETKSWYFTE  
 NMERNCRAPC  
 1401 NIQMEDPTFK ENYRFHAING YIMDTLPGLV MAQDQRIRWY  
 LLSMGSNENI  
 1451 HSIHFSGHVF TVRKKEEYKM ALYNLYPGVF ETVEMLP  
 SKA  
 1501 EHLHAGMSTL FLVYSNKCQT PLGMASGHIR DFQITASGQY  
 GQWAPKLARL  
 1551 HYSGSINAWS TKEPFSWIKV DLLAPMIIHG IKTQGARQKF  
 SSLYISQFII  
 1601 MYSLDGKKWQ TYRGNSTGTL MVFFGNVDSS GIKHNIFNPP  
 IIARYIRLHP  
 1651 THYSIRSTLR MELMGCDLNS CSMPLGMESK AISDAQITAS  
 SYFTNMFATW  
 1701 SPSKARLHLQ GRSNAWRPQV NNPKEWLQVD FQKTMKVTGV  
 TTQGVKSLLT  
 1751 SMYVKEFLIS SSQDGHQWTL FFQNGKVQVF QGNQDSFTPV  
 VNSLDPPLLT  
 1801 RYLRIHPQSW VHQIALRMEV LGCEAQDLYD KTHTCPPCPA  
 PELLGGPSVF  
 1851 LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG  
 VEVHNAKTKP  
 1901 REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNAKALPAP  
 IEKTISKAKG  
 1951 QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW  
 ESNGQPENNY  
 2001 KTTTPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA  
 LHNHYTQKSL  
 2051 SLSPGK\*

**pSYN FVIII 268 protein sequence (FVIII Fc with 144 AE-XTEN at amino acid 18) SEQ ID NO: 114**

```

1  MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQGAP
   TSESATPESG
51  PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS
   ESATPESGPG
101 TSTEPSEGS A PGSPAGSPTS TEEGTSESAT PESGPGSEPA
   TSGSETPGTS
151 ESATPESGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL
   GELPVDARFP
201 PRVPKSFPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL
   LGPTIQAEVY
251 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR
   EKEDDKVFPG
301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG
   LIGALLVCRE
351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD
   AASARAWPKM
401 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL
   EGHTFLVRNH
451 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME
   AYVKVDSCPE
501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI
   RSVAKKHPKT
551 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR
   KYKKVRFMAY
601 TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR
   PYNIYPHGIT
651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP
   TKSDPRCLTR
701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR
   NVILFSVFDE
751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV
   FDSLQLSVCL
801 HEVAYWYILS IGAQTDFLSV FFSGYT FKHK MVYEDTLTLF
   PFSGETVFM S
851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED
   SYEDISAYLL
901 SKNNAIEPRS FSQNPPVLKR HQAEITRTTL QSDQEEIDYD
   DTISVEMKKE
951 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP
   HVLNRNRAQSG
1001 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA
   EVEDNIMVTF
1051 RNQASRPYSF YSSLISYEED QRQGAEP RKN FVKPNETKTY
   FWKVQH HMAP
1101 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP
   AHGRQVT VQE
1151 FALFFTIFDE TKS WYFTENM ERNCRAPCNI QMEDPTFKEN
   YRFHAINGYI

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1201 MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV  
 RKKEEYKMAL  
 1251 YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL  
 VYSNKCQTPL  
 1301 GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK  
 EPFSWIKVDL  
 1351 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY  
 RGNSTGTLMV  
 1401 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME  
 LMGCDLNSCS  
 1451 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR  
 SNAWRPQVNN  
 1501 PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS  
 QDGHQWTLFF  
 1551 QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH  
 QIALRMEVLG  
 1601 CEAQDLYDKT HTPPCPAPE LLGGPSVFLF PPKPKDTLMI  
 SRTPEVTCVV  
 1651 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV  
 SVLTVLHQDW  
 1701 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP  
 SRDELTKNQV  
 1751 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDS DGS  
 FFLYSKLTVD  
 1801 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK\*

**pSYN FVIII 269 protein sequence (FVIII Fc with 72 AE-XTEN at amino acid 18) SEQ ID NO: 115**

1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQGAP  
 TSESATPESG  
 51 PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS  
 ESATPESGPG  
 101 TSTEPSEGS A PGASSSDLGE LPVDARFPPR VPKSFPFNTS  
 VVYKKTLEVE  
 151 FTDHLFNIAK PRPPWMGLLG PTIQAEVYDT VVITLKNMAS  
 HPVSLHAVGV  
 201 SYWKASEGAE YDDQTSQREK EDDKVFP GGS HTYVWQVLKE  
 NGPMASDPLC  
 251 LTYSYLSHVD LVKDLNSGLI GALLVCREGS LAKEKTQTLH  
 KFILLFAVFD  
 301 EGKSWHSETK NSLMQDRDAA SARAWPKMHT VNGYVNRSLP  
 GLIGCHRKSV  
 351 YWHVIGMGT T PEVHSIFLEG HTFLVRNHRQ ASLEISPITF  
 LTAQTLLMDL  
 401 GQFLLFCHIS SHQHDGMEAY VKVDSCPEEP QLRMKNNEEA  
 EDYDDDLTDS  
 451 EMDVVRFD D NSPSFIQIRS VAKKHPKTWV HYIAAEEEDW  
 DYAPLVLAPD  
 501 DRSYKSQYLN NGPQRIGRKY KKVRFMAYTD ETFKTREAIQ  
 HESGILGPLL

551 YGEVGDTHLLI IFKNQASRPY NIYPHGITDV RPLYSRRLPK  
 GVKHLKDFPI  
 601 LPGEIFKYKW TVTVEDGPTK SDPRCLTRY Y SSFVNMERDL  
 ASGLIGPLLI  
 651 CYKESVDQRG NQIMSDKRN ILFSVFDENR SWYLTENIQR  
 FLPNPAGVQL  
 701 EDPEFQASNI MHSINGYVFD SLQLSVCLHE VAYWYILSIG  
 AQTDFLSVFF  
 751 SGYTFKHKHMV YEDTLTLFPF SGETVFMSME NPGLWILGCH  
 NSDFRNRGMT  
 801 ALLKVSSCDK NTGDYYEDSY EDISAYLLSK NNAIEPRSFS  
 QNPPVLKRHQ  
 851 AEITRTHLQS DQEEIDYDDT ISVEMKKEDF DIYDEDENQS  
 PRSFQKKTRH  
 901 YFIAAVERLW DYGMSSSPHV LRNRAQSGSV PQFKKVVVFQE  
 FTDGSFTQPL  
 951 YRGELNEHLG LLGPYIRAEV EDNIMVTFRN QASRPYSFYS  
 SLISYEEDQR  
 1001 QGAEPKRFV KPNETKTYFW KVQHMAPTK DEFDCAWAY  
 FSDVDLEKDV  
 1051 HSGLIGPLLV CHTNTLNPAH GRQVTVQEFA LFFTIFDETK  
 SWYFTENMER  
 1101 NCRAPCNIQM EDPTFKENYR FHAINGYIMD TLPGLVMAQD  
 QRIRWYLLSM  
 1151 GSNENIHSIH FSGHVFTVRK KEEYKMALYN LYPGVFETVE  
 MLPSKAGIWR  
 1201 VECLIGEHLH AGMSTLFLVY SNKCQTPLGM ASGHIRDFQI  
 TASGQYGQWA  
 1251 PKLARLHYS SINAWSTKEP FSWIKVDLLA PMIIHGKTQ  
 GARQKFSSLY  
 1301 ISQFIIMYSL DGKKWQTYRG NSTGTLMVFF GNVDSSGIKH  
 NIFNPPIAR  
 1351 YIRLHPHYS IRSTLRMELM GCDLNSCSMP LGMESKAISD  
 AQITASSYFT  
 1401 NMFATWSPSK ARLHLQGRSN AWRPQVNNPK EWLQVDFQKT  
 MKVTGVTTQG  
 1451 VKSLLTSMYV KEFLISSQD GHQWTLFFQN GKVKVFQGNQ  
 DSFTPVVNSL  
 1501 DPPLLTRYLR IHPQSWVHQI ALRMEVLGCE AQDLYDKTHT  
 CPPCPAPELL  
 1551 GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF  
 NWYVDGVEVH  
 1601 NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN  
 KALPAPIEKT  
 1651 ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS  
 DIAVEWESNG  
 1701 QPENNYKTHP PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC  
 SVMHEALHNN  
 1751 YTQKSLSLSP GK\*

**pSYNFVIII 271 protein sequence (FVIII Fc with 42 AE-XTEN at amino acid 18) SEQ ID NO: 116**

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1  MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQGAP
   GSPAGSPTST
51  EEGTSESATP ESGPGSEPAT SGSETPASSS DLGELPVDAR
   FPPRVPKSFP
101 FNTSVVYKKT LFVEFTDHLF NIAKPRPPWM GLLGPTIQAE
   VYDTVVITLK
151 NMASHPVSLH AVGVSYWKAS EGAEYDDQTS QREKEDDKVF
   PGGSHTYVWQ
201 VLKENGPMAS DPLCLTYSYL SHVDLVKDLN SGLIGALLVC
   REGSLAKEKT
251 QTLHKFILLF AVFDEGKSWH SETKNLSMQD RDAASARAWP
   KMHTVNGYVN
301 RSLPGLIGCH RKSVMYWHVIG MGTTPVHESI FLEGHTFLVR
   NHRQASLEIS
351 PITFLTAQTL LMDLGQFLLF CHISSHQHDG MEAYVKVDSC
   PEEPQLRMKN
401 NEEAEDYDDD LTDSEMDVVR FDDDNPSFSI QIRSVAKKHP
   KTWVHYIAAE
451 EEDWDYAPLV LAPDDRSYKS QYLNNGPQRI GRKYKKVRFM
   AYTDETFKTR
501 EAIQHESGIL GPLLYGEVGD TLLIIFKNQA SRPYNIYPHG
   ITDVRPLYSR
551 RLPKGVKHLK DFPILPGEIF KYKWTVTVED GPTKSDPRCL
   TRYYSFVNM
601 ERDLASGLIG PLLICYKESV DQRGNQIMSD KRNVILFSVF
   DENRSWYLTE
651 NIQRFLPNPA GVQLEDPEFQ ASNIMHSING YVFDSLQLSV
   CLHEVAYWYI
701 LSIGAQTDFL SVFFSGYTFK HKMVYEDTLT LFPFSGETVF
   MSMENPGLWI
751 LGCHNSDFRN RGMTALLKVS SCDKNTGDYY EDSYEDISAY
   LLSKNNAIEP
801 RSFSQNPPVL KRHQAEITRT TLQSDQEEID YDDTISVEMK
   KEDFDIYDED
851 ENQSPRSFQK KTRHYFIAAV ERLWDYGMSS SPHVLRNRAQ
   SGSVPQFKKV
901 VFQEFTDGSF TQPLYRGELN EHLGLLGPYI RAEVEDNIMV
   TFRNQASRPY
951 SFYSSLISYE EDQRQGAEPR KNFVKPNETK TYFWKVQHMM
   APTKDEFDCK
1001 AWAYFSDVDL EKDVHSGDIG PLLVCHTNLT NPAHGRQVTV
   QEFALFFTIF
1051 DETKSWYFTE NMERNCRAPC NIQMEDPTFK ENYRFHAING
   YIMDTLPGLV
1101 MAQDQRIRWY LLSMGSNENI HSIHFSGHVF TVRKKEEYKM
   ALYNLYPGVF
1151 ETVEMLPSKA GIWRVECLIG EHLHAGMSTL FLVYSNKCQT
   PLGMASGHIR

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1201 DFQITASGQY GQWAPKLARL HYSGSINAWs TKEPFSWIKV  
 DLLAPMIIHG  
 1251 IKTQGARQKF SSLYISQFII MYSLDGKKWQ TYRGNSTGTL  
 MVFFGNVDSS  
 1301 GIKHNIFNPP IIARYIRLHP THYSIRSTLR MELMGCDLNS  
 CSMPLGMESK  
 1351 AISDAQITAS SYFTNMFATW SPSKARLHLQ GRSNAWRPQV  
 NNPKEWLQVD  
 1401 FQKTMKVTGV TTQGVKSLLT SMYVKEFLIS SSQDGHQWTL  
 FFQNGKVKVF  
 1451 QGNQDSFTPV VNSLDPPLLT RYLRIHPQSW VHQIALRMEV  
 LGCEAQDLYD  
 1501 KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC  
 VVVDVSHEDP  
 1551 EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ  
 DWLNGKEYKC  
 1601 KVSNAKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN  
 QVSLTCLVKG  
 1651 FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT  
 VDKSRWQQGN  
 1701 VFSCSVMHEA LHNHYTQKSL SLSPGK\*

**pSYN FVIII protein sequence 272 ( FVIII with 144 AE XTEN at amino acid 18 and 244 AE XTEN in B-domain- no Fc) SEQ ID NO: 117**

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQGAP  
 TSESATPESG  
 51 PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS  
 ESATPESGPG  
 101 TSTEPSEGS A PGSPAGSPTS TEEGTSESAT PESGPGSEPA  
 TSGSETPGTS  
 151 ESATPESGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL  
 GELPVDARFP  
 201 PRVPKSFPFN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL  
 LGPTIQAEVY  
 251 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR  
 EKEDDKVFPG  
 301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG  
 LIGALLVCRE  
 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD  
 AASARAWPKM  
 401 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL  
 EGHTFLVRNH  
 451 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME  
 AYVKVDSCPE  
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI  
 RSVAKKHPKT  
 551 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR  
 KYKKVRFMAY  
 601 TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR  
 PYNIPHGIT

651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP  
 TKSDPRCLTR  
 701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR  
 NVILFSVFDE  
 751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV  
 FDSLQLSVCL  
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFFKHK MUYEDTLTLF  
 PFSGETVFMS  
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYED  
 SYEDISAYLL  
 901 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET  
 PGTSESATPE  
 951 SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG  
 SPAGSPTSTE  
 1001 EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP  
 AGSPTSTEEG  
 1051 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES  
 ATPESGPGTS  
 1101 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP  
 TSTEEGTSTE  
 1151 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE  
 SGPGTSTEPS  
 1201 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE  
 MKKEDFDIYD  
 1251 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR  
 AQSGSVPPQFK  
 1301 KVVFEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI  
 MVTFRNQASR  
 1351 PYSFYSSLIS YEEDQRQGA E PRKNFVKPNE TKTYFWKVQH  
 HMAPTKDEFD  
 1401 CKAWAYFSDV DLEKDVHSG L IGPLLVC HTN TLNPAHGRQV  
 TVQEFALFFT  
 1451 IFDETKSWYF TENMERN CRA PCNIQMEDPT FKENYRFHAI  
 NGYIMDTLPG  
 1501 LVMAQDQRIR WYLLSMGSNE NIHSIHFS GH VFTVRKKEEY  
 KMALYNLYPG  
 1551 VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC  
 QTPLGMASGH  
 1601 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI  
 KVDLLAPMII  
 1651 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG  
 TLMVFFGNVD  
 1701 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL  
 NSCSMPLGME  
 1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP  
 QVNNPKEWLQ  
 1801 VDFQKTMKVT GVT TQGVKSL LTS MYVKEFL ISSSQDGHQW  
 TLFFQNGKVK  
 1851 VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM  
 EVLGCEAQDL  
 1901 Y\*

**pSYN VWF 031 protein sequence ( VWF D1D2D'D3- 48aa long thrombin cleavable GS****linker-Fc) SEQ ID NO: 118**

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1   MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS
   DFVNTFDGSM
51  YSFAGYCSYL LAGGCQKRSE SIIGDFQNGK RVSLSVYLGE
   FFDIHLFVNG
101 TVTQGDQRRS MPYASKGLYL ETEAGYYKLS GEAYGFVARI
   DGSGNFQVLL
151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS
   WALSSGEQWC
201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL
   VDPEPFVALC
251 EKTLCCEAGG LECACPALLE YARTCAQEGM VLYGWDTHSA
   CSPVCPAGME
301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG
   LCVESTECPC
351 VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECVL
   TGQSHFKSFD
401 NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC
   TRSVTVRLPG
451 LHNSLVKLKH GAGVAMDQD IQLPLLKGDL RIQHTVTASV
   RLSYGEDLQM
501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG
   LAEPRVEDFG
551 NAWKLHGDCQ DLQKQHSDDC ALNPRMTRFS EEACAVLTSP
   TFEACHRAVS
601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV
   AWREPGRCEL
651 NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP
   PGLYMDERGD
701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM
   SGVPGSLLPD
751 AVLSSPLSHR SKRSLSCRPP MVKLVCPADN LRAEGLECTK
   TCQNYDLECM
801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE
   TVKIGCNTCV
851 CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ
   YVLVQDYCGS
901 NPGTFRILVG NKGCSHPSVK CKKRVITLVE GGEIELFDGE
   VNVKRPMKDE
951 THFEVVESGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE
   KVCGLCGNFD
1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD
   SSPATCHNNI
1051 MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS
   CESIGDCAAF
1101 CDTIAAYAHV CAQHKGKVTW RTATLCPQSC EERNLRENGY
   EAEWRYNSCA

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1151 PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC  
 VDPEDCPVCE  
 1201 VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP  
 ISGGGGSGGG  
 1251 GSGGGGSGGG GSGGGGSGGG GSLVPRGSGG GSGGGGSDK  
 THTCPPCPAP  
 1301 ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE  
 VKFNWYVDGV  
 1351 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK  
 VSNKALPAPI  
 1401 EKTISKAKGQ PREPQVYTLF PSRDELTKNQ VSLTCLVKGF  
 YPSDIAVEWE  
 1451 SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV  
 FSCSVMHEAL  
 1501 HNHYTQKSLS LSPGK\*

**pSYN VWF 034 protein sequence ( VWF D1D2D'D3- 288AE XTEN- 35aa long thrombin cleavable GS linker-Fc) SEQ ID NO: 119**

1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS  
 DFVNTFDGSM  
 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE  
 FFDIHLFVNG  
 101 TVTQGDQRV MPYASKGLYL ETEAGYYKLS GEAYGFVARI  
 DGSGNFQVLL  
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS  
 WALSSGEQWC  
 201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL  
 VDPEPFVALC  
 251 EKTLCCEAGG LECACPALLE YARTCAQEGM VLYGWTDHSA  
 CSPVCPAGME  
 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG  
 LCVESTECPC  
 351 VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV  
 TGQSHFKSFD  
 401 NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC  
 TRSVTVRLPG  
 451 LHNSLVKLKH GAGVAMDGQD IQLPLLKGD LRIQHTVTASV  
 RLSYGEDLQM  
 501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG  
 LAEPRVEDFG  
 551 NAWKLHGDCQ DLQKQHSDPC ALNPRMTRFS EEACAVLTSP  
 TFEACHRAVS  
 601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV  
 AWREPGRCEL  
 651 NCPKGQVYLQ CGTPCNLTCT SLSYPDEECN EACLEGCFCP  
 PGLYMDERGD  
 701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM  
 SGVPGSLLPD  
 751 AVLSSPLSHR SKRSLSCRPP MVKLVC PADN LRAEGLECTK  
 TCQNYDLECM

801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE  
 TVKIGCNTCV  
 851 CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGEQ  
 YVLVQDYCGS  
 901 NPGTFRILVG NKGCSHPSVK CKKRVITLVE GGEIELFDGE  
 VNVKRPMKDE  
 951 THFEVVESGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE  
 KVCGLCGNFD  
 1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD  
 SSPATCHNNI  
 1051 MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS  
 CESIGDCAAF  
 1101 CDTIAAYAHV CAQHKGKVVW RTATLCPQSC EERNLRENGY  
 EAEWRYNCSA  
 1151 PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC  
 VDPEDCPVCE  
 1201 VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP  
 ISGTSESATP  
 1251 ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP  
 GTSESATPES  
 1301 GPGTSTEPSE GSAPGSPAGS PTSTEEGTSE SATPESGPGS  
 EPATSGSETP  
 1351 GTSESATPES GPGSPAGSPT STEEGSPAGS PTSTEEGTST  
 EPSEGSAPGT  
 1401 SESATPESGP GTSESATPES GPGTSESATP ESGPGSEPAT  
 SGSETPGSEP  
 1451 ATSGSETPGS PAGSPTSTEE GTSTEPSEGS APGTSTEPSE  
 GSAPGSEPAT  
 1501 SGSETPGTSE SATPESGPGT STEPSEGSAP DIGGGGGSGG  
 GGS LVPRGSG  
 1551 GDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV  
 TCVVVDVSHE  
 1601 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL  
 HQDWLNGKEY  
 1651 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT  
 KNQVSLTCLV  
 1701 KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK  
 LTVDKSRWQQ  
 1751 GNVFSCSVMH EALHNHYTQK SLSLSPGK\*

**pSYN VWF 036 protein sequence ( VWF D1D2D'D-98aa long thrombin cleavable GS**

**linker-Fc) SEQ ID NO: 120**

1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS  
 DFVNTFDGSM  
 51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE  
 FFDIHLFVNG  
 101 TVTQGDQRV S MPYASKGLYL ETEAGYYKLS GEAYGFVARI  
 DGSGNFQVLL  
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS  
 WALSSGEQWC



201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL  
 VDPEPFVALC  
 251 EKTLCCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA  
 CSPVCPAGME  
 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG  
 LCVESTECPC  
 351 VHS GKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECVL  
 TGQSHFKSFD  
 401 NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC  
 TRSVTVRLPG  
 451 LHNSLVKLKH GAGVAMDGD IQLPLLKGD LRIQHTVTASV  
 RLSYGEDLQM  
 501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG  
 LAEPRVEDFG  
 551 NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP  
 TFEACHRAVS  
 601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV  
 AWREPGRCEL  
 651 NCPKGQVYLQ CGTPCNLTCT SLSYPDEECN EACLEGCFCP  
 PGLYMDERGD  
 701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM  
 SGVPGSLLPD  
 751 AVLSSPLSHR SKRSLSCRPP MVKLVC PADN LRAEGLECTK  
 TCQNYDLECM  
 801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE  
 TVKIGCNTCV  
 851 CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGEQ  
 YVLVQDYCGS  
 901 NPGTFRILVG NKGCSHPSVK CKKRVTLVE GGEIELFDGE  
 VNVKRPMKDE  
 951 THFEVVESGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE  
 KVCGLCGNFD  
 1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD  
 SSPATCHNNI  
 1051 MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS  
 CESIGDCAAF  
 1101 CDTIAAYAHV CAQH GKVV TW RTATLCPQSC EERNLRENGY  
 EAEWRYNSCA  
 1151 PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC  
 VDPEDCPVCE  
 1201 VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP  
 ISGGGGSGGG  
 1251 GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG  
 GSGGGGSGGG  
 1301 GSGGGGSGGG GSGGGGSGGG GSLVPRGSGG GSGGGGSGDK  
 THTCPPCPAP  
 1351 ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE  
 VKFNWYVDGV  
 1401 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK  
 VSNKALPAPI

1451 EKTISKAKGQ PREPQVYTL P SRDELTKNQ VSLTCLVKGF  
 YPSDIAVEWE  
 1501 SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV  
 FSCSVMHEAL  
 1551 HNHYTQKSLS LSPGK\*

**pSYN Fc-015 protein sequence ( IgG-Fc domain) SEQ ID NO: 121**

1 METDTLLLWV LLLWVPGSTG DKHTCPPCP APELLGGPSV  
 FLFPPKPKDT  
 51 LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK  
 PREEQYNSTY  
 101 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK  
 GQPREPQVYT  
 151 LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN  
 YKTPPVLDSD  
 201 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS  
 LSLSPGK\*

Example 15: FVIII-XTEN-Fc:VWF-Fc heterodimers have maintained normal FVIII specific activity as compared to wild type BDD-FVIII.

[0383] The FVIII specific activity of FVIII-XTEN-Fc:VWF-Fc heterodimers were determined. Heterodimers were purified using a two-step chromatography process. A weak anion exchange resin was used, followed by affinity chromatography. The final purified product had acceptable purity by SEC-HPLC. The specific activity was compared to B-domain deleted FVIII (BDD-FVIII), as measured by FVIII chromogenic assay and A280 concentration. The data are presented in Table 26. All tested molecules had demonstrated comparable FVIII specific activities to BDD-FVIII. Purity and the presence of each moiety of the molecules were confirmed by SDS-PAGE and western blotting.

**Table 26: FVIII specific activity of FVIII-XTEN-Fc:VWF-Fc heterodimers**

| Construct                                     | FVIII 207<br>scBDDFVIII | FVIII-66<br>dcBDD<br>FVIII) | FVIII<br>155 /<br>vWF31 | FVIII<br>155 /<br>vWF39 | FVIII<br>169 /<br>vWF31 | FVIII<br>205 /<br>vWF31 | FVIII<br>169 /<br>vWF34 |
|-----------------------------------------------|-------------------------|-----------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Measured<br>Specific<br>Activity<br>(IU/nmol) | 1473                    | 1592                        | 1534                    | 1796                    | 1511                    | 1345                    | 1505                    |

[0384] The half-lives of rFVIII-XTEN/D'D3 and BDD-FVIII were compared in HemA Mice (Figure 15; Table 27). As Figure 15 shows, rFVIII-XTEN/D'D3

achieved a half-life that was four fold longer than the half-life achieved by BDD-FVIII.

**Table 27: rFVIII-XTEN/D'D3 and BDD-FVIII in Hema mice**

| Treatment        | 5 minutes Recovery (%) | HL (hr) | MRT (hr) | Cl (mL/hr/kg) | Vss (mL/kg) | AUC <sub>D</sub> (hr*kg*mIU/mL/mIU) |
|------------------|------------------------|---------|----------|---------------|-------------|-------------------------------------|
| BDD-FVIII        | 89                     | 7.6     | 11       | 4.5           | 49.2        | 0.22                                |
| rFVIII-Fc        | 78                     | 16      | 20       | 2.9           | 57.8        | 0.35                                |
| rFVIII-XTEN/D'D3 | 86                     | 30      | 36       | 1.8           | 63.4        | 0.57                                |

Example 16: FVIII-XTEN-Fc:VWF-Fc heterodimer's potency (FVIII activity) in hemostasis as measured by one stage aPTT assay

[0385] The potency of FVIII-XTEN-Fc:VWF-Fc heterodimers in hemostasis was evaluated by their FVIII specific aPTT activity as summarized in Table 28. As demonstrated by Table 28, while the addition of the VWF D'D3 fragment and the insertion of XTEN into the intra-domains of FVIII reduce the FVIII specific aPTT activity of the heterodimers (as indicated by the FVIII155/VWF031 data and the FVIII205/VWF031 data), XTEN insertions in the FVIII B domain region or C-terminus of the VWF D'D3 fragment have no negative effect on the FVIII specific aPTT activity (as indicated by the FVIII169/VWF031 data and the FVIII169/VWF034 data). Compared to dual-chain BDD-FVIII (dcBDD-FVIII), FVIII155/VWF031, FVIII169/VWF031, FVIII169/VWF034 and VWF205/VWF031 showed reduction of specific aPTT activity by 2.5-fold, 2.8-fold, 2.6-fold and 5.5-fold, respectively.

**Table 28: FVIII specific aPTT activity of FVIII-XTEN-Fc:VWF-Fc heterodimers**

| Construct                                 | FVIII 207 scBDD-FVIII | FVIII-66 dcBDD-FVIII | FVIII 155 / VWF31 | FVIII 169 / VWF31 | FVIII 205 / VWF31 | FVIII 169 / VWF34 |
|-------------------------------------------|-----------------------|----------------------|-------------------|-------------------|-------------------|-------------------|
| Measured Specific aPTT Activity (IU/nmol) | 818 ± 153             | 1188 ± 213           | 448 ± 111         | 416 ± 70          | 214 ± 38          | 436 ± 189         |

FVIII specific aPTT assay

[0386] FVIII variants were diluted with aPTT buffer (0.15 M NaCl, 0.05 M Tris-HCl, 1% BSA, pH 7.4) to the linear assay range (200- 1.6 mU/mL). 50  $\mu$ L of diluted samples or standards were sequentially mixed with 50  $\mu$ L of 37°C naïve human HemA pooled plasma, 50  $\mu$ L of 37°C aPTT reagent (ACTIN® FSL activated cephaloplastin reagent - Dade Behring, reference # B4219-2) and incubated at 37 °C for 4 minutes. 50  $\mu$ L of 20 mM CaCl<sub>2</sub> (Dade Behring [reference # ORFO37]) was then added to the reaction mixture to start the clotting reactions. Using the clotting time of each sample (the length of time from the addition of CaCl<sub>2</sub> until the onset of clot formation), the aPTT activity was calculated against the standard that was generated with the 8<sup>th</sup> international standard FVIII concentrate. Specific aPTT activity was calculated against the protein concentration of each molecule that measured by OD280.

Example 17: *In vivo* efficacy of FVIII-XTEN-Fc:VWF-Fc heterodimer in HemA mice Tail Clip bleeding model

[0387] To further access the hemostasis potency of the heterodimers, the acute efficacy of FVIII169/VWF034 and FVIII205/VWF031 was evaluated in comparison with BDD-FVIII in the HemA mice Tail clip bleeding model. HemA mice were treated with a single IV injection of BDD-FVIII at 200, 65 and 20 IU/kg to generate the post tail clip injury blood loss control level. Blood loss from mice treated with 200 IU/kg of FVIII169/VWF034 or FVIII205/VWF031 was compared to that of the BDD-FVIII treated control group mice to estimate their potency on hemostasis. Vehicle treated animals were used to generate blood loss baseline for the model. As shown in Figure 16, significant reduction in blood loss was observed from all FVIII treatment groups compared to that of the vehicle treated animals ( $p < 0.05$ ). Both FVIII169/VWF034 and FVIII205/VWF031 are efficacious in the HemA mice Tail Clip model. Compared to BDD-FVIII, about 3 fold lower potency was observed for FVIII169/VWF034, as demonstrated by the similar blood loss reduction achieved by 65 IU/kg BDD-FVIII and 200 IU/kg FVIII169/VWF034. As for FVIII205/VWF034, a 10 fold potency reduction has been observed, as demonstrated by the similar blood loss reduction achieved by 20 IU/kg BDD-FVIII and 200 IU/kg FVIII205/VWF031.

[0388] Even though FVIII69/VWF034 and FVIII205/VWF031 had similar specific FVIII chromogenic activity compared to rBDD-FVIII, their FVIII aPTT activity and *in vivo* potency were both reduced due to the modifications of the molecules. Those data indicate that the aPTT activity of a FVIII molecule is a more accurate measurement on predicating its *in vivo* potency on hemostasis than the FVIII chromogenic activity.

HemA mice Tail clip bleeding model

[0389] 8-10 weeks old male HemA mice were used for the study. Prior to tail clip injury, mice were anesthetized with a 50 mg/kg Ketamine/0.5 mg/kg Dexmedetomidine cocktail and placed on a 37°C heating pad to help maintain the body temperature. The tails of the mice were then be immersed in 37°C water for 10 minutes to dilate the lateral vein. After vein dilation, rFVIII or vehicle solution were injected via the tail vein and 5 min later, the distal 1 cm of the tail was cut off using a #11 scalpel with straight edge. The shed blood was collected into 13 ml of 37°C warm saline for 30 minutes and the mice were then euthanized while still under anesthesia by bilateral thoracotomy. Blood loss was quantified gravimetrically by weight change of the blood collection tubes before and after blood was collected in gram, which translated into milliliter (mL) of blood loss volume (1g weight change = 1 mL blood loss).

[0390] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0391] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as

exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

[0392] All patents and publications cited herein are incorporated by reference herein in their entirety.

[0393] The term “comprise” and variants of the term such as “comprises” or “comprising” are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

[0394] Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge in Australia.

## CLAIMS

1. A chimeric protein comprising (i) a von Willebrand Factor (VWF) fragment comprising the D' domain and the D3 domain of VWF, (ii) an extended length polypeptide (XTEN) sequence, and (iii) a factor VIII (FVIII) protein, wherein the VWF fragment and the XTEN sequence are linked by an optional linker and wherein the VWF fragment is linked to or associated with the FVIII protein by a covalent bond.
2. The chimeric protein of claim 1, which comprises
  - (a) a single polypeptide chain comprising the VWF fragment, the XTEN sequence and the FVIII protein;
  - (b) two polypeptide chains comprising a first polypeptide chain and a second polypeptide chain, wherein the first polypeptide chain comprises the FVIII protein and the second polypeptide chain comprises the VWF fragment and the XTEN sequence; or
  - (c) two polypeptide chains comprising a first polypeptide chain and a second polypeptide chain, wherein the first polypeptide chain comprises the FVIII protein and the XTEN sequence and the second polypeptide chain comprises the VWF fragment.
3. The chimeric protein of claim 1 or claim 2, further comprising (iv) an immunoglobulin (Ig) constant region or a portion thereof linked to either the VWF fragment, the XTEN sequence, the FVIII protein, or any combination thereof.
4. The chimeric protein of claim 1, comprising a formula selected from the group consisting of:
  - (a) V-X-FVIII;
  - (b) FVIII-X-V;
  - (c) V-X:FVIII;
  - (d) X-V:FVIII;
  - (e) FVIII:V-X;
  - (f) FVIII:X-V;
  - (g) V-L2-X-L1-F1:FVIII-L3-F2;
  - (h) V-L2-X-L1-F1:F2-L3-FVIII;
  - (i) F1-L1-X-L2-V: FVIII-L3-F2;

- (j) F1-L1-X-L2-V:F2-L3-FVIII;
- (k) V-L2-X-L1-F1-L4-FVIII-L3-F2;
- (l) F2-L3-FVIII-L4-F1-L1-X-L2-V;
- (m) FVIII-L3-F2-L4-V-L2-X-L1-F1; and
- (n) F1-L1-X-L2-V-L4-F2-L3-FVIII,

wherein V comprises a VWF fragment;

each of L1, L2, and L3 comprises an optional linker;

L4 is an optional linker;

FVIII comprises a FVIII protein;

X comprises one or more XTEN sequences;

F1 comprises an optional Ig constant region or a portion thereof;

F2 comprises an optional additional Ig constant region or a portion thereof;

(-) is a peptide bond or one or more amino acids; and

(:) is a covalent bond.

5. The chimeric protein of claim 3 or claim 4, wherein the Ig constant region or a portion thereof comprises a first Fc region, which is linked to the XTEN sequence or the VWF fragment.
6. The chimeric protein of any one of claims 3 to 5, wherein the Ig constant region or a portion thereof is linked to the XTEN sequence by a linker.
7. The chimeric protein of claim 6, wherein the linker between the Ig constant region or a portion thereof and the XTEN sequence comprises a cleavable linker.
8. The chimeric protein of any one of claims 3 to 7, further comprising an additional Ig constant region or a portion thereof.
9. The chimeric protein of claim 8, wherein the additional Ig constant region or a portion thereof comprises a second Fc region.
10. The chimeric protein of claim 8 or claim 9, wherein the additional Ig constant region or a portion thereof is linked to the FVIII protein.
11. The chimeric protein of claim 9 or claim 10, wherein the second Fc region is further linked to the VWF fragment by a linker.



12. The chimeric protein of claim 9 or claim 10, wherein the additional Ig constant region or a portion thereof is associated with the first Ig constant region or a portion thereof.

13. The chimeric protein of claim 12, wherein the additional Ig constant region or a portion thereof is associated with the first Ig constant region or a portion thereof by a covalent bond.

14. The chimeric protein of claim 13, wherein the covalent bond is a disulfide bond.

15. The chimeric protein of any one of claims 1 to 14, wherein the half-life of the FVIII protein, is extended compared to a FVIII protein without the VWF fragment or compared to wild type FVIII.

16. The chimeric protein of any one of claims 1 to 15, which further comprises a second XTEN sequence, which is inserted immediately downstream of one or more insertion sites in the FVIII protein.

17. The chimeric protein of claim 1, comprising a formula selected from the group consisting of:

- (1) FVIII(X1)-L1-F1:V-L2-X2-L3-F2;
- (2) FVIII(X1)-L1-F1:F2-L3-X2-L2-V;
- (3) F1-L1-FVIII(X1):V-L2-X2-L3-F2;
- (4) F1-L1-FVIII(X1):F2-L3-X2-L2-V;
- (5) FVIII(X1)-L1-F1-L4-V-L2-X2-L3-F2;
- (6) FVIII(X1)-L1-F1-L4-F2-L3-X2-L2-V;
- (7) F1-L1-FVIII(X1)-L4-V-L2-X2-L3-F2; and
- (8) F1-L1-FVIII(X1)-L4-F2-L3-X2-L2-V;

wherein FVIII(X1) comprises the FVIII protein and a second XTEN sequence, wherein the second XTEN sequence is linked to the N-terminus or C-terminus of the FVIII protein or inserted immediately downstream of one or more amino acids ("one or more insertion site") in the FVIII protein;

each of L1, L2, or L3 comprises an optional linker;

L4 is a linker;

X2 comprises the XTEN sequence;

F1 comprises an Ig constant region or a portion thereof;

F2 comprises an optional additional Ig constant region or a portion thereof, and

V comprises the VWF fragment;

(-) is a peptide bond or one or more amino acids; and

(:) comprises a covalent bond.

18. The chimeric protein of any one of claims 1 to 17, wherein the VWF fragment does not bind to a VWF clearance receptor or is capable of protecting the FVIII protein from cleavage by one or more protease, protecting the FVIII protein from activation, stabilizing the heavy chain and/or the light chain of the FVIII protein, or preventing clearance of the FVIII protein by one or more scavenger receptors.

19. The chimeric protein of any one of claims 8 to 18, wherein the Ig constant region or a portion thereof and the additional Ig constant region or a portion thereof are identical.

20. A chimeric protein comprising a first polypeptide chain and a second polypeptide chain, wherein the first polypeptide chain comprises a VWF fragment, a first XTEN sequence, and a first Ig constant region or a portion thereof and the second polypeptide chain comprises a FVIII protein, a second XTEN sequence, and a second Ig constant region or a portion thereof, wherein the first polypeptide chain is linked to or associated with the second polypeptide chain through a covalent bond, and wherein the second XTEN is linked to the C-terminus or N-terminus of the FVIII protein or is inserted immediately downstream of one or more insertion sites in the FVIII protein.

21. The method of any one of claims 16 to 20, wherein the second XTEN sequence is inserted in the FVIII protein immediately downstream of amino acid 745 corresponding to the mature FVIII protein (SEQ ID NO: 4).

22. The chimeric protein of any one of claims 1 to 21, wherein the FVIII protein comprises B domain or a portion thereof.

23. The chimeric protein of claim 22, wherein the XTEN sequence in the second polypeptide chain or the third polypeptide chain is inserted within the B domain or a portion thereof.

24. The chimeric protein of any one of claims 1 to 23, wherein the FVIII protein comprises single chain FVIII.

25. The chimeric protein of any one of claims 1 to 24, wherein the VWF fragment is a monomer.

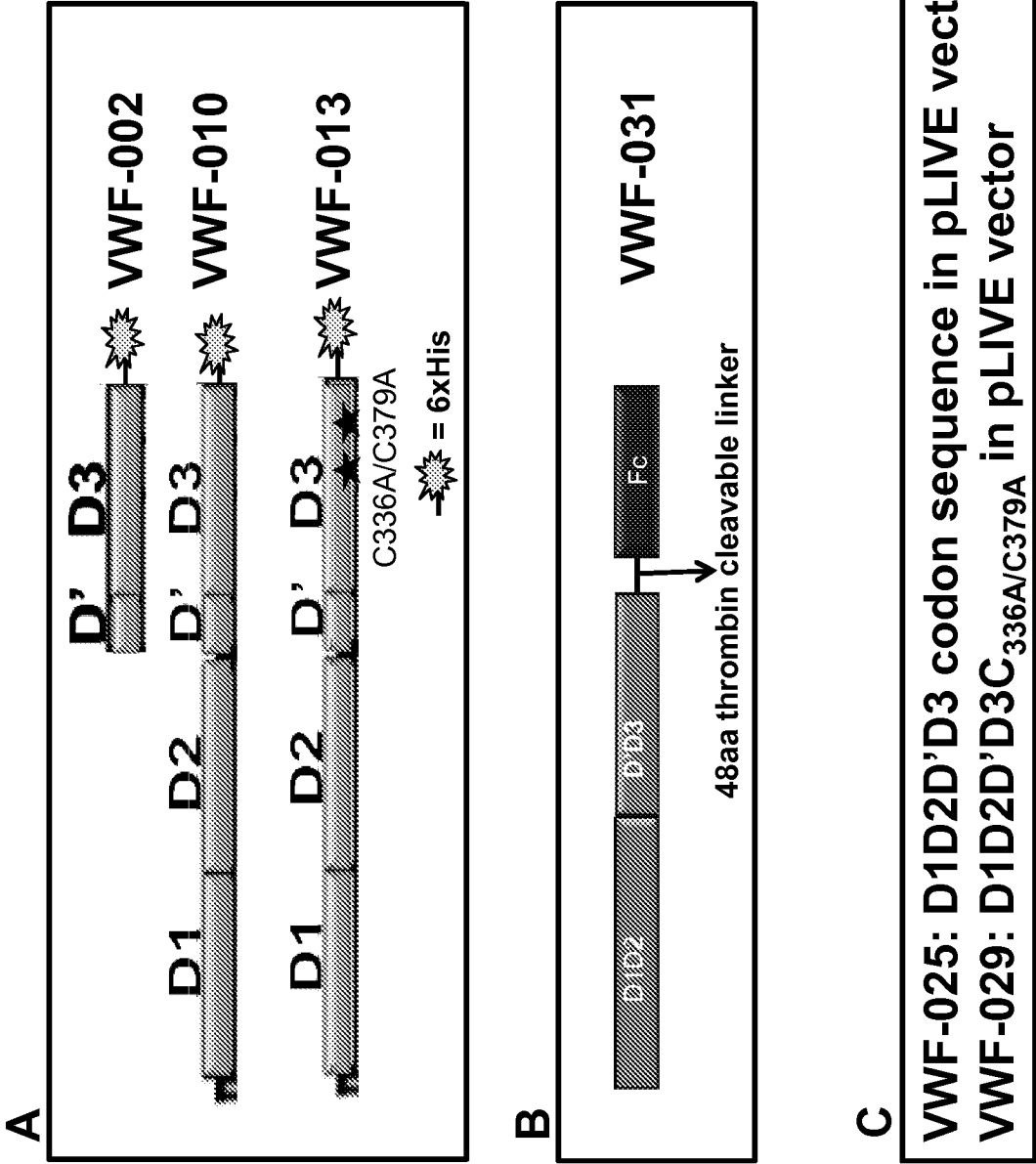
26. The chimeric protein of any one of claims 1 to 24, comprising at least two VWF fragments, at least three VWF fragments, at least four VWF fragments, at least five VWF fragments, or at least six VWF fragments.
27. The chimeric protein of any one of claims 1 to 26, wherein the VWF fragment comprises an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 1240 of SEQ ID NO: 2.
28. The chimeric protein of claim 27, wherein the VWF fragment consists essentially of or consists of amino acids 764 to 1240 of SEQ ID NO: 2.
29. The chimeric protein of any one of claims 1 to 28, wherein the VWF fragment contains an amino acid other than cysteine substituted for a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 of SEQ ID NO: 2.
30. The chimeric protein of any one of claims 1 to 29, wherein the VWF fragment consists essentially of or consists of: (1) the D' and D3 domains of VWF; (2) the D1, D', and D3 domains of VWF; (3) the D2, D', and D3 domains of VWF; (4) the D1, D2, D', and D3 domains of VWF; or (5) the D1, D2, D', D3, and A1 domains of VWF.
31. The chimeric protein of any one of claims 1 to 30, wherein one or more of the XTEN sequences comprise at least one motif selected from SEQ ID NOs: 141-170.
32. The chimeric protein of any one of claims 1 to 31, wherein one or more of the XTEN sequences are selected from the group consisting of AE42, AE72, AE144, AG144, AE288, AG288, AE576, AG576, AE864, AG864, and any combination thereof.
33. The chimeric protein of claim 32, wherein the XTEN sequence is selected from the group consisting of SEQ ID NO: 36; SEQ ID NO: 37; SEQ ID NO: 38; SEQ ID NO: 39; SEQ ID NO: 40; SEQ ID NO: 41; SEQ ID NO: 42; SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, and any combination thereof.
34. The chimeric protein of claim 33, wherein the XTEN sequence is AE144 or AE288.

35. A polynucleotide or a set of polynucleotides encoding the chimeric protein of any one of claims 1 to 34.
36. A vector comprising the polynucleotide or the set of polynucleotides of claim 35 and one or more promoters operably linked to the polynucleotide or the set of polynucleotides.
37. A host cell comprising the polynucleotide or the set of polynucleotides of claim 35 or the vector of claim 36.
38. The host cell of claim 37, which is a mammalian cell.
39. The host cell of claim 38, wherein the mammalian cell is selected from the group consisting of HEK293 cell, CHO cell, and BHK cell.
40. A pharmaceutical composition comprising the chimeric protein of any one of claims 1 to 34, the polynucleotide or the set of polynucleotides of claim 35, the vector of claim 36, or the host cell of any one of claims 37 to 39, and a pharmaceutically acceptable carrier.
41. A method of treating a bleeding disease or disorder in a subject in need thereof comprising administering an effective amount of the chimeric protein of any one of claims 1 to 34, the polynucleotide or the set of polynucleotides of claim 35, the vector of claim 36, the host cell of any one of claims 37 to 39, or the composition of claim 40, wherein the bleeding disease or disorder is selected from the group consisting of a bleeding coagulation disorder, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, and bleeding in the iliopsoas sheath.
42. The method of claim 41, wherein the chimeric protein of any one of claims 1 to 34, the polynucleotide or the set of polynucleotides of claim 35, the vector of claim 36, the host cell of any one of claims 37 to 39, or the composition of claim 40 is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intravenous administration, subcutaneous administration, intramuscular administration, intradermal administration, intrathecal administration, subdural administration and oral administration.

43. A method of making a chimeric protein, comprising culturing the host cell of any one of claims 37 to 39 under suitable medium and expressing the chimeric protein in the host cell.

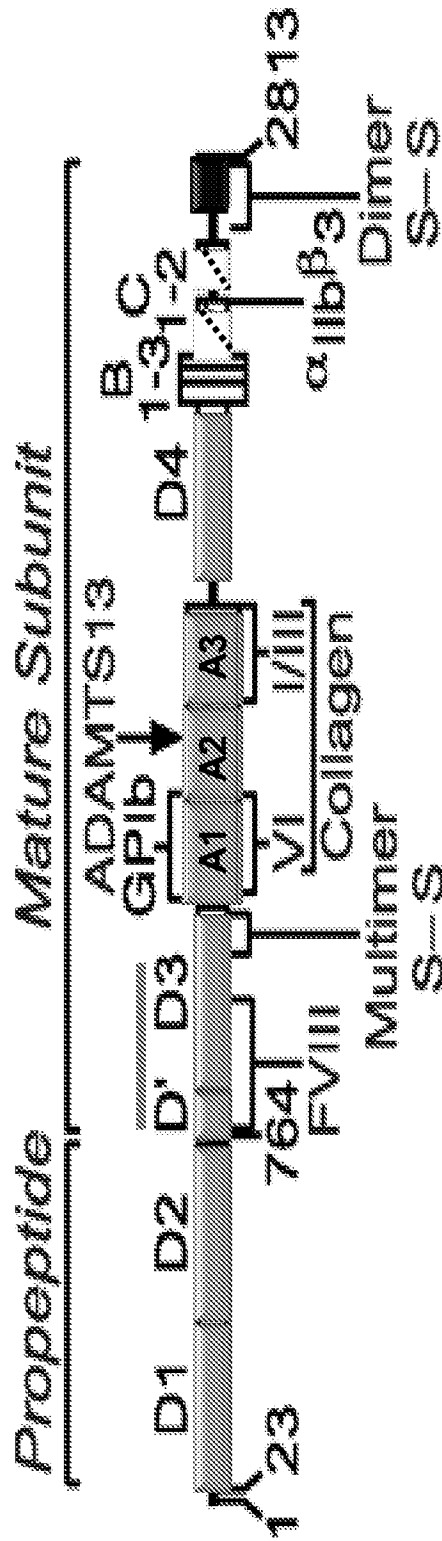
Date: 19 January 2018

Figure 1: Different VWF Constructs



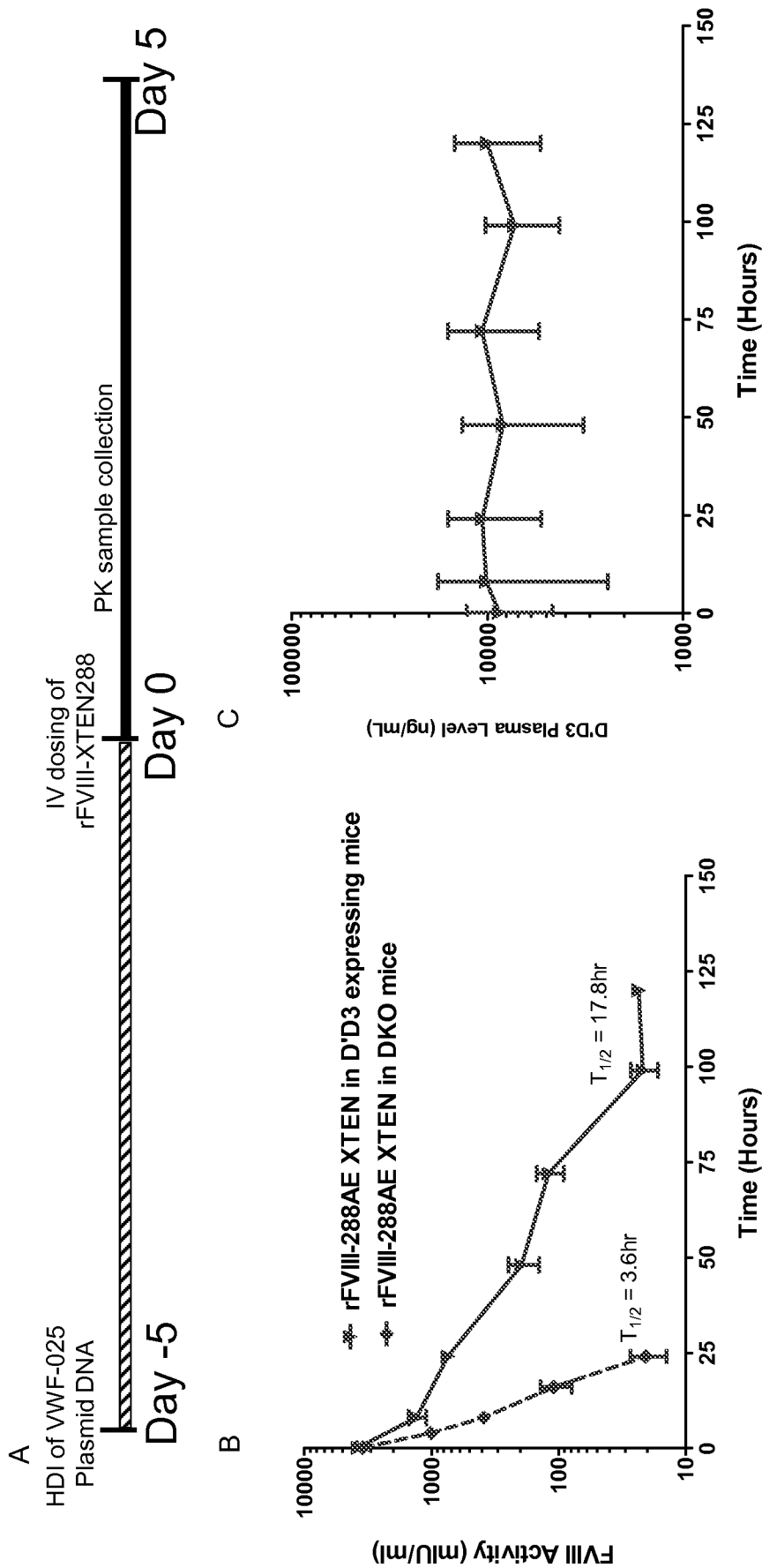
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Figure 1D. Von Willebrand Factor



- ~ 250 kDa protein, forms multimers ( > 20 MDa) by disulfide bonding
  - Associates with FVIII (95-98%) in non-covalent complex
    - Protects FVIII from protease cleavage/activation
    - Stabilizes heavy & light chain
    - Prevents clearance of FVIII by scavenger receptors
  - Clearance of FVIII-vWF complex through vWF-receptors
    - Prevents pinocytosis and recycling of rFVIII-Fc?
- Extends half-life**  
**Limits half-life**

Figure 2: rFVIII-XTEN PK in VWF D'D3 Expressing Mice



5 fold additional half-life extension of FVIII-XTEN from VWF D'D3 fragment



Figure 3: FVIII<sub>FC</sub>/VWF heterodimer Constructs (variable linker)

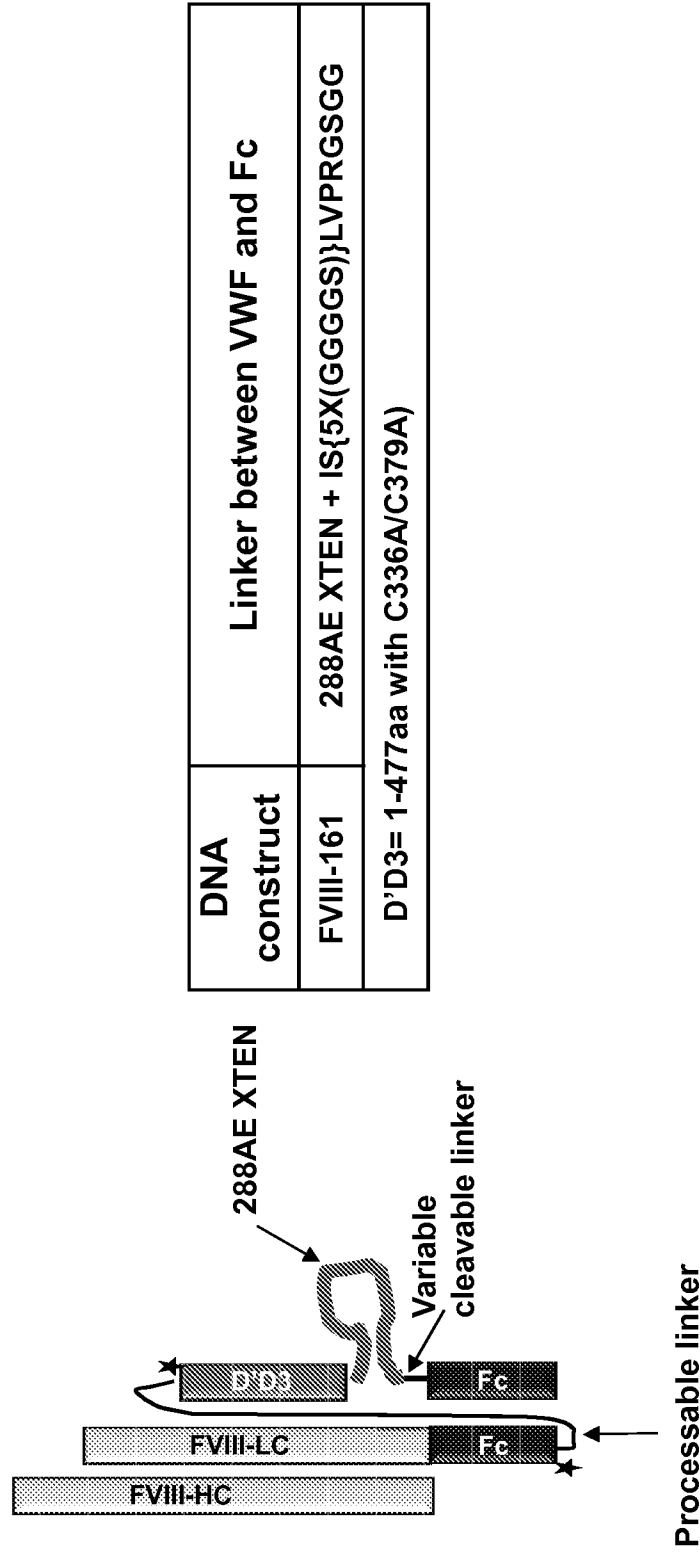


Figure 4: FVIII/VWF Constructs with XTEN insertions

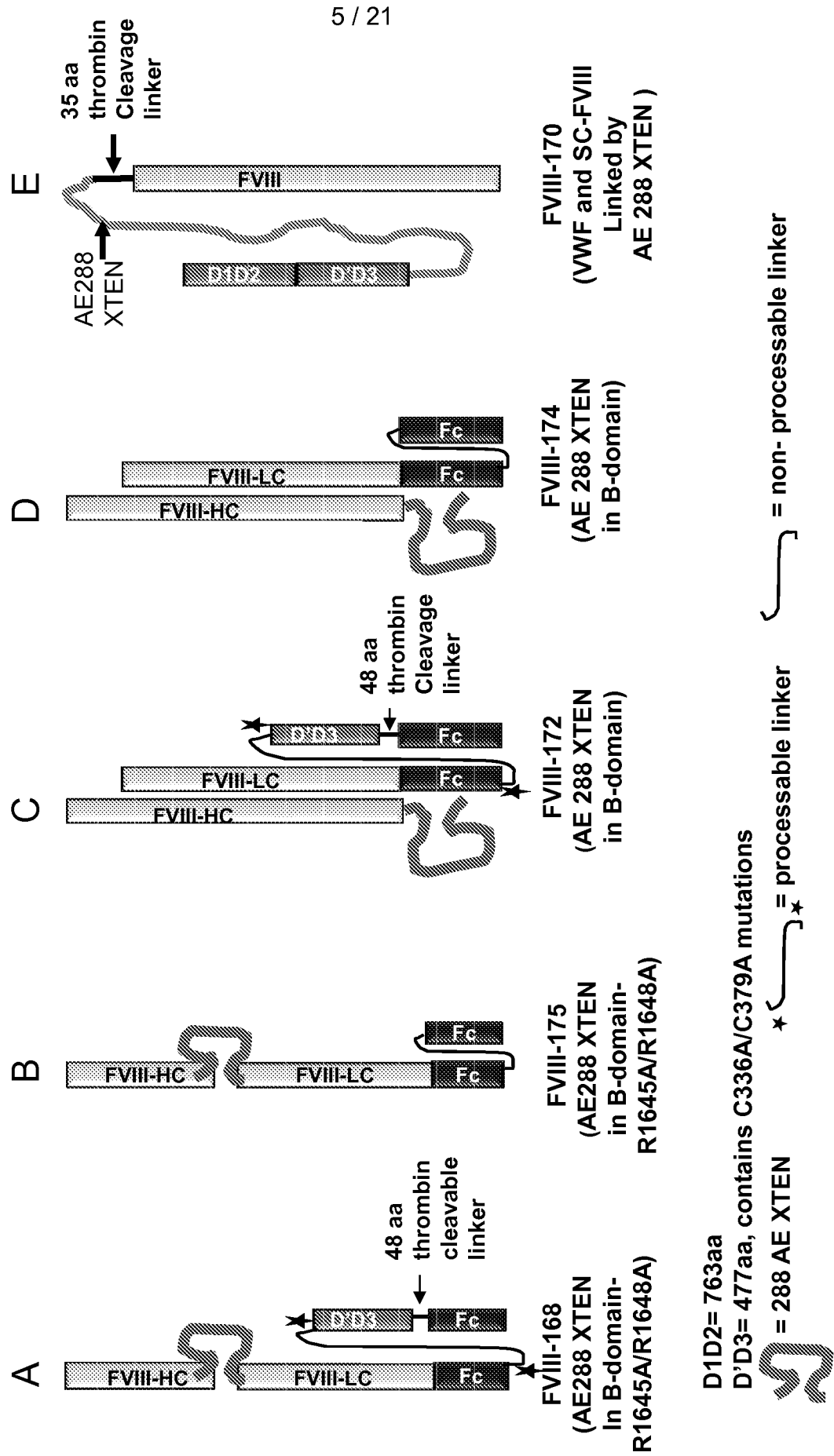


Figure 5: XTEN insertion improves the PK of FVIII/VWF heterodimers

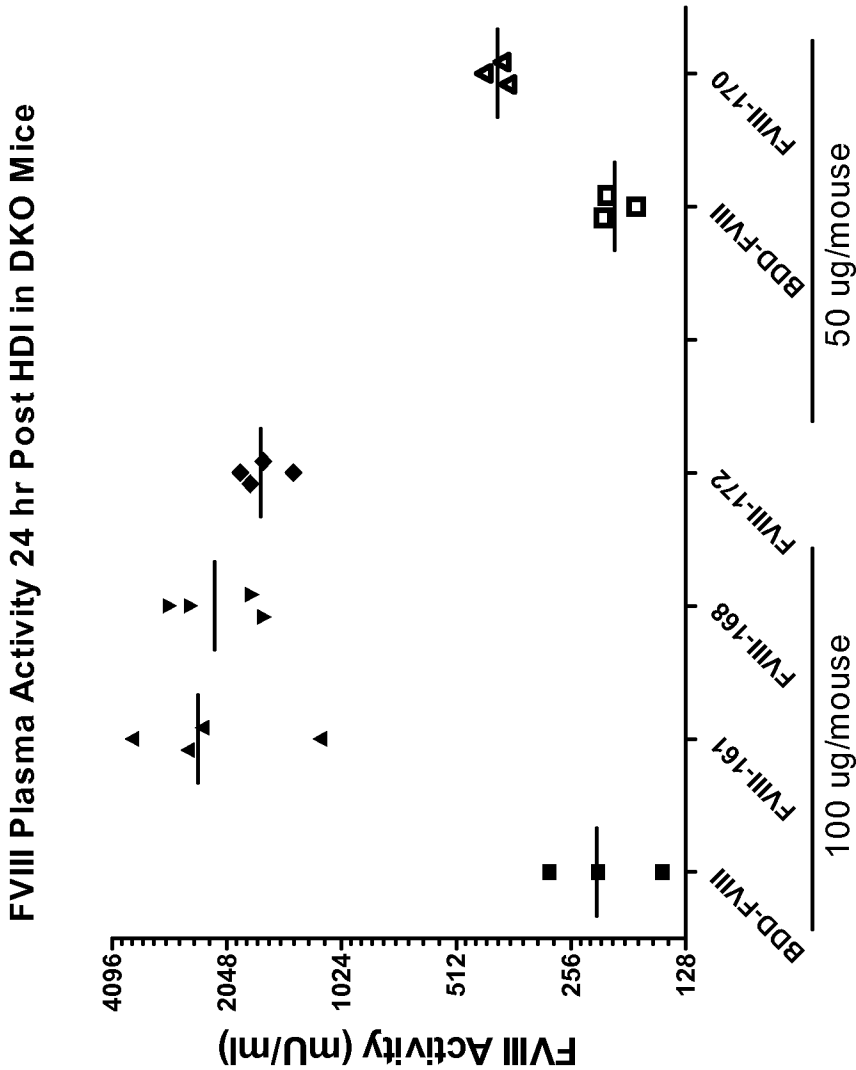


Figure 6: FVIII Constructs (Co-transfection system)

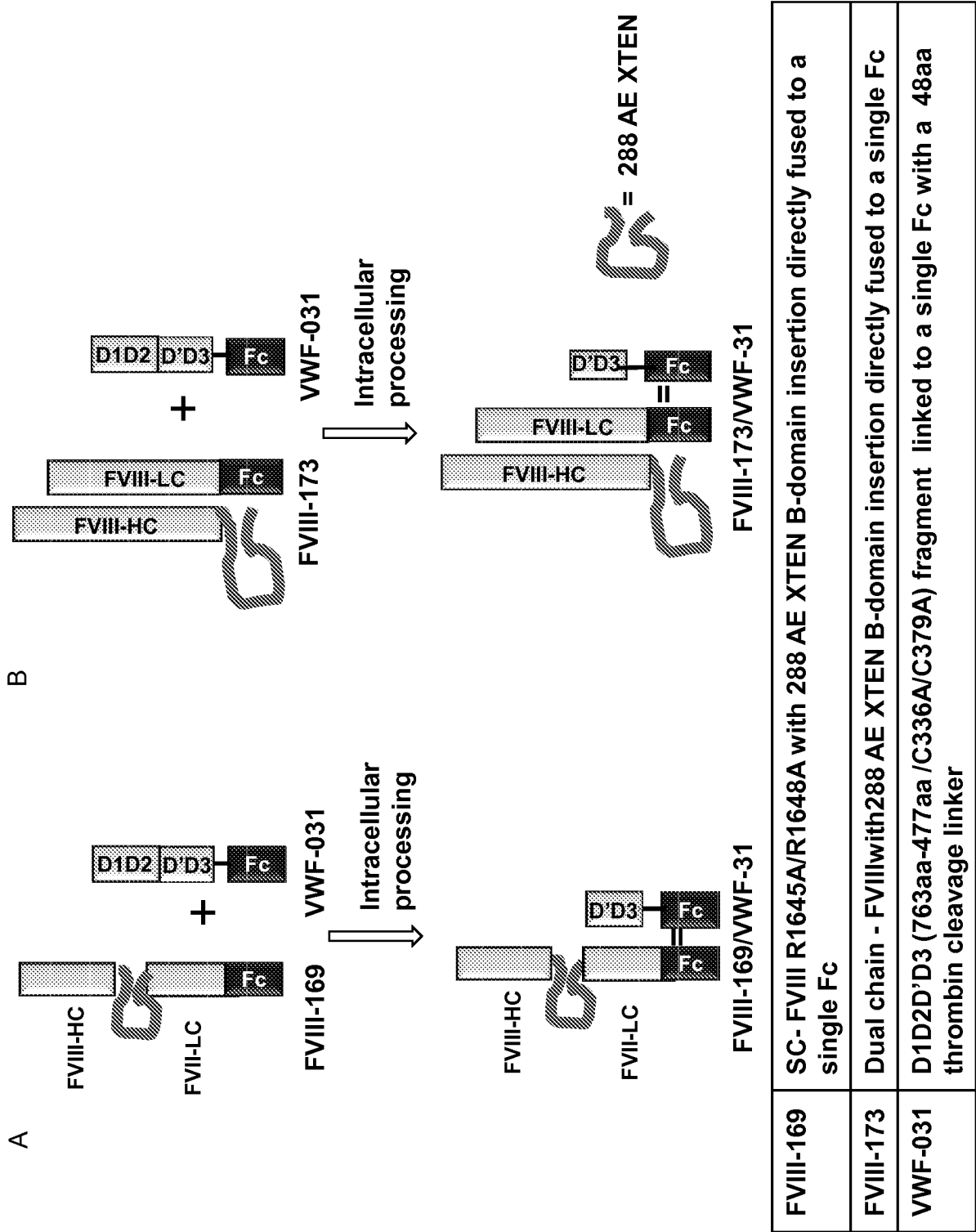
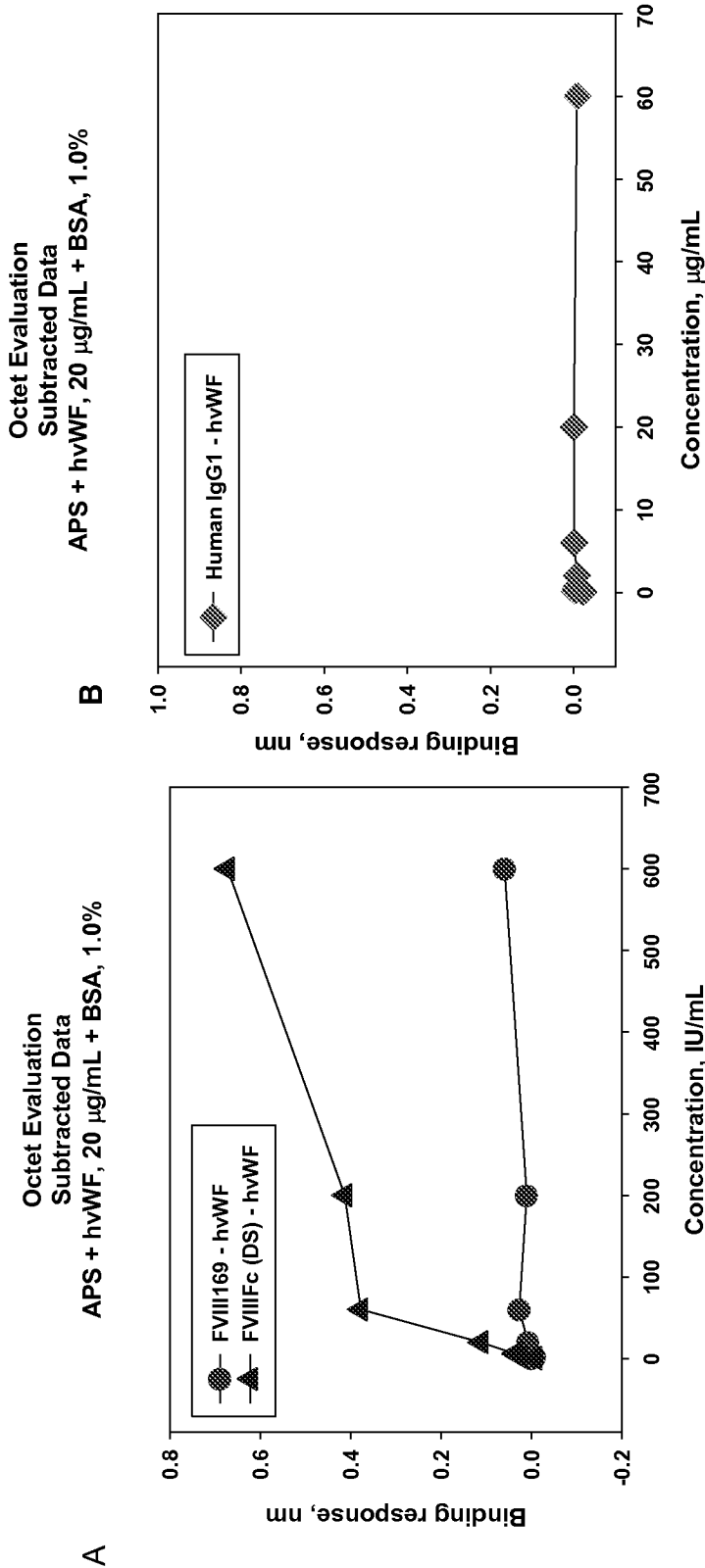


Figure 7: FVIII-169/VWF-31 has no detectable binding toward immobilized hVWF



| Analyte           | APS Probe + hVWF | Protein G Probe |
|-------------------|------------------|-----------------|
| FVIII-169/VWF-031 | -                | +               |
| rFVIII Fc         | +                | +               |
| Human IgG1        | -                | +               |

Figure 8A: FVIII-169/VWF-031 PK in HemaA

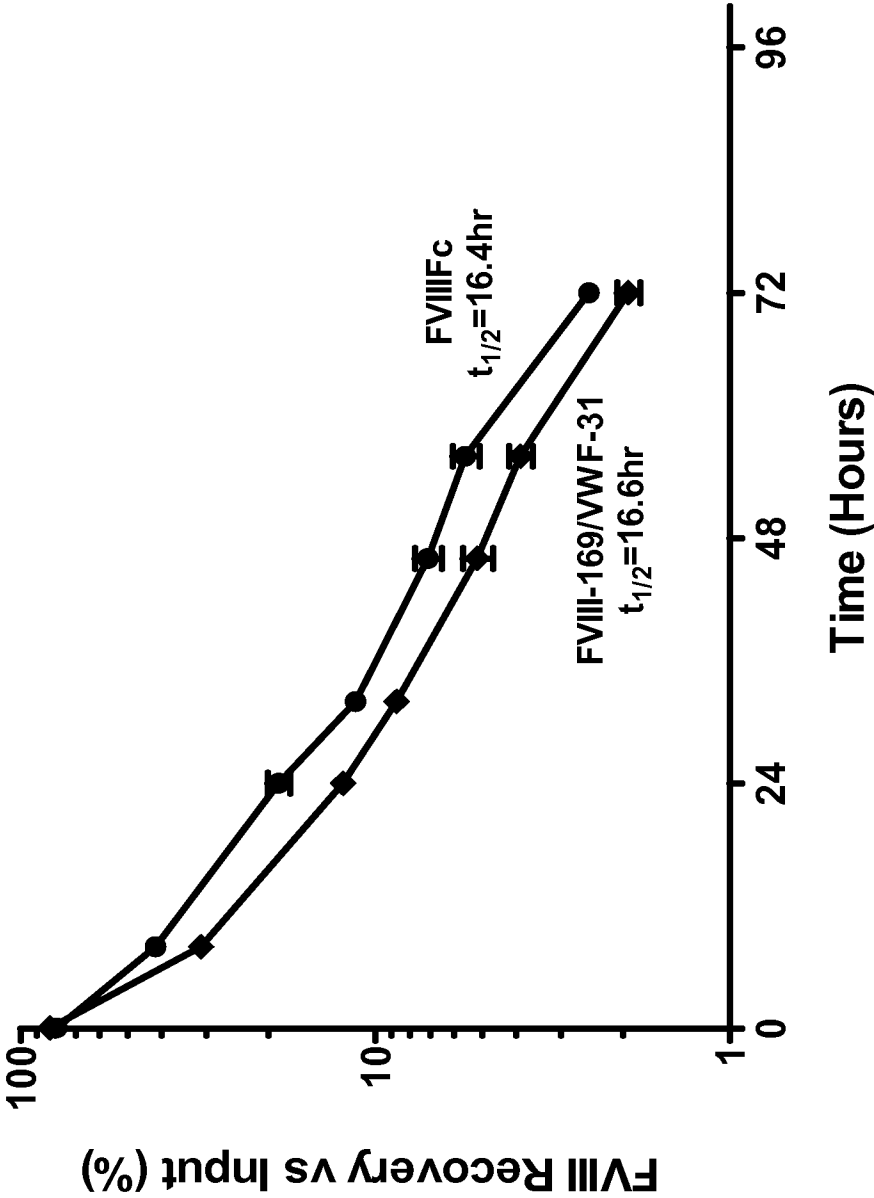


Figure 8B: FVIII-169/VWF-031 PK in FVIII/VWF DKO Mice

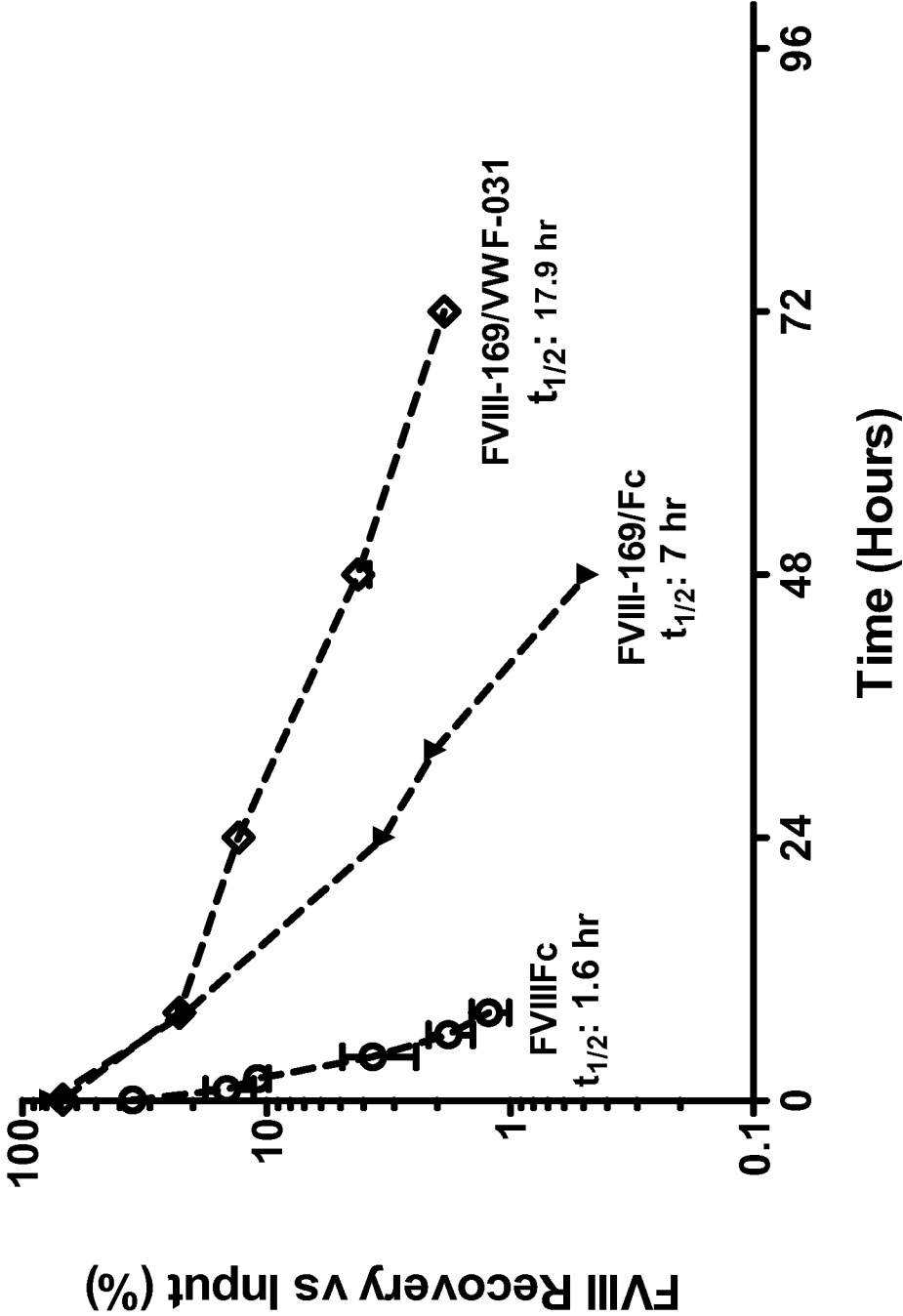


Figure 9A: FVIII-XTEN variants half-life in D'D3 expressing FVIII/VWF DKO mice

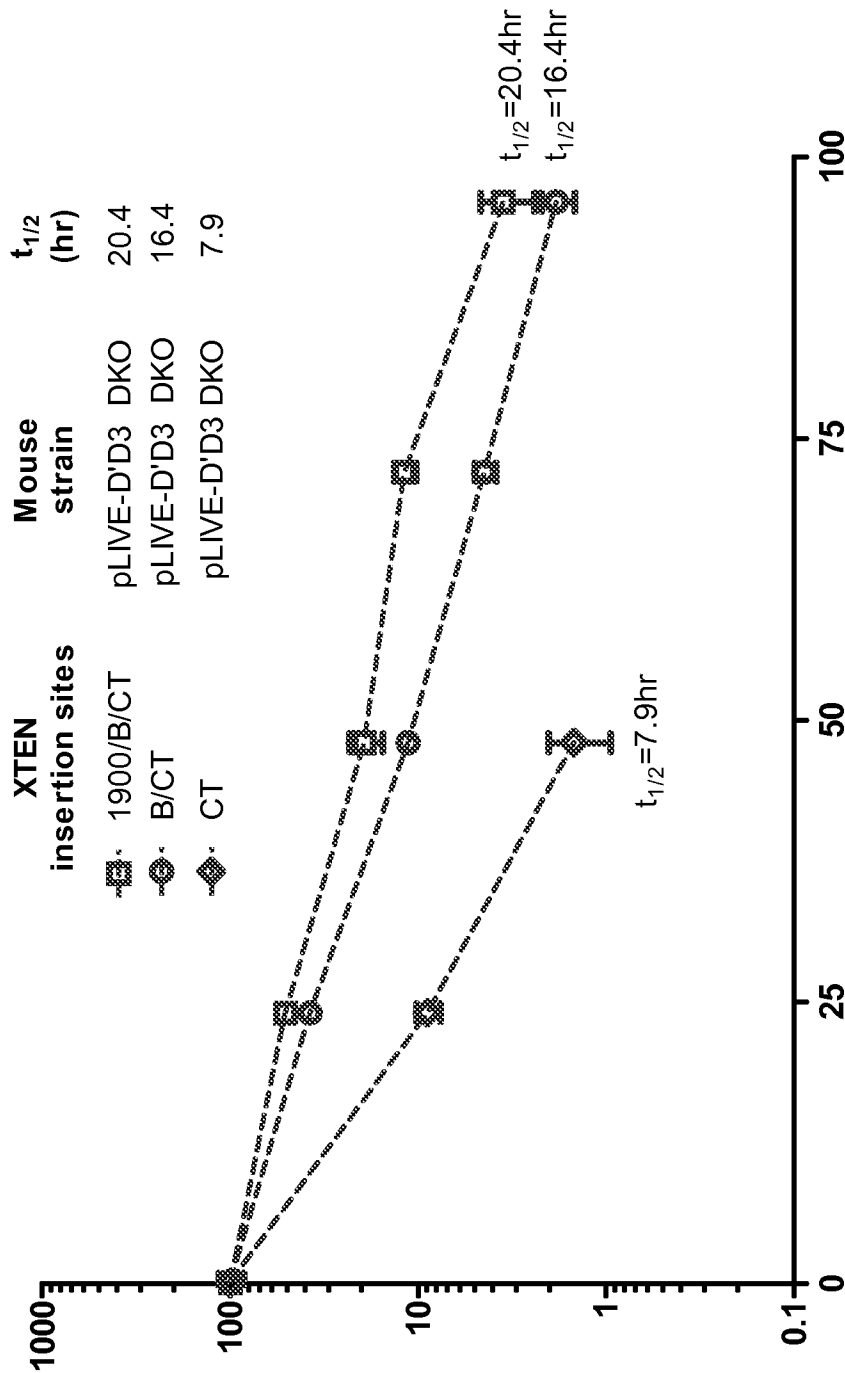




Figure 9B: PK of FVIII-XTEN with three XTEN insertions

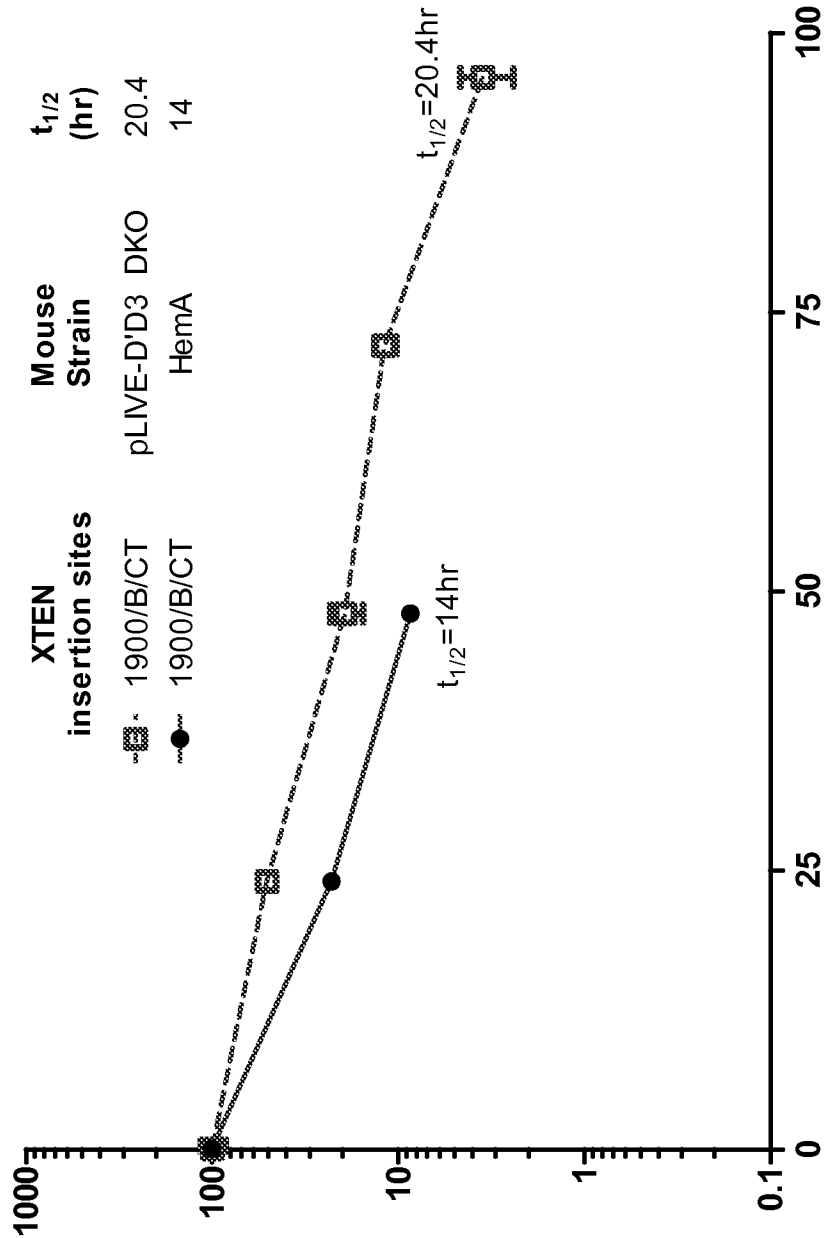


Figure 10: FVIII Fc activity in mouse DKO plasma

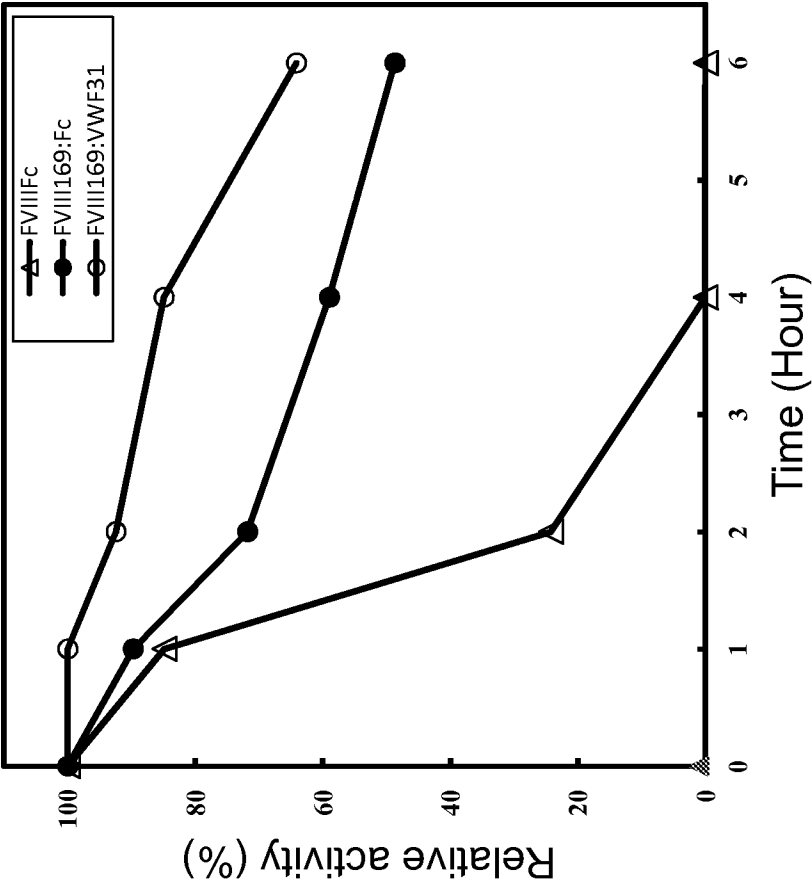


Figure 11: Additive effect of Fc, XTEN and VWF-D'D3 fragment on FVIII half-life extension

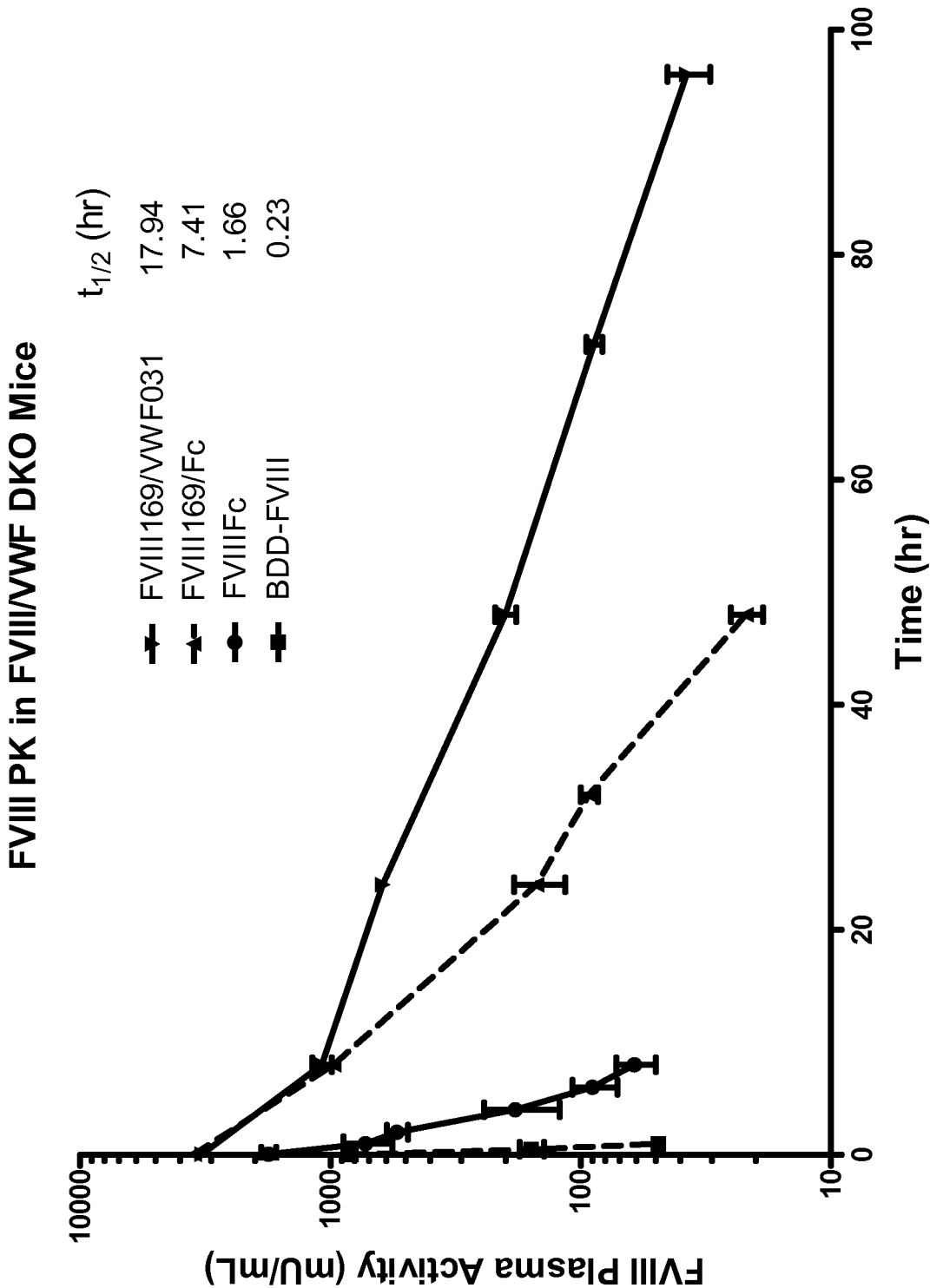


Figure 12A: Effect of different XTEN on rFVIII-XTEN/VWF heterodimer PK in Hema mice

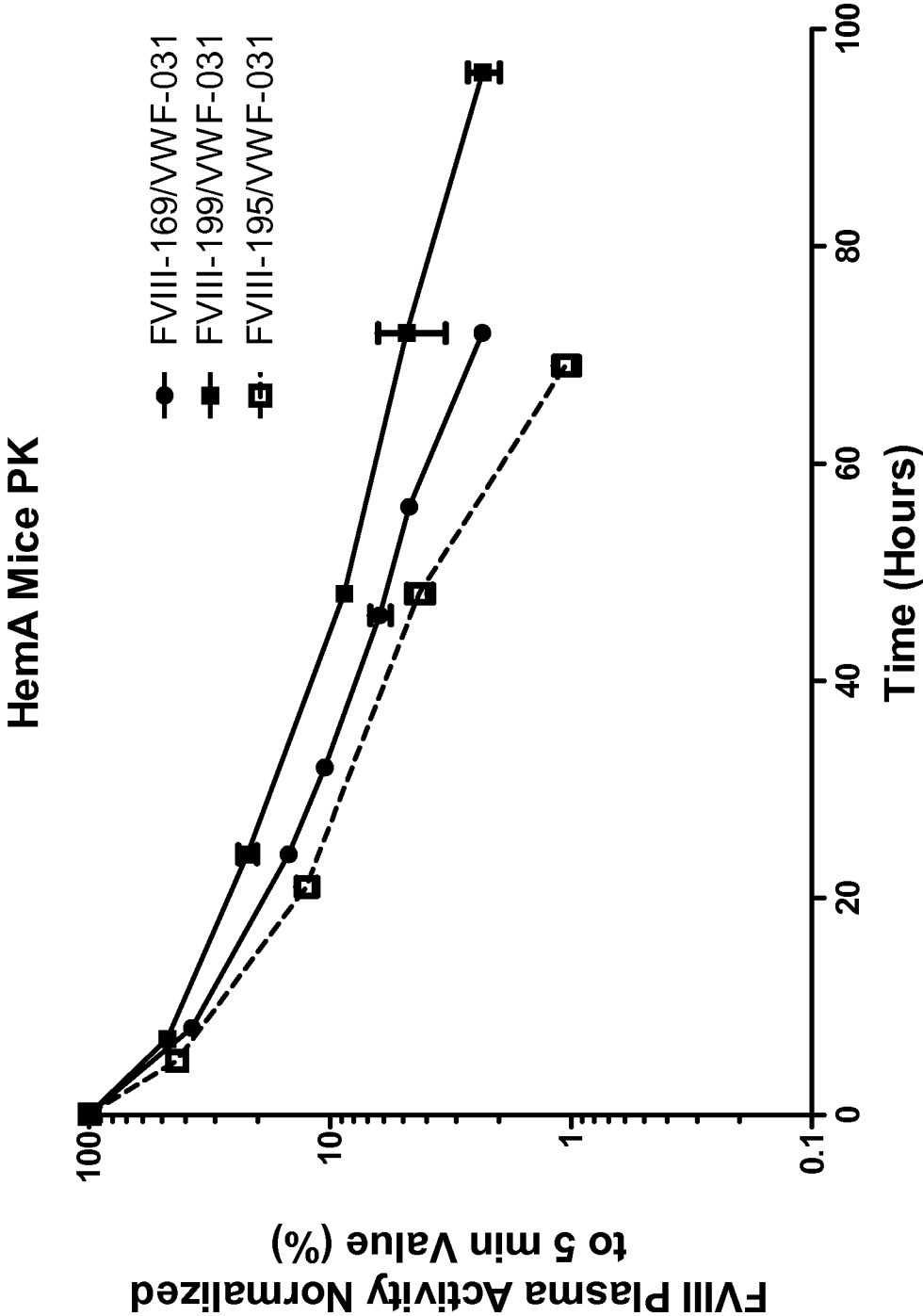


Figure 12B: Effect of different XTEN on rFVIII-XTEN/VWF heterodimer PK in Hema mice

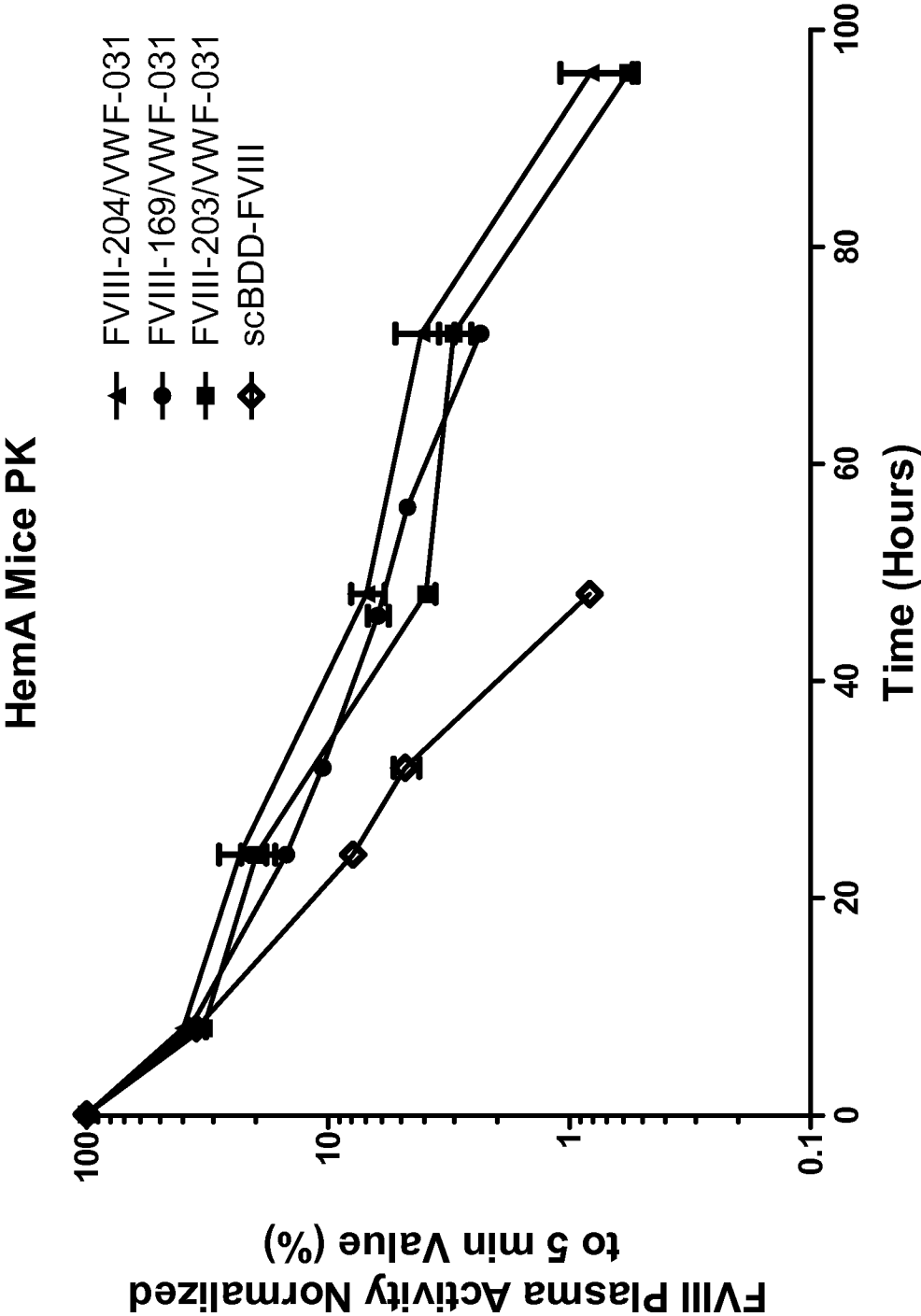


Figure 12C: Effect of different XTEN on rFVIII-XTEN/VWF heterodimer PK in Hema mice

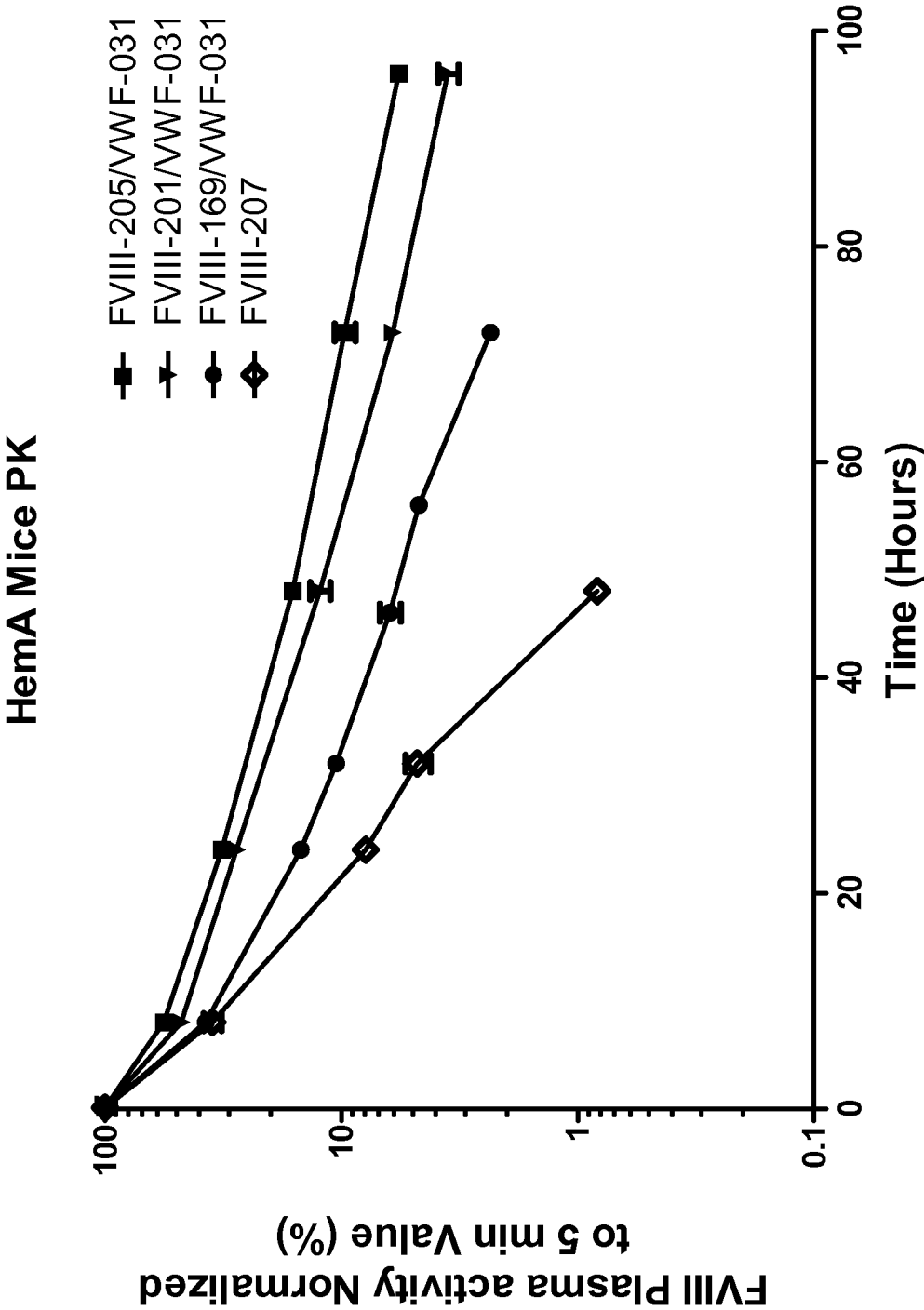


Figure 13: rFVIII-XTEN/VWF-XTEN heterodimer PK in FVIII/VWF DKO mice

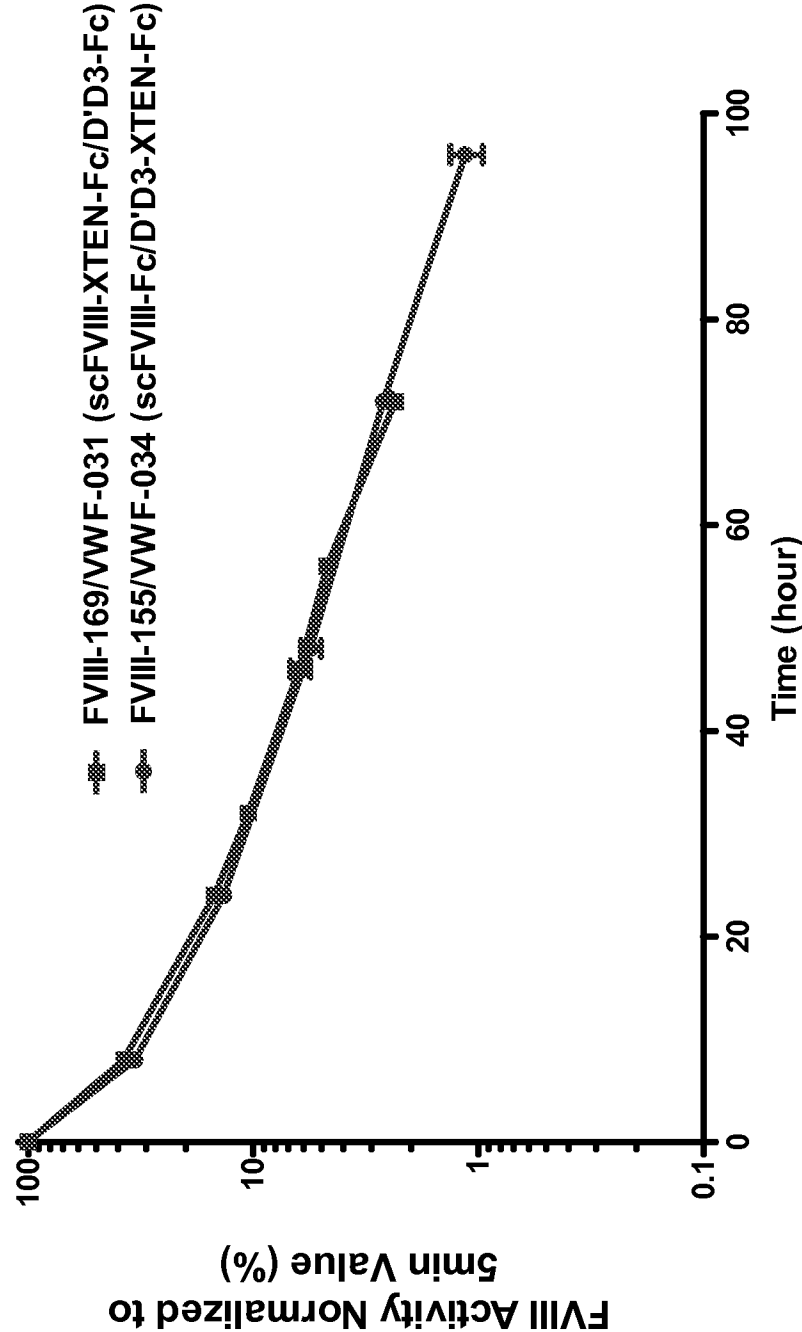


Figure 14. rFVIII-XTEN/VWF Heterodimer PK In Hema Mice

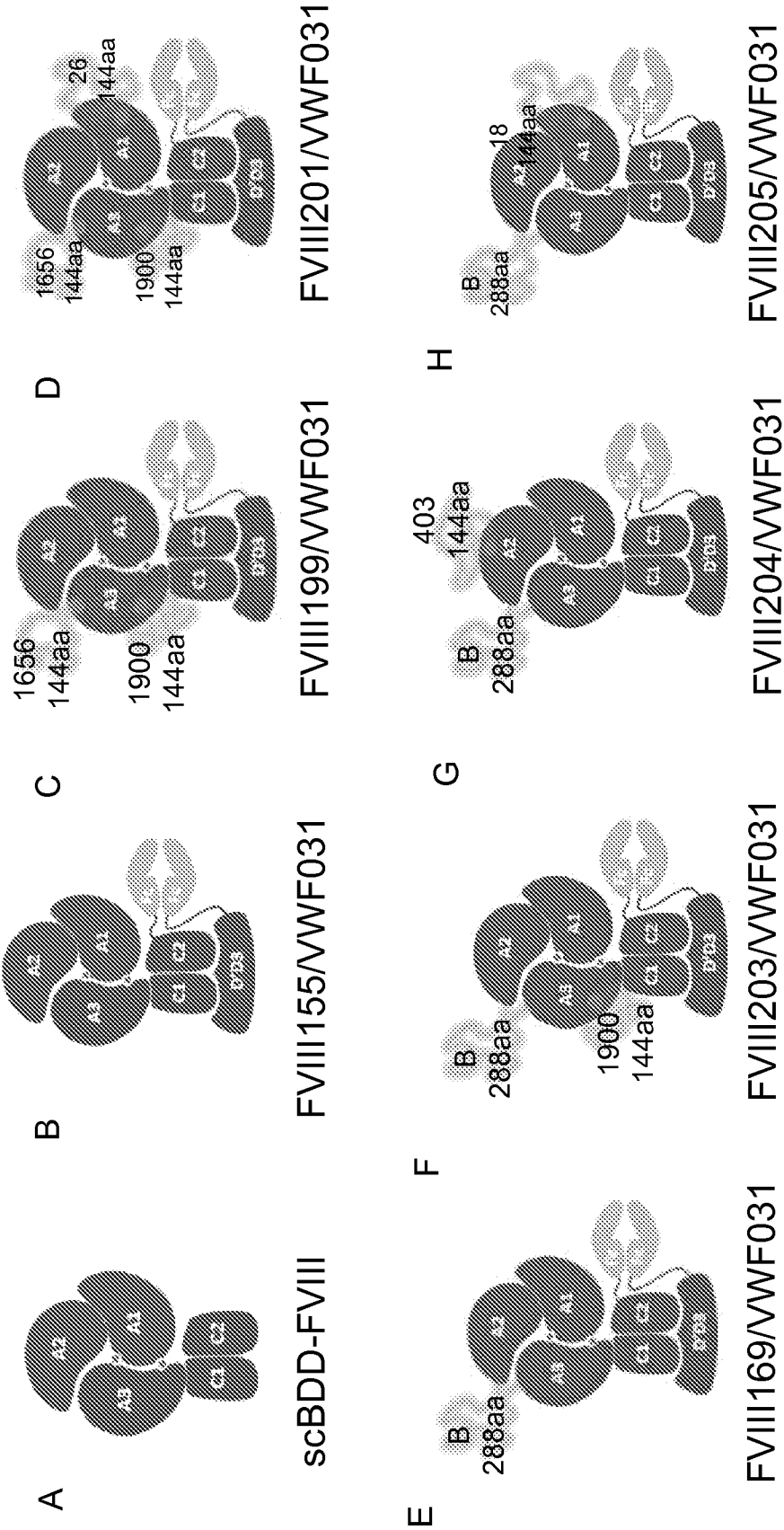




**Figure 15:**  
**Half-life extension in HemA mice by rFVIII-XTEN/VWF**

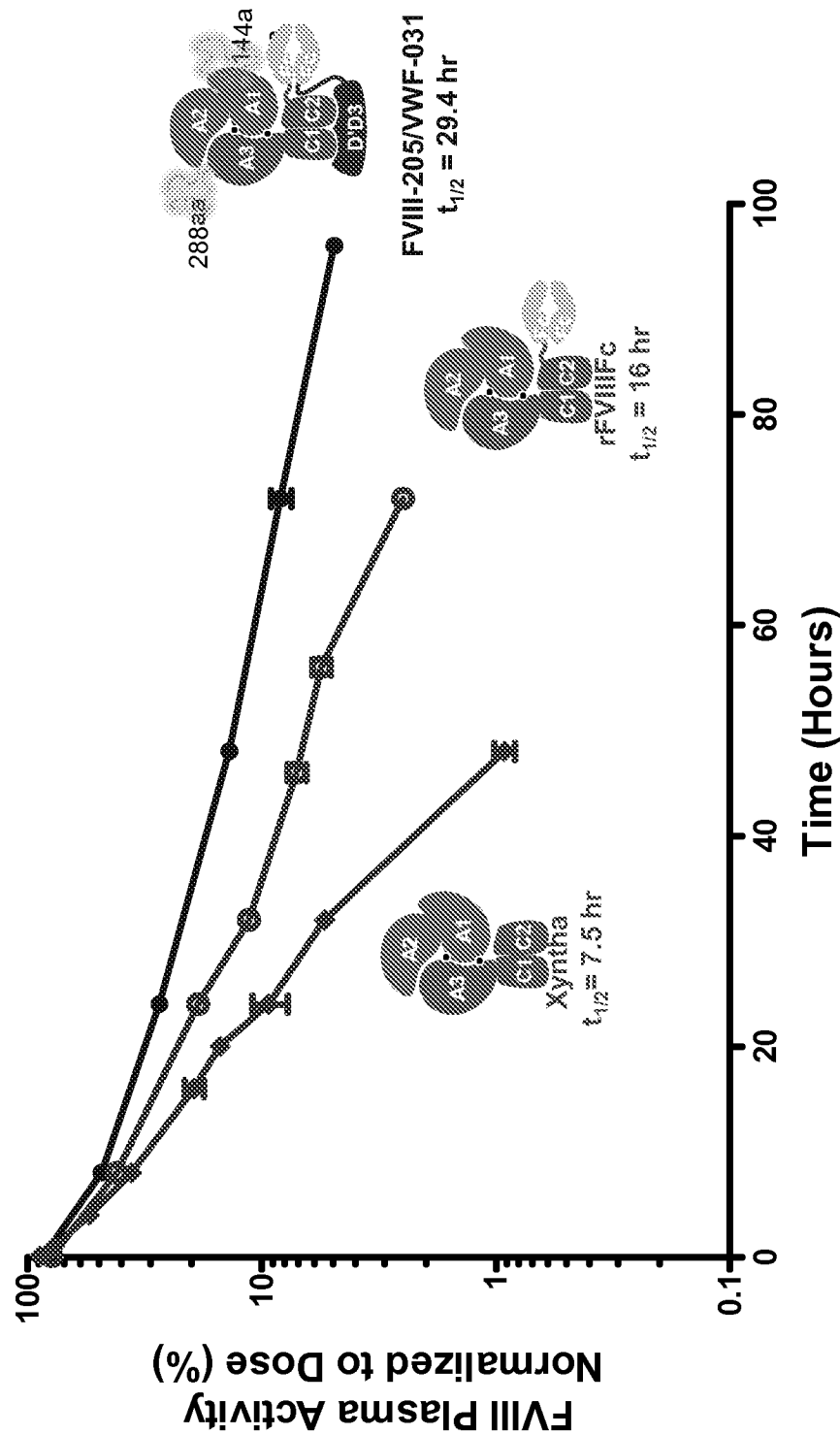
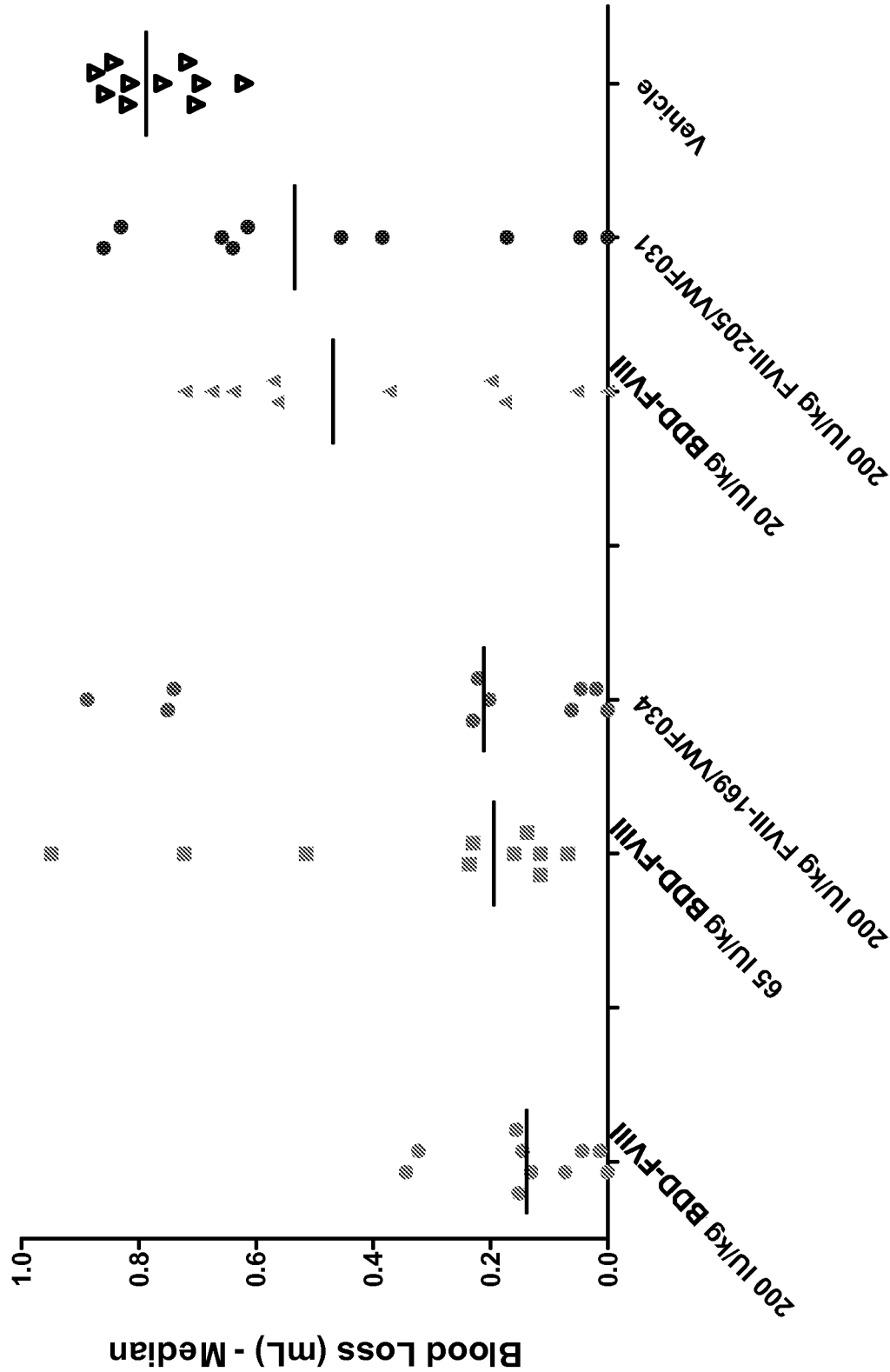


Figure 16: Acute efficacy of FVIII-XTEN-Fc : VWF-Fc heterodimer in Hema mice Tail Clip bleeding model



<110> Biogen Idec MA Inc.  
Chhabra, Ekta Seth  
Liu, Tongyao

<120> FACTOR VIII COMPLEX WITH XTEN AND VON WILLEBRAND FACTOR PROTEIN,  
AND USES THEREOF

<130> 2159.365PC06/EKS/C-K/E-H

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| tccattgaca tcaacgatgt gcaggaccag tgctcctgct gctctccgac acggacggag   | 8340 |

cccatgcagg tggccctgca ctgcaccaat ggctctgttg tgtaccatga ggttctcaat 8400

gccatggagt gcaaattgctc ccccaggaag tgcagcaagt ga 8442

<210> 2  
<211> 2813  
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<213> Homo sapiens

<220>  
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<222> (1)..(22)  
<223> VWF Signal Peptide

<220>  
<221> misc\_feature  
<222> (23)..(763)  
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<220>  
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<222> (764)..(866)  
<223> VWF D' Domain

<220>  
<221> misc\_feature  
<222> (867)..(1240)  
<223> VWF D3 Domain

<220>  
<221> misc\_feature  
<222> (1241)..(1479)  
<223> VWF A1 Domain

<220>  
<221> misc\_feature  
<222> (2016)..(2016)  
<223> Xaa can be any naturally occurring amino acid

&lt;400&gt; 2

Met Ile Pro Ala Arg Phe Ala Gly Val Leu Leu Ala Leu Ala Leu Ile  
 1 5 10 15

Leu Pro Gly Thr Leu Cys Ala Glu Gly Thr Arg Gly Arg Ser Ser Thr  
 20 25 30

Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly  
 35 40 45

Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly  
 50 55 60

Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys  
 65 70 75 80

Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu  
 85 90 95

Phe Val Asn Gly Thr Val Thr Gln Gly Asp Gln Arg Val Ser Met Pro  
 100 105 110

Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys  
 115 120 125

Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly  
 130 135 140

Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 145 |     | 150 |     | 155 |     | 160 |     |     |     |     |     |     |     |     |     |
| Leu | Cys | Gly | Asn | Phe | Asn | Ile | Phe | Ala | Glu | Asp | Asp | Phe | Met | Thr | Gln |
|     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Glu | Gly | Thr | Leu | Thr | Ser | Asp | Pro | Tyr | Asp | Phe | Ala | Asn | Ser | Trp | Ala |
|     |     |     | 180 |     |     |     |     |     | 185 |     |     |     |     | 190 |     |
| Leu | Ser | Ser | Gly | Glu | Gln | Trp | Cys | Glu | Arg | Ala | Ser | Pro | Pro | Ser | Ser |
|     |     |     | 195 |     |     |     |     |     | 200 |     |     |     |     | 205 |     |
| Ser | Cys | Asn | Ile | Ser | Ser | Gly | Glu | Met | Gln | Lys | Gly | Leu | Trp | Glu | Gln |
|     |     |     | 210 |     |     |     |     |     | 215 |     |     |     |     | 220 |     |
| Cys | Gln | Leu | Leu | Lys | Ser | Thr | Ser | Val | Phe | Ala | Arg | Cys | His | Pro | Leu |
|     |     |     | 225 |     |     |     |     |     | 230 |     |     |     |     | 235 | 240 |
| Val | Asp | Pro | Glu | Pro | Phe | Val | Ala | Leu | Cys | Glu | Lys | Thr | Leu | Cys | Glu |
|     |     |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Cys | Ala | Gly | Gly | Leu | Glu | Cys | Ala | Cys | Pro | Ala | Leu | Leu | Glu | Tyr | Ala |
|     |     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |
| Arg | Thr | Cys | Ala | Gln | Glu | Gly | Met | Val | Leu | Tyr | Gly | Trp | Thr | Asp | His |
|     |     |     | 275 |     |     |     |     |     | 280 |     |     |     |     | 285 |     |
| Ser | Ala | Cys | Ser | Pro | Val | Cys | Pro | Ala | Gly | Met | Glu | Tyr | Arg | Gln | Cys |
|     |     |     | 290 |     |     |     |     |     | 295 |     |     |     |     | 300 |     |
| Val | Ser | Pro | Cys | Ala | Arg | Thr | Cys | Gln | Ser | Leu | His | Ile | Asn | Glu | Met |



465 470 475 480

Arg Ile Gln His Thr Val Thr Ala Ser Val Arg Leu Ser Tyr Gly Glu  
485 490 495

Asp Leu Gln Met Asp Trp Asp Gly Arg Gly Arg Leu Leu Val Lys Leu  
500 505 510

Ser Pro Val Tyr Ala Gly Lys Thr Cys Gly Leu Cys Gly Asn Tyr Asn  
515 520 525

Gly Asn Gln Gly Asp Asp Phe Leu Thr Pro Ser Gly Leu Ala Glu Pro  
530 535 540

Arg Val Glu Asp Phe Gly Asn Ala Trp Lys Leu His Gly Asp Cys Gln  
545 550 555 560

Asp Leu Gln Lys Gln His Ser Asp Pro Cys Ala Leu Asn Pro Arg Met  
565 570 575

Thr Arg Phe Ser Glu Glu Ala Cys Ala Val Leu Thr Ser Pro Thr Phe  
580 585 590

Glu Ala Cys His Arg Ala Val Ser Pro Leu Pro Tyr Leu Arg Asn Cys  
595 600 605

Arg Tyr Asp Val Cys Ser Cys Ser Asp Gly Arg Glu Cys Leu Cys Gly  
610 615 620

Ala Leu Ala Ser Tyr Ala Ala Ala Cys Ala Gly Arg Gly Val Arg Val

625 630 635 640

Ala Trp Arg Glu Pro Gly Arg Cys Glu Leu Asn Cys Pro Lys Gly Gln  
645 650 655

Val Tyr Leu Gln Cys Gly Thr Pro Cys Asn Leu Thr Cys Arg Ser Leu  
660 665 670

Ser Tyr Pro Asp Glu Glu Cys Asn Glu Ala Cys Leu Glu Gly Cys Phe  
675 680 685

Cys Pro Pro Gly Leu Tyr Met Asp Glu Arg Gly Asp Cys Val Pro Lys  
690 695 700

Ala Gln Cys Pro Cys Tyr Tyr Asp Gly Glu Ile Phe Gln Pro Glu Asp  
705 710 715 720

Ile Phe Ser Asp His His Thr Met Cys Tyr Cys Glu Asp Gly Phe Met  
725 730 735

His Cys Thr Met Ser Gly Val Pro Gly Ser Leu Leu Pro Asp Ala Val  
740 745 750

Leu Ser Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg  
755 760 765

Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp Asn Leu Arg Ala Glu  
770 775 780

Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr Asp Leu Glu Cys Met



785                      790                      795                      800

Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro Pro Gly Met Val Arg  
                             805                      810                      815

His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys Pro Cys Phe His Gln  
                             820                      825                      830

Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr  
                             835                      840                      845

Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp  
                             850                      855                      860

Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly  
 865                      870                      875                      880

Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp  
                             885                      890                      895

Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys  
                             900                      905                      910

Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu  
                             915                      920                      925

Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys  
                             930                      935                      940

Arg Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Arg

|                                                                 |      |      |     |
|-----------------------------------------------------------------|------|------|-----|
| 945                                                             | 950  | 955  | 960 |
| Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg |      |      |     |
| 965                                                             | 970  | 975  |     |
| His Leu Ser Ile Ser Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val |      |      |     |
| 980                                                             | 985  | 990  |     |
| Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln Asn Asn Asp Leu Thr |      |      |     |
| 995                                                             | 1000 | 1005 |     |
| Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val Asp Phe Gly Asn     |      |      |     |
| 1010                                                            | 1015 | 1020 |     |
| Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg Lys Val Pro     |      |      |     |
| 1025                                                            | 1030 | 1035 |     |
| Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met Lys Gln     |      |      |     |
| 1040                                                            | 1045 | 1050 |     |
| Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val Phe     |      |      |     |
| 1055                                                            | 1060 | 1065 |     |
| Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val     |      |      |     |
| 1070                                                            | 1075 | 1080 |     |
| Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala     |      |      |     |
| 1085                                                            | 1090 | 1095 |     |
| Cys Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln     |      |      |     |

1100

1105

1110

His Gly Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln

1115

1120

1125

Ser Cys Glu Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Cys Glu

1130

1135

1140

Trp Arg Tyr Asn Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln

1145

1150

1155

His Pro Glu Pro Leu Ala Cys Pro Val Gln Cys Val Glu Gly Cys

1160

1165

1170

His Ala His Cys Pro Pro Gly Lys Ile Leu Asp Glu Leu Leu Gln

1175

1180

1185

Thr Cys Val Asp Pro Glu Asp Cys Pro Val Cys Glu Val Ala Gly

1190

1195

1200

Arg Arg Phe Ala Ser Gly Lys Lys Val Thr Leu Asn Pro Ser Asp

1205

1210

1215

Pro Glu His Cys Gln Ile Cys His Cys Asp Val Val Asn Leu Thr

1220

1225

1230

Cys Glu Ala Cys Gln Glu Pro Gly Gly Leu Val Val Pro Pro Thr

1235

1240

1245

Asp Ala Pro Val Ser Pro Thr Thr Leu Tyr Val Glu Asp Ile Ser

1250

1255

1260

Glu Pro Pro Leu His Asp Phe Tyr Cys Ser Arg Leu Leu Asp Leu

1265

1270

1275

Val Phe Leu Leu Asp Gly Ser Ser Arg Leu Ser Glu Ala Glu Phe

1280

1285

1290

Glu Val Leu Lys Ala Phe Val Val Asp Met Met Glu Arg Leu Arg

1295

1300

1305

Ile Ser Gln Lys Trp Val Arg Val Ala Val Val Glu Tyr His Asp

1310

1315

1320

Gly Ser His Ala Tyr Ile Gly Leu Lys Asp Arg Lys Arg Pro Ser

1325

1330

1335

Glu Leu Arg Arg Ile Ala Ser Gln Val Lys Tyr Ala Gly Ser Gln

1340

1345

1350

Val Ala Ser Thr Ser Glu Val Leu Lys Tyr Thr Leu Phe Gln Ile

1355

1360

1365

Phe Ser Lys Ile Asp Arg Pro Glu Ala Ser Arg Ile Ala Leu Leu

1370

1375

1380

Leu Met Ala Ser Gln Glu Pro Gln Arg Met Ser Arg Asn Phe Val

1385

1390

1395

Arg Tyr Val Gln Gly Leu Lys Lys Lys Lys Val Ile Val Ile Pro

1400

1405

1410

Val Gly Ile Gly Pro His Ala Asn Leu Lys Gln Ile Arg Leu Ile

1415

1420

1425

Glu Lys Gln Ala Pro Glu Asn Lys Ala Phe Val Leu Ser Ser Val

1430

1435

1440

Asp Glu Leu Glu Gln Gln Arg Asp Glu Ile Val Ser Tyr Leu Cys

1445

1450

1455

Asp Leu Ala Pro Glu Ala Pro Pro Pro Thr Leu Pro Pro Asp Met

1460

1465

1470

Ala Gln Val Thr Val Gly Pro Gly Leu Leu Gly Val Ser Thr Leu

1475

1480

1485

Gly Pro Lys Arg Asn Ser Met Val Leu Asp Val Ala Phe Val Leu

1490

1495

1500

Glu Gly Ser Asp Lys Ile Gly Glu Ala Asp Phe Asn Arg Ser Lys

1505

1510

1515

Glu Phe Met Glu Glu Val Ile Gln Arg Met Asp Val Gly Gln Asp

1520

1525

1530

Ser Ile His Val Thr Val Leu Gln Tyr Ser Tyr Met Val Thr Val

1535

1540

1545

Glu Tyr Pro Phe Ser Glu Ala Gln Ser Lys Gly Asp Ile Leu Gln

1550

1555

1560

Arg Val Arg Glu Ile Arg Tyr Gln Gly Gly Asn Arg Thr Asn Thr  
 1565 1570 1575

Gly Leu Ala Leu Arg Tyr Leu Ser Asp His Ser Phe Leu Val Ser  
 1580 1585 1590

Gln Gly Asp Arg Glu Gln Ala Pro Asn Leu Val Tyr Met Val Thr  
 1595 1600 1605

Gly Asn Pro Ala Ser Asp Glu Ile Lys Arg Leu Pro Gly Asp Ile  
 1610 1615 1620

Gln Val Val Pro Ile Gly Val Gly Pro Asn Ala Asn Val Gln Glu  
 1625 1630 1635

Leu Glu Arg Ile Gly Trp Pro Asn Ala Pro Ile Leu Ile Gln Asp  
 1640 1645 1650

Phe Glu Thr Leu Pro Arg Glu Ala Pro Asp Leu Val Leu Gln Arg  
 1655 1660 1665

Cys Cys Ser Gly Glu Gly Leu Gln Ile Pro Thr Leu Ser Pro Ala  
 1670 1675 1680

Pro Asp Cys Ser Gln Pro Leu Asp Val Ile Leu Leu Leu Asp Gly  
 1685 1690 1695

Ser Ser Ser Phe Pro Ala Ser Tyr Phe Asp Glu Met Lys Ser Phe

1700

1705

1710

Ala Lys Ala Phe Ile Ser Lys Ala Asn Ile Gly Pro Arg Leu Thr  
 1715 1720 1725

Gln Val Ser Val Leu Gln Tyr Gly Ser Ile Thr Thr Ile Asp Val  
 1730 1735 1740

Pro Trp Asn Val Val Pro Glu Lys Ala His Leu Leu Ser Leu Val  
 1745 1750 1755

Asp Val Met Gln Arg Glu Gly Gly Pro Ser Gln Ile Gly Asp Ala  
 1760 1765 1770

Leu Gly Phe Ala Val Arg Tyr Leu Thr Ser Glu Met His Gly Ala  
 1775 1780 1785

Arg Pro Gly Ala Ser Lys Ala Val Val Ile Leu Val Thr Asp Val  
 1790 1795 1800

Ser Val Asp Ser Val Asp Ala Ala Ala Asp Ala Ala Arg Ser Asn  
 1805 1810 1815

Arg Val Thr Val Phe Pro Ile Gly Ile Gly Asp Arg Tyr Asp Ala  
 1820 1825 1830

Ala Gln Leu Arg Ile Leu Ala Gly Pro Ala Gly Asp Ser Asn Val  
 1835 1840 1845

Val Lys Leu Gln Arg Ile Glu Asp Leu Pro Thr Met Val Thr Leu

1850

1855

1860

Gly Asn Ser Phe Leu His Lys Leu Cys Ser Gly Phe Val Arg Ile

1865

1870

1875

Cys Met Asp Glu Asp Gly Asn Glu Lys Arg Pro Gly Asp Val Trp

1880

1885

1890

Thr Leu Pro Asp Gln Cys His Thr Val Thr Cys Gln Pro Asp Gly

1895

1900

1905

Gln Thr Leu Leu Lys Ser His Arg Val Asn Cys Asp Arg Gly Leu

1910

1915

1920

Arg Pro Ser Cys Pro Asn Ser Gln Ser Pro Val Lys Val Glu Glu

1925

1930

1935

Thr Cys Gly Cys Arg Trp Thr Cys Pro Cys Val Cys Thr Gly Ser

1940

1945

1950

Ser Thr Arg His Ile Val Thr Phe Asp Gly Gln Asn Phe Lys Leu

1955

1960

1965

Thr Gly Ser Cys Ser Tyr Val Leu Phe Gln Asn Lys Glu Gln Asp

1970

1975

1980

Leu Glu Val Ile Leu His Asn Gly Ala Cys Ser Pro Gly Ala Arg

1985

1990

1995

Gln Gly Cys Met Lys Ser Ile Glu Val Lys His Ser Ala Leu Ser



2000

2005

2010

Val Glu Xaa His Ser Asp Met Glu Val Thr Val Asn Gly Arg Leu  
 2015 2020 2025

Val Ser Val Pro Tyr Val Gly Gly Asn Met Glu Val Asn Val Tyr  
 2030 2035 2040

Gly Ala Ile Met His Glu Val Arg Phe Asn His Leu Gly His Ile  
 2045 2050 2055

Phe Thr Phe Thr Pro Gln Asn Asn Glu Phe Gln Leu Gln Leu Ser  
 2060 2065 2070

Pro Lys Thr Phe Ala Ser Lys Thr Tyr Gly Leu Cys Gly Ile Cys  
 2075 2080 2085

Asp Glu Asn Gly Ala Asn Asp Phe Met Leu Arg Asp Gly Thr Val  
 2090 2095 2100

Thr Thr Asp Trp Lys Thr Leu Val Gln Glu Trp Thr Val Gln Arg  
 2105 2110 2115

Pro Gly Gln Thr Cys Gln Pro Ile Leu Glu Glu Gln Cys Leu Val  
 2120 2125 2130

Pro Asp Ser Ser His Cys Gln Val Leu Leu Leu Pro Leu Phe Ala  
 2135 2140 2145

Glu Cys His Lys Val Leu Ala Pro Ala Thr Phe Tyr Ala Ile Cys

2150

2155

2160

Gln Gln Asp Ser Cys His Gln Glu Gln Val Cys Glu Val Ile Ala  
 2165 2170 2175

Ser Tyr Ala His Leu Cys Arg Thr Asn Gly Val Cys Val Asp Trp  
 2180 2185 2190

Arg Thr Pro Asp Phe Cys Ala Met Ser Cys Pro Pro Ser Leu Val  
 2195 2200 2205

Tyr Asn His Cys Glu His Gly Cys Pro Arg His Cys Asp Gly Asn  
 2210 2215 2220

Val Ser Ser Cys Gly Asp His Pro Ser Glu Gly Cys Phe Cys Pro  
 2225 2230 2235

Pro Asp Lys Val Met Leu Glu Gly Ser Cys Val Pro Glu Glu Ala  
 2240 2245 2250

Cys Thr Gln Cys Ile Gly Glu Asp Gly Val Gln His Gln Phe Leu  
 2255 2260 2265

Glu Ala Trp Val Pro Asp His Gln Pro Cys Gln Ile Cys Thr Cys  
 2270 2275 2280

Leu Ser Gly Arg Lys Val Asn Cys Thr Thr Gln Pro Cys Pro Thr  
 2285 2290 2295

Ala Lys Ala Pro Thr Cys Gly Leu Cys Glu Val Ala Arg Leu Arg

2300

2305

2310

Gln Asn Ala Asp Gln Cys Cys Pro Glu Tyr Glu Cys Val Cys Asp  
 2315 2320 2325

Pro Val Ser Cys Asp Leu Pro Pro Val Pro His Cys Glu Arg Gly  
 2330 2335 2340

Leu Gln Pro Thr Leu Thr Asn Pro Gly Glu Cys Arg Pro Asn Phe  
 2345 2350 2355

Thr Cys Ala Cys Arg Lys Glu Glu Cys Lys Arg Val Ser Pro Pro  
 2360 2365 2370

Ser Cys Pro Pro His Arg Leu Pro Thr Leu Arg Lys Thr Gln Cys  
 2375 2380 2385

Cys Asp Glu Tyr Glu Cys Ala Cys Asn Cys Val Asn Ser Thr Val  
 2390 2395 2400

Ser Cys Pro Leu Gly Tyr Leu Ala Ser Thr Ala Thr Asn Asp Cys  
 2405 2410 2415

Gly Cys Thr Thr Thr Thr Cys Leu Pro Asp Lys Val Cys Val His  
 2420 2425 2430

Arg Ser Thr Ile Tyr Pro Val Gly Gln Phe Trp Glu Glu Gly Cys  
 2435 2440 2445

Asp Val Cys Thr Cys Thr Asp Met Glu Asp Ala Val Met Gly Leu

2450

2455

2460

Arg Val Ala Gln Cys Ser Gln Lys Pro Cys Glu Asp Ser Cys Arg  
 2465 2470 2475

Ser Gly Phe Thr Tyr Val Leu His Glu Gly Glu Cys Cys Gly Arg  
 2480 2485 2490

Cys Leu Pro Ser Ala Cys Glu Val Val Thr Gly Ser Pro Arg Gly  
 2495 2500 2505

Asp Ser Gln Ser Ser Trp Lys Ser Val Gly Ser Gln Trp Ala Ser  
 2510 2515 2520

Pro Glu Asn Pro Cys Leu Ile Asn Glu Cys Val Arg Val Lys Glu  
 2525 2530 2535

Glu Val Phe Ile Gln Gln Arg Asn Val Ser Cys Pro Gln Leu Glu  
 2540 2545 2550

Val Pro Val Cys Pro Ser Gly Phe Gln Leu Ser Cys Lys Thr Ser  
 2555 2560 2565

Ala Cys Cys Pro Ser Cys Arg Cys Glu Arg Met Glu Ala Cys Met  
 2570 2575 2580

Leu Asn Gly Thr Val Ile Gly Pro Gly Lys Thr Val Met Ile Asp  
 2585 2590 2595

Val Cys Thr Thr Cys Arg Cys Met Val Gln Val Gly Val Ile Ser

2600

2605

2610

Gly Phe Lys Leu Glu Cys Arg Lys Thr Thr Cys Asn Pro Cys Pro  
 2615 2620 2625

Leu Gly Tyr Lys Glu Glu Asn Asn Thr Gly Glu Cys Cys Gly Arg  
 2630 2635 2640

Cys Leu Pro Thr Ala Cys Thr Ile Gln Leu Arg Gly Gly Gln Ile  
 2645 2650 2655

Met Thr Leu Lys Arg Asp Glu Thr Leu Gln Asp Gly Cys Asp Thr  
 2660 2665 2670

His Phe Cys Lys Val Asn Glu Arg Gly Glu Tyr Phe Trp Glu Lys  
 2675 2680 2685

Arg Val Thr Gly Cys Pro Pro Phe Asp Glu His Lys Cys Leu Ala  
 2690 2695 2700

Glu Gly Gly Lys Ile Met Lys Ile Pro Gly Thr Cys Cys Asp Thr  
 2705 2710 2715

Cys Glu Glu Pro Glu Cys Asn Asp Ile Thr Ala Arg Leu Gln Tyr  
 2720 2725 2730

Val Lys Val Gly Ser Cys Lys Ser Glu Val Glu Val Asp Ile His  
 2735 2740 2745

Tyr Cys Gln Gly Lys Cys Ala Ser Lys Ala Met Tyr Ser Ile Asp

2750

2755

2760

Ile Asn Asp Val Gln Asp Gln Cys Ser Cys Cys Ser Pro Thr Arg

2765

2770

2775

Thr Glu Pro Met Gln Val Ala Leu His Cys Thr Asn Gly Ser Val

2780

2785

2790

Val Tyr His Glu Val Leu Asn Ala Met Glu Cys Lys Cys Ser Pro

2795

2800

2805

Arg Lys Cys Ser Lys

2810

&lt;210&gt; 3

&lt;211&gt; 19

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 3

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe

1

5

10

15

Cys Phe Ser

&lt;210&gt; 4

&lt;211&gt; 2332

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 4

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Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser Trp Asp Tyr
1           5           10           15

Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro
           20           25           30

Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys
           35           40           45

Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile Ala Lys Pro
           50           55           60

Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val
65           70           75           80

Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val
           85           90           95

Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala
           100           105           110

Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val
           115           120           125

Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn
           130           135           140

Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser
145           150           155           160

```

```

His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu
      165              170              175

Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu
      180              185              190

His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp
      195              200              205

His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser
      210              215              220

Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg
      225              230              235              240

Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His
      245              250              255

Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu
      260              265              270

Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile
      275              280              285

Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly
      290              295              300

Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met
      305              310              315              320

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|
| Glu | Ala | Tyr | Val | Lys | Val | Asp | Ser | Cys | Pro | Glu | Glu | Pro | Gln | Leu | Arg |  |  |  |
|     |     |     |     | 325 |     |     |     | 330 |     |     |     |     | 335 |     |     |  |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
| Met | Lys | Asn | Asn | Glu | Glu | Ala | Glu | Asp | Tyr | Asp | Asp | Asp | Leu | Thr | Asp |  |  |  |
|     |     |     |     | 340 |     |     |     | 345 |     |     |     |     | 350 |     |     |  |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
| Ser | Glu | Met | Asp | Val | Val | Arg | Phe | Asp | Asp | Asp | Asn | Ser | Pro | Ser | Phe |  |  |  |
|     |     |     |     | 355 |     |     |     | 360 |     |     |     |     | 365 |     |     |  |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
| Ile | Gln | Ile | Arg | Ser | Val | Ala | Lys | Lys | His | Pro | Lys | Thr | Trp | Val | His |  |  |  |
|     |     |     |     | 370 |     |     |     | 375 |     |     |     |     | 380 |     |     |  |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
| Tyr | Ile | Ala | Ala | Glu | Glu | Glu | Asp | Trp | Asp | Tyr | Ala | Pro | Leu | Val | Leu |  |  |  |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |  |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
| Ala | Pro | Asp | Asp | Arg | Ser | Tyr | Lys | Ser | Gln | Tyr | Leu | Asn | Asn | Gly | Pro |  |  |  |
|     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |  |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
| Gln | Arg | Ile | Gly | Arg | Lys | Tyr | Lys | Lys | Val | Arg | Phe | Met | Ala | Tyr | Thr |  |  |  |
|     |     |     |     | 420 |     |     |     | 425 |     |     |     |     | 430 |     |     |  |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
| Asp | Glu | Thr | Phe | Lys | Thr | Arg | Glu | Ala | Ile | Gln | His | Glu | Ser | Gly | Ile |  |  |  |
|     |     |     |     | 435 |     |     |     | 440 |     |     |     |     | 445 |     |     |  |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
| Leu | Gly | Pro | Leu | Leu | Tyr | Gly | Glu | Val | Gly | Asp | Thr | Leu | Leu | Ile | Ile |  |  |  |
|     |     |     |     | 450 |     |     |     | 455 |     |     |     | 460 |     |     |     |  |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
| Phe | Lys | Asn | Gln | Ala | Ser | Arg | Pro | Tyr | Asn | Ile | Tyr | Pro | His | Gly | Ile |  |  |  |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     | 480 |     |  |  |  |

Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys

485

490

495

His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys

500

505

510

Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys

515

520

525

Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala

530

535

540

Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp

545

550

555

560

Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe

565

570

575

Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln

580

585

590

Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe

595

600

605

Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser

610

615

620

Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu

625

630

635

640

Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr

645

650

655

Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro

660

665

670

Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp

675

680

685

Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala

690

695

700

Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu

705

710

715

720

Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala

725

730

735

Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Pro Ser Thr Arg

740

745

750

Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Asp Ile Glu Lys

755

760

765

Thr Asp Pro Trp Phe Ala His Arg Thr Pro Met Pro Lys Ile Gln Asn

770

775

780

Val Ser Ser Ser Asp Leu Leu Met Leu Leu Arg Gln Ser Pro Thr Pro

785

790

795

800

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Gly | Leu | Ser | Leu | Ser | Asp | Leu | Gln | Glu | Ala | Lys | Tyr | Glu | Thr | Phe |
|     |     |     |     | 805 |     |     |     |     | 810 |     |     |     |     | 815 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Asp | Asp | Pro | Ser | Pro | Gly | Ala | Ile | Asp | Ser | Asn | Asn | Ser | Leu | Ser |
|     |     |     |     | 820 |     |     |     | 825 |     |     |     |     | 830 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Met | Thr | His | Phe | Arg | Pro | Gln | Leu | His | His | Ser | Gly | Asp | Met | Val |
|     |     |     |     | 835 |     |     |     | 840 |     |     |     |     | 845 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Phe | Thr | Pro | Glu | Ser | Gly | Leu | Gln | Leu | Arg | Leu | Asn | Glu | Lys | Leu | Gly |
|     |     |     |     | 850 |     |     |     | 855 |     |     |     |     | 860 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr | Thr | Ala | Ala | Thr | Glu | Leu | Lys | Lys | Leu | Asp | Phe | Lys | Val | Ser | Ser |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 865 |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 880 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr | Ser | Asn | Asn | Leu | Ile | Ser | Thr | Ile | Pro | Ser | Asp | Asn | Leu | Ala | Ala |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 885 |     |     |     |     |     |     |     |     |     | 895 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Thr | Asp | Asn | Thr | Ser | Ser | Leu | Gly | Pro | Pro | Ser | Met | Pro | Val | His |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 900 |     |     |     |     |     |     |     |     |     | 910 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Asp | Ser | Gln | Leu | Asp | Thr | Thr | Leu | Phe | Gly | Lys | Lys | Ser | Ser | Pro |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 915 |     |     |     |     |     |     |     |     |     | 925 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Thr | Glu | Ser | Gly | Gly | Pro | Leu | Ser | Leu | Ser | Glu | Glu | Asn | Asn | Asp |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 930 |     |     |     |     |     |     |     |     |     | 940 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Lys | Leu | Leu | Glu | Ser | Gly | Leu | Met | Asn | Ser | Gln | Glu | Ser | Ser | Trp |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 945 |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 960 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Lys | Asn | Val | Ser | Ser | Thr | Glu | Ser | Gly | Arg | Leu | Phe | Lys | Gly | Lys |
|     |     |     |     | 965 |     |     |     |     | 970 |     |     |     |     | 975 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ala | His | Gly | Pro | Ala | Leu | Leu | Thr | Lys | Asp | Asn | Ala | Leu | Phe | Lys |
|     |     |     | 980 |     |     |     |     | 985 |     |     |     |     | 990 |     |     |

|     |     |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|
| Val | Ser | Ile | Ser | Leu | Leu | Lys | Thr  | Asn | Lys | Thr | Ser | Asn | Asn  | Ser | Ala |
|     |     |     | 995 |     |     |     | 1000 |     |     |     |     |     | 1005 |     |     |

|     |      |     |     |     |     |      |     |     |     |     |      |     |     |     |  |
|-----|------|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|--|
| Thr | Asn  | Arg | Lys | Thr | His | Ile  | Asp | Gly | Pro | Ser | Leu  | Leu | Ile | Glu |  |
|     | 1010 |     |     |     |     | 1015 |     |     |     |     | 1020 |     |     |     |  |

|     |      |     |     |     |     |      |     |     |     |     |      |     |     |     |  |
|-----|------|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|--|
| Asn | Ser  | Pro | Ser | Val | Trp | Gln  | Asn | Ile | Leu | Glu | Ser  | Asp | Thr | Glu |  |
|     | 1025 |     |     |     |     | 1030 |     |     |     |     | 1035 |     |     |     |  |

|     |      |     |     |     |     |      |     |     |     |     |      |     |     |     |  |
|-----|------|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|--|
| Phe | Lys  | Lys | Val | Thr | Pro | Leu  | Ile | His | Asp | Arg | Met  | Leu | Met | Asp |  |
|     | 1040 |     |     |     |     | 1045 |     |     |     |     | 1050 |     |     |     |  |

|     |      |     |     |     |     |      |     |     |     |     |      |     |     |     |  |
|-----|------|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|--|
| Lys | Asn  | Ala | Thr | Ala | Leu | Arg  | Leu | Asn | His | Met | Ser  | Asn | Lys | Thr |  |
|     | 1055 |     |     |     |     | 1060 |     |     |     |     | 1065 |     |     |     |  |

|     |      |     |     |     |     |      |     |     |     |     |      |     |     |     |  |
|-----|------|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|--|
| Thr | Ser  | Ser | Lys | Asn | Met | Glu  | Met | Val | Gln | Gln | Lys  | Lys | Glu | Gly |  |
|     | 1070 |     |     |     |     | 1075 |     |     |     |     | 1080 |     |     |     |  |

|     |      |     |     |     |     |      |     |     |     |     |      |     |     |     |  |
|-----|------|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|--|
| Pro | Ile  | Pro | Pro | Asp | Ala | Gln  | Asn | Pro | Asp | Met | Ser  | Phe | Phe | Lys |  |
|     | 1085 |     |     |     |     | 1090 |     |     |     |     | 1095 |     |     |     |  |

|     |      |     |     |     |     |      |     |     |     |     |      |     |     |     |  |
|-----|------|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|--|
| Met | Leu  | Phe | Leu | Pro | Glu | Ser  | Ala | Arg | Trp | Ile | Gln  | Arg | Thr | His |  |
|     | 1100 |     |     |     |     | 1105 |     |     |     |     | 1110 |     |     |     |  |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Gly  | Lys | Asn | Ser | Leu | Asn | Ser  | Gly | Gln | Gly | Pro | Ser  | Pro | Lys | Gln |
| 1115 |     |     |     |     |     | 1120 |     |     |     |     | 1125 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Val | Ser | Leu | Gly | Pro | Glu  | Lys | Ser | Val | Glu | Gly  | Gln | Asn | Phe |
| 1130 |     |     |     |     |     | 1135 |     |     |     |     | 1140 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Ser | Glu | Lys | Asn | Lys | Val  | Val | Val | Gly | Lys | Gly  | Glu | Phe | Thr |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     | 1155 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Asp | Val | Gly | Leu | Lys | Glu  | Met | Val | Phe | Pro | Ser  | Ser | Arg | Asn |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Phe | Leu | Thr | Asn | Leu | Asp  | Asn | Leu | His | Glu | Asn  | Asn | Thr | His |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Gln | Glu | Lys | Lys | Ile | Gln  | Glu | Glu | Ile | Glu | Lys  | Lys | Glu | Thr |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     | 1200 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Ile | Gln | Glu | Asn | Val | Val  | Leu | Pro | Gln | Ile | His  | Thr | Val | Thr |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     | 1215 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Thr | Lys | Asn | Phe | Met | Lys  | Asn | Leu | Phe | Leu | Leu  | Ser | Thr | Arg |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Asn | Val | Glu | Gly | Ser | Tyr  | Asp | Gly | Ala | Tyr | Ala  | Pro | Val | Leu |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Asp | Phe | Arg | Ser | Leu | Asn  | Asp | Ser | Thr | Asn | Arg  | Thr | Lys | Lys |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|
| His  | Thr | Ala | His | Phe | Ser | Lys  | Lys | Gly | Glu | Glu | Glu | Asn  | Leu | Glu |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     |     | 1275 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gly  | Leu | Gly | Asn | Gln | Thr | Lys  | Gln | Ile | Val | Glu | Lys | Tyr  | Ala | Cys |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     |     | 1290 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Thr  | Thr | Arg | Ile | Ser | Pro | Asn  | Thr | Ser | Gln | Gln | Asn | Phe  | Val | Thr |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     |     | 1305 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gln  | Arg | Ser | Lys | Arg | Ala | Leu  | Lys | Gln | Phe | Arg | Leu | Pro  | Leu | Glu |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     |     | 1320 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Glu  | Thr | Glu | Leu | Glu | Lys | Arg  | Ile | Ile | Val | Asp | Asp | Thr  | Ser | Thr |
| 1325 |     |     |     |     |     | 1330 |     |     |     |     |     | 1335 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gln  | Trp | Ser | Lys | Asn | Met | Lys  | His | Leu | Thr | Pro | Ser | Thr  | Leu | Thr |
| 1340 |     |     |     |     |     | 1345 |     |     |     |     |     | 1350 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gln  | Ile | Asp | Tyr | Asn | Glu | Lys  | Glu | Lys | Gly | Ala | Ile | Thr  | Gln | Ser |
| 1355 |     |     |     |     |     | 1360 |     |     |     |     |     | 1365 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Pro  | Leu | Ser | Asp | Cys | Leu | Thr  | Arg | Ser | His | Ser | Ile | Pro  | Gln | Ala |
| 1370 |     |     |     |     |     | 1375 |     |     |     |     |     | 1380 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Asn  | Arg | Ser | Pro | Leu | Pro | Ile  | Ala | Lys | Val | Ser | Ser | Phe  | Pro | Ser |
| 1385 |     |     |     |     |     | 1390 |     |     |     |     |     | 1395 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ile  | Arg | Pro | Ile | Tyr | Leu | Thr  | Arg | Val | Leu | Phe | Gln | Asp  | Asn | Ser |
| 1400 |     |     |     |     |     | 1405 |     |     |     |     |     | 1410 |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ser  | His | Leu | Pro | Ala | Ala | Ser  | Tyr | Arg | Lys | Lys | Asp  | Ser | Gly | Val |
| 1415 |     |     |     |     |     | 1420 |     |     |     |     | 1425 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Glu | Ser | Ser | His | Phe | Leu  | Gln | Gly | Ala | Lys | Lys  | Asn | Asn | Leu |
| 1430 |     |     |     |     |     | 1435 |     |     |     |     | 1440 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Leu | Ala | Ile | Leu | Thr | Leu  | Glu | Met | Thr | Gly | Asp  | Gln | Arg | Glu |
| 1445 |     |     |     |     |     | 1450 |     |     |     |     | 1455 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Gly | Ser | Leu | Gly | Thr | Ser  | Ala | Thr | Asn | Ser | Val  | Thr | Tyr | Lys |
| 1460 |     |     |     |     |     | 1465 |     |     |     |     | 1470 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Val | Glu | Asn | Thr | Val | Leu  | Pro | Lys | Pro | Asp | Leu  | Pro | Lys | Thr |
| 1475 |     |     |     |     |     | 1480 |     |     |     |     | 1485 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | Lys | Val | Glu | Leu | Leu  | Pro | Lys | Val | His | Ile  | Tyr | Gln | Lys |
| 1490 |     |     |     |     |     | 1495 |     |     |     |     | 1500 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Leu | Phe | Pro | Thr | Glu | Thr  | Ser | Asn | Gly | Ser | Pro  | Gly | His | Leu |
| 1505 |     |     |     |     |     | 1510 |     |     |     |     | 1515 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Leu | Val | Glu | Gly | Ser | Leu  | Leu | Gln | Gly | Thr | Glu  | Gly | Ala | Ile |
| 1520 |     |     |     |     |     | 1525 |     |     |     |     | 1530 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Trp | Asn | Glu | Ala | Asn | Arg  | Pro | Gly | Lys | Val | Pro  | Phe | Leu | Arg |
| 1535 |     |     |     |     |     | 1540 |     |     |     |     | 1545 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Ala | Thr | Glu | Ser | Ser | Ala  | Lys | Thr | Pro | Ser | Lys  | Leu | Leu | Asp |
| 1550 |     |     |     |     |     | 1555 |     |     |     |     | 1560 |     |     |     |



|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Pro  | Leu | Ala | Trp | Asp | Asn | His  | Tyr | Gly | Thr | Gln | Ile  | Pro | Lys | Glu |
| 1565 |     |     |     |     |     | 1570 |     |     |     |     | 1575 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Trp | Lys | Ser | Gln | Glu | Lys  | Ser | Pro | Glu | Lys | Thr  | Ala | Phe | Lys |
| 1580 |     |     |     |     |     | 1585 |     |     |     |     | 1590 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Lys | Asp | Thr | Ile | Leu | Ser  | Leu | Asn | Ala | Cys | Glu  | Ser | Asn | His |
| 1595 |     |     |     |     |     | 1600 |     |     |     |     | 1605 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Ile | Ala | Ala | Ile | Asn | Glu  | Gly | Gln | Asn | Lys | Pro  | Glu | Ile | Glu |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Thr | Trp | Ala | Lys | Gln | Gly  | Arg | Thr | Glu | Arg | Leu  | Cys | Ser | Gln |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Pro | Pro | Val | Leu | Lys | Arg  | His | Gln | Arg | Glu | Ile  | Thr | Arg | Thr |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Leu | Gln | Ser | Asp | Gln | Glu  | Glu | Ile | Asp | Tyr | Asp  | Asp | Thr | Ile |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Val | Glu | Met | Lys | Lys | Glu  | Asp | Phe | Asp | Ile | Tyr  | Asp | Glu | Asp |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Asn | Gln | Ser | Pro | Arg | Ser  | Phe | Gln | Lys | Lys | Thr  | Arg | His | Tyr |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Ile | Ala | Ala | Val | Glu | Arg  | Leu | Trp | Asp | Tyr | Gly  | Met | Ser | Ser |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ser  | Pro | His | Val | Leu | Arg | Asn  | Arg | Ala | Gln | Ser | Gly  | Ser | Val | Pro |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     | 1725 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Phe | Lys | Lys | Val | Val | Phe  | Gln | Glu | Phe | Thr | Asp  | Gly | Ser | Phe |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     | 1740 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Gln | Pro | Leu | Tyr | Arg | Gly  | Glu | Leu | Asn | Glu | His  | Leu | Gly | Leu |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     | 1755 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Gly | Pro | Tyr | Ile | Arg | Ala  | Glu | Val | Glu | Asp | Asn  | Ile | Met | Val |
| 1760 |     |     |     |     |     | 1765 |     |     |     |     | 1770 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Phe | Arg | Asn | Gln | Ala | Ser  | Arg | Pro | Tyr | Ser | Phe  | Tyr | Ser | Ser |
| 1775 |     |     |     |     |     | 1780 |     |     |     |     | 1785 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Ile | Ser | Tyr | Glu | Glu | Asp  | Gln | Arg | Gln | Gly | Ala  | Glu | Pro | Arg |
| 1790 |     |     |     |     |     | 1795 |     |     |     |     | 1800 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Asn | Phe | Val | Lys | Pro | Asn  | Glu | Thr | Lys | Thr | Tyr  | Phe | Trp | Lys |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     | 1815 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Gln | His | His | Met | Ala | Pro  | Thr | Lys | Asp | Glu | Phe  | Asp | Cys | Lys |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     | 1830 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Trp | Ala | Tyr | Phe | Ser | Asp  | Val | Asp | Leu | Glu | Lys  | Asp | Val | His |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     | 1845 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | Leu | Ile | Gly | Pro | Leu  | Leu | Val | Cys | His | Thr  | Asn | Thr | Leu |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     | 1860 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Asn  | Pro | Ala | His | Gly | Arg | Gln  | Val | Thr | Val | Gln | Glu  | Phe | Ala | Leu |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     | 1875 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Phe | Thr | Ile | Phe | Asp | Glu  | Thr | Lys | Ser | Trp | Tyr  | Phe | Thr | Glu |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     | 1890 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Met | Glu | Arg | Asn | Cys | Arg  | Ala | Pro | Cys | Asn | Ile  | Gln | Met | Glu |
| 1895 |     |     |     |     |     | 1900 |     |     |     |     | 1905 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Pro | Thr | Phe | Lys | Glu | Asn  | Tyr | Arg | Phe | His | Ala  | Ile | Asn | Gly |
| 1910 |     |     |     |     |     | 1915 |     |     |     |     | 1920 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Ile | Met | Asp | Thr | Leu | Pro  | Gly | Leu | Val | Met | Ala  | Gln | Asp | Gln |
| 1925 |     |     |     |     |     | 1930 |     |     |     |     | 1935 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Ile | Arg | Trp | Tyr | Leu | Leu  | Ser | Met | Gly | Ser | Asn  | Glu | Asn | Ile |
| 1940 |     |     |     |     |     | 1945 |     |     |     |     | 1950 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| His  | Ser | Ile | His | Phe | Ser | Gly  | His | Val | Phe | Thr | Val  | Arg | Lys | Lys |
| 1955 |     |     |     |     |     | 1960 |     |     |     |     | 1965 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Glu | Tyr | Lys | Met | Ala | Leu  | Tyr | Asn | Leu | Tyr | Pro  | Gly | Val | Phe |
| 1970 |     |     |     |     |     | 1975 |     |     |     |     | 1980 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Thr | Val | Glu | Met | Leu | Pro  | Ser | Lys | Ala | Gly | Ile  | Trp | Arg | Val |
| 1985 |     |     |     |     |     | 1990 |     |     |     |     | 1995 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Cys | Leu | Ile | Gly | Glu | His  | Leu | His | Ala | Gly | Met  | Ser | Thr | Leu |
| 2000 |     |     |     |     |     | 2005 |     |     |     |     | 2010 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Phe  | Leu | Val | Tyr | Ser | Asn | Lys  | Cys | Gln | Thr | Pro | Leu  | Gly | Met | Ala |
| 2015 |     |     |     |     |     | 2020 |     |     |     |     | 2025 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | His | Ile | Arg | Asp | Phe  | Gln | Ile | Thr | Ala | Ser  | Gly | Gln | Tyr |
| 2030 |     |     |     |     |     | 2035 |     |     |     |     | 2040 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Gln | Trp | Ala | Pro | Lys | Leu  | Ala | Arg | Leu | His | Tyr  | Ser | Gly | Ser |
| 2045 |     |     |     |     |     | 2050 |     |     |     |     | 2055 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Asn | Ala | Trp | Ser | Thr | Lys  | Glu | Pro | Phe | Ser | Trp  | Ile | Lys | Val |
| 2060 |     |     |     |     |     | 2065 |     |     |     |     | 2070 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Leu | Leu | Ala | Pro | Met | Ile  | Ile | His | Gly | Ile | Lys  | Thr | Gln | Gly |
| 2075 |     |     |     |     |     | 2080 |     |     |     |     | 2085 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Arg | Gln | Lys | Phe | Ser | Ser  | Leu | Tyr | Ile | Ser | Gln  | Phe | Ile | Ile |
| 2090 |     |     |     |     |     | 2095 |     |     |     |     | 2100 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Tyr | Ser | Leu | Asp | Gly | Lys  | Lys | Trp | Gln | Thr | Tyr  | Arg | Gly | Asn |
| 2105 |     |     |     |     |     | 2110 |     |     |     |     | 2115 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Thr | Gly | Thr | Leu | Met | Val  | Phe | Phe | Gly | Asn | Val  | Asp | Ser | Ser |
| 2120 |     |     |     |     |     | 2125 |     |     |     |     | 2130 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ile | Lys | His | Asn | Ile | Phe  | Asn | Pro | Pro | Ile | Ile  | Ala | Arg | Tyr |
| 2135 |     |     |     |     |     | 2140 |     |     |     |     | 2145 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Arg | Leu | His | Pro | Thr | His  | Tyr | Ser | Ile | Arg | Ser  | Thr | Leu | Arg |
| 2150 |     |     |     |     |     | 2155 |     |     |     |     | 2160 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Met  | Glu | Leu | Met | Gly | Cys | Asp  | Leu | Asn | Ser | Cys | Ser  | Met | Pro | Leu |
| 2165 |     |     |     |     |     | 2170 |     |     |     |     | 2175 |     |     |     |
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| Gly  | Met | Glu | Ser | Lys | Ala | Ile  | Ser | Asp | Ala | Gln | Ile  | Thr | Ala | Ser |
| 2180 |     |     |     |     |     | 2185 |     |     |     |     | 2190 |     |     |     |
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| Ser  | Tyr | Phe | Thr | Asn | Met | Phe  | Ala | Thr | Trp | Ser | Pro  | Ser | Lys | Ala |
| 2195 |     |     |     |     |     | 2200 |     |     |     |     | 2205 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Leu | His | Leu | Gln | Gly | Arg  | Ser | Asn | Ala | Trp | Arg  | Pro | Gln | Val |
| 2210 |     |     |     |     |     | 2215 |     |     |     |     | 2220 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Asn | Pro | Lys | Glu | Trp | Leu  | Gln | Val | Asp | Phe | Gln  | Lys | Thr | Met |
| 2225 |     |     |     |     |     | 2230 |     |     |     |     | 2235 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Val | Thr | Gly | Val | Thr | Thr  | Gln | Gly | Val | Lys | Ser  | Leu | Leu | Thr |
| 2240 |     |     |     |     |     | 2245 |     |     |     |     | 2250 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Met | Tyr | Val | Lys | Glu | Phe  | Leu | Ile | Ser | Ser | Ser  | Gln | Asp | Gly |
| 2255 |     |     |     |     |     | 2260 |     |     |     |     | 2265 |     |     |     |
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| His  | Gln | Trp | Thr | Leu | Phe | Phe  | Gln | Asn | Gly | Lys | Val  | Lys | Val | Phe |
| 2270 |     |     |     |     |     | 2275 |     |     |     |     | 2280 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Gly | Asn | Gln | Asp | Ser | Phe  | Thr | Pro | Val | Val | Asn  | Ser | Leu | Asp |
| 2285 |     |     |     |     |     | 2290 |     |     |     |     | 2295 |     |     |     |
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| Pro  | Pro | Leu | Leu | Thr | Arg | Tyr  | Leu | Arg | Ile | His | Pro  | Gln | Ser | Trp |
| 2300 |     |     |     |     |     | 2305 |     |     |     |     | 2310 |     |     |     |

Val His Gln Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu Ala

2315

2320

2325

Gln Asp Leu Tyr

2330

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|                                                                    |      |
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Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile Ala Lys Pro  
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Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val  
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Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val  
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Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala  
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Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val  
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Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn  
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Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser  
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His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu  
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Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu  
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His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp  
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His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser  
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Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg  
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Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His  
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Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu  
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Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile  
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Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly  
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Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met  
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Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg  
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Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp  
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Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe  
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Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His  
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Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu  
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Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro  
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Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr  
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Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile  
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Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile  
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Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys  
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His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys  
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Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln  
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Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe  
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Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser  
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Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu  
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Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr  
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Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro  
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Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp  
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Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala  
 690 695 700

Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu  
 705 710 715 720



Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala  
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Ile Glu Pro Arg Ser Phe Ser Gln Asn Pro Pro Val Leu Lys Arg His  
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Gln Arg Glu Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile  
755 760 765

Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu Asp Phe Asp  
770 775 780

Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys  
785 790 795 800

Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly  
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Met Ser Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly Ser  
820 825 830

Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser  
835 840 845

Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly Leu  
850 855 860

Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr  
865 870 875 880

Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile  
885 890 895

Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe  
900 905 910

Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His His  
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Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe  
930 935 940

Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro  
945 950 955 960

Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly Arg Gln  
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Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr  
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Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala Pro  
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Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn Tyr Arg  
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Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr Leu Pro Gly Leu  
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|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Val  | Met | Ala | Gln | Asp | Gln | Arg  | Ile | Arg | Trp | Tyr | Leu  | Leu | Ser | Met |
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|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ser | Asn | Glu | Asn | Ile | His  | Ser | Ile | His | Phe | Ser  | Gly | His | Val |
| 1055 |     |     |     |     |     | 1060 |     |     |     |     | 1065 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Thr | Val | Arg | Lys | Lys | Glu  | Glu | Tyr | Lys | Met | Ala  | Leu | Tyr | Asn |
| 1070 |     |     |     |     |     | 1075 |     |     |     |     | 1080 |     |     |     |
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| Leu  | Tyr | Pro | Gly | Val | Phe | Glu  | Thr | Val | Glu | Met | Leu  | Pro | Ser | Lys |
| 1085 |     |     |     |     |     | 1090 |     |     |     |     | 1095 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Gly | Ile | Trp | Arg | Val | Glu  | Cys | Leu | Ile | Gly | Glu  | His | Leu | His |
| 1100 |     |     |     |     |     | 1105 |     |     |     |     | 1110 |     |     |     |
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| Ala  | Gly | Met | Ser | Thr | Leu | Phe  | Leu | Val | Tyr | Ser | Asn  | Lys | Cys | Gln |
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| Thr  | Pro | Leu | Gly | Met | Ala | Ser  | Gly | His | Ile | Arg | Asp  | Phe | Gln | Ile |
| 1130 |     |     |     |     |     | 1135 |     |     |     |     | 1140 |     |     |     |
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| Thr  | Ala | Ser | Gly | Gln | Tyr | Gly  | Gln | Trp | Ala | Pro | Lys  | Leu | Ala | Arg |
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| Leu  | His | Tyr | Ser | Gly | Ser | Ile  | Asn | Ala | Trp | Ser | Thr  | Lys | Glu | Pro |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Ser | Trp | Ile | Lys | Val | Asp  | Leu | Leu | Ala | Pro | Met  | Ile | Ile | His |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |

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| Gly  | Ile | Lys | Thr | Gln | Gly | Ala  | Arg | Gln | Lys | Phe | Ser  | Ser | Leu | Tyr |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     | 1200 |     |     |     |
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| Ile  | Ser | Gln | Phe | Ile | Ile | Met  | Tyr | Ser | Leu | Asp | Gly  | Lys | Lys | Trp |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     | 1215 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Thr | Tyr | Arg | Gly | Asn | Ser  | Thr | Gly | Thr | Leu | Met  | Val | Phe | Phe |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
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| Gly  | Asn | Val | Asp | Ser | Ser | Gly  | Ile | Lys | His | Asn | Ile  | Phe | Asn | Pro |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
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| Pro  | Ile | Ile | Ala | Arg | Tyr | Ile  | Arg | Leu | His | Pro | Thr  | His | Tyr | Ser |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |
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| Ile  | Arg | Ser | Thr | Leu | Arg | Met  | Glu | Leu | Met | Gly | Cys  | Asp | Leu | Asn |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     | 1275 |     |     |     |
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| Ser  | Cys | Ser | Met | Pro | Leu | Gly  | Met | Glu | Ser | Lys | Ala  | Ile | Ser | Asp |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     | 1290 |     |     |     |
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| Ala  | Gln | Ile | Thr | Ala | Ser | Ser  | Tyr | Phe | Thr | Asn | Met  | Phe | Ala | Thr |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     | 1305 |     |     |     |
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| Trp  | Ser | Pro | Ser | Lys | Ala | Arg  | Leu | His | Leu | Gln | Gly  | Arg | Ser | Asn |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     | 1320 |     |     |     |
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| Ala  | Trp | Arg | Pro | Gln | Val | Asn  | Asn | Pro | Lys | Glu | Trp  | Leu | Gln | Val |
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Ser Ser Ser Gln Asp Gly His Gln Trp Thr Leu Phe Phe Gln Asn  
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Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp Ser Phe Thr Pro  
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Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg  
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| acctcagtcg tgtacaaaaa gactctgttt gtagaattca cggatcacct tttcaacatc   | 240  |
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| accactcctg aagtgcactc aatattcctc gaaggtcaca catttcttgt gaggaaccat   | 900  |
| cgccaggcgt ccttggaat ctcgccaata actttcctta ctgctcaaac actcttgatg    | 960  |
| gaccttggaac agtttctact gttttgtcat atctcttccc accaacaatga tggcatggaa | 1020 |
| gcttatgtca aagtagacag ctgtccagag gaaccccaac tacgaatgaa aaataatgaa   | 1080 |
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| gatgacaact ctccttcctt tatccaaatt cgctcagttg ccaagaagca tcctaaaact   | 1200 |
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| caggatcaaa ggattcgatg gtatctgctc agcatgggca gcaatgaaaa catccattct | 3240 |
| attcatttca gtggacatgt gttcactgta cgaaaaaaag aggagtataa aatggcactg | 3300 |
| tacaatctct atccagggtg ttttgagaca gtggaaatgt taccatccaa agctggaatt | 3360 |
| tggcgggtgg aatgccttat tggcgagcat ctacatgctg ggatgagcac actttttctg | 3420 |
| gtgtacagca ataagtgtca gactcccctg ggaatggctt ctggacacat tagagatttt | 3480 |
| cagattacag cttcaggaca atatggacag tgggccccaa agctggccag acttcattat | 3540 |
| tccggatcaa tcaatgcctg gagcaccaag gagccctttt cttggatcaa ggtggatctg | 3600 |
| ttggcaccaa tgattattca cggcatcaag acccagggtg cccgtcagaa gttctccagc | 3660 |



|                                                                    |      |
|--------------------------------------------------------------------|------|
| ctctacatct ctcagtttat catcatgtat agtcttgatg ggaagaagtg gcagacttat  | 3720 |
| cgaggaaatt ccactggaac cttaatggtc ttctttggca atgtggattc atctgggata  | 3780 |
| aaacacaata tttttaaccc tccaattatt gctcgataca tccgtttgca cccaactcat  | 3840 |
| tatagcattc gcagcactct tcgcatggag ttgatgggct gtgatttaaa tagttgcagc  | 3900 |
| atgccattgg gaatggagag taaagcaata tcagatgcac agattactgc ttcacacctac | 3960 |
| tttaccaata tgtttgccac ctggtctcct tcaaaagctc gacttcacct ccaagggagg  | 4020 |
| agtaatgcct ggagacctca ggtgaataat ccaaaagagt ggctgcaagt ggacttccag  | 4080 |
| aagacaatga aagtcacagg agtaactact cagggagtaa aatctctgct taccagcatg  | 4140 |
| tatgtgaagg agttcctcat ctccagcagt caagatggcc atcagtggac tctctttttt  | 4200 |
| cagaatggca aagtaaaggt ttttcagga aatcaagact ccttcacacc tgtggtgaac   | 4260 |
| tctctagacc caccgttact gactcgctac cttcgaattc acccccagag ttgggtgcac  | 4320 |
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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Leu | Asp | Pro | Arg | Ser | Phe | Leu | Leu | Arg | Asn | Pro | Asn | Asp | Lys | Tyr |
| 1   |     |     | 5   |     |     |     |     |     | 10  |     |     |     |     | 15  |     |

Glu Pro Phe Trp Glu Asp Glu Glu Lys

20

25

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Arg Arg Arg Arg

1

<210> 10

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 10

Arg Lys Arg Arg Lys Arg

1

5

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<211> 5

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<223> cleavage site

&lt;400&gt; 11

Arg Arg Arg Arg Ser

1 5

&lt;210&gt; 12

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; cleavage site

&lt;400&gt; 12

Thr Gln Ser Phe Asn Asp Phe Thr Arg

1 5

&lt;210&gt; 13

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; cleavage site

&lt;400&gt; 13

Ser Val Ser Gln Thr Ser Lys Leu Thr Arg

1 5 10

&lt;210&gt; 14

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

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<400> 14

Asp Phe Leu Ala Glu Gly Gly Gly Val Arg

1 5 10

<210> 15

<211> 7

<212> PRT

<213> Artificial Sequence

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<400> 15

Thr Thr Lys Ile Lys Pro Arg

1 5

<210> 16

<211> 5

<212> PRT

<213> Artificial Sequence

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<400> 16

Leu Val Pro Arg Gly

1 5

<210> 17

<211> 5

<212> PRT

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Ala Leu Arg Pro Arg  
1 5

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<400> 18

Lys Leu Thr Arg Ala Glu Thr  
1 5

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<400> 19

Asp Phe Thr Arg Val Val Gly  
1 5

<210> 20  
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<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 20

Thr Met Thr Arg Ile Val Gly Gly

1 5

<210> 21

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 21

Ser Pro Phe Arg Ser Thr Gly Gly

1 5

<210> 22

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 22

Leu Gln Val Arg Ile Val Gly Gly

1 5

<210> 23

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 23

Pro Leu Gly Arg Ile Val Gly Gly

1 5

<210> 24

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 24

Ile Glu Gly Arg Thr Val Gly Gly

1 5

<210> 25

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 25

Leu Thr Pro Arg Ser Leu Leu Val

1 5

<210> 26  
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<220>  
<223> cleavage site

<400> 26

Leu Gly Pro Val Ser Gly Val Pro  
1 5

<210> 27  
<211> 8  
<212> PRT  
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<220>  
<223> cleavage site

<400> 27

Val Ala Gly Asp Ser Leu Glu Glu  
1 5

<210> 28  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

<400> 28



Gly Pro Ala Gly Leu Gly Gly Ala

1 5

<210> 29

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 29

Gly Pro Ala Gly Leu Arg Gly Ala

1 5

<210> 30

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 30

Ala Pro Leu Gly Leu Arg Leu Arg

1 5

<210> 31

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

&lt;400&gt; 31

Pro Ala Leu Pro Leu Val Ala Gln

1 5

&lt;210&gt; 32

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; cleavage site

&lt;400&gt; 32

Glu Asn Leu Tyr Phe Gln Gly

1 5

&lt;210&gt; 33

&lt;211&gt; 8

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; cleavage site

&lt;400&gt; 33

Asp Asp Asp Lys Ile Val Gly Gly

1 5

&lt;210&gt; 34

&lt;211&gt; 8

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> cleavage site

<400> 34

Leu Glu Val Leu Phe Gln Gly Pro

1 5

<210> 35

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 35

Leu Pro Lys Thr Gly Ser Glu Ser

1 5

<210> 36

<211> 42

<212> PRT

<213> Artificial Sequence

<220>

<223> AE42

<400> 36

Gly Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly

1 5 10 15

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala

20 25 30

Thr Ser Gly Ser Glu Thr Pro Ala Ser Ser

35

40

<210> 37

<211> 143

<212> PRT

<213> Artificial Sequence

<220>

<223> AE144

<400> 37

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu

1

5

10

15

Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly

20

25

30

Ser Glu Thr Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu

35

40

45

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Glu Pro

50

55

60

Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Glu Pro Ala Thr Ser Gly

65

70

75

80

Ser Glu Thr Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro

85

90

95

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu

100

105

110

Ser Ala Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser  
 115 120 125

Glu Thr Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 130 135 140

<210> 38

<211> 144

<212> PRT

<213> Artificial Sequence

<220>

<223> AG144

<400> 38

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr  
 1 5 10 15

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser Thr  
 20 25 30

Gly Thr Gly Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro  
 35 40 45

Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro  
 50 55 60

Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala  
 65 70 75 80

Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro  
85 90 95

Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser Pro  
100 105 110

Ser Ala Ser Thr Gly Thr Gly Pro Gly Thr Pro Gly Ser Gly Thr Ala  
115 120 125

Ser Ser Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro  
130 135 140

<210> 39

<211> 288

<212> PRT

<213> Artificial Sequence

<220>

<223> AE288

<400> 39

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro  
1 5 10 15

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro  
20 25 30

Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro  
35 40 45

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr  
50 55 60

Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr  
 65 70 75 80

Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
 85 90 95

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu  
 100 105 110

Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Pro Ala Gly Ser Pro Thr  
 115 120 125

Ser Thr Glu Glu Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu  
 130 135 140

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu  
 145 150 155 160

Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 165 170 175

Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
 180 185 190

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Glu Pro  
 195 200 205

Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Pro Ala Gly Ser Pro Thr  
 210 215 220

Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 225 230 235 240

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Glu Pro  
 245 250 255

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 260 265 270

Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 275 280 285

<210> 40

<211> 288

<212> PRT

<213> Artificial Sequence

<220>

<223> AG288

<400> 40

Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser  
 1 5 10 15

Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr  
 20 25 30

Ala Ser Ser Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser  
 35 40 45



Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser  
 50 55 60

Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr  
 65 70 75 80

Ala Ser Ser Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser  
 85 90 95

Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser  
 100 105 110

Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser Pro Ser Ala Ser  
 115 120 125

Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser  
 130 135 140

Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser  
 145 150 155 160

Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser  
 165 170 175

Thr Gly Thr Gly Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly  
 180 185 190

Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser  
 195 200 205

Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly  
 210 215 220

Ala Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly  
 225 230 235 240

Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser  
 245 250 255

Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Thr Pro Gly Ser Gly Thr  
 260 265 270

Ala Ser Ser Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser  
 275 280 285

<210> 41  
 <211> 576  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> AE576

<400> 41

Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu  
 1 5 10 15

Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu  
 20 25 30

Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu  
 35 40 45

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr  
 50 55 60

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 65 70 75 80

Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro  
 85 90 95

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Pro Ala  
 100 105 110

Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro  
 115 120 125

Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 130 135 140

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala  
 145 150 155 160

Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu  
 165 170 175

Gly Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 180 185 190

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr  
 195 200 205

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 210 215 220

Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro  
 225 230 235 240

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr  
 245 250 255

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 260 265 270

Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
 275 280 285

Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu  
 290 295 300

Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly  
 305 310 315 320

Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
 325 330 335

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr  
 340 345 350

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu  
 355 360 365

Gly Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 370 375 380

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr  
 385 390 395 400

Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr  
 405 410 415

Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 420 425 430

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro  
 435 440 445

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 450 455 460

Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro  
 465 470 475 480

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr  
 485 490 495

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 500 505 510

Glu Ser Gly Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu  
 515 520 525

Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala  
 530 535 540

Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro  
 545 550 555 560

Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 565 570 575

<210> 42

<211> 576

<212> PRT

<213> Artificial Sequence

<220>

<223> AG576

<400> 42

Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser  
 1 5 10 15

Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser  
 20 25 30

Thr Gly Thr Gly Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly  
 35 40 45

Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser  
 50 55 60

Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser  
65 70 75 80

Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser  
85 90 95

Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Thr Pro  
100 105 110

Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser Pro Gly Thr Ser  
115 120 125

Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser  
130 135 140

Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser  
145 150 155 160

Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Thr Pro Gly Ser Gly Thr  
165 170 175

Ala Ser Ser Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser  
180 185 190

Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser  
195 200 205

Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly  
210 215 220

Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser  
 225 230 235 240

Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Thr Pro  
 245 250 255

Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr Pro Ser Gly  
 260 265 270

Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser  
 275 280 285

Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser  
 290 295 300

Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser  
 305 310 315 320

Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser  
 325 330 335

Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser  
 340 345 350

Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser  
 355 360 365

Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser  
 370 375 380



Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Thr Pro  
 385 390 395 400

Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr Pro Ser Gly  
 405 410 415

Ala Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser  
 420 425 430

Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Thr Pro  
 435 440 445

Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr Pro Ser Gly  
 450 455 460

Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser  
 465 470 475 480

Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser  
 485 490 495

Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser  
 500 505 510

Ser Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser  
 515 520 525

Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser  
 530 535 540

Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser Pro Ser Ala Ser  
545 550 555 560

Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser  
565 570 575

<210> 43

<211> 864

<212> PRT

<213> Artificial Sequence

<220>

<223> AE864

<400> 43

Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu  
1 5 10 15

Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu  
20 25 30

Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu  
35 40 45

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr  
50 55 60

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro  
65 70 75 80

Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro  
85 90 95

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Pro Ala  
 100 105 110

Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro  
 115 120 125

Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 130 135 140

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala  
 145 150 155 160

Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu  
 165 170 175

Gly Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 180 185 190

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr  
 195 200 205

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 210 215 220

Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro  
 225 230 235 240

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr  
 245 250 255

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 260 265 270

Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
 275 280 285

Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu  
 290 295 300

Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly  
 305 310 315 320

Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
 325 330 335

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr  
 340 345 350

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu  
 355 360 365

Gly Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 370 375 380

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr  
 385 390 395 400

Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr  
 405 410 415

Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 420 425 430

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro  
 435 440 445

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 450 455 460

Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro  
 465 470 475 480

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr  
 485 490 495

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 500 505 510

Glu Ser Gly Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu  
 515 520 525

Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala  
 530 535 540

Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro  
 545 550 555 560

Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
 565 570 575

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro  
580 585 590

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro  
595 600 605

Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro  
610 615 620

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr  
625 630 635 640

Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr  
645 650 655

Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
660 665 670

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu  
675 680 685

Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Pro Ala Gly Ser Pro Thr  
690 695 700

Ser Thr Glu Glu Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu  
705 710 715 720

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu  
725 730 735

Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro  
740 745 750

Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
755 760 765

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Glu Pro  
770 775 780

Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Pro Ala Gly Ser Pro Thr  
785 790 795 800

Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
805 810 815

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Glu Pro  
820 825 830

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro  
835 840 845

Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
850 855 860

<210> 44

<211> 864

<212> PRT

<213> Artificial Sequence

<220>

&lt;223&gt; AG864

&lt;400&gt; 44

Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser Pro  
1 5 10 15

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser Pro Ser Ala Ser Thr  
20 25 30

Gly Thr Gly Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro  
35 40 45

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro  
50 55 60

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser  
65 70 75 80

Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro  
85 90 95

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Thr Pro Gly  
100 105 110

Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
115 120 125

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
130 135 140



Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr  
 145 150 155 160

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 165 170 175

Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro  
 180 185 190

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro  
 195 200 205

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser Pro Ser Ala Ser Thr  
 210 215 220

Gly Thr Gly Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro  
 225 230 235 240

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ala Ser Pro  
 245 250 255

Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 260 265 270

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 275 280 285

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser Pro  
 290 295 300

Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 305 310 315 320

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 325 330 335

Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Thr Pro Gly  
 340 345 350

Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 355 360 365

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 370 375 380

Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser Thr  
 385 390 395 400

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala  
 405 410 415

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 420 425 430

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr  
 435 440 445

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala  
 450 455 460

Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro  
 465 470 475 480

Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro  
 485 490 495

Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 500 505 510

Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro  
 515 520 525

Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro  
 530 535 540

Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 545 550 555 560

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 565 570 575

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr  
 580 585 590

Pro Ser Gly Ala Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr Ala  
 595 600 605

Ser Ser Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro  
 610 615 620

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr  
 625 630 635 640

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala  
 645 650 655

Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro  
 660 665 670

Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro  
 675 680 685

Gly Thr Ser Ser Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr Ala  
 690 695 700

Ser Ser Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro  
 705 710 715 720

Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser Pro  
 725 730 735

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 740 745 750

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 755 760 765

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro  
 770 775 780

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 785 790 795 800

Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro  
 805 810 815

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr  
 820 825 830

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala  
 835 840 845

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 850 855 860

<210> 45

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 45

Thr Gln Ser Phe Asn Asp Phe Thr Arg

1 5

<210> 46

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 46

Ser Val Ser Gln Thr Ser Lys Leu Thr Arg

1 5 10

<210> 47

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 47

Asp Phe Leu Ala Glu Gly Gly Gly Val Arg

1 5 10

<210> 48

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<400> 48

Thr Thr Lys Ile Lys Pro Arg

1 5

<210> 49

<211> 5

<212> PRT

<213> Artificial Sequence

<220>  
<223> cleavage site  
  
<400> 49

Leu Val Pro Arg Gly  
1 5

<210> 50  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site  
  
<400> 50

Ala Leu Arg Pro Arg  
1 5

<210> 51  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> sortase recognition motif

<220>  
<221> misc\_feature  
<222> (3)..(3)  
<223> Xaa can be any naturally occurring amino acid  
  
<400> 51

Leu Pro Xaa Thr Gly

1 5

<210> 52

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> polypeptide

<400> 52

Pro Lys Asn Ser Ser Met Ile Ser Asn Thr Pro

1 5 10

<210> 53

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> polypeptide

<400> 53

His Gln Ser Leu Gly Thr Gln

1 5

<210> 54

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> polypeptide



&lt;400&gt; 54

His Gln Asn Leu Ser Asp Gly Lys

1 5

&lt;210&gt; 55

&lt;211&gt; 8

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; polypeptide

&lt;400&gt; 55

His Gln Asn Ile Ser Asp Gly Lys

1 5

&lt;210&gt; 56

&lt;211&gt; 8

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; polypeptide

&lt;400&gt; 56

Val Ile Ser Ser His Leu Gly Gln

1 5

&lt;210&gt; 57

&lt;211&gt; 4

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> linker

<220>

<221> REPEAT

<222> (1)..(4)

<223> Repeat from 1 to 100 times

<400> 57

Gly Gly Gly Ser

1

<210> 58

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> linker

<220>

<221> REPEAT

<222> (1)..(3)

<223> May be repeated 1 to 100 times

<220>

<221> REPEAT

<222> (4)..(8)

<223> May be repeated 1 to 100 times

<400> 58

Gly Gly Ser Gly Gly Gly Gly Ser

1

5

<210> 59

<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> linker

<400> 59

Ser Gly Gly Ser Gly Gly Ser  
1 5

<210> 60  
<211> 15  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> linker

<400> 60

Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Gly  
1 5 10 15

<210> 61  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> linker

<400> 61

Gly Gly Ser Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10 15

<210> 62  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> linker

<400> 62

Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly  
1 5 10 15

Gly Ser

<210> 63  
<211> 15  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> linker

<400> 63

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10 15

<210> 64  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> linker

<220>  
<221> REPEAT  
<222> (1)..(5)  
<223> May be repeated 1-20 times

<400> 64

Gly Gly Gly Gly Ser  
1 5

<210> 65  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

<220>  
<221> MISC\_FEATURE  
<222> (4)..(5)  
<223> Cleavage site

<400> 65

Lys Leu Thr Arg Ala Glu Thr  
1 5

<210> 66  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

<220>  
<221> MISC\_FEATURE  
<222> (4)..(5)  
<223> Cleavage site

<400> 66

Asp Phe Thr Arg Val Val Gly  
1 5

<210> 67  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

<220>  
<221> MISC\_FEATURE  
<222> (4)..(5)  
<223> Cleavage Site

<400> 67

Thr Met Thr Arg Ile Val Gly Gly  
1 5

<210> 68  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> Cleavage site

<400> 68

Ser Pro Phe Arg Ser Thr Gly Gly

1 5

<210> 69

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> Cleavage site

<400> 69

Leu Gln Val Arg Ile Val Gly Gly

1 5

<210> 70

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> Cleavage site

<400> 70

Pro Leu Gly Arg Ile Val Gly Gly

1 5

<210> 71

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> Cleavage site

<400> 71

Ile Glu Gly Arg Thr Val Gly Gly

1 5

<210> 72

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site



<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> cleavage site

<400> 72

Leu Thr Pro Arg Ser Leu Leu Val

1 5

<210> 73

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> cleavage site

<400> 73

Leu Gly Pro Val Ser Gly Val Pro

1 5

<210> 74

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> Cleavage site

<400> 74

Val Ala Gly Asp Ser Leu Glu Glu

1 5

<210> 75

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> Cleavage site

<400> 75

Gly Pro Ala Gly Leu Gly Gly Ala

1 5

<210> 76

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> Cleavage site

<400> 76

Gly Pro Ala Gly Leu Arg Gly Ala

1 5

<210> 77

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> Cleavage site

<400> 77

Ala Pro Leu Gly Leu Arg Leu Arg

1 5

<210> 78

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (4)..(5)

<223> Cleavage site

<400> 78

Pro Ala Leu Pro Leu Val Ala Gln

1 5

<210> 79

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>

<221> MISC\_FEATURE

<222> (6)..(7)

<223> Cleavage site

<400> 79

Glu Asn Leu Tyr Phe Gln Gly

1 5

<210> 80

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> cleavage site

<220>  
<221> MISC\_FEATURE  
<222> (4)..(5)  
<223> Cleavage site  
  
<400> 80  
  
Asp Asp Asp Lys Ile Val Gly Gly  
1 5

<210> 81  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

<220>  
<221> MISC\_FEATURE  
<222> (6)..(7)  
<223> Cleavage site

<400> 81

Leu Glu Val Leu Phe Gln Gly Pro  
1 5

<210> 82  
<211> 8  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

&lt;220&gt;

&lt;221&gt; MISC\_FEATURE

&lt;222&gt; (4)..(5)

&lt;223&gt; Cleavage site

&lt;400&gt; 82

Leu Pro Lys Thr Gly Ser Glu Ser

1 5

&lt;210&gt; 83

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; cleavage site

&lt;400&gt; 83

Thr Gln Ser Phe Asn Asp Phe Thr Arg

1 5

&lt;210&gt; 84

&lt;211&gt; 10

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; cleavage site

&lt;400&gt; 84

Ser Val Ser Gln Thr Ser Lys Leu Thr Arg

1 5 10

<210> 85  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

<400> 85

Asp Phe Leu Ala Glu Gly Gly Gly Val Arg  
1 5 10

<210> 86  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

<400> 86

Thr Thr Lys Ile Lys Pro Arg  
1 5

<210> 87  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

<400> 87

Leu Val Pro Arg Gly

1 5

<210> 88  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> cleavage site

<400> 88

Ala Leu Arg Pro Arg Val Val Gly Gly Ala

1 5 10

<210> 89  
<211> 1896  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> FVIII 198

<400> 89

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe

1 5 10 15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser  
20 25 30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg  
35 40 45

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val



50

55

60

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile  
 65 70 75 80

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln  
 85 90 95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser  
 100 105 110

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser  
 115 120 125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp  
 130 135 140

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu  
 145 150 155 160

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser  
 165 170 175

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile  
 180 185 190

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr  
 195 200 205

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly

210

215

220

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp  
225 230 235 240

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr  
245 250 255

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val  
260 265 270

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile  
275 280 285

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser  
290 295 300

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met  
305 310 315 320

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His  
325 330 335

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro  
340 345 350

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp  
355 360 365

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser

370

375

380

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr  
385 390 395 400

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro  
405 410 415

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn  
420 425 430

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met  
435 440 445

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu  
450 455 460

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu  
465 470 475 480

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro  
485 490 495

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys  
500 505 510

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe  
515 520 525

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp

530

535

540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg  
 545 550 555 560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu  
 565 570 575

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val  
 580 585 590

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu  
 595 600 605

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp  
 610 615 620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val  
 625 630 635 640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp  
 645 650 655

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe  
 660 665 670

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr  
 675 680 685

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro

690

695

700

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly  
 705 710 715 720

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp  
 725 730 735

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys  
 740 745 750

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Pro  
 755 760 765

Ser Thr Arg Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Asp  
 770 775 780

Ile Glu Lys Thr Asp Pro Trp Phe Ala His Arg Thr Pro Met Pro Lys  
 785 790 795 800

Ile Gln Asn Val Ser Ser Ser Asp Leu Leu Met Leu Leu Arg Gln Ser  
 805 810 815

Pro Thr Pro His Gly Leu Ser Leu Ser Asp Leu Gln Glu Ala Lys Tyr  
 820 825 830

Glu Thr Phe Ser Asp Asp Pro Ser Pro Gly Ala Ile Asp Ser Asn Asn  
 835 840 845

Ser Leu Ser Glu Met Thr His Phe Arg Pro Gln Leu His His Ser Gly

850

855

860

Asp Met Val Phe Thr Pro Glu Ser Gly Leu Gln Leu Arg Leu Asn Glu

865

870

875

880

Lys Leu Gly Thr Thr Ala Ala Thr Glu Leu Lys Lys Leu Asp Phe Lys

885

890

895

Val Ser Ser Thr Ser Asn Asn Leu Ile Ser Thr Ile Pro Ser Asp Asn

900

905

910

Leu Ala Ala Gly Thr Asp Asn Thr Ser Ser Leu Gly Pro Pro Ser Met

915

920

925

Pro Val His Tyr Asp Ser Gln Leu Asp Thr Thr Leu Phe Gly Lys Lys

930

935

940

Ser Ser Pro Leu Thr Glu Ser Gly Gly Pro Leu Ser Leu Ser Glu Glu

945

950

955

960

Asn Asn Asp Ser Lys Leu Leu Glu Ser Gly Leu Met Asn Ser Gln Glu

965

970

975

Ser Ser Trp Gly Lys Asn Val Ser Ser Glu Ile Thr Arg Thr Thr Leu

980

985

990

Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu

995

1000

1005

Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln

1010

1015

1020

Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala  
 1025 1030 1035

Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His  
 1040 1045 1050

Val Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys  
 1055 1060 1065

Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro  
 1070 1075 1080

Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly Pro  
 1085 1090 1095

Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg  
 1100 1105 1110

Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser  
 1115 1120 1125

Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe  
 1130 1135 1140

Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His  
 1145 1150 1155

His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala

1160

1165

1170

Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu  
 1175 1180 1185

Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala  
 1190 1195 1200

His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr  
 1205 1210 1215

Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu  
 1220 1225 1230

Arg Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr  
 1235 1240 1245

Phe Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met  
 1250 1255 1260

Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg  
 1265 1270 1275

Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile  
 1280 1285 1290

His Phe Ser Gly His Val Phe Thr Val Arg Lys Lys Glu Glu Tyr  
 1295 1300 1305

Lys Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val



1310

1315

1320

Glu Met Leu Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys Leu

1325

1330

1335

Ile Gly Glu His Leu His Ala Gly Met Ser Thr Leu Phe Leu Val

1340

1345

1350

Tyr Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His

1355

1360

1365

Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp

1370

1375

1380

Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala

1385

1390

1395

Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu

1400

1405

1410

Ala Pro Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln

1415

1420

1425

Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser

1430

1435

1440

Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly

1445

1450

1455

Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys

1460

1465

1470

His Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu  
 1475 1480 1485

His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu Leu  
 1490 1495 1500

Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Glu  
 1505 1510 1515

Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe  
 1520 1525 1530

Thr Asn Met Phe Ala Thr Trp Ser Pro Ser Lys Ala Arg Leu His  
 1535 1540 1545

Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln Val Asn Asn Pro  
 1550 1555 1560

Lys Glu Trp Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val Thr  
 1565 1570 1575

Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr  
 1580 1585 1590

Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His Gln Trp  
 1595 1600 1605

Thr Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn

1610

1615

1620

Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro Leu  
 1625 1630 1635

Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln  
 1640 1645 1650

Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln Asp Leu  
 1655 1660 1665

Tyr Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu  
 1670 1675 1680

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 1685 1690 1695

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val  
 1700 1705 1710

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
 1715 1720 1725

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu  
 1730 1735 1740

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu  
 1745 1750 1755

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser

1760

1765

1770

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala  
 1775 1780 1785

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser  
 1790 1795 1800

Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
 1805 1810 1815

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn  
 1820 1825 1830

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
 1835 1840 1845

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys  
 1850 1855 1860

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 1865 1870 1875

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
 1880 1885 1890

Pro Gly Lys  
 1895

&lt;211&gt; 99

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; ESC48- Fwd - VWF-D'D3 with VIII signal and BsiW1 site

&lt;400&gt; 90

tcgcgacgta cggccgccac catgcaaata gagctctcca cctgcttctt tctgtgcctt 60

ttgcgattct gctttagcct atcctgtcgg ccccccattg 99

&lt;210&gt; 91

&lt;211&gt; 74

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; ESC51- Rev- VWF D'D3 (1-477 amino acid) with 6His and Not 1 site

&lt;400&gt; 91

tgacctcgag cggccgctca gtggtgatgg tgatgatgcg gctcctggca ggcttcacag 60

gtgaggttga caac 74

&lt;210&gt; 92

&lt;211&gt; 32

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; ESC 89-fwd with Nhe1site

&lt;400&gt; 92

ctcactatag ggagacccaa gctggctagc cg 32

&lt;210&gt; 93

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> ESC 91-rev with Sal1

<400> 93

ctggatcccg ggagtcgact cgtcagtggt gatggtgatg atg

43

<210> 94

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> linker

<400> 94

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly

1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly

20 25 30

Gly Gly Ser Gly Gly Gly Gly Ser

35 40

<210> 95

<211> 92

<212> DNA

<213> Artificial Sequence

<220>

<223> LW 22-FWD-VWF-D'D3 with FVIII signal sequence and BsiW1 site

<400> 95  
gcgccggccg tacgatgcaa atagagctct ccacctgctt ctttctgtgc cttttgcgat 60  
  
tctgctttag cctatcctgt cggcccccca tg 92

<210> 96  
<211> 47  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> LW 23-Rev- Fc with stop codon and Not1 site

<400> 96  
tcatcaatgt atcttatcat gtctgaattc gcggccgctc atttacc 47

<210> 97  
<211> 41  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> LW24- Fwd- VWF D1D2D'D3 cloning oligo with BsiW1 site

<400> 97  
gcgccggccg tacgatgatt cctgccagat ttgccggggt g 41

<210> 98  
<211> 41  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> LW27-Rev-VWF D'D3 oligo with EcoRV

<400> 98

ccaccgccag atatacggctc ctggcaggct tcacaggtga g

41

&lt;210&gt; 99

&lt;211&gt; 1240

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; VWF-D1D2D'D3 protein sequence 1

&lt;400&gt; 99

Met Ile Pro Ala Arg Phe Ala Gly Val Leu Leu Ala Leu Ala Leu Ile

1 5 10 15

Leu Pro Gly Thr Leu Cys Ala Glu Gly Thr Arg Gly Arg Ser Ser Thr

20 25 30

Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly

35 40 45

Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly

50 55 60

Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys

65 70 75 80

Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu

85 90 95

Phe Val Asn Gly Thr Val Thr Gln Gly Asp Gln Arg Val Ser Met Pro

100 105 110



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Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys
      115              120              125

Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly
      130              135              140

Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly
      145              150              155              160

Leu Cys Gly Asn Phe Asn Ile Phe Ala Glu Asp Asp Phe Met Thr Gln
              165              170              175

Glu Gly Thr Leu Thr Ser Asp Pro Tyr Asp Phe Ala Asn Ser Trp Ala
              180              185              190

Leu Ser Ser Gly Glu Gln Trp Cys Glu Arg Ala Ser Pro Pro Ser Ser
      195              200              205

Ser Cys Asn Ile Ser Ser Gly Glu Met Gln Lys Gly Leu Trp Glu Gln
      210              215              220

Cys Gln Leu Leu Lys Ser Thr Ser Val Phe Ala Arg Cys His Pro Leu
      225              230              235              240

Val Asp Pro Glu Pro Phe Val Ala Leu Cys Glu Lys Thr Leu Cys Glu
              245              250              255

Cys Ala Gly Gly Leu Glu Cys Ala Cys Pro Ala Leu Leu Glu Tyr Ala
      260              265              270

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Thr | Cys | Ala | Gln | Glu | Gly | Met | Val | Leu | Tyr | Gly | Trp | Thr | Asp | His |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Ala | Cys | Ser | Pro | Val | Cys | Pro | Ala | Gly | Met | Glu | Tyr | Arg | Gln | Cys |
|     |     | 290 |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val | Ser | Pro | Cys | Ala | Arg | Thr | Cys | Gln | Ser | Leu | His | Ile | Asn | Glu | Met |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cys | Gln | Glu | Arg | Cys | Val | Asp | Gly | Cys | Ser | Cys | Pro | Glu | Gly | Gln | Leu |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Asp | Glu | Gly | Leu | Cys | Val | Glu | Ser | Thr | Glu | Cys | Pro | Cys | Val | His |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Gly | Lys | Arg | Tyr | Pro | Pro | Gly | Thr | Ser | Leu | Ser | Arg | Asp | Cys | Asn |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr | Cys | Ile | Cys | Arg | Asn | Ser | Gln | Trp | Ile | Cys | Ser | Asn | Glu | Glu | Cys |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Gly | Glu | Cys | Leu | Val | Thr | Gly | Gln | Ser | His | Phe | Lys | Ser | Phe | Asp |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asn | Arg | Tyr | Phe | Thr | Phe | Ser | Gly | Ile | Cys | Gln | Tyr | Leu | Leu | Ala | Arg |
|     |     |     | 405 |     |     |     |     | 410 |     |     |     |     |     | 415 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Cys | Gln | Asp | His | Ser | Phe | Ser | Ile | Val | Ile | Glu | Thr | Val | Gln | Cys |
|     |     | 420 |     |     |     |     |     | 425 |     |     |     |     | 430 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Asp | Asp | Arg | Asp | Ala | Val | Cys | Thr | Arg | Ser | Val | Thr | Val | Arg | Leu |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Gly | Leu | His | Asn | Ser | Leu | Val | Lys | Leu | Lys | His | Gly | Ala | Gly | Val |
|     |     | 450 |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Met | Asp | Gly | Gln | Asp | Ile | Gln | Leu | Pro | Leu | Leu | Lys | Gly | Asp | Leu |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Ile | Gln | His | Thr | Val | Thr | Ala | Ser | Val | Arg | Leu | Ser | Tyr | Gly | Glu |
|     |     |     | 485 |     |     |     |     |     | 490 |     |     |     |     | 495 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Leu | Gln | Met | Asp | Trp | Asp | Gly | Arg | Gly | Arg | Leu | Leu | Val | Lys | Leu |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     |     |     | 510 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Pro | Val | Tyr | Ala | Gly | Lys | Thr | Cys | Gly | Leu | Cys | Gly | Asn | Tyr | Asn |
|     |     | 515 |     |     |     |     |     | 520 |     |     |     |     | 525 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Asn | Gln | Gly | Asp | Asp | Phe | Leu | Thr | Pro | Ser | Gly | Leu | Ala | Glu | Pro |
|     | 530 |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Val | Glu | Asp | Phe | Gly | Asn | Ala | Trp | Lys | Leu | His | Gly | Asp | Cys | Gln |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Leu | Gln | Lys | Gln | His | Ser | Asp | Pro | Cys | Ala | Leu | Asn | Pro | Arg | Met |
|     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr | Arg | Phe | Ser | Glu | Glu | Ala | Cys | Ala | Val | Leu | Thr | Ser | Pro | Thr | Phe |
|     |     |     | 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ala | Cys | His | Arg | Ala | Val | Ser | Pro | Leu | Pro | Tyr | Leu | Arg | Asn | Cys |
|     |     | 595 |     |     |     |     | 600 |     |     |     |     | 605 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Tyr | Asp | Val | Cys | Ser | Cys | Ser | Asp | Gly | Arg | Glu | Cys | Leu | Cys | Gly |
|     | 610 |     |     |     |     | 615 |     |     |     |     | 620 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Leu | Ala | Ser | Tyr | Ala | Ala | Ala | Cys | Ala | Gly | Arg | Gly | Val | Arg | Val |
| 625 |     |     |     |     | 630 |     |     |     |     | 635 |     |     |     | 640 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Trp | Arg | Glu | Pro | Gly | Arg | Cys | Glu | Leu | Asn | Cys | Pro | Lys | Gly | Gln |
|     |     |     | 645 |     |     |     |     |     | 650 |     |     |     |     | 655 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val | Tyr | Leu | Gln | Cys | Gly | Thr | Pro | Cys | Asn | Leu | Thr | Cys | Arg | Ser | Leu |
|     |     | 660 |     |     |     |     |     | 665 |     |     |     |     | 670 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Tyr | Pro | Asp | Glu | Glu | Cys | Asn | Glu | Ala | Cys | Leu | Glu | Gly | Cys | Phe |
|     |     | 675 |     |     |     |     |     | 680 |     |     |     |     | 685 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cys | Pro | Pro | Gly | Leu | Tyr | Met | Asp | Glu | Arg | Gly | Asp | Cys | Val | Pro | Lys |
|     | 690 |     |     |     |     | 695 |     |     |     |     | 700 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Gln | Cys | Pro | Cys | Tyr | Tyr | Asp | Gly | Glu | Ile | Phe | Gln | Pro | Glu | Asp |
| 705 |     |     |     |     | 710 |     |     |     |     | 715 |     |     |     | 720 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ile | Phe | Ser | Asp | His | His | Thr | Met | Cys | Tyr | Cys | Glu | Asp | Gly | Phe | Met |
|     |     |     | 725 |     |     |     |     |     | 730 |     |     |     |     | 735 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| His | Cys | Thr | Met | Ser | Gly | Val | Pro | Gly | Ser | Leu | Leu | Pro | Asp | Ala | Val |
|     |     | 740 |     |     |     |     |     | 745 |     |     |     |     | 750 |     |     |

Leu Ser Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg

755

760

765

Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp Asn Leu Arg Ala Glu

770

775

780

Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr Asp Leu Glu Cys Met

785

790

795

800

Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro Pro Gly Met Val Arg

805

810

815

His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys Pro Cys Phe His Gln

820

825

830

Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr

835

840

845

Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp

850

855

860

Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly

865

870

875

880

Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp

885

890

895

Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys

900

905

910

Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu

915

920

925

Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys

930

935

940

Arg Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Arg

945

950

955

960

Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg

965

970

975

His Leu Ser Ile Ser Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val

980

985

990

Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln Asn Asn Asp Leu Thr

995

1000

1005

Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val Asp Phe Gly Asn

1010

1015

1020

Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg Lys Val Pro

1025

1030

1035

Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met Lys Gln

1040

1045

1050

Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val Phe

1055

1060

1065

|         |                     |                     |             |
|---------|---------------------|---------------------|-------------|
| Gln Asp | Cys Asn Lys Leu Val | Asp Pro Glu Pro Tyr | Leu Asp Val |
| 1070    | 1075                | 1080                |             |
|         |                     |                     |             |
| Cys Ile | Tyr Asp Thr Cys Ser | Cys Glu Ser Ile Gly | Asp Cys Ala |
| 1085    | 1090                | 1095                |             |
|         |                     |                     |             |
| Cys Phe | Cys Asp Thr Ile Ala | Ala Tyr Ala His Val | Cys Ala Gln |
| 1100    | 1105                | 1110                |             |
|         |                     |                     |             |
| His Gly | Lys Val Val Thr Trp | Arg Thr Ala Thr Leu | Cys Pro Gln |
| 1115    | 1120                | 1125                |             |
|         |                     |                     |             |
| Ser Cys | Glu Glu Arg Asn Leu | Arg Glu Asn Gly Tyr | Glu Cys Glu |
| 1130    | 1135                | 1140                |             |
|         |                     |                     |             |
| Trp Arg | Tyr Asn Ser Cys Ala | Pro Ala Cys Gln Val | Thr Cys Gln |
| 1145    | 1150                | 1155                |             |
|         |                     |                     |             |
| His Pro | Glu Pro Leu Ala Cys | Pro Val Gln Cys Val | Glu Gly Cys |
| 1160    | 1165                | 1170                |             |
|         |                     |                     |             |
| His Ala | His Cys Pro Pro Gly | Lys Ile Leu Asp Glu | Leu Leu Gln |
| 1175    | 1180                | 1185                |             |
|         |                     |                     |             |
| Thr Cys | Val Asp Pro Glu Asp | Cys Pro Val Cys Glu | Val Ala Gly |
| 1190    | 1195                | 1200                |             |
|         |                     |                     |             |
| Arg Arg | Phe Ala Ser Gly Lys | Lys Val Thr Leu Asn | Pro Ser Asp |
| 1205    | 1210                | 1215                |             |

Pro Glu His Cys Gln Ile Cys His Cys Asp Val Val Asn Leu Thr  
 1220 1225 1230

Cys Glu Ala Cys Gln Glu Pro  
 1235 1240

<210> 100

<211> 477

<212> PRT

<213> Artificial Sequence

<220>

<223> VWF-D'D3 protein sequence 2

<400> 100

Ser Leu Ser Cys Arg Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp  
 1 5 10 15

Asn Leu Arg Ala Glu Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr  
 20 25 30

Asp Leu Glu Cys Met Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro  
 35 40 45

Pro Gly Met Val Arg His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys  
 50 55 60

Pro Cys Phe His Gln Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys  
 65 70 75 80

Ile Gly Cys Asn Thr Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr



85

90

95

Asp His Val Cys Asp Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr  
100 105 110

Leu Thr Phe Asp Gly Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr  
115 120 125

Val Leu Val Gln Asp Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile  
130 135 140

Leu Val Gly Asn Lys Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys  
145 150 155 160

Arg Val Thr Ile Leu Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly  
165 170 175

Glu Val Asn Val Lys Arg Pro Met Lys Asp Glu Thr His Phe Glu Val  
180 185 190

Val Glu Ser Gly Arg Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser  
195 200 205

Val Val Trp Asp Arg His Leu Ser Ile Ser Val Val Leu Lys Gln Thr  
210 215 220

Tyr Gln Glu Lys Val Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln  
225 230 235 240

Asn Asn Asp Leu Thr Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val

245

250

255

Asp Phe Gly Asn Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg  
260 265 270

Lys Val Pro Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met  
275 280 285

Lys Gln Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val  
290 295 300

Phe Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val  
305 310 315 320

Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala Cys  
325 330 335

Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln His Gly  
340 345 350

Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln Ser Cys Glu  
355 360 365

Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Cys Glu Trp Arg Tyr Asn  
370 375 380

Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln His Pro Glu Pro Leu  
385 390 395 400

Ala Cys Pro Val Gln Cys Val Glu Gly Cys His Ala His Cys Pro Pro

405

410

415

Gly Lys Ile Leu Asp Glu Leu Leu Gln Thr Cys Val Asp Pro Glu Asp  
420 425 430

Cys Pro Val Cys Glu Val Ala Gly Arg Arg Phe Ala Ser Gly Lys Lys  
435 440 445

Val Thr Leu Asn Pro Ser Asp Pro Glu His Cys Gln Ile Cys His Cys  
450 455 460

Asp Val Val Asn Leu Thr Cys Glu Ala Cys Gln Glu Pro  
465 470 475

<210> 101

<211> 2754

<212> PRT

<213> Artificial Sequence

<220>

<223> pSYN-FVIII-161

<400> 101

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe  
1 5 10 15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser  
20 25 30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg  
35 40 45

```

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
    50                      55                      60

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
65                      70                      75                      80

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
                      85                      90                      95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
                      100                     105                     110

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
                      115                     120                     125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
    130                      135                      140

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
145                      150                      155                      160

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
                      165                      170                      175

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
                      180                      185                      190

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
    195                      200                      205

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Thr | Leu | His | Lys | Phe | Ile | Leu | Leu | Phe | Ala | Val | Phe | Asp | Glu | Gly |
| 210 |     |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Lys | Ser | Trp | His | Ser | Glu | Thr | Lys | Asn | Ser | Leu | Met | Gln | Asp | Arg | Asp |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Ala | Ser | Ala | Arg | Ala | Trp | Pro | Lys | Met | His | Thr | Val | Asn | Gly | Tyr |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val | Asn | Arg | Ser | Leu | Pro | Gly | Leu | Ile | Gly | Cys | His | Arg | Lys | Ser | Val |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Trp | His | Val | Ile | Gly | Met | Gly | Thr | Thr | Pro | Glu | Val | His | Ser | Ile |
|     |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Phe | Leu | Glu | Gly | His | Thr | Phe | Leu | Val | Arg | Asn | His | Arg | Gln | Ala | Ser |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 290 |     |     |     |     |     | 295 |     |     |     |     |     |     | 300 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Glu | Ile | Ser | Pro | Ile | Thr | Phe | Leu | Thr | Ala | Gln | Thr | Leu | Leu | Met |
| 305 |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Leu | Gly | Gln | Phe | Leu | Leu | Phe | Cys | His | Ile | Ser | Ser | His | Gln | His |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Gly | Met | Glu | Ala | Tyr | Val | Lys | Val | Asp | Ser | Cys | Pro | Glu | Glu | Pro |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gln | Leu | Arg | Met | Lys | Asn | Asn | Glu | Glu | Ala | Glu | Asp | Tyr | Asp | Asp | Asp |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |

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Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
    370                375                380

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
385                390                395                400

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
                405                410                415

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
                420                425                430

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
    435                440                445

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
    450                455                460

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
465                470                475                480

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
                485                490                495

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
                500                505                510

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
    515                520                525

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Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
    530                535                540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
545                550                555                560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
                565                570                575

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
                580                585                590

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
    595                600                605

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
    610                615                620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
625                630                635                640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
                645                650                655

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
                660                665                670

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
    675                680                685

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Phe | Pro | Phe | Ser | Gly | Glu | Thr | Val | Phe | Met | Ser | Met | Glu | Asn | Pro |
| 690 |     |     |     |     |     | 695 |     |     |     |     | 700 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Leu | Trp | Ile | Leu | Gly | Cys | His | Asn | Ser | Asp | Phe | Arg | Asn | Arg | Gly |
| 705 |     |     |     |     | 710 |     |     |     |     | 715 |     |     |     | 720 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Met | Thr | Ala | Leu | Leu | Lys | Val | Ser | Ser | Cys | Asp | Lys | Asn | Thr | Gly | Asp |
|     |     |     |     | 725 |     |     |     |     | 730 |     |     |     |     | 735 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Tyr | Glu | Asp | Ser | Tyr | Glu | Asp | Ile | Ser | Ala | Tyr | Leu | Leu | Ser | Lys |
|     |     | 740 |     |     |     |     |     | 745 |     |     |     |     | 750 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asn | Asn | Ala | Ile | Glu | Pro | Arg | Ser | Phe | Ser | Gln | Asn | Pro | Pro | Val | Leu |
|     | 755 |     |     |     |     |     | 760 |     |     |     |     | 765 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Lys | Arg | His | Gln | Arg | Glu | Ile | Thr | Arg | Thr | Thr | Leu | Gln | Ser | Asp | Gln |
| 770 |     |     |     |     | 775 |     |     |     |     |     | 780 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Glu | Ile | Asp | Tyr | Asp | Asp | Thr | Ile | Ser | Val | Glu | Met | Lys | Lys | Glu |
| 785 |     |     |     |     | 790 |     |     |     |     | 795 |     |     |     |     | 800 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Phe | Asp | Ile | Tyr | Asp | Glu | Asp | Glu | Asn | Gln | Ser | Pro | Arg | Ser | Phe |
|     |     |     | 805 |     |     |     |     |     | 810 |     |     |     |     | 815 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gln | Lys | Lys | Thr | Arg | His | Tyr | Phe | Ile | Ala | Ala | Val | Glu | Arg | Leu | Trp |
|     |     |     | 820 |     |     |     |     | 825 |     |     |     |     | 830 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Tyr | Gly | Met | Ser | Ser | Ser | Pro | His | Val | Leu | Arg | Asn | Arg | Ala | Gln |
|     | 835 |     |     |     |     |     | 840 |     |     |     |     | 845 |     |     |     |



Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr  
850 855 860

Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His  
865 870 875 880

Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile  
885 890 895

Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser  
900 905 910

Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg  
915 920 925

Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val  
930 935 940

Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp  
945 950 955 960

Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu  
965 970 975

Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala His  
980 985 990

Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe  
995 1000 1005

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Asp  | Glu | Thr | Lys | Ser | Trp | Tyr  | Phe | Thr | Glu | Asn | Met  | Glu | Arg | Asn |
| 1010 |     |     |     |     |     | 1015 |     |     |     |     | 1020 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Arg | Ala | Pro | Cys | Asn | Ile  | Gln | Met | Glu | Asp | Pro  | Thr | Phe | Lys |
| 1025 |     |     |     |     |     | 1030 |     |     |     |     | 1035 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Asn | Tyr | Arg | Phe | His | Ala  | Ile | Asn | Gly | Tyr | Ile  | Met | Asp | Thr |
| 1040 |     |     |     |     |     | 1045 |     |     |     |     | 1050 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Pro | Gly | Leu | Val | Met | Ala  | Gln | Asp | Gln | Arg | Ile  | Arg | Trp | Tyr |
| 1055 |     |     |     |     |     | 1060 |     |     |     |     | 1065 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Leu | Ser | Met | Gly | Ser | Asn  | Glu | Asn | Ile | His | Ser  | Ile | His | Phe |
| 1070 |     |     |     |     |     | 1075 |     |     |     |     | 1080 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | His | Val | Phe | Thr | Val  | Arg | Lys | Lys | Glu | Glu  | Tyr | Lys | Met |
| 1085 |     |     |     |     |     | 1090 |     |     |     |     | 1095 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Leu | Tyr | Asn | Leu | Tyr | Pro  | Gly | Val | Phe | Glu | Thr  | Val | Glu | Met |
| 1100 |     |     |     |     |     | 1105 |     |     |     |     | 1110 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Pro | Ser | Lys | Ala | Gly | Ile  | Trp | Arg | Val | Glu | Cys  | Leu | Ile | Gly |
| 1115 |     |     |     |     |     | 1120 |     |     |     |     | 1125 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | His | Leu | His | Ala | Gly | Met  | Ser | Thr | Leu | Phe | Leu  | Val | Tyr | Ser |
| 1130 |     |     |     |     |     | 1135 |     |     |     |     | 1140 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Lys | Cys | Gln | Thr | Pro | Leu  | Gly | Met | Ala | Ser | Gly  | His | Ile | Arg |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     | 1155 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|
| Asp  | Phe | Gln | Ile | Thr | Ala | Ser  | Gly | Gln | Tyr | Gly | Gln | Trp  | Ala | Pro |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     |     | 1170 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Lys  | Leu | Ala | Arg | Leu | His | Tyr  | Ser | Gly | Ser | Ile | Asn | Ala  | Trp | Ser |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     |     | 1185 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Thr  | Lys | Glu | Pro | Phe | Ser | Trp  | Ile | Lys | Val | Asp | Leu | Leu  | Ala | Pro |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     |     | 1200 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Met  | Ile | Ile | His | Gly | Ile | Lys  | Thr | Gln | Gly | Ala | Arg | Gln  | Lys | Phe |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     |     | 1215 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ser  | Ser | Leu | Tyr | Ile | Ser | Gln  | Phe | Ile | Ile | Met | Tyr | Ser  | Leu | Asp |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     |     | 1230 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gly  | Lys | Lys | Trp | Gln | Thr | Tyr  | Arg | Gly | Asn | Ser | Thr | Gly  | Thr | Leu |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     |     | 1245 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Met  | Val | Phe | Phe | Gly | Asn | Val  | Asp | Ser | Ser | Gly | Ile | Lys  | His | Asn |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     |     | 1260 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ile  | Phe | Asn | Pro | Pro | Ile | Ile  | Ala | Arg | Tyr | Ile | Arg | Leu  | His | Pro |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     |     | 1275 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Thr  | His | Tyr | Ser | Ile | Arg | Ser  | Thr | Leu | Arg | Met | Glu | Leu  | Met | Gly |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     |     | 1290 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Cys  | Asp | Leu | Asn | Ser | Cys | Ser  | Met | Pro | Leu | Gly | Met | Glu  | Ser | Lys |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     |     | 1305 |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ala  | Ile | Ser | Asp | Ala | Gln | Ile  | Thr | Ala | Ser | Ser | Tyr  | Phe | Thr | Asn |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     | 1320 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Phe | Ala | Thr | Trp | Ser | Pro  | Ser | Lys | Ala | Arg | Leu  | His | Leu | Gln |
| 1325 |     |     |     |     |     | 1330 |     |     |     |     | 1335 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Arg | Ser | Asn | Ala | Trp | Arg  | Pro | Gln | Val | Asn | Asn  | Pro | Lys | Glu |
| 1340 |     |     |     |     |     | 1345 |     |     |     |     | 1350 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Leu | Gln | Val | Asp | Phe | Gln  | Lys | Thr | Met | Lys | Val  | Thr | Gly | Val |
| 1355 |     |     |     |     |     | 1360 |     |     |     |     | 1365 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Thr | Gln | Gly | Val | Lys | Ser  | Leu | Leu | Thr | Ser | Met  | Tyr | Val | Lys |
| 1370 |     |     |     |     |     | 1375 |     |     |     |     | 1380 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Phe | Leu | Ile | Ser | Ser | Ser  | Gln | Asp | Gly | His | Gln  | Trp | Thr | Leu |
| 1385 |     |     |     |     |     | 1390 |     |     |     |     | 1395 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Phe | Gln | Asn | Gly | Lys | Val  | Lys | Val | Phe | Gln | Gly  | Asn | Gln | Asp |
| 1400 |     |     |     |     |     | 1405 |     |     |     |     | 1410 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Phe | Thr | Pro | Val | Val | Asn  | Ser | Leu | Asp | Pro | Pro  | Leu | Leu | Thr |
| 1415 |     |     |     |     |     | 1420 |     |     |     |     | 1425 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Tyr | Leu | Arg | Ile | His | Pro  | Gln | Ser | Trp | Val | His  | Gln | Ile | Ala |
| 1430 |     |     |     |     |     | 1435 |     |     |     |     | 1440 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Arg | Met | Glu | Val | Leu | Gly  | Cys | Glu | Ala | Gln | Asp  | Leu | Tyr | Asp |
| 1445 |     |     |     |     |     | 1450 |     |     |     |     | 1455 |     |     |     |

|         |         |         |     |         |         |     |         |      |  |
|---------|---------|---------|-----|---------|---------|-----|---------|------|--|
| Lys Thr | His Thr | Cys Pro | Pro | Cys Pro | Ala Pro | Glu | Leu Leu | Gly  |  |
| 1460    |         |         |     | 1465    |         |     |         | 1470 |  |
|         |         |         |     |         |         |     |         |      |  |
| Gly Pro | Ser Val | Phe Leu | Phe | Pro Pro | Lys Pro | Lys | Asp Thr | Leu  |  |
| 1475    |         |         |     | 1480    |         |     |         | 1485 |  |
|         |         |         |     |         |         |     |         |      |  |
| Met Ile | Ser Arg | Thr Pro | Glu | Val Thr | Cys Val | Val | Val Asp | Val  |  |
| 1490    |         |         |     | 1495    |         |     |         | 1500 |  |
|         |         |         |     |         |         |     |         |      |  |
| Ser His | Glu Asp | Pro Glu | Val | Lys Phe | Asn Trp | Tyr | Val Asp | Gly  |  |
| 1505    |         |         |     | 1510    |         |     |         | 1515 |  |
|         |         |         |     |         |         |     |         |      |  |
| Val Glu | Val His | Asn Ala | Lys | Thr Lys | Pro Arg | Glu | Glu Gln | Tyr  |  |
| 1520    |         |         |     | 1525    |         |     |         | 1530 |  |
|         |         |         |     |         |         |     |         |      |  |
| Asn Ser | Thr Tyr | Arg Val | Val | Ser Val | Leu Thr | Val | Leu His | Gln  |  |
| 1535    |         |         |     | 1540    |         |     |         | 1545 |  |
|         |         |         |     |         |         |     |         |      |  |
| Asp Trp | Leu Asn | Gly Lys | Glu | Tyr Lys | Cys Lys | Val | Ser Asn | Lys  |  |
| 1550    |         |         |     | 1555    |         |     |         | 1560 |  |
|         |         |         |     |         |         |     |         |      |  |
| Ala Leu | Pro Ala | Pro Ile | Glu | Lys Thr | Ile Ser | Lys | Ala Lys | Gly  |  |
| 1565    |         |         |     | 1570    |         |     |         | 1575 |  |
|         |         |         |     |         |         |     |         |      |  |
| Gln Pro | Arg Glu | Pro Gln | Val | Tyr Thr | Leu Pro | Pro | Ser Arg | Asp  |  |
| 1580    |         |         |     | 1585    |         |     |         | 1590 |  |
|         |         |         |     |         |         |     |         |      |  |
| Glu Leu | Thr Lys | Asn Gln | Val | Ser Leu | Thr Cys | Leu | Val Lys | Gly  |  |
| 1595    |         |         |     | 1600    |         |     |         | 1605 |  |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Phe  | Tyr | Pro | Ser | Asp | Ile | Ala  | Val | Glu | Trp | Glu | Ser  | Asn | Gly | Gln |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Glu | Asn | Asn | Tyr | Lys | Thr  | Thr | Pro | Pro | Val | Leu  | Asp | Ser | Asp |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ser | Phe | Phe | Leu | Tyr | Ser  | Lys | Leu | Thr | Val | Asp  | Lys | Ser | Arg |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Gln | Gln | Gly | Asn | Val | Phe  | Ser | Cys | Ser | Val | Met  | His | Glu | Ala |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | His | Asn | His | Tyr | Thr | Gln  | Lys | Ser | Leu | Ser | Leu  | Ser | Pro | Gly |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Arg | Arg | Arg | Arg | Ser | Gly  | Gly | Gly | Gly | Ser | Gly  | Gly | Gly | Gly |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | Gly | Gly | Gly | Ser | Gly  | Gly | Gly | Gly | Ser | Gly  | Gly | Gly | Gly |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | Gly | Gly | Gly | Ser | Arg  | Lys | Arg | Arg | Lys | Arg  | Ser | Leu | Ser |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     | 1725 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Arg | Pro | Pro | Met | Val | Lys  | Leu | Val | Cys | Pro | Ala  | Asp | Asn | Leu |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     | 1740 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Ala | Glu | Gly | Leu | Glu | Cys  | Thr | Lys | Thr | Cys | Gln  | Asn | Tyr | Asp |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     | 1755 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Leu  | Glu | Cys | Met | Ser | Met | Gly  | Cys | Val | Ser | Gly | Cys  | Leu | Cys | Pro |
| 1760 |     |     |     |     |     | 1765 |     |     |     |     | 1770 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gly | Met | Val | Arg | His | Glu  | Asn | Arg | Cys | Val | Ala  | Leu | Glu | Arg |
| 1775 |     |     |     |     |     | 1780 |     |     |     |     | 1785 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Pro | Cys | Phe | His | Gln | Gly  | Lys | Glu | Tyr | Ala | Pro  | Gly | Glu | Thr |
| 1790 |     |     |     |     |     | 1795 |     |     |     |     | 1800 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Lys | Ile | Gly | Cys | Asn | Thr  | Cys | Val | Cys | Arg | Asp  | Arg | Lys | Trp |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     | 1815 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Cys | Thr | Asp | His | Val | Cys  | Asp | Ala | Thr | Cys | Ser  | Thr | Ile | Gly |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     | 1830 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Ala | His | Tyr | Leu | Thr | Phe  | Asp | Gly | Leu | Lys | Tyr  | Leu | Phe | Pro |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     | 1845 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Glu | Cys | Gln | Tyr | Val | Leu  | Val | Gln | Asp | Tyr | Cys  | Gly | Ser | Asn |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     | 1860 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gly | Thr | Phe | Arg | Ile | Leu  | Val | Gly | Asn | Lys | Gly  | Cys | Ser | His |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     | 1875 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Ser | Val | Lys | Cys | Lys | Lys  | Arg | Val | Thr | Ile | Leu  | Val | Glu | Gly |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     | 1890 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Glu | Ile | Glu | Leu | Phe | Asp  | Gly | Glu | Val | Asn | Val  | Lys | Arg | Pro |
| 1895 |     |     |     |     |     | 1900 |     |     |     |     | 1905 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Met  | Lys | Asp | Glu | Thr | His | Phe  | Glu | Val | Val | Glu | Ser  | Gly | Arg | Tyr |
| 1910 |     |     |     |     |     | 1915 |     |     |     |     | 1920 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Ile | Leu | Leu | Leu | Gly | Lys  | Ala | Leu | Ser | Val | Val  | Trp | Asp | Arg |
| 1925 |     |     |     |     |     | 1930 |     |     |     |     | 1935 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| His  | Leu | Ser | Ile | Ser | Val | Val  | Leu | Lys | Gln | Thr | Tyr  | Gln | Glu | Lys |
| 1940 |     |     |     |     |     | 1945 |     |     |     |     | 1950 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Cys | Gly | Leu | Cys | Gly | Asn  | Phe | Asp | Gly | Ile | Gln  | Asn | Asn | Asp |
| 1955 |     |     |     |     |     | 1960 |     |     |     |     | 1965 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Thr | Ser | Ser | Asn | Leu | Gln  | Val | Glu | Glu | Asp | Pro  | Val | Asp | Phe |
| 1970 |     |     |     |     |     | 1975 |     |     |     |     | 1980 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Asn | Ser | Trp | Lys | Val | Ser  | Ser | Gln | Cys | Ala | Asp  | Thr | Arg | Lys |
| 1985 |     |     |     |     |     | 1990 |     |     |     |     | 1995 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Pro | Leu | Asp | Ser | Ser | Pro  | Ala | Thr | Cys | His | Asn  | Asn | Ile | Met |
| 2000 |     |     |     |     |     | 2005 |     |     |     |     | 2010 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Gln | Thr | Met | Val | Asp | Ser  | Ser | Cys | Arg | Ile | Leu  | Thr | Ser | Asp |
| 2015 |     |     |     |     |     | 2020 |     |     |     |     | 2025 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Phe | Gln | Asp | Cys | Asn | Lys  | Leu | Val | Asp | Pro | Glu  | Pro | Tyr | Leu |
| 2030 |     |     |     |     |     | 2035 |     |     |     |     | 2040 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Val | Cys | Ile | Tyr | Asp | Thr  | Cys | Ser | Cys | Glu | Ser  | Ile | Gly | Asp |
| 2045 |     |     |     |     |     | 2050 |     |     |     |     | 2055 |     |     |     |



|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Cys  | Ala | Ala | Phe | Cys | Asp | Thr  | Ile | Ala | Ala | Tyr | Ala  | His | Val | Cys |
| 2060 |     |     |     |     |     | 2065 |     |     |     |     | 2070 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Gln | His | Gly | Lys | Val | Val  | Thr | Trp | Arg | Thr | Ala  | Thr | Leu | Cys |
| 2075 |     |     |     |     |     | 2080 |     |     |     |     | 2085 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gln | Ser | Cys | Glu | Glu | Arg  | Asn | Leu | Arg | Glu | Asn  | Gly | Tyr | Glu |
| 2090 |     |     |     |     |     | 2095 |     |     |     |     | 2100 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Glu | Trp | Arg | Tyr | Asn | Ser  | Cys | Ala | Pro | Ala | Cys  | Gln | Val | Thr |
| 2105 |     |     |     |     |     | 2110 |     |     |     |     | 2115 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Gln | His | Pro | Glu | Pro | Leu  | Ala | Cys | Pro | Val | Gln  | Cys | Val | Glu |
| 2120 |     |     |     |     |     | 2125 |     |     |     |     | 2130 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Cys | His | Ala | His | Cys | Pro  | Pro | Gly | Lys | Ile | Leu  | Asp | Glu | Leu |
| 2135 |     |     |     |     |     | 2140 |     |     |     |     | 2145 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Gln | Thr | Cys | Val | Asp | Pro  | Glu | Asp | Cys | Pro | Val  | Cys | Glu | Val |
| 2150 |     |     |     |     |     | 2155 |     |     |     |     | 2160 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Gly | Arg | Arg | Phe | Ala | Ser  | Gly | Lys | Lys | Val | Thr  | Leu | Asn | Pro |
| 2165 |     |     |     |     |     | 2170 |     |     |     |     | 2175 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Asp | Pro | Glu | His | Cys | Gln  | Ile | Cys | His | Cys | Asp  | Val | Val | Asn |
| 2180 |     |     |     |     |     | 2185 |     |     |     |     | 2190 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Thr | Cys | Glu | Ala | Cys | Gln  | Glu | Pro | Ile | Ser | Gly  | Thr | Ser | Glu |
| 2195 |     |     |     |     |     | 2200 |     |     |     |     | 2205 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ser  | Ala | Thr | Pro | Glu | Ser | Gly  | Pro | Gly | Ser | Glu | Pro  | Ala | Thr | Ser |
| 2210 |     |     |     |     |     | 2215 |     |     |     |     | 2220 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ser | Glu | Thr | Pro | Gly | Thr  | Ser | Glu | Ser | Ala | Thr  | Pro | Glu | Ser |
| 2225 |     |     |     |     |     | 2230 |     |     |     |     | 2235 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Pro | Gly | Ser | Glu | Pro | Ala  | Thr | Ser | Gly | Ser | Glu  | Thr | Pro | Gly |
| 2240 |     |     |     |     |     | 2245 |     |     |     |     | 2250 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Ser | Glu | Ser | Ala | Thr | Pro  | Glu | Ser | Gly | Pro | Gly  | Thr | Ser | Thr |
| 2255 |     |     |     |     |     | 2260 |     |     |     |     | 2265 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Pro | Ser | Glu | Gly | Ser | Ala  | Pro | Gly | Ser | Pro | Ala  | Gly | Ser | Pro |
| 2270 |     |     |     |     |     | 2275 |     |     |     |     | 2280 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Ser | Thr | Glu | Glu | Gly | Thr  | Ser | Glu | Ser | Ala | Thr  | Pro | Glu | Ser |
| 2285 |     |     |     |     |     | 2290 |     |     |     |     | 2295 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Pro | Gly | Ser | Glu | Pro | Ala  | Thr | Ser | Gly | Ser | Glu  | Thr | Pro | Gly |
| 2300 |     |     |     |     |     | 2305 |     |     |     |     | 2310 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Ser | Glu | Ser | Ala | Thr | Pro  | Glu | Ser | Gly | Pro | Gly  | Ser | Pro | Ala |
| 2315 |     |     |     |     |     | 2320 |     |     |     |     | 2325 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ser | Pro | Thr | Ser | Thr | Glu  | Glu | Gly | Ser | Pro | Ala  | Gly | Ser | Pro |
| 2330 |     |     |     |     |     | 2335 |     |     |     |     | 2340 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Ser | Thr | Glu | Glu | Gly | Thr  | Ser | Thr | Glu | Pro | Ser  | Glu | Gly | Ser |
| 2345 |     |     |     |     |     | 2350 |     |     |     |     | 2355 |     |     |     |

|                             |                     |             |
|-----------------------------|---------------------|-------------|
| Ala Pro Gly Thr Ser Glu Ser | Ala Thr Pro Glu Ser | Gly Pro Gly |
| 2360                        | 2365                | 2370        |
| Thr Ser Glu Ser Ala Thr Pro | Glu Ser Gly Pro Gly | Thr Ser Glu |
| 2375                        | 2380                | 2385        |
| Ser Ala Thr Pro Glu Ser Gly | Pro Gly Ser Glu Pro | Ala Thr Ser |
| 2390                        | 2395                | 2400        |
| Gly Ser Glu Thr Pro Gly Ser | Glu Pro Ala Thr Ser | Gly Ser Glu |
| 2405                        | 2410                | 2415        |
| Thr Pro Gly Ser Pro Ala Gly | Ser Pro Thr Ser Thr | Glu Glu Gly |
| 2420                        | 2425                | 2430        |
| Thr Ser Thr Glu Pro Ser Glu | Gly Ser Ala Pro Gly | Thr Ser Thr |
| 2435                        | 2440                | 2445        |
| Glu Pro Ser Glu Gly Ser Ala | Pro Gly Ser Glu Pro | Ala Thr Ser |
| 2450                        | 2455                | 2460        |
| Gly Ser Glu Thr Pro Gly Thr | Ser Glu Ser Ala Thr | Pro Glu Ser |
| 2465                        | 2470                | 2475        |
| Gly Pro Gly Thr Ser Thr Glu | Pro Ser Glu Gly Ser | Ala Pro Asp |
| 2480                        | 2485                | 2490        |
| Ser Gly Gly Gly Gly Ser Gly | Gly Gly Gly Ser Gly | Gly Gly Gly |
| 2495                        | 2500                | 2505        |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ser  | Gly | Gly | Gly | Gly | Ser | Gly  | Gly | Gly | Gly | Ser | Leu  | Val | Pro | Arg |
| 2510 |     |     |     |     |     | 2515 |     |     |     |     | 2520 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ser | Gly | Gly | Asp | Lys | Thr  | His | Thr | Cys | Pro | Pro  | Cys | Pro | Ala |
| 2525 |     |     |     |     |     | 2530 |     |     |     |     | 2535 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Glu | Leu | Leu | Gly | Gly | Pro  | Ser | Val | Phe | Leu | Phe  | Pro | Pro | Lys |
| 2540 |     |     |     |     |     | 2545 |     |     |     |     | 2550 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Lys | Asp | Thr | Leu | Met | Ile  | Ser | Arg | Thr | Pro | Glu  | Val | Thr | Cys |
| 2555 |     |     |     |     |     | 2560 |     |     |     |     | 2565 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Val | Val | Asp | Val | Ser | His  | Glu | Asp | Pro | Glu | Val  | Lys | Phe | Asn |
| 2570 |     |     |     |     |     | 2575 |     |     |     |     | 2580 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Tyr | Val | Asp | Gly | Val | Glu  | Val | His | Asn | Ala | Lys  | Thr | Lys | Pro |
| 2585 |     |     |     |     |     | 2590 |     |     |     |     | 2595 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Glu | Glu | Gln | Tyr | Asn | Ser  | Thr | Tyr | Arg | Val | Val  | Ser | Val | Leu |
| 2600 |     |     |     |     |     | 2605 |     |     |     |     | 2610 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Val | Leu | His | Gln | Asp | Trp  | Leu | Asn | Gly | Lys | Glu  | Tyr | Lys | Cys |
| 2615 |     |     |     |     |     | 2620 |     |     |     |     | 2625 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Val | Ser | Asn | Lys | Ala | Leu  | Pro | Ala | Pro | Ile | Glu  | Lys | Thr | Ile |
| 2630 |     |     |     |     |     | 2635 |     |     |     |     | 2640 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Lys | Ala | Lys | Gly | Gln | Pro  | Arg | Glu | Pro | Gln | Val  | Tyr | Thr | Leu |
| 2645 |     |     |     |     |     | 2650 |     |     |     |     | 2655 |     |     |     |

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr  
 2660 2665 2670

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 2675 2680 2685

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 2690 2695 2700

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
 2705 2710 2715

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser  
 2720 2725 2730

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
 2735 2740 2745

Ser Leu Ser Pro Gly Lys  
 2750

<210> 102

<211> 2242

<212> PRT

<213> Artificial Sequence

<220>

<223> pSYN-FVIII-170 protein sequence

<400> 102

Ser Leu Ser Cys Arg Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 5   | 10  | 15  |     |     |     |     |     |     |     |     |     |     |     |     |
| Asn | Leu | Arg | Ala | Glu | Gly | Leu | Glu | Cys | Thr | Lys | Thr | Cys | Gln | Asn | Tyr |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Asp | Leu | Glu | Cys | Met | Ser | Met | Gly | Cys | Val | Ser | Gly | Cys | Leu | Cys | Pro |
|     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |
| Pro | Gly | Met | Val | Arg | His | Glu | Asn | Arg | Cys | Val | Ala | Leu | Glu | Arg | Cys |
|     |     |     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |
| Pro | Cys | Phe | His | Gln | Gly | Lys | Glu | Tyr | Ala | Pro | Gly | Glu | Thr | Val | Lys |
| 65  |     |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |
| Ile | Gly | Cys | Asn | Thr | Cys | Val | Cys | Arg | Asp | Arg | Lys | Trp | Asn | Cys | Thr |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
| Asp | His | Val | Cys | Asp | Ala | Thr | Cys | Ser | Thr | Ile | Gly | Met | Ala | His | Tyr |
|     |     |     |     | 100 |     |     |     |     | 105 |     |     |     | 110 |     |     |
| Leu | Thr | Phe | Asp | Gly | Leu | Lys | Tyr | Leu | Phe | Pro | Gly | Glu | Cys | Gln | Tyr |
|     |     |     |     | 115 |     |     |     |     | 120 |     |     |     | 125 |     |     |
| Val | Leu | Val | Gln | Asp | Tyr | Cys | Gly | Ser | Asn | Pro | Gly | Thr | Phe | Arg | Ile |
|     |     |     |     | 130 |     |     |     | 135 |     |     |     | 140 |     |     |     |
| Leu | Val | Gly | Asn | Lys | Gly | Cys | Ser | His | Pro | Ser | Val | Lys | Cys | Lys | Lys |
| 145 |     |     |     |     |     | 150 |     |     |     | 155 |     |     |     |     | 160 |
| Arg | Val | Thr | Ile | Leu | Val | Glu | Gly | Gly | Glu | Ile | Glu | Leu | Phe | Asp | Gly |

165

170

175

Glu Val Asn Val Lys Arg Pro Met Lys Asp Glu Thr His Phe Glu Val  
180 185 190

Val Glu Ser Gly Arg Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser  
195 200 205

Val Val Trp Asp Arg His Leu Ser Ile Ser Val Val Leu Lys Gln Thr  
210 215 220

Tyr Gln Glu Lys Val Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln  
225 230 235 240

Asn Asn Asp Leu Thr Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val  
245 250 255

Asp Phe Gly Asn Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg  
260 265 270

Lys Val Pro Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met  
275 280 285

Lys Gln Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val  
290 295 300

Phe Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val  
305 310 315 320

Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala Ala

325

330

335

Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln His Gly  
340 345 350

Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln Ser Cys Glu  
355 360 365

Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Ala Glu Trp Arg Tyr Asn  
370 375 380

Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln His Pro Glu Pro Leu  
385 390 395 400

Ala Cys Pro Val Gln Cys Val Glu Gly Cys His Ala His Cys Pro Pro  
405 410 415

Gly Lys Ile Leu Asp Glu Leu Leu Gln Thr Cys Val Asp Pro Glu Asp  
420 425 430

Cys Pro Val Cys Glu Val Ala Gly Arg Arg Phe Ala Ser Gly Lys Lys  
435 440 445

Val Thr Leu Asn Pro Ser Asp Pro Glu His Cys Gln Ile Cys His Cys  
450 455 460

Asp Val Val Asn Leu Thr Cys Glu Ala Cys Gln Glu Pro Ile Ser Gly  
465 470 475 480

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala



485

490

495

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu  
500 505 510

Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly  
515 520 525

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu  
530 535 540

Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser  
545 550 555 560

Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
565 570 575

Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser  
580 585 590

Ala Thr Pro Glu Ser Gly Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser  
595 600 605

Thr Glu Glu Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly  
610 615 620

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser  
625 630 635 640

Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu

645

650

655

Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
660 665 670

Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Glu Pro Ala  
675 680 685

Thr Ser Gly Ser Glu Thr Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser  
690 695 700

Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly  
705 710 715 720

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Glu Pro Ala  
725 730 735

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu  
740 745 750

Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Asp  
755 760 765

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
770 775 780

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Val Pro Arg Gly Ser  
785 790 795 800

Gly Gly Ala Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu

805

810

815

Ser Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala  
820 825 830

Arg Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val  
835 840 845

Val Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn  
850 855 860

Ile Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile  
865 870 875 880

Gln Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala  
885 890 895

Ser His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala  
900 905 910

Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu  
915 920 925

Asp Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val  
930 935 940

Leu Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr  
945 950 955 960

Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu

965

970

975

Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys

980

985

990

Thr Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu

995

1000

1005

Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp

1010

1015

1020

Arg Asp Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val

1025

1030

1035

Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His

1040

1045

1050

Arg Lys Ser Val Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro

1055

1060

1065

Glu Val His Ser Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg

1070

1075

1080

Asn His Arg Gln Ala Ser Leu Glu Ile Ser Pro Ile Thr Phe Leu

1085

1090

1095

Thr Ala Gln Thr Leu Leu Met Asp Leu Gly Gln Phe Leu Leu Phe

1100

1105

1110

Cys His Ile Ser Ser His Gln His Asp Gly Met Glu Ala Tyr Val

1115

1120

1125

Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg Met Lys Asn  
 1130 1135 1140

Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp Ser Glu  
 1145 1150 1155

Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe Ile  
 1160 1165 1170

Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His  
 1175 1180 1185

Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val  
 1190 1195 1200

Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn  
 1205 1210 1215

Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met  
 1220 1225 1230

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His  
 1235 1240 1245

Glu Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp  
 1250 1255 1260

Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn

1265

1270

1275

Ile Tyr Pro His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg  
 1280 1285 1290

Arg Leu Pro Lys Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu  
 1295 1300 1305

Pro Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val Thr Val Glu Asp  
 1310 1315 1320

Gly Pro Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser  
 1325 1330 1335

Ser Phe Val Asn Met Glu Arg Asp Leu Ala Ser Gly Leu Ile Gly  
 1340 1345 1350

Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly Asn  
 1355 1360 1365

Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe Ser Val Phe  
 1370 1375 1380

Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln Arg Phe  
 1385 1390 1395

Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe Gln  
 1400 1405 1410

Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser

1415

1420

1425

Leu Gln    Leu Ser Val Cys Leu    His Glu Val Ala Tyr    Trp Tyr Ile

1430

1435

1440

Leu Ser    Ile Gly Ala Gln Thr    Asp Phe Leu Ser Val    Phe Phe Ser

1445

1450

1455

Gly Tyr    Thr Phe Lys His Lys    Met Val Tyr Glu Asp    Thr Leu Thr

1460

1465

1470

Leu Phe    Pro Phe Ser Gly Glu    Thr Val Phe Met Ser    Met Glu Asn

1475

1480

1485

Pro Gly    Leu Trp Ile Leu Gly    Cys His Asn Ser Asp    Phe Arg Asn

1490

1495

1500

Arg Gly    Met Thr Ala Leu Leu    Lys Val Ser Ser Cys    Asp Lys Asn

1505

1510

1515

Thr Gly    Asp Tyr Tyr Glu Asp    Ser Tyr Glu Asp Ile    Ser Ala Tyr

1520

1525

1530

Leu Leu    Ser Lys Asn Asn Ala    Ile Glu Pro Arg Ser    Phe Ser Gln

1535

1540

1545

Asn Pro    Pro Val Leu Lys Arg    His Gln Arg Glu Ile    Thr Arg Thr

1550

1555

1560

Thr Leu    Gln Ser Asp Gln Glu    Glu Ile Asp Tyr Asp    Asp Thr Ile

1565

1570

1575

Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp  
 1580 1585 1590

Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr  
 1595 1600 1605

Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser  
 1610 1615 1620

Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro  
 1625 1630 1635

Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser Phe  
 1640 1645 1650

Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly Leu  
 1655 1660 1665

Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val  
 1670 1675 1680

Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser  
 1685 1690 1695

Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg  
 1700 1705 1710

Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys



1715

1720

1725

Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys  
 1730 1735 1740

Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His  
 1745 1750 1755

Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu  
 1760 1765 1770

Asn Pro Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu  
 1775 1780 1785

Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu  
 1790 1795 1800

Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu  
 1805 1810 1815

Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly  
 1820 1825 1830

Tyr Ile Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asp Gln  
 1835 1840 1845

Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile  
 1850 1855 1860

His Ser Ile His Phe Ser Gly His Val Phe Thr Val Arg Lys Lys

1865

1870

1875

Glu Glu Tyr Lys Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe

1880

1885

1890

Glu Thr Val Glu Met Leu Pro Ser Lys Ala Gly Ile Trp Arg Val

1895

1900

1905

Glu Cys Leu Ile Gly Glu His Leu His Ala Gly Met Ser Thr Leu

1910

1915

1920

Phe Leu Val Tyr Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala

1925

1930

1935

Ser Gly His Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr

1940

1945

1950

Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly Ser

1955

1960

1965

Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val

1970

1975

1980

Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Lys Thr Gln Gly

1985

1990

1995

Ala Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile

2000

2005

2010

Met Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn

2015

2020

2025

Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser Ser  
 2030 2035 2040

Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr  
 2045 2050 2055

Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg  
 2060 2065 2070

Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro Leu  
 2075 2080 2085

Gly Met Glu Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser  
 2090 2095 2100

Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser Pro Ser Lys Ala  
 2105 2110 2115

Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln Val  
 2120 2125 2130

Asn Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln Lys Thr Met  
 2135 2140 2145

Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu Thr  
 2150 2155 2160

Ser Met Tyr Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly

2165

2170

2175

His Gln Trp Thr Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe

2180

2185

2190

Gln Gly Asn Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu Asp

2195

2200

2205

Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp

2210

2215

2220

Val His Gln Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu Ala

2225

2230

2235

Gln Asp Leu Tyr

2240

&lt;210&gt; 103

&lt;211&gt; 1959

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; pSYN-FVIII-169 mature Protein sequence

&lt;400&gt; 103

Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser Trp Asp Tyr

1

5

10

15

Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro

20

25

30

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Val | Pro | Lys | Ser | Phe | Pro | Phe | Asn | Thr | Ser | Val | Val | Tyr | Lys | Lys |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr | Leu | Phe | Val | Glu | Phe | Thr | Asp | His | Leu | Phe | Asn | Ile | Ala | Lys | Pro |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Pro | Pro | Trp | Met | Gly | Leu | Leu | Gly | Pro | Thr | Ile | Gln | Ala | Glu | Val |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Asp | Thr | Val | Val | Ile | Thr | Leu | Lys | Asn | Met | Ala | Ser | His | Pro | Val |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     | 95  |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Leu | His | Ala | Val | Gly | Val | Ser | Tyr | Trp | Lys | Ala | Ser | Glu | Gly | Ala |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Tyr | Asp | Asp | Gln | Thr | Ser | Gln | Arg | Glu | Lys | Glu | Asp | Asp | Lys | Val |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Phe | Pro | Gly | Gly | Ser | His | Thr | Tyr | Val | Trp | Gln | Val | Leu | Lys | Glu | Asn |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Pro | Met | Ala | Ser | Asp | Pro | Leu | Cys | Leu | Thr | Tyr | Ser | Tyr | Leu | Ser |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     | 160 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| His | Val | Asp | Leu | Val | Lys | Asp | Leu | Asn | Ser | Gly | Leu | Ile | Gly | Ala | Leu |
|     |     |     | 165 |     |     |     |     |     | 170 |     |     |     | 175 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Val | Cys | Arg | Glu | Gly | Ser | Leu | Ala | Lys | Glu | Lys | Thr | Gln | Thr | Leu |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |

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His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp
      195                200                205

His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser
      210                215                220

Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg
225                230                235                240

Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His
      245                250                255

Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu
      260                265                270

Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile
      275                280                285

Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly
      290                295                300

Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met
305                310                315                320

Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg
      325                330                335

Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp
      340                345                350

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Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe
      355              360              365

Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His
      370              375              380

Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu
385              390              395              400

Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro
      405              410              415

Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr
      420              425              430

Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile
      435              440              445

Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile
      450              455              460

Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile
465              470              475              480

Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys
      485              490              495

His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys
      500              505              510

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Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys

515

520

525

Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala

530

535

540

Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp

545

550

555

560

Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe

565

570

575

Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln

580

585

590

Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe

595

600

605

Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser

610

615

620

Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu

625

630

635

640

Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr

645

650

655

Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro

660

665

670



Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp

675

680

685

Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala

690

695

700

Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu

705

710

715

720

Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala

725

730

735

Ile Glu Pro Arg Ser Phe Ser Gln Asn Gly Ala Pro Gly Thr Ser Glu

740

745

750

Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly

755

760

765

Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro

770

775

780

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu

785

790

795

800

Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu

805

810

815

Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu

820

825

830

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Thr | Ser | Glu | Ser | Ala | Thr | Pro | Glu | Ser | Gly | Pro | Gly | Ser | Glu | Pro |
|     |     | 835 |     |     |     |     | 840 |     |     |     |     | 845 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Thr | Ser | Gly | Ser | Glu | Thr | Pro | Gly | Thr | Ser | Glu | Ser | Ala | Thr | Pro |
|     |     | 850 |     |     |     |     | 855 |     |     |     |     | 860 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Ser | Gly | Pro | Gly | Ser | Pro | Ala | Gly | Ser | Pro | Thr | Ser | Thr | Glu | Glu |
| 865 |     |     |     |     | 870 |     |     |     |     | 875 |     |     |     |     | 880 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Ser | Pro | Ala | Gly | Ser | Pro | Thr | Ser | Thr | Glu | Glu | Gly | Thr | Ser | Thr |
|     |     |     |     | 885 |     |     |     |     | 890 |     |     |     |     | 895 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Pro | Ser | Glu | Gly | Ser | Ala | Pro | Gly | Thr | Ser | Glu | Ser | Ala | Thr | Pro |
|     |     |     | 900 |     |     |     |     | 905 |     |     |     |     | 910 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Ser | Gly | Pro | Gly | Thr | Ser | Glu | Ser | Ala | Thr | Pro | Glu | Ser | Gly | Pro |
|     |     | 915 |     |     |     |     |     | 920 |     |     |     |     | 925 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Thr | Ser | Glu | Ser | Ala | Thr | Pro | Glu | Ser | Gly | Pro | Gly | Ser | Glu | Pro |
|     | 930 |     |     |     |     |     | 935 |     |     |     |     | 940 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Thr | Ser | Gly | Ser | Glu | Thr | Pro | Gly | Ser | Glu | Pro | Ala | Thr | Ser | Gly |
| 945 |     |     |     |     |     |     | 950 |     |     |     | 955 |     |     |     | 960 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Glu | Thr | Pro | Gly | Ser | Pro | Ala | Gly | Ser | Pro | Thr | Ser | Thr | Glu | Glu |
|     |     |     |     | 965 |     |     |     |     | 970 |     |     |     |     | 975 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Thr | Ser | Thr | Glu | Pro | Ser | Glu | Gly | Ser | Ala | Pro | Gly | Thr | Ser | Thr |
|     |     |     |     | 980 |     |     |     |     | 985 |     |     |     |     | 990 |     |

Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Glu Pro Ala Thr Ser Gly

995

1000

1005

Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly

1010

1015

1020

Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Ala Ser

1025

1030

1035

Ser Pro Pro Val Leu Lys Arg His Gln Ala Glu Ile Thr Arg Thr

1040

1045

1050

Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile

1055

1060

1065

Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp

1070

1075

1080

Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr

1085

1090

1095

Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser

1100

1105

1110

Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro

1115

1120

1125

Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser Phe

1130

1135

1140

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Thr  | Gln | Pro | Leu | Tyr | Arg | Gly  | Glu | Leu | Asn | Glu | His  | Leu | Gly | Leu |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     | 1155 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Gly | Pro | Tyr | Ile | Arg | Ala  | Glu | Val | Glu | Asp | Asn  | Ile | Met | Val |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Phe | Arg | Asn | Gln | Ala | Ser  | Arg | Pro | Tyr | Ser | Phe  | Tyr | Ser | Ser |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Ile | Ser | Tyr | Glu | Glu | Asp  | Gln | Arg | Gln | Gly | Ala  | Glu | Pro | Arg |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     | 1200 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Asn | Phe | Val | Lys | Pro | Asn  | Glu | Thr | Lys | Thr | Tyr  | Phe | Trp | Lys |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     | 1215 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Gln | His | His | Met | Ala | Pro  | Thr | Lys | Asp | Glu | Phe  | Asp | Cys | Lys |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Trp | Ala | Tyr | Phe | Ser | Asp  | Val | Asp | Leu | Glu | Lys  | Asp | Val | His |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | Leu | Ile | Gly | Pro | Leu  | Leu | Val | Cys | His | Thr  | Asn | Thr | Leu |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Pro | Ala | His | Gly | Arg | Gln  | Val | Thr | Val | Gln | Glu  | Phe | Ala | Leu |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     | 1275 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Phe | Thr | Ile | Phe | Asp | Glu  | Thr | Lys | Ser | Trp | Tyr  | Phe | Thr | Glu |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     | 1290 |     |     |     |



|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Gly  | Gln | Trp | Ala | Pro | Lys | Leu  | Ala | Arg | Leu | His | Tyr  | Ser | Gly | Ser |
| 1445 |     |     |     |     |     | 1450 |     |     |     |     | 1455 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Asn | Ala | Trp | Ser | Thr | Lys  | Glu | Pro | Phe | Ser | Trp  | Ile | Lys | Val |
| 1460 |     |     |     |     |     | 1465 |     |     |     |     | 1470 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Leu | Leu | Ala | Pro | Met | Ile  | Ile | His | Gly | Ile | Lys  | Thr | Gln | Gly |
| 1475 |     |     |     |     |     | 1480 |     |     |     |     | 1485 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Arg | Gln | Lys | Phe | Ser | Ser  | Leu | Tyr | Ile | Ser | Gln  | Phe | Ile | Ile |
| 1490 |     |     |     |     |     | 1495 |     |     |     |     | 1500 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Tyr | Ser | Leu | Asp | Gly | Lys  | Lys | Trp | Gln | Thr | Tyr  | Arg | Gly | Asn |
| 1505 |     |     |     |     |     | 1510 |     |     |     |     | 1515 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Thr | Gly | Thr | Leu | Met | Val  | Phe | Phe | Gly | Asn | Val  | Asp | Ser | Ser |
| 1520 |     |     |     |     |     | 1525 |     |     |     |     | 1530 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ile | Lys | His | Asn | Ile | Phe  | Asn | Pro | Pro | Ile | Ile  | Ala | Arg | Tyr |
| 1535 |     |     |     |     |     | 1540 |     |     |     |     | 1545 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Arg | Leu | His | Pro | Thr | His  | Tyr | Ser | Ile | Arg | Ser  | Thr | Leu | Arg |
| 1550 |     |     |     |     |     | 1555 |     |     |     |     | 1560 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Glu | Leu | Met | Gly | Cys | Asp  | Leu | Asn | Ser | Cys | Ser  | Met | Pro | Leu |
| 1565 |     |     |     |     |     | 1570 |     |     |     |     | 1575 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Met | Glu | Ser | Lys | Ala | Ile  | Ser | Asp | Ala | Gln | Ile  | Thr | Ala | Ser |
| 1580 |     |     |     |     |     | 1585 |     |     |     |     | 1590 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ser  | Tyr | Phe | Thr | Asn | Met | Phe  | Ala | Thr | Trp | Ser | Pro  | Ser | Lys | Ala |
| 1595 |     |     |     |     |     | 1600 |     |     |     |     | 1605 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Leu | His | Leu | Gln | Gly | Arg  | Ser | Asn | Ala | Trp | Arg  | Pro | Gln | Val |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Asn | Pro | Lys | Glu | Trp | Leu  | Gln | Val | Asp | Phe | Gln  | Lys | Thr | Met |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Val | Thr | Gly | Val | Thr | Thr  | Gln | Gly | Val | Lys | Ser  | Leu | Leu | Thr |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Met | Tyr | Val | Lys | Glu | Phe  | Leu | Ile | Ser | Ser | Ser  | Gln | Asp | Gly |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| His  | Gln | Trp | Thr | Leu | Phe | Phe  | Gln | Asn | Gly | Lys | Val  | Lys | Val | Phe |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Gly | Asn | Gln | Asp | Ser | Phe  | Thr | Pro | Val | Val | Asn  | Ser | Leu | Asp |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Pro | Leu | Leu | Thr | Arg | Tyr  | Leu | Arg | Ile | His | Pro  | Gln | Ser | Trp |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | His | Gln | Ile | Ala | Leu | Arg  | Met | Glu | Val | Leu | Gly  | Cys | Glu | Ala |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     | 1725 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Asp | Leu | Tyr | Asp | Lys | Thr  | His | Thr | Cys | Pro | Pro  | Cys | Pro | Ala |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     | 1740 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|
| Pro  | Glu | Leu | Leu | Gly | Gly | Pro  | Ser | Val | Phe | Leu | Phe | Pro  | Pro | Lys |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     |     | 1755 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Pro  | Lys | Asp | Thr | Leu | Met | Ile  | Ser | Arg | Thr | Pro | Glu | Val  | Thr | Cys |
| 1760 |     |     |     |     |     | 1765 |     |     |     |     |     | 1770 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Val  | Val | Val | Asp | Val | Ser | His  | Glu | Asp | Pro | Glu | Val | Lys  | Phe | Asn |
| 1775 |     |     |     |     |     | 1780 |     |     |     |     |     | 1785 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Trp  | Tyr | Val | Asp | Gly | Val | Glu  | Val | His | Asn | Ala | Lys | Thr  | Lys | Pro |
| 1790 |     |     |     |     |     | 1795 |     |     |     |     |     | 1800 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Arg  | Glu | Glu | Gln | Tyr | Asn | Ser  | Thr | Tyr | Arg | Val | Val | Ser  | Val | Leu |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     |     | 1815 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Thr  | Val | Leu | His | Gln | Asp | Trp  | Leu | Asn | Gly | Lys | Glu | Tyr  | Lys | Cys |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     |     | 1830 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Lys  | Val | Ser | Asn | Lys | Ala | Leu  | Pro | Ala | Pro | Ile | Glu | Lys  | Thr | Ile |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     |     | 1845 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ser  | Lys | Ala | Lys | Gly | Gln | Pro  | Arg | Glu | Pro | Gln | Val | Tyr  | Thr | Leu |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     |     | 1860 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Pro  | Pro | Ser | Arg | Asp | Glu | Leu  | Thr | Lys | Asn | Gln | Val | Ser  | Leu | Thr |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     |     | 1875 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Cys  | Leu | Val | Lys | Gly | Phe | Tyr  | Pro | Ser | Asp | Ile | Ala | Val  | Glu | Trp |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     |     | 1890 |     |     |



Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 1895 1900 1905

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr  
 1910 1915 1920

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser  
 1925 1930 1935

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
 1940 1945 1950

Ser Leu Ser Pro Gly Lys  
 1955

<210> 104

<211> 1959

<212> PRT

<213> Artificial Sequence

<220>

<223> pSYN-FVIII-173 mature Protein

<400> 104

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Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro  
 20 25 30

Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys

35

40

45

Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile Ala Lys Pro  
50 55 60

Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val  
65 70 75 80

Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val  
85 90 95

Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala  
100 105 110

Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val  
115 120 125

Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn  
130 135 140

Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser  
145 150 155 160

His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu  
165 170 175

Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu  
180 185 190

His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp

195

200

205

His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser  
 210 215 220

Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg  
 225 230 235 240

Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His  
 245 250 255

Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu  
 260 265 270

Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile  
 275 280 285

Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly  
 290 295 300

Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met  
 305 310 315 320

Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg  
 325 330 335

Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp  
 340 345 350

Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe

355

360

365

Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His  
370 375 380

Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu  
385 390 395 400

Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro  
405 410 415

Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr  
420 425 430

Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile  
435 440 445

Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile  
450 455 460

Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile  
465 470 475 480

Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys  
485 490 495

His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys  
500 505 510

Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys

515

520

525

Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala  
 530 535 540

Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp  
 545 550 555 560

Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe  
 565 570 575

Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln  
 580 585 590

Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe  
 595 600 605

Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser  
 610 615 620

Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu  
 625 630 635 640

Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr  
 645 650 655

Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro  
 660 665 670

Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp

675

680

685

Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala  
690 695 700

Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu  
705 710 715 720

Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala  
725 730 735

Ile Glu Pro Arg Ser Phe Ser Gln Asn Gly Ala Pro Gly Thr Ser Glu  
740 745 750

Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly  
755 760 765

Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
770 775 780

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu  
785 790 795 800

Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu  
805 810 815

Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu  
820 825 830

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro

835

840

845

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro  
850 855 860

Glu Ser Gly Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu  
865 870 875 880

Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Thr  
885 890 895

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro  
900 905 910

Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
915 920 925

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro  
930 935 940

Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Glu Pro Ala Thr Ser Gly  
945 950 955 960

Ser Glu Thr Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu  
965 970 975

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr  
980 985 990

Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Glu Pro Ala Thr Ser Gly

995

1000

1005

Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
 1010 1015 1020

Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Ala Ser  
 1025 1030 1035

Ser Pro Pro Val Leu Lys Arg His Gln Arg Glu Ile Thr Arg Thr  
 1040 1045 1050

Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile  
 1055 1060 1065

Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp  
 1070 1075 1080

Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr  
 1085 1090 1095

Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser  
 1100 1105 1110

Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro  
 1115 1120 1125

Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser Phe  
 1130 1135 1140

Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly Leu



1145

1150

1155

Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val  
 1160 1165 1170

Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser  
 1175 1180 1185

Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg  
 1190 1195 1200

Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys  
 1205 1210 1215

Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys  
 1220 1225 1230

Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His  
 1235 1240 1245

Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu  
 1250 1255 1260

Asn Pro Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu  
 1265 1270 1275

Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu  
 1280 1285 1290

Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu

1295

1300

1305

Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly

1310

1315

1320

Tyr Ile Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asp Gln

1325

1330

1335

Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile

1340

1345

1350

His Ser Ile His Phe Ser Gly His Val Phe Thr Val Arg Lys Lys

1355

1360

1365

Glu Glu Tyr Lys Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe

1370

1375

1380

Glu Thr Val Glu Met Leu Pro Ser Lys Ala Gly Ile Trp Arg Val

1385

1390

1395

Glu Cys Leu Ile Gly Glu His Leu His Ala Gly Met Ser Thr Leu

1400

1405

1410

Phe Leu Val Tyr Ser Asn Lys Cys Gln Thr Pro Leu Gly Met Ala

1415

1420

1425

Ser Gly His Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr

1430

1435

1440

Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly Ser

1445

1450

1455

Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys Val

1460

1465

1470

Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Lys Thr Gln Gly

1475

1480

1485

Ala Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile

1490

1495

1500

Met Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn

1505

1510

1515

Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser Ser

1520

1525

1530

Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr

1535

1540

1545

Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu Arg

1550

1555

1560

Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro Leu

1565

1570

1575

Gly Met Glu Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser

1580

1585

1590

Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser Pro Ser Lys Ala

1595

1600

1605

Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln Val  
 1610 1615 1620

Asn Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln Lys Thr Met  
 1625 1630 1635

Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu Thr  
 1640 1645 1650

Ser Met Tyr Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly  
 1655 1660 1665

His Gln Trp Thr Leu Phe Phe Gln Asn Gly Lys Val Lys Val Phe  
 1670 1675 1680

Gln Gly Asn Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu Asp  
 1685 1690 1695

Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser Trp  
 1700 1705 1710

Val His Gln Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu Ala  
 1715 1720 1725

Gln Asp Leu Tyr Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
 1730 1735 1740

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys

1745

1750

1755

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys  
 1760 1765 1770

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn  
 1775 1780 1785

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro  
 1790 1795 1800

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu  
 1805 1810 1815

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
 1820 1825 1830

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile  
 1835 1840 1845

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
 1850 1855 1860

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr  
 1865 1870 1875

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 1880 1885 1890

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro

1895

1900

1905

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr

1910

1915

1920

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser

1925

1930

1935

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu

1940

1945

1950

Ser Leu Ser Pro Gly Lys

1955

&lt;210&gt; 105

&lt;211&gt; 1984

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; FVIII 195 protein sequence

&lt;400&gt; 105

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe

1

5

10

15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser

20

25

30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg

35

40

45

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Pro | Pro | Arg | Val | Pro | Lys | Ser | Phe | Pro | Phe | Asn | Thr | Ser | Val | Val |
| 50  |     |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Lys | Lys | Thr | Leu | Phe | Val | Glu | Phe | Thr | Asp | His | Leu | Phe | Asn | Ile |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Lys | Pro | Arg | Pro | Pro | Trp | Met | Gly | Leu | Leu | Gly | Pro | Thr | Ile | Gln |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Glu | Val | Tyr | Asp | Thr | Val | Val | Ile | Thr | Leu | Lys | Asn | Met | Ala | Ser |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| His | Pro | Val | Ser | Leu | His | Ala | Val | Gly | Val | Ser | Tyr | Trp | Lys | Ala | Ser |
|     |     |     | 115 |     |     |     |     | 120 |     |     |     | 125 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Gly | Ala | Glu | Tyr | Asp | Asp | Gln | Thr | Ser | Gln | Arg | Glu | Lys | Glu | Asp |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Lys | Val | Phe | Pro | Gly | Gly | Ser | His | Thr | Tyr | Val | Trp | Gln | Val | Leu |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Lys | Glu | Asn | Gly | Pro | Met | Ala | Ser | Asp | Pro | Leu | Cys | Leu | Thr | Tyr | Ser |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Leu | Ser | His | Val | Asp | Leu | Val | Lys | Asp | Leu | Asn | Ser | Gly | Leu | Ile |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Ala | Leu | Leu | Val | Cys | Arg | Glu | Gly | Ser | Leu | Ala | Lys | Glu | Lys | Thr |
|     |     |     | 195 |     |     |     | 200 |     |     |     |     | 205 |     |     |     |

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly

210

215

220

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp

225

230

235

240

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr

245

250

255

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val

260

265

270

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile

275

280

285

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser

290

295

300

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met

305

310

315

320

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His

325

330

335

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro

340

345

350

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp

355

360

365



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Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
    370              375              380

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
385              390              395              400

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
          405              410              415

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
          420              425              430

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
    435              440              445

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
    450              455              460

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
465              470              475              480

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
          485              490              495

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
          500              505              510

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
    515              520              525

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Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
530                      535                      540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
545                      550                      555                      560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
565                      570                      575

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
580                      585                      590

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
595                      600                      605

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
610                      615                      620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
625                      630                      635                      640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
645                      650                      655

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
660                      665                      670

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
675                      680                      685

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Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro

690

695

700

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly

705

710

715

720

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp

725

730

735

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys

740

745

750

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Pro Pro Val Leu

755

760

765

Lys Arg His Gln Arg Glu Ile Thr Arg Thr Thr Leu Gln Gly Ala Pro

770

775

780

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser Pro

785

790

795

800

Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser

805

810

815

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro

820

825

830

Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Thr Pro Gly

835

840

845

Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
850 855 860

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
865 870 875 880

Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser Thr  
885 890 895

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala  
900 905 910

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
915 920 925

Ala Ser Ser Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser  
930 935 940

Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn  
945 950 955 960

Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala  
965 970 975

Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val  
980 985 990

Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val  
995 1000 1005

|         |                     |                     |                 |
|---------|---------------------|---------------------|-----------------|
| Val Phe | Gln Glu Phe Thr     | Asp Gly Ser Phe Thr | Gln Pro Leu Tyr |
| 1010    |                     | 1015                | 1020            |
|         |                     |                     |                 |
| Arg Gly | Glu Leu Asn Glu His | Leu Gly Leu Leu Gly | Pro Tyr Ile     |
| 1025    |                     | 1030                | 1035            |
|         |                     |                     |                 |
| Arg Ala | Glu Val Glu Asp Asn | Ile Met Val Thr Phe | Arg Asn Gln     |
| 1040    |                     | 1045                | 1050            |
|         |                     |                     |                 |
| Ala Ser | Arg Pro Tyr Ser Phe | Tyr Ser Ser Leu Ile | Ser Tyr Glu     |
| 1055    |                     | 1060                | 1065            |
|         |                     |                     |                 |
| Glu Asp | Gln Arg Gln Gly Ala | Glu Pro Arg Lys Asn | Phe Val Lys     |
| 1070    |                     | 1075                | 1080            |
|         |                     |                     |                 |
| Pro Asn | Glu Thr Lys Thr Tyr | Phe Trp Lys Val Gln | His His Met     |
| 1085    |                     | 1090                | 1095            |
|         |                     |                     |                 |
| Ala Pro | Thr Lys Asp Glu Phe | Asp Cys Lys Ala Trp | Ala Tyr Phe     |
| 1100    |                     | 1105                | 1110            |
|         |                     |                     |                 |
| Ser Asp | Val Asp Leu Glu Lys | Asp Val His Ser Gly | Leu Ile Gly     |
| 1115    |                     | 1120                | 1125            |
|         |                     |                     |                 |
| Pro Leu | Leu Val Cys His Thr | Asn Thr Leu Asn Pro | Ala His Gly     |
| 1130    |                     | 1135                | 1140            |
|         |                     |                     |                 |
| Arg Gln | Val Thr Val Gln Glu | Phe Ala Leu Phe Phe | Thr Ile Phe     |
| 1145    |                     | 1150                | 1155            |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Asp  | Glu | Thr | Lys | Ser | Trp | Tyr  | Phe | Thr | Glu | Asn | Met  | Glu | Arg | Asn |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Arg | Gly | Ala | Pro | Thr | Ser  | Glu | Ser | Ala | Thr | Pro  | Glu | Ser | Gly |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gly | Ser | Glu | Pro | Ala | Thr  | Ser | Gly | Ser | Glu | Thr  | Pro | Gly | Thr |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     | 1200 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Glu | Ser | Ala | Thr | Pro | Glu  | Ser | Gly | Pro | Gly | Ser  | Glu | Pro | Ala |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     | 1215 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Ser | Gly | Ser | Glu | Thr | Pro  | Gly | Thr | Ser | Glu | Ser  | Ala | Thr | Pro |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Ser | Gly | Pro | Gly | Thr | Ser  | Thr | Glu | Pro | Ser | Glu  | Gly | Ser | Ala |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gly | Thr | Ser | Glu | Ser | Ala  | Thr | Pro | Glu | Ser | Gly  | Pro | Gly | Ser |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Ala | Gly | Ser | Pro | Thr | Ser  | Thr | Glu | Glu | Gly | Ser  | Pro | Ala | Gly |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     | 1275 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Pro | Thr | Ser | Thr | Glu | Glu  | Gly | Ser | Pro | Ala | Gly  | Ser | Pro | Thr |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     | 1290 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Thr | Glu | Glu | Gly | Thr | Ser  | Glu | Ser | Ala | Thr | Pro  | Glu | Ser | Gly |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     | 1305 |     |     |     |



|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Asp  | Phe | Gln | Ile | Thr | Ala | Ser  | Gly | Gln | Tyr | Gly | Gln  | Trp | Ala | Pro |
| 1460 |     |     |     |     |     | 1465 |     |     |     |     | 1470 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Leu | Ala | Arg | Leu | His | Tyr  | Ser | Gly | Ser | Ile | Asn  | Ala | Trp | Ser |
| 1475 |     |     |     |     |     | 1480 |     |     |     |     | 1485 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Lys | Glu | Pro | Phe | Ser | Trp  | Ile | Lys | Val | Asp | Leu  | Leu | Ala | Pro |
| 1490 |     |     |     |     |     | 1495 |     |     |     |     | 1500 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Ile | Ile | His | Gly | Ile | Lys  | Thr | Gln | Gly | Ala | Arg  | Gln | Lys | Phe |
| 1505 |     |     |     |     |     | 1510 |     |     |     |     | 1515 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Ser | Leu | Tyr | Ile | Ser | Gln  | Phe | Ile | Ile | Met | Tyr  | Ser | Leu | Asp |
| 1520 |     |     |     |     |     | 1525 |     |     |     |     | 1530 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Lys | Lys | Trp | Gln | Thr | Tyr  | Arg | Gly | Asn | Ser | Thr  | Gly | Thr | Leu |
| 1535 |     |     |     |     |     | 1540 |     |     |     |     | 1545 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Val | Phe | Phe | Gly | Asn | Val  | Asp | Ser | Ser | Gly | Ile  | Lys | His | Asn |
| 1550 |     |     |     |     |     | 1555 |     |     |     |     | 1560 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Phe | Asn | Pro | Pro | Ile | Ile  | Ala | Arg | Tyr | Ile | Arg  | Leu | His | Pro |
| 1565 |     |     |     |     |     | 1570 |     |     |     |     | 1575 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | His | Tyr | Ser | Ile | Arg | Ser  | Thr | Leu | Arg | Met | Glu  | Leu | Met | Gly |
| 1580 |     |     |     |     |     | 1585 |     |     |     |     | 1590 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Asp | Leu | Asn | Ser | Cys | Ser  | Met | Pro | Leu | Gly | Met  | Glu | Ser | Lys |
| 1595 |     |     |     |     |     | 1600 |     |     |     |     | 1605 |     |     |     |



|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ala  | Ile | Ser | Asp | Ala | Gln | Ile  | Thr | Ala | Ser | Ser | Tyr  | Phe | Thr | Asn |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Phe | Ala | Thr | Trp | Ser | Pro  | Ser | Lys | Ala | Arg | Leu  | His | Leu | Gln |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Arg | Ser | Asn | Ala | Trp | Arg  | Pro | Gln | Val | Asn | Asn  | Pro | Lys | Glu |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Leu | Gln | Val | Asp | Phe | Gln  | Lys | Thr | Met | Lys | Val  | Thr | Gly | Val |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Thr | Gln | Gly | Val | Lys | Ser  | Leu | Leu | Thr | Ser | Met  | Tyr | Val | Lys |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Phe | Leu | Ile | Ser | Ser | Ser  | Gln | Asp | Gly | His | Gln  | Trp | Thr | Leu |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Phe | Gln | Asn | Gly | Lys | Val  | Lys | Val | Phe | Gln | Gly  | Asn | Gln | Asp |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Phe | Thr | Pro | Val | Val | Asn  | Ser | Leu | Asp | Pro | Pro  | Leu | Leu | Thr |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     | 1725 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Tyr | Leu | Arg | Ile | His | Pro  | Gln | Ser | Trp | Val | His  | Gln | Ile | Ala |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     | 1740 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Arg | Met | Glu | Val | Leu | Gly  | Cys | Glu | Ala | Gln | Asp  | Leu | Tyr | Asp |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     | 1755 |     |     |     |

|         |         |         |      |         |         |      |         |     |  |
|---------|---------|---------|------|---------|---------|------|---------|-----|--|
| Lys Thr | His Thr | Cys Pro | Pro  | Cys Pro | Ala Pro | Glu  | Leu Leu | Gly |  |
| 1760    |         |         | 1765 |         |         | 1770 |         |     |  |
|         |         |         |      |         |         |      |         |     |  |
| Gly Pro | Ser Val | Phe Leu | Phe  | Pro Pro | Lys Pro | Lys  | Asp Thr | Leu |  |
| 1775    |         |         | 1780 |         |         | 1785 |         |     |  |
|         |         |         |      |         |         |      |         |     |  |
| Met Ile | Ser Arg | Thr Pro | Glu  | Val Thr | Cys Val | Val  | Val Asp | Val |  |
| 1790    |         |         | 1795 |         |         | 1800 |         |     |  |
|         |         |         |      |         |         |      |         |     |  |
| Ser His | Glu Asp | Pro Glu | Val  | Lys Phe | Asn Trp | Tyr  | Val Asp | Gly |  |
| 1805    |         |         | 1810 |         |         | 1815 |         |     |  |
|         |         |         |      |         |         |      |         |     |  |
| Val Glu | Val His | Asn Ala | Lys  | Thr Lys | Pro Arg | Glu  | Glu Gln | Tyr |  |
| 1820    |         |         | 1825 |         |         | 1830 |         |     |  |
|         |         |         |      |         |         |      |         |     |  |
| Asn Ser | Thr Tyr | Arg Val | Val  | Ser Val | Leu Thr | Val  | Leu His | Gln |  |
| 1835    |         |         | 1840 |         |         | 1845 |         |     |  |
|         |         |         |      |         |         |      |         |     |  |
| Asp Trp | Leu Asn | Gly Lys | Glu  | Tyr Lys | Cys Lys | Val  | Ser Asn | Lys |  |
| 1850    |         |         | 1855 |         |         | 1860 |         |     |  |
|         |         |         |      |         |         |      |         |     |  |
| Ala Leu | Pro Ala | Pro Ile | Glu  | Lys Thr | Ile Ser | Lys  | Ala Lys | Gly |  |
| 1865    |         |         | 1870 |         |         | 1875 |         |     |  |
|         |         |         |      |         |         |      |         |     |  |
| Gln Pro | Arg Glu | Pro Gln | Val  | Tyr Thr | Leu Pro | Pro  | Ser Arg | Asp |  |
| 1880    |         |         | 1885 |         |         | 1890 |         |     |  |
|         |         |         |      |         |         |      |         |     |  |
| Glu Leu | Thr Lys | Asn Gln | Val  | Ser Leu | Thr Cys | Leu  | Val Lys | Gly |  |
| 1895    |         |         | 1900 |         |         | 1905 |         |     |  |

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln  
1910 1915 1920

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
1925 1930 1935

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg  
1940 1945 1950

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
1955 1960 1965

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
1970 1975 1980

Lys

<210> 106

<211> 2134

<212> PRT

<213> Artificial Sequence

<220>

<223> FVIII 196 protein sequence

<400> 106

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe  
1 5 10 15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser

20

25

30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Gly Ala Pro  
 35 40 45

Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser Pro  
 50 55 60

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 65 70 75 80

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 85 90 95

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro  
 100 105 110

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 115 120 125

Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro  
 130 135 140

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr  
 145 150 155 160

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala  
 165 170 175

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro

180

185

190

Ala Ser Ser Asp Ala Arg Phe Pro Pro Arg Val Pro Lys Ser Phe Pro  
 195 200 205

Phe Asn Thr Ser Val Val Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr  
 210 215 220

Asp His Leu Phe Asn Ile Ala Lys Pro Arg Pro Pro Trp Met Gly Leu  
 225 230 235 240

Leu Gly Pro Thr Ile Gln Ala Glu Val Tyr Asp Thr Val Val Ile Thr  
 245 250 255

Leu Lys Asn Met Ala Ser His Pro Val Ser Leu His Ala Val Gly Val  
 260 265 270

Ser Tyr Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser  
 275 280 285

Gln Arg Glu Lys Glu Asp Asp Lys Val Phe Pro Gly Gly Ser His Thr  
 290 295 300

Tyr Val Trp Gln Val Leu Lys Glu Asn Gly Pro Met Ala Ser Asp Pro  
 305 310 315 320

Leu Cys Leu Thr Tyr Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp  
 325 330 335

Leu Asn Ser Gly Leu Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser

340

345

350

Leu Ala Lys Glu Lys Thr Gln Thr Leu His Lys Phe Ile Leu Leu Phe  
355 360 365

Ala Val Phe Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser  
370 375 380

Leu Met Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala Trp Pro Lys Met  
385 390 395 400

His Thr Val Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly  
405 410 415

Cys His Arg Lys Ser Val Tyr Trp His Val Ile Gly Met Gly Thr Thr  
420 425 430

Pro Glu Val His Ser Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg  
435 440 445

Asn His Arg Gln Ala Ser Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr  
450 455 460

Ala Gln Thr Leu Leu Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His  
465 470 475 480

Ile Ser Ser His Gln His Asp Gly Met Glu Ala Tyr Val Lys Val Asp  
485 490 495

Ser Cys Pro Glu Glu Pro Gln Leu Arg Met Lys Asn Asn Glu Glu Ala

500

505

510

Glu Asp Tyr Asp Asp Asp Leu Thr Asp Ser Glu Met Asp Val Val Arg  
515 520 525

Phe Asp Asp Asp Asn Ser Pro Ser Phe Ile Gln Ile Arg Ser Val Ala  
530 535 540

Lys Lys His Pro Lys Thr Trp Val His Tyr Ile Ala Ala Glu Glu Glu  
545 550 555 560

Asp Trp Asp Tyr Ala Pro Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr  
565 570 575

Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr  
580 585 590

Lys Lys Val Arg Phe Met Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg  
595 600 605

Glu Ala Ile Gln His Glu Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly  
610 615 620

Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg  
625 630 635 640

Pro Tyr Asn Ile Tyr Pro His Gly Ile Thr Asp Val Arg Pro Leu Tyr  
645 650 655

Ser Arg Arg Leu Pro Lys Gly Val Lys His Leu Lys Asp Phe Pro Ile

660

665

670

Leu Pro Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val Thr Val Glu Asp  
675 680 685

Gly Pro Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser  
690 695 700

Phe Val Asn Met Glu Arg Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu  
705 710 715 720

Leu Ile Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly Asn Gln Ile Met  
725 730 735

Ser Asp Lys Arg Asn Val Ile Leu Phe Ser Val Phe Asp Glu Asn Arg  
740 745 750

Ser Trp Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala  
755 760 765

Gly Val Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser Asn Ile Met His  
770 775 780

Ser Ile Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser Val Cys Leu  
785 790 795 800

His Glu Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp  
805 810 815

Phe Leu Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val



820

825

830

Tyr Glu Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe  
835 840 845

Met Ser Met Glu Asn Pro Gly Leu Trp Ile Leu Gly Cys His Asn Ser  
850 855 860

Asp Phe Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys  
865 870 875 880

Asp Lys Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser  
885 890 895

Ala Tyr Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser  
900 905 910

Gln Asn Pro Pro Val Leu Lys Arg His Gln Arg Glu Ile Thr Arg Thr  
915 920 925

Thr Leu Gln Gly Ala Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser  
930 935 940

Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala  
945 950 955 960

Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr  
965 970 975

Ser Ser Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr

980

985

990

Gly Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala  
 995 1000 1005

Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly  
 1010 1015 1020

Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 1025 1030 1035

Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser  
 1040 1045 1050

Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ala  
 1055 1060 1065

Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Ala Ser Ser Ser Asp  
 1070 1075 1080

Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys  
 1085 1090 1095

Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro  
 1100 1105 1110

Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val  
 1115 1120 1125

Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu

1130

1135

1140

Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val  
 1145 1150 1155

Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr  
 1160 1165 1170

Arg Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile  
 1175 1180 1185

Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln  
 1190 1195 1200

Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Glu  
 1205 1210 1215

Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys  
 1220 1225 1230

Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His His Met  
 1235 1240 1245

Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe  
 1250 1255 1260

Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly  
 1265 1270 1275

Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly

1280

1285

1290

Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe  
 1295 1300 1305

Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn  
 1310 1315 1320

Cys Arg Gly Ala Pro Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
 1325 1330 1335

Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr  
 1340 1345 1350

Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala  
 1355 1360 1365

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro  
 1370 1375 1380

Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala  
 1385 1390 1395

Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser  
 1400 1405 1410

Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala Gly  
 1415 1420 1425

Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala Gly Ser Pro Thr

1430

1435

1440

Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
 1445 1450 1455

Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ala  
 1460 1465 1470

Ser Ser Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys  
 1475 1480 1485

Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr  
 1490 1495 1500

Leu Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr  
 1505 1510 1515

Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe  
 1520 1525 1530

Ser Gly His Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met  
 1535 1540 1545

Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met  
 1550 1555 1560

Leu Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys Leu Ile Gly  
 1565 1570 1575

Glu His Leu His Ala Gly Met Ser Thr Leu Phe Leu Val Tyr Ser

1580

1585

1590

Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile Arg  
 1595 1600 1605

Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro  
 1610 1615 1620

Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser  
 1625 1630 1635

Thr Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala Pro  
 1640 1645 1650

Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe  
 1655 1660 1665

Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp  
 1670 1675 1680

Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu  
 1685 1690 1695

Met Val Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His Asn  
 1700 1705 1710

Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro  
 1715 1720 1725

Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly

1730

1735

1740

Cys Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Glu Ser Lys  
 1745 1750 1755

Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn  
 1760 1765 1770

Met Phe Ala Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu Gln  
 1775 1780 1785

Gly Arg Ser Asn Ala Trp Arg Pro Gln Val Asn Asn Pro Lys Glu  
 1790 1795 1800

Trp Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val Thr Gly Val  
 1805 1810 1815

Thr Thr Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr Val Lys  
 1820 1825 1830

Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr Leu  
 1835 1840 1845

Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp  
 1850 1855 1860

Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr  
 1865 1870 1875

Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala

1880

1885

1890

Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr Asp

1895

1900

1905

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly

1910

1915

1920

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu

1925

1930

1935

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val

1940

1945

1950

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly

1955

1960

1965

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr

1970

1975

1980

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln

1985

1990

1995

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys

2000

2005

2010

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly

2015

2020

2025

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp



2030

2035

2040

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly

2045

2050

2055

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln

2060

2065

2070

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp

2075

2080

2085

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg

2090

2095

2100

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala

2105

2110

2115

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly

2120

2125

2130

Lys

<210> 107

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<212> PRT

<213> Artificial Sequence

<220>

<223> FVIII 199 protein sequence

<400> 107

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Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe
1           5           10           15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
          20           25           30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
          35           40           45

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
          50           55           60

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
65           70           75           80

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
          85           90           95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
          100          105          110

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
          115          120          125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
          130          135          140

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
145          150          155          160

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Glu | Asn | Gly | Pro | Met | Ala | Ser | Asp | Pro | Leu | Cys | Leu | Thr | Tyr | Ser |
| 165 |     |     |     | 170 |     |     |     | 175 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Leu | Ser | His | Val | Asp | Leu | Val | Lys | Asp | Leu | Asn | Ser | Gly | Leu | Ile |
| 180 |     |     |     | 185 |     |     |     | 190 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Ala | Leu | Leu | Val | Cys | Arg | Glu | Gly | Ser | Leu | Ala | Lys | Glu | Lys | Thr |
| 195 |     |     |     | 200 |     |     |     | 205 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gln | Thr | Leu | His | Lys | Phe | Ile | Leu | Leu | Phe | Ala | Val | Phe | Asp | Glu | Gly |
| 210 |     |     |     | 215 |     |     |     | 220 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Lys | Ser | Trp | His | Ser | Glu | Thr | Lys | Asn | Ser | Leu | Met | Gln | Asp | Arg | Asp |
| 225 |     |     |     | 230 |     |     |     | 235 |     |     |     | 240 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Ala | Ser | Ala | Arg | Ala | Trp | Pro | Lys | Met | His | Thr | Val | Asn | Gly | Tyr |
| 245 |     |     |     | 250 |     |     |     | 255 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val | Asn | Arg | Ser | Leu | Pro | Gly | Leu | Ile | Gly | Cys | His | Arg | Lys | Ser | Val |
| 260 |     |     |     | 265 |     |     |     | 270 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Trp | His | Val | Ile | Gly | Met | Gly | Thr | Thr | Pro | Glu | Val | His | Ser | Ile |
| 275 |     |     |     | 280 |     |     |     | 285 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Phe | Leu | Glu | Gly | His | Thr | Phe | Leu | Val | Arg | Asn | His | Arg | Gln | Ala | Ser |
| 290 |     |     |     | 295 |     |     |     | 300 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Glu | Ile | Ser | Pro | Ile | Thr | Phe | Leu | Thr | Ala | Gln | Thr | Leu | Leu | Met |
| 305 |     |     |     | 310 |     |     |     | 315 |     |     |     | 320 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Leu | Gly | Gln | Phe | Leu | Leu | Phe | Cys | His | Ile | Ser | Ser | His | Gln | His |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Gly | Met | Glu | Ala | Tyr | Val | Lys | Val | Asp | Ser | Cys | Pro | Glu | Glu | Pro |
|     |     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gln | Leu | Arg | Met | Lys | Asn | Asn | Glu | Glu | Ala | Glu | Asp | Tyr | Asp | Asp | Asp |
|     |     |     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Thr | Asp | Ser | Glu | Met | Asp | Val | Val | Arg | Phe | Asp | Asp | Asp | Asn | Ser |
|     |     |     |     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Ser | Phe | Ile | Gln | Ile | Arg | Ser | Val | Ala | Lys | Lys | His | Pro | Lys | Thr |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 385 |     |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     | 400 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Trp | Val | His | Tyr | Ile | Ala | Ala | Glu | Glu | Glu | Asp | Trp | Asp | Tyr | Ala | Pro |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 405 |     |     |     |     |     | 410 |     |     |     | 415 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Val | Leu | Ala | Pro | Asp | Asp | Arg | Ser | Tyr | Lys | Ser | Gln | Tyr | Leu | Asn |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 420 |     |     |     |     |     | 425 |     |     |     | 430 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asn | Gly | Pro | Gln | Arg | Ile | Gly | Arg | Lys | Tyr | Lys | Lys | Val | Arg | Phe | Met |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 435 |     |     |     |     |     | 440 |     |     |     | 445 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Tyr | Thr | Asp | Glu | Thr | Phe | Lys | Thr | Arg | Glu | Ala | Ile | Gln | His | Glu |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 450 |     |     |     |     |     | 455 |     |     |     | 460 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Gly | Ile | Leu | Gly | Pro | Leu | Leu | Tyr | Gly | Glu | Val | Gly | Asp | Thr | Leu |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 465 |     |     |     |     |     | 470 |     |     |     | 475 |     |     |     |     | 480 |

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Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
      485              490              495

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
      500              505              510

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
      515              520              525

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
      530              535              540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
545              550              555              560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
      565              570              575

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
      580              585              590

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
      595              600              605

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
      610              615              620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
625              630              635              640

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Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp

645

650

655

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe

660

665

670

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr

675

680

685

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro

690

695

700

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly

705

710

715

720

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp

725

730

735

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys

740

745

750

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Pro Pro Val Leu

755

760

765

Lys Arg His Gln Ala Glu Ile Thr Arg Thr Thr Leu Gln Gly Ala Pro

770

775

780

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser Pro

785

790

795

800

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|
| Gly | Thr | Ser | Ser | Thr | Gly | Ser | Pro | Gly | Ala | Ser | Pro | Gly | Thr | Ser | Ser |  |  |
|     |     |     |     | 805 |     |     |     |     | 810 |     |     |     |     | 815 |     |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
| Thr | Gly | Ser | Pro | Gly | Ala | Ser | Pro | Gly | Thr | Ser | Ser | Thr | Gly | Ser | Pro |  |  |
|     |     |     | 820 |     |     |     |     | 825 |     |     |     |     | 830 |     |     |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
| Gly | Ser | Ser | Pro | Ser | Ala | Ser | Thr | Gly | Thr | Gly | Pro | Gly | Thr | Pro | Gly |  |  |
|     |     | 835 |     |     |     |     | 840 |     |     |     |     | 845 |     |     |     |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
| Ser | Gly | Thr | Ala | Ser | Ser | Ser | Pro | Gly | Ala | Ser | Pro | Gly | Thr | Ser | Ser |  |  |
|     | 850 |     |     |     |     | 855 |     |     |     |     | 860 |     |     |     |     |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
| Thr | Gly | Ser | Pro | Gly | Ala | Ser | Pro | Gly | Thr | Ser | Ser | Thr | Gly | Ser | Pro |  |  |
| 865 |     |     |     |     | 870 |     |     |     |     | 875 |     |     |     |     | 880 |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
| Gly | Ala | Ser | Pro | Gly | Thr | Ser | Ser | Thr | Gly | Ser | Pro | Gly | Ser | Ser | Thr |  |  |
|     |     |     |     | 885 |     |     |     |     | 890 |     |     |     |     | 895 |     |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
| Pro | Ser | Gly | Ala | Thr | Gly | Ser | Pro | Gly | Ser | Ser | Thr | Pro | Ser | Gly | Ala |  |  |
|     |     |     | 900 |     |     |     |     | 905 |     |     |     |     |     | 910 |     |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
| Thr | Gly | Ser | Pro | Gly | Ala | Ser | Pro | Gly | Thr | Ser | Ser | Thr | Gly | Ser | Pro |  |  |
|     |     | 915 |     |     |     |     |     | 920 |     |     |     |     | 925 |     |     |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
| Ala | Ser | Ser | Ser | Asp | Gln | Glu | Glu | Ile | Asp | Tyr | Asp | Asp | Thr | Ile | Ser |  |  |
|     | 930 |     |     |     |     | 935 |     |     |     |     | 940 |     |     |     |     |  |  |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |
| Val | Glu | Met | Lys | Lys | Glu | Asp | Phe | Asp | Ile | Tyr | Asp | Glu | Asp | Glu | Asn |  |  |
| 945 |     |     |     |     | 950 |     |     |     |     | 955 |     |     |     |     | 960 |  |  |

|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
|-----|------|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|------|
| Gln | Ser  | Pro | Arg | Ser | Phe | Gln | Lys | Lys | Thr  | Arg | His | Tyr | Phe | Ile  | Ala  |
|     |      |     |     | 965 |     |     |     |     | 970  |     |     |     |     | 975  |      |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
| Ala | Val  | Glu | Arg | Leu | Trp | Asp | Tyr | Gly | Met  | Ser | Ser | Ser | Pro | His  | Val  |
|     |      |     |     | 980 |     |     |     |     | 985  |     |     |     |     | 990  |      |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
| Leu | Arg  | Asn | Arg | Ala | Gln | Ser | Gly | Ser | Val  | Pro | Gln | Phe | Lys | Lys  | Val  |
|     |      |     |     | 995 |     |     |     |     | 1000 |     |     |     |     | 1005 |      |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
| Val | Phe  | Gln | Glu | Phe | Thr | Asp | Gly | Ser | Phe  | Thr | Gln | Pro | Leu | Tyr  |      |
|     | 1010 |     |     |     |     |     |     |     |      |     |     |     |     | 1015 |      |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      | 1020 |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
| Arg | Gly  | Glu | Leu | Asn | Glu | His | Leu | Gly | Leu  | Leu | Gly | Pro | Tyr | Ile  |      |
|     | 1025 |     |     |     |     |     |     |     |      |     |     |     |     | 1030 |      |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      | 1035 |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
| Arg | Ala  | Glu | Val | Glu | Asp | Asn | Ile | Met | Val  | Thr | Phe | Arg | Asn | Gln  |      |
|     | 1040 |     |     |     |     |     |     |     |      |     |     |     |     | 1045 |      |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      | 1050 |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
| Ala | Ser  | Arg | Pro | Tyr | Ser | Phe | Tyr | Ser | Ser  | Leu | Ile | Ser | Tyr | Glu  |      |
|     | 1055 |     |     |     |     |     |     |     |      |     |     |     |     | 1060 |      |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      | 1065 |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
| Glu | Asp  | Gln | Arg | Gln | Gly | Ala | Glu | Pro | Arg  | Lys | Asn | Phe | Val | Lys  |      |
|     | 1070 |     |     |     |     |     |     |     |      |     |     |     |     | 1075 |      |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      | 1080 |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
| Pro | Asn  | Glu | Thr | Lys | Thr | Tyr | Phe | Trp | Lys  | Val | Gln | His | His | Met  |      |
|     | 1085 |     |     |     |     |     |     |     |      |     |     |     |     | 1090 |      |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      | 1095 |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      |      |
| Ala | Pro  | Thr | Lys | Asp | Glu | Phe | Asp | Cys | Lys  | Ala | Trp | Ala | Tyr | Phe  |      |
|     | 1100 |     |     |     |     |     |     |     |      |     |     |     |     | 1105 |      |
|     |      |     |     |     |     |     |     |     |      |     |     |     |     |      | 1110 |



|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ser  | Asp | Val | Asp | Leu | Glu | Lys  | Asp | Val | His | Ser | Gly  | Leu | Ile | Gly |
| 1115 |     |     |     |     |     | 1120 |     |     |     |     | 1125 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Leu | Leu | Val | Cys | His | Thr  | Asn | Thr | Leu | Asn | Pro  | Ala | His | Gly |
| 1130 |     |     |     |     |     | 1135 |     |     |     |     | 1140 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Gln | Val | Thr | Val | Gln | Glu  | Phe | Ala | Leu | Phe | Phe  | Thr | Ile | Phe |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     | 1155 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Glu | Thr | Lys | Ser | Trp | Tyr  | Phe | Thr | Glu | Asn | Met  | Glu | Arg | Asn |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Arg | Gly | Ala | Pro | Thr | Ser  | Glu | Ser | Ala | Thr | Pro  | Glu | Ser | Gly |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gly | Ser | Glu | Pro | Ala | Thr  | Ser | Gly | Ser | Glu | Thr  | Pro | Gly | Thr |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     | 1200 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Glu | Ser | Ala | Thr | Pro | Glu  | Ser | Gly | Pro | Gly | Ser  | Glu | Pro | Ala |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     | 1215 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Ser | Gly | Ser | Glu | Thr | Pro  | Gly | Thr | Ser | Glu | Ser  | Ala | Thr | Pro |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Ser | Gly | Pro | Gly | Thr | Ser  | Thr | Glu | Pro | Ser | Glu  | Gly | Ser | Ala |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gly | Thr | Ser | Glu | Ser | Ala  | Thr | Pro | Glu | Ser | Gly  | Pro | Gly | Ser |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Pro  | Ala | Gly | Ser | Pro | Thr | Ser  | Thr | Glu | Glu | Gly | Ser  | Pro | Ala | Gly |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     | 1275 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Pro | Thr | Ser | Thr | Glu | Glu  | Gly | Ser | Pro | Ala | Gly  | Ser | Pro | Thr |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     | 1290 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Thr | Glu | Glu | Gly | Thr | Ser  | Glu | Ser | Ala | Thr | Pro  | Glu | Ser | Gly |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     | 1305 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gly | Thr | Ser | Thr | Glu | Pro  | Ser | Glu | Gly | Ser | Ala  | Pro | Gly | Ala |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     | 1320 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Ser | Ala | Pro | Cys | Asn | Ile  | Gln | Met | Glu | Asp | Pro  | Thr | Phe | Lys |
| 1325 |     |     |     |     |     | 1330 |     |     |     |     | 1335 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Asn | Tyr | Arg | Phe | His | Ala  | Ile | Asn | Gly | Tyr | Ile  | Met | Asp | Thr |
| 1340 |     |     |     |     |     | 1345 |     |     |     |     | 1350 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Pro | Gly | Leu | Val | Met | Ala  | Gln | Asp | Gln | Arg | Ile  | Arg | Trp | Tyr |
| 1355 |     |     |     |     |     | 1360 |     |     |     |     | 1365 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Leu | Ser | Met | Gly | Ser | Asn  | Glu | Asn | Ile | His | Ser  | Ile | His | Phe |
| 1370 |     |     |     |     |     | 1375 |     |     |     |     | 1380 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | His | Val | Phe | Thr | Val  | Arg | Lys | Lys | Glu | Glu  | Tyr | Lys | Met |
| 1385 |     |     |     |     |     | 1390 |     |     |     |     | 1395 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Leu | Tyr | Asn | Leu | Tyr | Pro  | Gly | Val | Phe | Glu | Thr  | Val | Glu | Met |
| 1400 |     |     |     |     |     | 1405 |     |     |     |     | 1410 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Leu  | Pro | Ser | Lys | Ala | Gly | Ile  | Trp | Arg | Val | Glu | Cys  | Leu | Ile | Gly |
| 1415 |     |     |     |     |     | 1420 |     |     |     |     | 1425 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | His | Leu | His | Ala | Gly | Met  | Ser | Thr | Leu | Phe | Leu  | Val | Tyr | Ser |
| 1430 |     |     |     |     |     | 1435 |     |     |     |     | 1440 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Lys | Cys | Gln | Thr | Pro | Leu  | Gly | Met | Ala | Ser | Gly  | His | Ile | Arg |
| 1445 |     |     |     |     |     | 1450 |     |     |     |     | 1455 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Phe | Gln | Ile | Thr | Ala | Ser  | Gly | Gln | Tyr | Gly | Gln  | Trp | Ala | Pro |
| 1460 |     |     |     |     |     | 1465 |     |     |     |     | 1470 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Leu | Ala | Arg | Leu | His | Tyr  | Ser | Gly | Ser | Ile | Asn  | Ala | Trp | Ser |
| 1475 |     |     |     |     |     | 1480 |     |     |     |     | 1485 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Lys | Glu | Pro | Phe | Ser | Trp  | Ile | Lys | Val | Asp | Leu  | Leu | Ala | Pro |
| 1490 |     |     |     |     |     | 1495 |     |     |     |     | 1500 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Ile | Ile | His | Gly | Ile | Lys  | Thr | Gln | Gly | Ala | Arg  | Gln | Lys | Phe |
| 1505 |     |     |     |     |     | 1510 |     |     |     |     | 1515 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Ser | Leu | Tyr | Ile | Ser | Gln  | Phe | Ile | Ile | Met | Tyr  | Ser | Leu | Asp |
| 1520 |     |     |     |     |     | 1525 |     |     |     |     | 1530 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Lys | Lys | Trp | Gln | Thr | Tyr  | Arg | Gly | Asn | Ser | Thr  | Gly | Thr | Leu |
| 1535 |     |     |     |     |     | 1540 |     |     |     |     | 1545 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Val | Phe | Phe | Gly | Asn | Val  | Asp | Ser | Ser | Gly | Ile  | Lys | His | Asn |
| 1550 |     |     |     |     |     | 1555 |     |     |     |     | 1560 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ile  | Phe | Asn | Pro | Pro | Ile | Ile  | Ala | Arg | Tyr | Ile | Arg  | Leu | His | Pro |
| 1565 |     |     |     |     |     | 1570 |     |     |     |     | 1575 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | His | Tyr | Ser | Ile | Arg | Ser  | Thr | Leu | Arg | Met | Glu  | Leu | Met | Gly |
| 1580 |     |     |     |     |     | 1585 |     |     |     |     | 1590 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Asp | Leu | Asn | Ser | Cys | Ser  | Met | Pro | Leu | Gly | Met  | Glu | Ser | Lys |
| 1595 |     |     |     |     |     | 1600 |     |     |     |     | 1605 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Ile | Ser | Asp | Ala | Gln | Ile  | Thr | Ala | Ser | Ser | Tyr  | Phe | Thr | Asn |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Phe | Ala | Thr | Trp | Ser | Pro  | Ser | Lys | Ala | Arg | Leu  | His | Leu | Gln |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Arg | Ser | Asn | Ala | Trp | Arg  | Pro | Gln | Val | Asn | Asn  | Pro | Lys | Glu |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Leu | Gln | Val | Asp | Phe | Gln  | Lys | Thr | Met | Lys | Val  | Thr | Gly | Val |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Thr | Gln | Gly | Val | Lys | Ser  | Leu | Leu | Thr | Ser | Met  | Tyr | Val | Lys |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Phe | Leu | Ile | Ser | Ser | Ser  | Gln | Asp | Gly | His | Gln  | Trp | Thr | Leu |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Phe | Gln | Asn | Gly | Lys | Val  | Lys | Val | Phe | Gln | Gly  | Asn | Gln | Asp |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ser  | Phe | Thr | Pro | Val | Val | Asn  | Ser | Leu | Asp | Pro | Pro  | Leu | Leu | Thr |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     | 1725 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Tyr | Leu | Arg | Ile | His | Pro  | Gln | Ser | Trp | Val | His  | Gln | Ile | Ala |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     | 1740 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Arg | Met | Glu | Val | Leu | Gly  | Cys | Glu | Ala | Gln | Asp  | Leu | Tyr | Asp |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     | 1755 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Thr | His | Thr | Cys | Pro | Pro  | Cys | Pro | Ala | Pro | Glu  | Leu | Leu | Gly |
| 1760 |     |     |     |     |     | 1765 |     |     |     |     | 1770 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Pro | Ser | Val | Phe | Leu | Phe  | Pro | Pro | Lys | Pro | Lys  | Asp | Thr | Leu |
| 1775 |     |     |     |     |     | 1780 |     |     |     |     | 1785 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Ile | Ser | Arg | Thr | Pro | Glu  | Val | Thr | Cys | Val | Val  | Val | Asp | Val |
| 1790 |     |     |     |     |     | 1795 |     |     |     |     | 1800 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | His | Glu | Asp | Pro | Glu | Val  | Lys | Phe | Asn | Trp | Tyr  | Val | Asp | Gly |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     | 1815 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Glu | Val | His | Asn | Ala | Lys  | Thr | Lys | Pro | Arg | Glu  | Glu | Gln | Tyr |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     | 1830 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Ser | Thr | Tyr | Arg | Val | Val  | Ser | Val | Leu | Thr | Val  | Leu | His | Gln |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     | 1845 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Trp | Leu | Asn | Gly | Lys | Glu  | Tyr | Lys | Cys | Lys | Val  | Ser | Asn | Lys |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     | 1860 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ala  | Leu | Pro | Ala | Pro | Ile | Glu  | Lys | Thr | Ile | Ser | Lys  | Ala | Lys | Gly |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     | 1875 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Gln  | Pro | Arg | Glu | Pro | Gln | Val  | Tyr | Thr | Leu | Pro | Pro  | Ser | Arg | Asp |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     | 1890 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Glu  | Leu | Thr | Lys | Asn | Gln | Val  | Ser | Leu | Thr | Cys | Leu  | Val | Lys | Gly |
| 1895 |     |     |     |     |     | 1900 |     |     |     |     | 1905 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Phe  | Tyr | Pro | Ser | Asp | Ile | Ala  | Val | Glu | Trp | Glu | Ser  | Asn | Gly | Gln |
| 1910 |     |     |     |     |     | 1915 |     |     |     |     | 1920 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Pro  | Glu | Asn | Asn | Tyr | Lys | Thr  | Thr | Pro | Pro | Val | Leu  | Asp | Ser | Asp |
| 1925 |     |     |     |     |     | 1930 |     |     |     |     | 1935 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Gly  | Ser | Phe | Phe | Leu | Tyr | Ser  | Lys | Leu | Thr | Val | Asp  | Lys | Ser | Arg |
| 1940 |     |     |     |     |     | 1945 |     |     |     |     | 1950 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Trp  | Gln | Gln | Gly | Asn | Val | Phe  | Ser | Cys | Ser | Val | Met  | His | Glu | Ala |
| 1955 |     |     |     |     |     | 1960 |     |     |     |     | 1965 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Leu  | His | Asn | His | Tyr | Thr | Gln  | Lys | Ser | Leu | Ser | Leu  | Ser | Pro | Gly |
| 1970 |     |     |     |     |     | 1975 |     |     |     |     | 1980 |     |     |     |

Lys

<210> 108

<211> 2134

<212> PRT

<213> Artificial Sequence

<220>

<223> FVIII 201 protein sequence

<400> 108

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe  
1 5 10 15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser  
20 25 30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Gly Ala Pro  
35 40 45

Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser Pro  
50 55 60

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser  
65 70 75 80

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
85 90 95

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro  
100 105 110

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser  
115 120 125

Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro

130

135

140

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr  
145 150 155 160

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala  
165 170 175

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
180 185 190

Ala Ser Ser Asp Ala Arg Phe Pro Pro Arg Val Pro Lys Ser Phe Pro  
195 200 205

Phe Asn Thr Ser Val Val Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr  
210 215 220

Asp His Leu Phe Asn Ile Ala Lys Pro Arg Pro Pro Trp Met Gly Leu  
225 230 235 240

Leu Gly Pro Thr Ile Gln Ala Glu Val Tyr Asp Thr Val Val Ile Thr  
245 250 255

Leu Lys Asn Met Ala Ser His Pro Val Ser Leu His Ala Val Gly Val  
260 265 270

Ser Tyr Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser  
275 280 285

Gln Arg Glu Lys Glu Asp Asp Lys Val Phe Pro Gly Gly Ser His Thr



290

295

300

Tyr Val Trp Gln Val Leu Lys Glu Asn Gly Pro Met Ala Ser Asp Pro  
 305 310 315 320

Leu Cys Leu Thr Tyr Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp  
 325 330 335

Leu Asn Ser Gly Leu Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser  
 340 345 350

Leu Ala Lys Glu Lys Thr Gln Thr Leu His Lys Phe Ile Leu Leu Phe  
 355 360 365

Ala Val Phe Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser  
 370 375 380

Leu Met Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala Trp Pro Lys Met  
 385 390 395 400

His Thr Val Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly  
 405 410 415

Cys His Arg Lys Ser Val Tyr Trp His Val Ile Gly Met Gly Thr Thr  
 420 425 430

Pro Glu Val His Ser Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg  
 435 440 445

Asn His Arg Gln Ala Ser Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr

450

455

460

Ala Gln Thr Leu Leu Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His

465

470

475

480

Ile Ser Ser His Gln His Asp Gly Met Glu Ala Tyr Val Lys Val Asp

485

490

495

Ser Cys Pro Glu Glu Pro Gln Leu Arg Met Lys Asn Asn Glu Glu Ala

500

505

510

Glu Asp Tyr Asp Asp Asp Leu Thr Asp Ser Glu Met Asp Val Val Arg

515

520

525

Phe Asp Asp Asp Asn Ser Pro Ser Phe Ile Gln Ile Arg Ser Val Ala

530

535

540

Lys Lys His Pro Lys Thr Trp Val His Tyr Ile Ala Ala Glu Glu Glu

545

550

555

560

Asp Trp Asp Tyr Ala Pro Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr

565

570

575

Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr

580

585

590

Lys Lys Val Arg Phe Met Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg

595

600

605

Glu Ala Ile Gln His Glu Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly

610

615

620

Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg

625

630

635

640

Pro Tyr Asn Ile Tyr Pro His Gly Ile Thr Asp Val Arg Pro Leu Tyr

645

650

655

Ser Arg Arg Leu Pro Lys Gly Val Lys His Leu Lys Asp Phe Pro Ile

660

665

670

Leu Pro Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val Thr Val Glu Asp

675

680

685

Gly Pro Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser

690

695

700

Phe Val Asn Met Glu Arg Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu

705

710

715

720

Leu Ile Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly Asn Gln Ile Met

725

730

735

Ser Asp Lys Arg Asn Val Ile Leu Phe Ser Val Phe Asp Glu Asn Arg

740

745

750

Ser Trp Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala

755

760

765

Gly Val Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser Asn Ile Met His

770

775

780

Ser Ile Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser Val Cys Leu  
785 790 795 800

His Glu Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp  
805 810 815

Phe Leu Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val  
820 825 830

Tyr Glu Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe  
835 840 845

Met Ser Met Glu Asn Pro Gly Leu Trp Ile Leu Gly Cys His Asn Ser  
850 855 860

Asp Phe Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys  
865 870 875 880

Asp Lys Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser  
885 890 895

Ala Tyr Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser  
900 905 910

Gln Asn Pro Pro Val Leu Lys Arg His Gln Ala Glu Ile Thr Arg Thr  
915 920 925

Thr Leu Gln Gly Ala Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser

930

935

940

Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala  
 945 950 955 960

Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr  
 965 970 975

Ser Ser Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr  
 980 985 990

Gly Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala  
 995 1000 1005

Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly  
 1010 1015 1020

Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 1025 1030 1035

Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser  
 1040 1045 1050

Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ala  
 1055 1060 1065

Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Ala Ser Ser Ser Asp  
 1070 1075 1080

Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met Lys

1085

1090

1095

Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro  
 1100 1105 1110

Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val  
 1115 1120 1125

Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu  
 1130 1135 1140

Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val  
 1145 1150 1155

Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr  
 1160 1165 1170

Arg Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile  
 1175 1180 1185

Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln  
 1190 1195 1200

Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Glu  
 1205 1210 1215

Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys  
 1220 1225 1230

Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His His Met

1235

1240

1245

Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe  
1250 1255 1260

Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly  
1265 1270 1275

Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly  
1280 1285 1290

Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe  
1295 1300 1305

Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn  
1310 1315 1320

Cys Arg Gly Ala Pro Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
1325 1330 1335

Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr  
1340 1345 1350

Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala  
1355 1360 1365

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro  
1370 1375 1380

Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala

1385

1390

1395

Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser  
 1400 1405 1410

Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala Gly  
 1415 1420 1425

Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala Gly Ser Pro Thr  
 1430 1435 1440

Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
 1445 1450 1455

Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ala  
 1460 1465 1470

Ser Ser Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys  
 1475 1480 1485

Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr  
 1490 1495 1500

Leu Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr  
 1505 1510 1515

Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe  
 1520 1525 1530

Ser Gly His Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met



1535

1540

1545

Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met

1550

1555

1560

Leu Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys Leu Ile Gly

1565

1570

1575

Glu His Leu His Ala Gly Met Ser Thr Leu Phe Leu Val Tyr Ser

1580

1585

1590

Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile Arg

1595

1600

1605

Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro

1610

1615

1620

Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser

1625

1630

1635

Thr Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala Pro

1640

1645

1650

Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe

1655

1660

1665

Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp

1670

1675

1680

Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu

1685

1690

1695

Met Val Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His Asn  
 1700 1705 1710

Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro  
 1715 1720 1725

Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly  
 1730 1735 1740

Cys Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Glu Ser Lys  
 1745 1750 1755

Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn  
 1760 1765 1770

Met Phe Ala Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu Gln  
 1775 1780 1785

Gly Arg Ser Asn Ala Trp Arg Pro Gln Val Asn Asn Pro Lys Glu  
 1790 1795 1800

Trp Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val Thr Gly Val  
 1805 1810 1815

Thr Thr Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr Val Lys  
 1820 1825 1830

Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr Leu

1835

1840

1845

Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp  
 1850 1855 1860

Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr  
 1865 1870 1875

Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala  
 1880 1885 1890

Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr Asp  
 1895 1900 1905

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly  
 1910 1915 1920

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
 1925 1930 1935

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val  
 1940 1945 1950

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 1955 1960 1965

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr  
 1970 1975 1980

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln

1985

1990

1995

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys  
 2000 2005 2010

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly  
 2015 2020 2025

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp  
 2030 2035 2040

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly  
 2045 2050 2055

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln  
 2060 2065 2070

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
 2075 2080 2085

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg  
 2090 2095 2100

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
 2105 2110 2115

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
 2120 2125 2130

Lys

&lt;210&gt; 109

&lt;211&gt; 2128

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; FVIII 203 protein sequence

&lt;400&gt; 109

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe

1 5 10 15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser

20 25 30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg

35 40 45

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val

50 55 60

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile

65 70 75 80

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln

85 90 95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser

100 105 110

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His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
      115              120              125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
      130              135              140

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
145              150              155              160

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
      165              170              175

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
      180              185              190

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
      195              200              205

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
      210              215              220

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
225              230              235              240

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
      245              250              255

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
      260              265              270

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Trp | His | Val | Ile | Gly | Met | Gly | Thr | Thr | Pro | Glu | Val | His | Ser | Ile |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Phe | Leu | Glu | Gly | His | Thr | Phe | Leu | Val | Arg | Asn | His | Arg | Gln | Ala | Ser |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Glu | Ile | Ser | Pro | Ile | Thr | Phe | Leu | Thr | Ala | Gln | Thr | Leu | Leu | Met |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Leu | Gly | Gln | Phe | Leu | Leu | Phe | Cys | His | Ile | Ser | Ser | His | Gln | His |
|     |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     | 335 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Gly | Met | Glu | Ala | Tyr | Val | Lys | Val | Asp | Ser | Cys | Pro | Glu | Glu | Pro |
|     |     | 340 |     |     |     |     |     | 345 |     |     |     |     | 350 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gln | Leu | Arg | Met | Lys | Asn | Asn | Glu | Glu | Ala | Glu | Asp | Tyr | Asp | Asp | Asp |
|     | 355 |     |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Thr | Asp | Ser | Glu | Met | Asp | Val | Val | Arg | Phe | Asp | Asp | Asp | Asn | Ser |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Ser | Phe | Ile | Gln | Ile | Arg | Ser | Val | Ala | Lys | Lys | His | Pro | Lys | Thr |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Trp | Val | His | Tyr | Ile | Ala | Ala | Glu | Glu | Glu | Asp | Trp | Asp | Tyr | Ala | Pro |
|     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Val | Leu | Ala | Pro | Asp | Asp | Arg | Ser | Tyr | Lys | Ser | Gln | Tyr | Leu | Asn |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |

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Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
      435              440              445

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
      450              455              460

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
465              470              475              480

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
      485              490              495

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
      500              505              510

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
      515              520              525

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
      530              535              540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
545              550              555              560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
      565              570              575

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
      580              585              590

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Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu

595

600

605

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp

610

615

620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val

625

630

635

640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp

645

650

655

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe

660

665

670

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr

675

680

685

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro

690

695

700

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly

705

710

715

720

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp

725

730

735

Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys

740

745

750

```

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Gly Ala Pro Gly
      755                760                765

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala
      770                775                780

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu
      785                790                795                800

Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly
      805                810                815

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu
      820                825                830

Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser
      835                840                845

Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly
      850                855                860

Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser
      865                870                875                880

Ala Thr Pro Glu Ser Gly Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser
      885                890                895

Thr Glu Glu Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly
      900                905                910

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Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser  
 915 920 925

Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu  
 930 935 940

Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
 945 950 955 960

Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Glu Pro Ala  
 965 970 975

Thr Ser Gly Ser Glu Thr Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser  
 980 985 990

Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly  
 995 1000 1005

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Glu Pro  
 1010 1015 1020

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr  
 1025 1030 1035

Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser  
 1040 1045 1050

Ala Pro Ala Ser Ser Pro Pro Val Leu Lys Arg His Gln Ala Glu  
 1055 1060 1065

|                                                             |      |      |
|-------------------------------------------------------------|------|------|
| Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr |      |      |
| 1070                                                        | 1075 | 1080 |
|                                                             |      |      |
| Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile |      |      |
| 1085                                                        | 1090 | 1095 |
|                                                             |      |      |
| Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys |      |      |
| 1100                                                        | 1105 | 1110 |
|                                                             |      |      |
| Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr |      |      |
| 1115                                                        | 1120 | 1125 |
|                                                             |      |      |
| Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser |      |      |
| 1130                                                        | 1135 | 1140 |
|                                                             |      |      |
| Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr |      |      |
| 1145                                                        | 1150 | 1155 |
|                                                             |      |      |
| Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu |      |      |
| 1160                                                        | 1165 | 1170 |
|                                                             |      |      |
| His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp |      |      |
| 1175                                                        | 1180 | 1185 |
|                                                             |      |      |
| Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser |      |      |
| 1190                                                        | 1195 | 1200 |
|                                                             |      |      |
| Phe Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly |      |      |
| 1205                                                        | 1210 | 1215 |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ala  | Glu | Pro | Arg | Lys | Asn | Phe  | Val | Lys | Pro | Asn | Glu  | Thr | Lys | Thr |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Phe | Trp | Lys | Val | Gln | His  | His | Met | Ala | Pro | Thr  | Lys | Asp | Glu |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Asp | Cys | Lys | Ala | Trp | Ala  | Tyr | Phe | Ser | Asp | Val  | Asp | Leu | Glu |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Asp | Val | His | Ser | Gly | Leu  | Ile | Gly | Pro | Leu | Leu  | Val | Cys | His |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     | 1275 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Asn | Thr | Leu | Asn | Pro | Ala  | His | Gly | Arg | Gln | Val  | Thr | Val | Gln |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     | 1290 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Phe | Ala | Leu | Phe | Phe | Thr  | Ile | Phe | Asp | Glu | Thr  | Lys | Ser | Trp |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     | 1305 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Phe | Thr | Glu | Asn | Met | Glu  | Arg | Asn | Cys | Arg | Gly  | Ala | Pro | Thr |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     | 1320 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Glu | Ser | Ala | Thr | Pro | Glu  | Ser | Gly | Pro | Gly | Ser  | Glu | Pro | Ala |
| 1325 |     |     |     |     |     | 1330 |     |     |     |     | 1335 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Ser | Gly | Ser | Glu | Thr | Pro  | Gly | Thr | Ser | Glu | Ser  | Ala | Thr | Pro |
| 1340 |     |     |     |     |     | 1345 |     |     |     |     | 1350 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Ser | Gly | Pro | Gly | Ser | Glu  | Pro | Ala | Thr | Ser | Gly  | Ser | Glu | Thr |
| 1355 |     |     |     |     |     | 1360 |     |     |     |     | 1365 |     |     |     |

|         |         |         |         |         |         |         |     |
|---------|---------|---------|---------|---------|---------|---------|-----|
| Pro Gly | Thr Ser | Glu Ser | Ala Thr | Pro Glu | Ser Gly | Pro Gly | Thr |
| 1370    |         |         | 1375    |         |         | 1380    |     |
|         |         |         |         |         |         |         |     |
| Ser Thr | Glu Pro | Ser Glu | Gly Ser | Ala Pro | Gly Thr | Ser Glu | Ser |
| 1385    |         |         | 1390    |         |         | 1395    |     |
|         |         |         |         |         |         |         |     |
| Ala Thr | Pro Glu | Ser Gly | Pro Gly | Ser Pro | Ala Gly | Ser Pro | Thr |
| 1400    |         |         | 1405    |         |         | 1410    |     |
|         |         |         |         |         |         |         |     |
| Ser Thr | Glu Glu | Gly Ser | Pro Ala | Gly Ser | Pro Thr | Ser Thr | Glu |
| 1415    |         |         | 1420    |         |         | 1425    |     |
|         |         |         |         |         |         |         |     |
| Glu Gly | Ser Pro | Ala Gly | Ser Pro | Thr Ser | Thr Glu | Glu Gly | Thr |
| 1430    |         |         | 1435    |         |         | 1440    |     |
|         |         |         |         |         |         |         |     |
| Ser Glu | Ser Ala | Thr Pro | Glu Ser | Gly Pro | Gly Thr | Ser Thr | Glu |
| 1445    |         |         | 1450    |         |         | 1455    |     |
|         |         |         |         |         |         |         |     |
| Pro Ser | Glu Gly | Ser Ala | Pro Gly | Ala Ser | Ser Ala | Pro Cys | Asn |
| 1460    |         |         | 1465    |         |         | 1470    |     |
|         |         |         |         |         |         |         |     |
| Ile Gln | Met Glu | Asp Pro | Thr Phe | Lys Glu | Asn Tyr | Arg Phe | His |
| 1475    |         |         | 1480    |         |         | 1485    |     |
|         |         |         |         |         |         |         |     |
| Ala Ile | Asn Gly | Tyr Ile | Met Asp | Thr Leu | Pro Gly | Leu Val | Met |
| 1490    |         |         | 1495    |         |         | 1500    |     |
|         |         |         |         |         |         |         |     |
| Ala Gln | Asp Gln | Arg Ile | Arg Trp | Tyr Leu | Leu Ser | Met Gly | Ser |
| 1505    |         |         | 1510    |         |         | 1515    |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Asn  | Glu | Asn | Ile | His | Ser | Ile  | His | Phe | Ser | Gly | His  | Val | Phe | Thr |
| 1520 |     |     |     |     |     | 1525 |     |     |     |     | 1530 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Arg | Lys | Lys | Glu | Glu | Tyr  | Lys | Met | Ala | Leu | Tyr  | Asn | Leu | Tyr |
| 1535 |     |     |     |     |     | 1540 |     |     |     |     | 1545 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gly | Val | Phe | Glu | Thr | Val  | Glu | Met | Leu | Pro | Ser  | Lys | Ala | Gly |
| 1550 |     |     |     |     |     | 1555 |     |     |     |     | 1560 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Trp | Arg | Val | Glu | Cys | Leu  | Ile | Gly | Glu | His | Leu  | His | Ala | Gly |
| 1565 |     |     |     |     |     | 1570 |     |     |     |     | 1575 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Ser | Thr | Leu | Phe | Leu | Val  | Tyr | Ser | Asn | Lys | Cys  | Gln | Thr | Pro |
| 1580 |     |     |     |     |     | 1585 |     |     |     |     | 1590 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Gly | Met | Ala | Ser | Gly | His  | Ile | Arg | Asp | Phe | Gln  | Ile | Thr | Ala |
| 1595 |     |     |     |     |     | 1600 |     |     |     |     | 1605 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | Gln | Tyr | Gly | Gln | Trp  | Ala | Pro | Lys | Leu | Ala  | Arg | Leu | His |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Ser | Gly | Ser | Ile | Asn | Ala  | Trp | Ser | Thr | Lys | Glu  | Pro | Phe | Ser |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Ile | Lys | Val | Asp | Leu | Leu  | Ala | Pro | Met | Ile | Ile  | His | Gly | Ile |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Thr | Gln | Gly | Ala | Arg | Gln  | Lys | Phe | Ser | Ser | Leu  | Tyr | Ile | Ser |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|
| Gln  | Phe | Ile | Ile | Met | Tyr | Ser  | Leu | Asp | Gly | Lys | Lys | Trp  | Gln | Thr |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     |     | 1680 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Tyr  | Arg | Gly | Asn | Ser | Thr | Gly  | Thr | Leu | Met | Val | Phe | Phe  | Gly | Asn |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     |     | 1695 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Val  | Asp | Ser | Ser | Gly | Ile | Lys  | His | Asn | Ile | Phe | Asn | Pro  | Pro | Ile |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     |     | 1710 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ile  | Ala | Arg | Tyr | Ile | Arg | Leu  | His | Pro | Thr | His | Tyr | Ser  | Ile | Arg |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     |     | 1725 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ser  | Thr | Leu | Arg | Met | Glu | Leu  | Met | Gly | Cys | Asp | Leu | Asn  | Ser | Cys |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     |     | 1740 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ser  | Met | Pro | Leu | Gly | Met | Glu  | Ser | Lys | Ala | Ile | Ser | Asp  | Ala | Gln |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     |     | 1755 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ile  | Thr | Ala | Ser | Ser | Tyr | Phe  | Thr | Asn | Met | Phe | Ala | Thr  | Trp | Ser |
| 1760 |     |     |     |     |     | 1765 |     |     |     |     |     | 1770 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Pro  | Ser | Lys | Ala | Arg | Leu | His  | Leu | Gln | Gly | Arg | Ser | Asn  | Ala | Trp |
| 1775 |     |     |     |     |     | 1780 |     |     |     |     |     | 1785 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Arg  | Pro | Gln | Val | Asn | Asn | Pro  | Lys | Glu | Trp | Leu | Gln | Val  | Asp | Phe |
| 1790 |     |     |     |     |     | 1795 |     |     |     |     |     | 1800 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gln  | Lys | Thr | Met | Lys | Val | Thr  | Gly | Val | Thr | Thr | Gln | Gly  | Val | Lys |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     |     | 1815 |     |     |



|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|
| Ser  | Leu | Leu | Thr | Ser | Met | Tyr  | Val | Lys | Glu | Phe | Leu | Ile  | Ser | Ser |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     |     | 1830 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ser  | Gln | Asp | Gly | His | Gln | Trp  | Thr | Leu | Phe | Phe | Gln | Asn  | Gly | Lys |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     |     | 1845 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Val  | Lys | Val | Phe | Gln | Gly | Asn  | Gln | Asp | Ser | Phe | Thr | Pro  | Val | Val |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     |     | 1860 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Asn  | Ser | Leu | Asp | Pro | Pro | Leu  | Leu | Thr | Arg | Tyr | Leu | Arg  | Ile | His |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     |     | 1875 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Pro  | Gln | Ser | Trp | Val | His | Gln  | Ile | Ala | Leu | Arg | Met | Glu  | Val | Leu |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     |     | 1890 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gly  | Cys | Glu | Ala | Gln | Asp | Leu  | Tyr | Asp | Lys | Thr | His | Thr  | Cys | Pro |
| 1895 |     |     |     |     |     | 1900 |     |     |     |     |     | 1905 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Pro  | Cys | Pro | Ala | Pro | Glu | Leu  | Leu | Gly | Gly | Pro | Ser | Val  | Phe | Leu |
| 1910 |     |     |     |     |     | 1915 |     |     |     |     |     | 1920 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Phe  | Pro | Pro | Lys | Pro | Lys | Asp  | Thr | Leu | Met | Ile | Ser | Arg  | Thr | Pro |
| 1925 |     |     |     |     |     | 1930 |     |     |     |     |     | 1935 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Glu  | Val | Thr | Cys | Val | Val | Val  | Asp | Val | Ser | His | Glu | Asp  | Pro | Glu |
| 1940 |     |     |     |     |     | 1945 |     |     |     |     |     | 1950 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Val  | Lys | Phe | Asn | Trp | Tyr | Val  | Asp | Gly | Val | Glu | Val | His  | Asn | Ala |
| 1955 |     |     |     |     |     | 1960 |     |     |     |     |     | 1965 |     |     |

|         |                     |                     |             |
|---------|---------------------|---------------------|-------------|
| Lys Thr | Lys Pro Arg Glu Glu | Gln Tyr Asn Ser Thr | Tyr Arg Val |
| 1970    | 1975                | 1980                |             |
|         |                     |                     |             |
| Val Ser | Val Leu Thr Val Leu | His Gln Asp Trp Leu | Asn Gly Lys |
| 1985    | 1990                | 1995                |             |
|         |                     |                     |             |
| Glu Tyr | Lys Cys Lys Val Ser | Asn Lys Ala Leu Pro | Ala Pro Ile |
| 2000    | 2005                | 2010                |             |
|         |                     |                     |             |
| Glu Lys | Thr Ile Ser Lys Ala | Lys Gly Gln Pro Arg | Glu Pro Gln |
| 2015    | 2020                | 2025                |             |
|         |                     |                     |             |
| Val Tyr | Thr Leu Pro Pro Ser | Arg Asp Glu Leu Thr | Lys Asn Gln |
| 2030    | 2035                | 2040                |             |
|         |                     |                     |             |
| Val Ser | Leu Thr Cys Leu Val | Lys Gly Phe Tyr Pro | Ser Asp Ile |
| 2045    | 2050                | 2055                |             |
|         |                     |                     |             |
| Ala Val | Glu Trp Glu Ser Asn | Gly Gln Pro Glu Asn | Asn Tyr Lys |
| 2060    | 2065                | 2070                |             |
|         |                     |                     |             |
| Thr Thr | Pro Pro Val Leu Asp | Ser Asp Gly Ser Phe | Phe Leu Tyr |
| 2075    | 2080                | 2085                |             |
|         |                     |                     |             |
| Ser Lys | Leu Thr Val Asp Lys | Ser Arg Trp Gln Gln | Gly Asn Val |
| 2090    | 2095                | 2100                |             |
|         |                     |                     |             |
| Phe Ser | Cys Ser Val Met His | Glu Ala Leu His Asn | His Tyr Thr |
| 2105    | 2110                | 2115                |             |

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

2120

2125

<210> 110

<211> 2128

<212> PRT

<213> Artificial Sequence

<220>

<223> FVIII 204 protein sequence

<400> 110

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe

1

5

10

15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser

20

25

30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg

35

40

45

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val

50

55

60

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile

65

70

75

80

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln

85

90

95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser

100

105

110

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser  
115 120 125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp  
130 135 140

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu  
145 150 155 160

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser  
165 170 175

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile  
180 185 190

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr  
195 200 205

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly  
210 215 220

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp  
225 230 235 240

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr  
245 250 255

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val

260

265

270

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile  
275 280 285

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser  
290 295 300

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met  
305 310 315 320

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His  
325 330 335

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro  
340 345 350

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp  
355 360 365

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser  
370 375 380

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr  
385 390 395 400

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro  
405 410 415

Leu Val Leu Ala Pro Asp Gly Ala Pro Thr Ser Thr Glu Pro Ser Glu

420

425

430

Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu

435

440

445

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr

450

455

460

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro

465

470

475

480

Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro

485

490

495

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro

500

505

510

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Thr Glu Pro Ser Glu

515

520

525

Gly Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro

530

535

540

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Glu

545

550

555

560

Ser Ala Thr Pro Glu Ser Gly Pro Gly Ala Ser Ser Asp Arg Ser Tyr

565

570

575

Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr

580

585

590

Lys Lys Val Arg Phe Met Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg  
595 600 605

Glu Ala Ile Gln His Glu Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly  
610 615 620

Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg  
625 630 635 640

Pro Tyr Asn Ile Tyr Pro His Gly Ile Thr Asp Val Arg Pro Leu Tyr  
645 650 655

Ser Arg Arg Leu Pro Lys Gly Val Lys His Leu Lys Asp Phe Pro Ile  
660 665 670

Leu Pro Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val Thr Val Glu Asp  
675 680 685

Gly Pro Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser  
690 695 700

Phe Val Asn Met Glu Arg Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu  
705 710 715 720

Leu Ile Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly Asn Gln Ile Met  
725 730 735

Ser Asp Lys Arg Asn Val Ile Leu Phe Ser Val Phe Asp Glu Asn Arg

740

745

750

Ser Trp Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala  
755 760 765

Gly Val Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser Asn Ile Met His  
770 775 780

Ser Ile Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser Val Cys Leu  
785 790 795 800

His Glu Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp  
805 810 815

Phe Leu Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val  
820 825 830

Tyr Glu Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe  
835 840 845

Met Ser Met Glu Asn Pro Gly Leu Trp Ile Leu Gly Cys His Asn Ser  
850 855 860

Asp Phe Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys  
865 870 875 880

Asp Lys Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser  
885 890 895

Ala Tyr Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser



900

905

910

Gln Asn Gly Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
915 920 925

Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser  
930 935 940

Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser  
945 950 955 960

Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
965 970 975

Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro  
980 985 990

Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr  
995 1000 1005

Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu  
1010 1015 1020

Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
1025 1030 1035

Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala  
1040 1045 1050

Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser

1055

1060

1065

Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser  
 1070 1075 1080

Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
 1085 1090 1095

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro  
 1100 1105 1110

Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Glu Pro Ala Thr Ser  
 1115 1120 1125

Gly Ser Glu Thr Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr  
 1130 1135 1140

Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly  
 1145 1150 1155

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Glu Pro  
 1160 1165 1170

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr  
 1175 1180 1185

Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser  
 1190 1195 1200

Ala Pro Ala Ser Ser Pro Pro Val Leu Lys Arg His Gln Ala Glu

1205

1210

1215

Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr  
 1220 1225 1230

Asp Asp Thr Ile Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile  
 1235 1240 1245

Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys  
 1250 1255 1260

Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr  
 1265 1270 1275

Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser  
 1280 1285 1290

Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr  
 1295 1300 1305

Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu  
 1310 1315 1320

His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp  
 1325 1330 1335

Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser  
 1340 1345 1350

Phe Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly

1355

1360

1365

Ala Glu Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr  
 1370 1375 1380

Tyr Phe Trp Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu  
 1385 1390 1395

Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu  
 1400 1405 1410

Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His  
 1415 1420 1425

Thr Asn Thr Leu Asn Pro Ala His Gly Arg Gln Val Thr Val Gln  
 1430 1435 1440

Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp  
 1445 1450 1455

Tyr Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn  
 1460 1465 1470

Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe His  
 1475 1480 1485

Ala Ile Asn Gly Tyr Ile Met Asp Thr Leu Pro Gly Leu Val Met  
 1490 1495 1500

Ala Gln Asp Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser

1505

1510

1515

Asn Glu Asn Ile His Ser Ile His Phe Ser Gly His Val Phe Thr

1520

1525

1530

Val Arg Lys Lys Glu Glu Tyr Lys Met Ala Leu Tyr Asn Leu Tyr

1535

1540

1545

Pro Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser Lys Ala Gly

1550

1555

1560

Ile Trp Arg Val Glu Cys Leu Ile Gly Glu His Leu His Ala Gly

1565

1570

1575

Met Ser Thr Leu Phe Leu Val Tyr Ser Asn Lys Cys Gln Thr Pro

1580

1585

1590

Leu Gly Met Ala Ser Gly His Ile Arg Asp Phe Gln Ile Thr Ala

1595

1600

1605

Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His

1610

1615

1620

Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser

1625

1630

1635

Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile

1640

1645

1650

Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser

1655

1660

1665

Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr  
 1670 1675 1680

Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn  
 1685 1690 1695

Val Asp Ser Ser Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile  
 1700 1705 1710

Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg  
 1715 1720 1725

Ser Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys  
 1730 1735 1740

Ser Met Pro Leu Gly Met Glu Ser Lys Ala Ile Ser Asp Ala Gln  
 1745 1750 1755

Ile Thr Ala Ser Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser  
 1760 1765 1770

Pro Ser Lys Ala Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp  
 1775 1780 1785

Arg Pro Gln Val Asn Asn Pro Lys Glu Trp Leu Gln Val Asp Phe  
 1790 1795 1800

Gln Lys Thr Met Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys

1805

1810

1815

Ser Leu Leu Thr Ser Met Tyr Val Lys Glu Phe Leu Ile Ser Ser

1820

1825

1830

Ser Gln Asp Gly His Gln Trp Thr Leu Phe Phe Gln Asn Gly Lys

1835

1840

1845

Val Lys Val Phe Gln Gly Asn Gln Asp Ser Phe Thr Pro Val Val

1850

1855

1860

Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His

1865

1870

1875

Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg Met Glu Val Leu

1880

1885

1890

Gly Cys Glu Ala Gln Asp Leu Tyr Asp Lys Thr His Thr Cys Pro

1895

1900

1905

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu

1910

1915

1920

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro

1925

1930

1935

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu

1940

1945

1950

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala

1955

1960

1965

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val  
 1970 1975 1980

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 1985 1990 1995

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
 2000 2005 2010

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln  
 2015 2020 2025

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln  
 2030 2035 2040

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 2045 2050 2055

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys  
 2060 2065 2070

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
 2075 2080 2085

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val  
 2090 2095 2100

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr



2105

2110

2115

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

2120

2125

&lt;210&gt; 111

&lt;211&gt; 2128

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; FVIII 205 protein sequence

&lt;400&gt; 111

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe

1

5

10

15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser

20

25

30

Trp Asp Tyr Met Gln Gly Ala Pro Thr Ser Glu Ser Ala Thr Pro Glu

35

40

45

Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly

50

55

60

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala

65

70

75

80

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu

85

90

95

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Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly
      100              105              110

Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser
      115              120              125

Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser
      130              135              140

Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly
145              150              155              160

Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala Gly
      165              170              175

Ser Pro Thr Ser Thr Glu Glu Gly Ala Ser Ser Ser Asp Leu Gly Glu
      180              185              190

Leu Pro Val Asp Ala Arg Phe Pro Pro Arg Val Pro Lys Ser Phe Pro
      195              200              205

Phe Asn Thr Ser Val Val Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr
      210              215              220

Asp His Leu Phe Asn Ile Ala Lys Pro Arg Pro Pro Trp Met Gly Leu
225              230              235              240

Leu Gly Pro Thr Ile Gln Ala Glu Val Tyr Asp Thr Val Val Ile Thr
      245              250              255

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Leu Lys Asn Met Ala Ser His Pro Val Ser Leu His Ala Val Gly Val
      260              265              270

Ser Tyr Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser
      275              280              285

Gln Arg Glu Lys Glu Asp Asp Lys Val Phe Pro Gly Gly Ser His Thr
      290              295              300

Tyr Val Trp Gln Val Leu Lys Glu Asn Gly Pro Met Ala Ser Asp Pro
305              310              315              320

Leu Cys Leu Thr Tyr Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp
      325              330              335

Leu Asn Ser Gly Leu Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser
      340              345              350

Leu Ala Lys Glu Lys Thr Gln Thr Leu His Lys Phe Ile Leu Leu Phe
      355              360              365

Ala Val Phe Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser
      370              375              380

Leu Met Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala Trp Pro Lys Met
385              390              395              400

His Thr Val Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly
      405              410              415

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | His | Arg | Lys | Ser | Val | Tyr | Trp | His | Val | Ile | Gly | Met | Gly | Thr | Thr |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Glu | Val | His | Ser | Ile | Phe | Leu | Glu | Gly | His | Thr | Phe | Leu | Val | Arg |
|     |     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asn | His | Arg | Gln | Ala | Ser | Leu | Glu | Ile | Ser | Pro | Ile | Thr | Phe | Leu | Thr |
|     |     |     |     |     |     |     |     | 450 |     |     |     |     | 455 |     | 460 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Gln | Thr | Leu | Leu | Met | Asp | Leu | Gly | Gln | Phe | Leu | Leu | Phe | Cys | His |
| 465 |     |     |     |     |     |     | 470 |     |     |     | 475 |     |     |     | 480 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ile | Ser | Ser | His | Gln | His | Asp | Gly | Met | Glu | Ala | Tyr | Val | Lys | Val | Asp |
|     |     |     |     |     |     |     | 485 |     |     |     | 490 |     |     | 495 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Cys | Pro | Glu | Glu | Pro | Gln | Leu | Arg | Met | Lys | Asn | Asn | Glu | Glu | Ala |
|     |     |     |     |     |     |     | 500 |     |     |     | 505 |     |     | 510 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Asp | Tyr | Asp | Asp | Asp | Leu | Thr | Asp | Ser | Glu | Met | Asp | Val | Val | Arg |
|     |     |     |     |     |     |     | 515 |     |     |     | 520 |     | 525 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Phe | Asp | Asp | Asp | Asn | Ser | Pro | Ser | Phe | Ile | Gln | Ile | Arg | Ser | Val | Ala |
|     |     |     |     |     |     |     | 530 |     |     |     | 535 |     | 540 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Lys | Lys | His | Pro | Lys | Thr | Trp | Val | His | Tyr | Ile | Ala | Ala | Glu | Glu | Glu |
| 545 |     |     |     |     |     |     | 550 |     |     |     | 555 |     |     | 560 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Trp | Asp | Tyr | Ala | Pro | Leu | Val | Leu | Ala | Pro | Asp | Asp | Arg | Ser | Tyr |
|     |     |     |     |     |     |     | 565 |     |     |     | 570 |     |     | 575 |     |

Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr

580

585

590

Lys Lys Val Arg Phe Met Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg

595

600

605

Glu Ala Ile Gln His Glu Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly

610

615

620

Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg

625

630

635

640

Pro Tyr Asn Ile Tyr Pro His Gly Ile Thr Asp Val Arg Pro Leu Tyr

645

650

655

Ser Arg Arg Leu Pro Lys Gly Val Lys His Leu Lys Asp Phe Pro Ile

660

665

670

Leu Pro Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val Thr Val Glu Asp

675

680

685

Gly Pro Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser

690

695

700

Phe Val Asn Met Glu Arg Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu

705

710

715

720

Leu Ile Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly Asn Gln Ile Met

725

730

735

Ser Asp Lys Arg Asn Val Ile Leu Phe Ser Val Phe Asp Glu Asn Arg

740

745

750

Ser Trp Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala

755

760

765

Gly Val Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser Asn Ile Met His

770

775

780

Ser Ile Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser Val Cys Leu

785

790

795

800

His Glu Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp

805

810

815

Phe Leu Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val

820

825

830

Tyr Glu Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe

835

840

845

Met Ser Met Glu Asn Pro Gly Leu Trp Ile Leu Gly Cys His Asn Ser

850

855

860

Asp Phe Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys

865

870

875

880

Asp Lys Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser

885

890

895

Ala Tyr Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser  
 900 905 910

Gln Asn Gly Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
 915 920 925

Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser  
 930 935 940

Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser  
 945 950 955 960

Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
 965 970 975

Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro  
 980 985 990

Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr  
 995 1000 1005

Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu  
 1010 1015 1020

Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
 1025 1030 1035

Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala  
 1040 1045 1050

|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|
| Gly  | Ser | Pro | Thr | Ser | Thr | Glu  | Glu | Gly | Thr | Ser | Thr | Glu  | Pro | Ser |
| 1055 |     |     |     |     |     | 1060 |     |     |     |     |     | 1065 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Glu  | Gly | Ser | Ala | Pro | Gly | Thr  | Ser | Glu | Ser | Ala | Thr | Pro  | Glu | Ser |
| 1070 |     |     |     |     |     | 1075 |     |     |     |     |     | 1080 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gly  | Pro | Gly | Thr | Ser | Glu | Ser  | Ala | Thr | Pro | Glu | Ser | Gly  | Pro | Gly |
| 1085 |     |     |     |     |     | 1090 |     |     |     |     |     | 1095 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Thr  | Ser | Glu | Ser | Ala | Thr | Pro  | Glu | Ser | Gly | Pro | Gly | Ser  | Glu | Pro |
| 1100 |     |     |     |     |     | 1105 |     |     |     |     |     | 1110 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ala  | Thr | Ser | Gly | Ser | Glu | Thr  | Pro | Gly | Ser | Glu | Pro | Ala  | Thr | Ser |
| 1115 |     |     |     |     |     | 1120 |     |     |     |     |     | 1125 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gly  | Ser | Glu | Thr | Pro | Gly | Ser  | Pro | Ala | Gly | Ser | Pro | Thr  | Ser | Thr |
| 1130 |     |     |     |     |     | 1135 |     |     |     |     |     | 1140 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Glu  | Glu | Gly | Thr | Ser | Thr | Glu  | Pro | Ser | Glu | Gly | Ser | Ala  | Pro | Gly |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     |     | 1155 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Thr  | Ser | Thr | Glu | Pro | Ser | Glu  | Gly | Ser | Ala | Pro | Gly | Ser  | Glu | Pro |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     |     | 1170 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ala  | Thr | Ser | Gly | Ser | Glu | Thr  | Pro | Gly | Thr | Ser | Glu | Ser  | Ala | Thr |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     |     | 1185 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Pro  | Glu | Ser | Gly | Pro | Gly | Thr  | Ser | Thr | Glu | Pro | Ser | Glu  | Gly | Ser |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     |     | 1200 |     |     |



|         |                     |                     |             |
|---------|---------------------|---------------------|-------------|
| Ala Pro | Ala Ser Ser Pro Pro | Val Leu Lys Arg His | Gln Ala Glu |
| 1205    | 1210                | 1215                |             |
|         |                     |                     |             |
| Ile Thr | Arg Thr Thr Leu Gln | Ser Asp Gln Glu Glu | Ile Asp Tyr |
| 1220    | 1225                | 1230                |             |
|         |                     |                     |             |
| Asp Asp | Thr Ile Ser Val Glu | Met Lys Lys Glu Asp | Phe Asp Ile |
| 1235    | 1240                | 1245                |             |
|         |                     |                     |             |
| Tyr Asp | Glu Asp Glu Asn Gln | Ser Pro Arg Ser Phe | Gln Lys Lys |
| 1250    | 1255                | 1260                |             |
|         |                     |                     |             |
| Thr Arg | His Tyr Phe Ile Ala | Ala Val Glu Arg Leu | Trp Asp Tyr |
| 1265    | 1270                | 1275                |             |
|         |                     |                     |             |
| Gly Met | Ser Ser Ser Pro His | Val Leu Arg Asn Arg | Ala Gln Ser |
| 1280    | 1285                | 1290                |             |
|         |                     |                     |             |
| Gly Ser | Val Pro Gln Phe Lys | Lys Val Val Phe Gln | Glu Phe Thr |
| 1295    | 1300                | 1305                |             |
|         |                     |                     |             |
| Asp Gly | Ser Phe Thr Gln Pro | Leu Tyr Arg Gly Glu | Leu Asn Glu |
| 1310    | 1315                | 1320                |             |
|         |                     |                     |             |
| His Leu | Gly Leu Leu Gly Pro | Tyr Ile Arg Ala Glu | Val Glu Asp |
| 1325    | 1330                | 1335                |             |
|         |                     |                     |             |
| Asn Ile | Met Val Thr Phe Arg | Asn Gln Ala Ser Arg | Pro Tyr Ser |
| 1340    | 1345                | 1350                |             |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Phe  | Tyr | Ser | Ser | Leu | Ile | Ser  | Tyr | Glu | Glu | Asp | Gln  | Arg | Gln | Gly |
| 1355 |     |     |     |     |     | 1360 |     |     |     |     | 1365 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Glu | Pro | Arg | Lys | Asn | Phe  | Val | Lys | Pro | Asn | Glu  | Thr | Lys | Thr |
| 1370 |     |     |     |     |     | 1375 |     |     |     |     | 1380 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Phe | Trp | Lys | Val | Gln | His  | His | Met | Ala | Pro | Thr  | Lys | Asp | Glu |
| 1385 |     |     |     |     |     | 1390 |     |     |     |     | 1395 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Asp | Cys | Lys | Ala | Trp | Ala  | Tyr | Phe | Ser | Asp | Val  | Asp | Leu | Glu |
| 1400 |     |     |     |     |     | 1405 |     |     |     |     | 1410 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Asp | Val | His | Ser | Gly | Leu  | Ile | Gly | Pro | Leu | Leu  | Val | Cys | His |
| 1415 |     |     |     |     |     | 1420 |     |     |     |     | 1425 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Asn | Thr | Leu | Asn | Pro | Ala  | His | Gly | Arg | Gln | Val  | Thr | Val | Gln |
| 1430 |     |     |     |     |     | 1435 |     |     |     |     | 1440 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Phe | Ala | Leu | Phe | Phe | Thr  | Ile | Phe | Asp | Glu | Thr  | Lys | Ser | Trp |
| 1445 |     |     |     |     |     | 1450 |     |     |     |     | 1455 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Phe | Thr | Glu | Asn | Met | Glu  | Arg | Asn | Cys | Arg | Ala  | Pro | Cys | Asn |
| 1460 |     |     |     |     |     | 1465 |     |     |     |     | 1470 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Gln | Met | Glu | Asp | Pro | Thr  | Phe | Lys | Glu | Asn | Tyr  | Arg | Phe | His |
| 1475 |     |     |     |     |     | 1480 |     |     |     |     | 1485 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Ile | Asn | Gly | Tyr | Ile | Met  | Asp | Thr | Leu | Pro | Gly  | Leu | Val | Met |
| 1490 |     |     |     |     |     | 1495 |     |     |     |     | 1500 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ala  | Gln | Asp | Gln | Arg | Ile | Arg  | Trp | Tyr | Leu | Leu | Ser  | Met | Gly | Ser |
| 1505 |     |     |     |     |     | 1510 |     |     |     |     | 1515 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Glu | Asn | Ile | His | Ser | Ile  | His | Phe | Ser | Gly | His  | Val | Phe | Thr |
| 1520 |     |     |     |     |     | 1525 |     |     |     |     | 1530 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Arg | Lys | Lys | Glu | Glu | Tyr  | Lys | Met | Ala | Leu | Tyr  | Asn | Leu | Tyr |
| 1535 |     |     |     |     |     | 1540 |     |     |     |     | 1545 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gly | Val | Phe | Glu | Thr | Val  | Glu | Met | Leu | Pro | Ser  | Lys | Ala | Gly |
| 1550 |     |     |     |     |     | 1555 |     |     |     |     | 1560 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Trp | Arg | Val | Glu | Cys | Leu  | Ile | Gly | Glu | His | Leu  | His | Ala | Gly |
| 1565 |     |     |     |     |     | 1570 |     |     |     |     | 1575 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Ser | Thr | Leu | Phe | Leu | Val  | Tyr | Ser | Asn | Lys | Cys  | Gln | Thr | Pro |
| 1580 |     |     |     |     |     | 1585 |     |     |     |     | 1590 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Gly | Met | Ala | Ser | Gly | His  | Ile | Arg | Asp | Phe | Gln  | Ile | Thr | Ala |
| 1595 |     |     |     |     |     | 1600 |     |     |     |     | 1605 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | Gln | Tyr | Gly | Gln | Trp  | Ala | Pro | Lys | Leu | Ala  | Arg | Leu | His |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Ser | Gly | Ser | Ile | Asn | Ala  | Trp | Ser | Thr | Lys | Glu  | Pro | Phe | Ser |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Ile | Lys | Val | Asp | Leu | Leu  | Ala | Pro | Met | Ile | Ile  | His | Gly | Ile |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |



|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Gln  | Lys | Thr | Met | Lys | Val | Thr  | Gly | Val | Thr | Thr | Gln  | Gly | Val | Lys |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     | 1815 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Leu | Leu | Thr | Ser | Met | Tyr  | Val | Lys | Glu | Phe | Leu  | Ile | Ser | Ser |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     | 1830 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gln | Asp | Gly | His | Gln | Trp  | Thr | Leu | Phe | Phe | Gln  | Asn | Gly | Lys |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     | 1845 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Lys | Val | Phe | Gln | Gly | Asn  | Gln | Asp | Ser | Phe | Thr  | Pro | Val | Val |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     | 1860 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Ser | Leu | Asp | Pro | Pro | Leu  | Leu | Thr | Arg | Tyr | Leu  | Arg | Ile | His |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     | 1875 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gln | Ser | Trp | Val | His | Gln  | Ile | Ala | Leu | Arg | Met  | Glu | Val | Leu |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     | 1890 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Cys | Glu | Ala | Gln | Asp | Leu  | Tyr | Asp | Lys | Thr | His  | Thr | Cys | Pro |
| 1895 |     |     |     |     |     | 1900 |     |     |     |     | 1905 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Cys | Pro | Ala | Pro | Glu | Leu  | Leu | Gly | Gly | Pro | Ser  | Val | Phe | Leu |
| 1910 |     |     |     |     |     | 1915 |     |     |     |     | 1920 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Pro | Pro | Lys | Pro | Lys | Asp  | Thr | Leu | Met | Ile | Ser  | Arg | Thr | Pro |
| 1925 |     |     |     |     |     | 1930 |     |     |     |     | 1935 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Val | Thr | Cys | Val | Val | Val  | Asp | Val | Ser | His | Glu  | Asp | Pro | Glu |
| 1940 |     |     |     |     |     | 1945 |     |     |     |     | 1950 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|
| Val  | Lys | Phe | Asn | Trp | Tyr | Val  | Asp | Gly | Val | Glu | Val | His  | Asn | Ala |
| 1955 |     |     |     |     |     | 1960 |     |     |     |     |     | 1965 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Lys  | Thr | Lys | Pro | Arg | Glu | Glu  | Gln | Tyr | Asn | Ser | Thr | Tyr  | Arg | Val |
| 1970 |     |     |     |     |     | 1975 |     |     |     |     |     | 1980 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Val  | Ser | Val | Leu | Thr | Val | Leu  | His | Gln | Asp | Trp | Leu | Asn  | Gly | Lys |
| 1985 |     |     |     |     |     | 1990 |     |     |     |     |     | 1995 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Glu  | Tyr | Lys | Cys | Lys | Val | Ser  | Asn | Lys | Ala | Leu | Pro | Ala  | Pro | Ile |
| 2000 |     |     |     |     |     | 2005 |     |     |     |     |     | 2010 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Glu  | Lys | Thr | Ile | Ser | Lys | Ala  | Lys | Gly | Gln | Pro | Arg | Glu  | Pro | Gln |
| 2015 |     |     |     |     |     | 2020 |     |     |     |     |     | 2025 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Val  | Tyr | Thr | Leu | Pro | Pro | Ser  | Arg | Asp | Glu | Leu | Thr | Lys  | Asn | Gln |
| 2030 |     |     |     |     |     | 2035 |     |     |     |     |     | 2040 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Val  | Ser | Leu | Thr | Cys | Leu | Val  | Lys | Gly | Phe | Tyr | Pro | Ser  | Asp | Ile |
| 2045 |     |     |     |     |     | 2050 |     |     |     |     |     | 2055 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ala  | Val | Glu | Trp | Glu | Ser | Asn  | Gly | Gln | Pro | Glu | Asn | Asn  | Tyr | Lys |
| 2060 |     |     |     |     |     | 2065 |     |     |     |     |     | 2070 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Thr  | Thr | Pro | Pro | Val | Leu | Asp  | Ser | Asp | Gly | Ser | Phe | Phe  | Leu | Tyr |
| 2075 |     |     |     |     |     | 2080 |     |     |     |     |     | 2085 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ser  | Lys | Leu | Thr | Val | Asp | Lys  | Ser | Arg | Trp | Gln | Gln | Gly  | Asn | Val |
| 2090 |     |     |     |     |     | 2095 |     |     |     |     |     | 2100 |     |     |

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr  
 2105 2110 2115

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
 2120 2125

<210> 112

<211> 2020

<212> PRT

<213> Artificial Sequence

<220>

<223> pSYN FVIII 266 protein sequence

<400> 112

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe  
 1 5 10 15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser  
 20 25 30

Trp Asp Tyr Met Gln Gly Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr  
 35 40 45

Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
 50 55 60

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Ala Ser Ser Ser  
 65 70 75 80

Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro Arg Val Pro

85

90

95

Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys Thr Leu Phe  
 100 105 110

Val Glu Phe Thr Asp His Leu Phe Asn Ile Ala Lys Pro Arg Pro Pro  
 115 120 125

Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val Tyr Asp Thr  
 130 135 140

Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val Ser Leu His  
 145 150 155 160

Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp  
 165 170 175

Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val Phe Pro Gly  
 180 185 190

Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn Gly Pro Met  
 195 200 205

Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser His Val Asp  
 210 215 220

Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu Leu Val Cys  
 225 230 235 240

Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu His Lys Phe



245

250

255

Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp His Ser Glu  
260 265 270

Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala  
275 280 285

Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg Ser Leu Pro  
290 295 300

Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His Val Ile Gly  
305 310 315 320

Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu Gly His Thr  
325 330 335

Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile Ser Pro Ile  
340 345 350

Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly Gln Phe Leu  
355 360 365

Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met Glu Ala Tyr  
370 375 380

Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg Met Lys Asn  
385 390 395 400

Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp Ser Glu Met

405

410

415

Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe Ile Gln Ile  
420 425 430

Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His Tyr Ile Ala  
435 440 445

Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu Ala Pro Asp  
450 455 460

Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg Ile  
465 470 475 480

Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr Asp Glu Thr  
485 490 495

Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile Leu Gly Pro  
500 505 510

Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn  
515 520 525

Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile Thr Asp Val  
530 535 540

Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys His Leu Lys  
545 550 555 560

Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val

565

570

575

Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg  
580 585 590

Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala Ser Gly Leu  
595 600 605

Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly  
610 615 620

Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe Ser Val Phe  
625 630 635 640

Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu  
645 650 655

Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser  
660 665 670

Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu  
675 680 685

Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly  
690 695 700

Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys  
705 710 715 720

His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly

725

730

735

Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp Ile Leu Gly  
740 745 750

Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala Leu Leu Lys  
755 760 765

Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr  
770 775 780

Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro  
785 790 795 800

Arg Ser Phe Ser Gln Asn Gly Ala Pro Gly Thr Ser Glu Ser Ala Thr  
805 810 815

Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr  
820 825 830

Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu  
835 840 845

Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr  
850 855 860

Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala  
865 870 875 880

Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser

885

890

895

Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser  
900 905 910

Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
915 920 925

Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro  
930 935 940

Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser  
945 950 955 960

Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
965 970 975

Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser  
980 985 990

Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser  
995 1000 1005

Gly Ser Glu Thr Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu  
1010 1015 1020

Thr Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly  
1025 1030 1035

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr

1040

1045

1050

Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Glu Pro Ala Thr Ser  
 1055 1060 1065

Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser  
 1070 1075 1080

Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Ala  
 1085 1090 1095

Ser Ser Pro Pro Val Leu Lys Arg His Gln Ala Glu Ile Thr Arg  
 1100 1105 1110

Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr  
 1115 1120 1125

Ile Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu  
 1130 1135 1140

Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His  
 1145 1150 1155

Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser  
 1160 1165 1170

Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly Ser Val  
 1175 1180 1185

Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser

1190

1195

1200

Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly  
 1205 1210 1215

Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met  
 1220 1225 1230

Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser  
 1235 1240 1245

Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro  
 1250 1255 1260

Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp  
 1265 1270 1275

Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys  
 1280 1285 1290

Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val  
 1295 1300 1305

His Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr  
 1310 1315 1320

Leu Asn Pro Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala  
 1325 1330 1335

Leu Phe Phe Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr

1340

1345

1350

Glu Asn Met Glu Arg Asn Cys Arg Ala Pro Cys Asn Ile Gln Met

1355

1360

1365

Glu Asp Pro Thr Phe Lys Glu Asn Tyr Arg Phe His Ala Ile Asn

1370

1375

1380

Gly Tyr Ile Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asp

1385

1390

1395

Gln Arg Ile Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn

1400

1405

1410

Ile His Ser Ile His Phe Ser Gly His Val Phe Thr Val Arg Lys

1415

1420

1425

Lys Glu Glu Tyr Lys Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val

1430

1435

1440

Phe Glu Thr Val Glu Met Leu Pro Ser Lys Ala Gly Ile Trp Arg

1445

1450

1455

Val Glu Cys Leu Ile Gly Glu His Leu His Ala Gly Met Ser Thr

1460

1465

1470

Leu Phe Leu Val Tyr Ser Asn Lys Cys Gln Thr Pro Leu Gly Met

1475

1480

1485

Ala Ser Gly His Ile Arg Asp Phe Gln Ile Thr Ala Ser Gly Gln



1490

1495

1500

Tyr Gly Gln Trp Ala Pro Lys Leu Ala Arg Leu His Tyr Ser Gly  
 1505 1510 1515

Ser Ile Asn Ala Trp Ser Thr Lys Glu Pro Phe Ser Trp Ile Lys  
 1520 1525 1530

Val Asp Leu Leu Ala Pro Met Ile Ile His Gly Ile Lys Thr Gln  
 1535 1540 1545

Gly Ala Arg Gln Lys Phe Ser Ser Leu Tyr Ile Ser Gln Phe Ile  
 1550 1555 1560

Ile Met Tyr Ser Leu Asp Gly Lys Lys Trp Gln Thr Tyr Arg Gly  
 1565 1570 1575

Asn Ser Thr Gly Thr Leu Met Val Phe Phe Gly Asn Val Asp Ser  
 1580 1585 1590

Ser Gly Ile Lys His Asn Ile Phe Asn Pro Pro Ile Ile Ala Arg  
 1595 1600 1605

Tyr Ile Arg Leu His Pro Thr His Tyr Ser Ile Arg Ser Thr Leu  
 1610 1615 1620

Arg Met Glu Leu Met Gly Cys Asp Leu Asn Ser Cys Ser Met Pro  
 1625 1630 1635

Leu Gly Met Glu Ser Lys Ala Ile Ser Asp Ala Gln Ile Thr Ala

1640

1645

1650

Ser Ser Tyr Phe Thr Asn Met Phe Ala Thr Trp Ser Pro Ser Lys  
 1655 1660 1665

Ala Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln  
 1670 1675 1680

Val Asn Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln Lys Thr  
 1685 1690 1695

Met Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu  
 1700 1705 1710

Thr Ser Met Tyr Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp  
 1715 1720 1725

Gly His Gln Trp Thr Leu Phe Phe Gln Asn Gly Lys Val Lys Val  
 1730 1735 1740

Phe Gln Gly Asn Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu  
 1745 1750 1755

Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser  
 1760 1765 1770

Trp Val His Gln Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu  
 1775 1780 1785

Ala Gln Asp Leu Tyr Asp Lys Thr His Thr Cys Pro Pro Cys Pro

1790

1795

1800

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro  
 1805 1810 1815

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr  
 1820 1825 1830

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe  
 1835 1840 1845

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys  
 1850 1855 1860

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
 1865 1870 1875

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys  
 1880 1885 1890

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr  
 1895 1900 1905

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr  
 1910 1915 1920

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu  
 1925 1930 1935

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu

1940

1945

1950

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro  
 1955 1960 1965

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu  
 1970 1975 1980

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
 1985 1990 1995

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser  
 2000 2005 2010

Leu Ser Leu Ser Pro Gly Lys  
 2015 2020

&lt;210&gt; 113

&lt;211&gt; 2056

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; pSYN FVIII 267 protein sequence

&lt;400&gt; 113

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe  
 1 5 10 15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser  
 20 25 30

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Trp Asp Tyr Met Gln Gly Ala Pro Thr Ser Glu Ser Ala Thr Pro Glu
      35              40              45

Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly
      50              55              60

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala
      65              70              75              80

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu
      85              90              95

Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly
      100             105             110

Ala Ser Ser Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro
      115             120             125

Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys
      130             135             140

Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile Ala Lys
      145             150             155             160

Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu
      165             170             175

Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro
      180             185             190

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Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly
      195                200                205

Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys
      210                215                220

Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu
      225                230                235                240

Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu
      245                250                255

Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala
      260                265                270

Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr
      275                280                285

Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser
      290                295                300

Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala
      305                310                315                320

Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn
      325                330                335

Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp
      340                345                350

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His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu

355

360

365

Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu

370

375

380

Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu

385

390

395

400

Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly

405

410

415

Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu

420

425

430

Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr

435

440

445

Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser

450

455

460

Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val

465

470

475

480

His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val

485

490

495

Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly

500

505

510

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Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr
      515                520                525

Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly
      530                535                540

Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile
545                550                555                560

Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly
      565                570                575

Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val
      580                585                590

Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr
      595                600                605

Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg
      610                615                620

Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu
625                630                635                640

Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val
      645                650                655

Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu
      660                665                670

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Ser | Val | Phe | Asp | Glu | Asn | Arg | Ser | Trp | Tyr | Leu | Thr | Glu | Asn | Ile |
|     |     | 675 |     |     |     |     | 680 |     |     |     |     | 685 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gln | Arg | Phe | Leu | Pro | Asn | Pro | Ala | Gly | Val | Gln | Leu | Glu | Asp | Pro | Glu |
|     |     | 690 |     |     |     | 695 |     |     |     |     | 700 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Phe | Gln | Ala | Ser | Asn | Ile | Met | His | Ser | Ile | Asn | Gly | Tyr | Val | Phe | Asp |
|     |     |     |     |     | 710 |     |     |     |     | 715 |     |     |     |     | 720 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Leu | Gln | Leu | Ser | Val | Cys | Leu | His | Glu | Val | Ala | Tyr | Trp | Tyr | Ile |
|     |     |     |     |     | 725 |     |     |     | 730 |     |     |     |     | 735 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Ser | Ile | Gly | Ala | Gln | Thr | Asp | Phe | Leu | Ser | Val | Phe | Phe | Ser | Gly |
|     |     |     | 740 |     |     |     |     | 745 |     |     |     |     | 750 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Thr | Phe | Lys | His | Lys | Met | Val | Tyr | Glu | Asp | Thr | Leu | Thr | Leu | Phe |
|     |     | 755 |     |     |     |     | 760 |     |     |     |     | 765 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Phe | Ser | Gly | Glu | Thr | Val | Phe | Met | Ser | Met | Glu | Asn | Pro | Gly | Leu |
|     |     | 770 |     |     |     |     | 775 |     |     |     |     | 780 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Trp | Ile | Leu | Gly | Cys | His | Asn | Ser | Asp | Phe | Arg | Asn | Arg | Gly | Met | Thr |
|     |     |     |     |     | 790 |     |     |     |     | 795 |     |     |     |     | 800 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Leu | Leu | Lys | Val | Ser | Ser | Cys | Asp | Lys | Asn | Thr | Gly | Asp | Tyr | Tyr |
|     |     |     |     | 805 |     |     |     |     |     | 810 |     |     |     | 815 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Asp | Ser | Tyr | Glu | Asp | Ile | Ser | Ala | Tyr | Leu | Leu | Ser | Lys | Asn | Asn |
|     |     |     |     | 820 |     |     |     |     | 825 |     |     |     |     | 830 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Ile | Glu | Pro | Arg | Ser | Phe | Ser | Gln | Asn | Gly | Ala | Pro | Gly | Thr | Ser |
| 835 |     |     |     | 840 |     |     |     | 845 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Ser | Ala | Thr | Pro | Glu | Ser | Gly | Pro | Gly | Ser | Glu | Pro | Ala | Thr | Ser |
| 850 |     |     |     | 855 |     |     |     | 860 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Ser | Glu | Thr | Pro | Gly | Thr | Ser | Glu | Ser | Ala | Thr | Pro | Glu | Ser | Gly |
| 865 |     |     |     | 870 |     |     |     | 875 |     |     |     | 880 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Gly | Ser | Glu | Pro | Ala | Thr | Ser | Gly | Ser | Glu | Thr | Pro | Gly | Thr | Ser |
|     |     |     |     | 885 |     |     |     | 890 |     |     |     | 895 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Ser | Ala | Thr | Pro | Glu | Ser | Gly | Pro | Gly | Thr | Ser | Thr | Glu | Pro | Ser |
|     |     |     |     | 900 |     |     |     | 905 |     |     |     | 910 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Gly | Ser | Ala | Pro | Gly | Ser | Pro | Ala | Gly | Ser | Pro | Thr | Ser | Thr | Glu |
| 915 |     |     |     | 920 |     |     |     | 925 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Gly | Thr | Ser | Glu | Ser | Ala | Thr | Pro | Glu | Ser | Gly | Pro | Gly | Ser | Glu |
| 930 |     |     |     | 935 |     |     |     | 940 |     |     |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Ala | Thr | Ser | Gly | Ser | Glu | Thr | Pro | Gly | Thr | Ser | Glu | Ser | Ala | Thr |
| 945 |     |     |     | 950 |     |     |     | 955 |     |     |     | 960 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Glu | Ser | Gly | Pro | Gly | Ser | Pro | Ala | Gly | Ser | Pro | Thr | Ser | Thr | Glu |
|     |     |     |     | 965 |     |     |     | 970 |     |     |     | 975 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Gly | Ser | Pro | Ala | Gly | Ser | Pro | Thr | Ser | Thr | Glu | Glu | Gly | Thr | Ser |
| 980 |     |     |     | 985 |     |     |     | 990 |     |     |     |     |     |     |     |

Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr

995

1000

1005

Pro Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser

1010

1015

1020

Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly

1025

1030

1035

Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Glu Pro

1040

1045

1050

Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Pro Ala Gly Ser Pro

1055

1060

1065

Thr Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser

1070

1075

1080

Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly

1085

1090

1095

Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu

1100

1105

1110

Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser

1115

1120

1125

Glu Gly Ser Ala Pro Ala Ser Ser Pro Pro Val Leu Lys Arg His

1130

1135

1140

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Gln  | Ala | Glu | Ile | Thr | Arg | Thr  | Thr | Leu | Gln | Ser | Asp  | Gln | Glu | Glu |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     | 1155 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Asp | Tyr | Asp | Asp | Thr | Ile  | Ser | Val | Glu | Met | Lys  | Lys | Glu | Asp |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Asp | Ile | Tyr | Asp | Glu | Asp  | Glu | Asn | Gln | Ser | Pro  | Arg | Ser | Phe |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Lys | Lys | Thr | Arg | His | Tyr  | Phe | Ile | Ala | Ala | Val  | Glu | Arg | Leu |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     | 1200 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Asp | Tyr | Gly | Met | Ser | Ser  | Ser | Pro | His | Val | Leu  | Arg | Asn | Arg |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     | 1215 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Gln | Ser | Gly | Ser | Val | Pro  | Gln | Phe | Lys | Lys | Val  | Val | Phe | Gln |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Phe | Thr | Asp | Gly | Ser | Phe  | Thr | Gln | Pro | Leu | Tyr  | Arg | Gly | Glu |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Asn | Glu | His | Leu | Gly | Leu  | Leu | Gly | Pro | Tyr | Ile  | Arg | Ala | Glu |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Glu | Asp | Asn | Ile | Met | Val  | Thr | Phe | Arg | Asn | Gln  | Ala | Ser | Arg |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     | 1275 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Tyr | Ser | Phe | Tyr | Ser | Ser  | Leu | Ile | Ser | Tyr | Glu  | Glu | Asp | Gln |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     | 1290 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Arg  | Gln | Gly | Ala | Glu | Pro | Arg  | Lys | Asn | Phe | Val | Lys  | Pro | Asn | Glu |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     | 1305 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Lys | Thr | Tyr | Phe | Trp | Lys  | Val | Gln | His | His | Met  | Ala | Pro | Thr |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     | 1320 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Asp | Glu | Phe | Asp | Cys | Lys  | Ala | Trp | Ala | Tyr | Phe  | Ser | Asp | Val |
| 1325 |     |     |     |     |     | 1330 |     |     |     |     | 1335 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Leu | Glu | Lys | Asp | Val | His  | Ser | Gly | Leu | Ile | Gly  | Pro | Leu | Leu |
| 1340 |     |     |     |     |     | 1345 |     |     |     |     | 1350 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Cys | His | Thr | Asn | Thr | Leu  | Asn | Pro | Ala | His | Gly  | Arg | Gln | Val |
| 1355 |     |     |     |     |     | 1360 |     |     |     |     | 1365 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Val | Gln | Glu | Phe | Ala | Leu  | Phe | Phe | Thr | Ile | Phe  | Asp | Glu | Thr |
| 1370 |     |     |     |     |     | 1375 |     |     |     |     | 1380 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Ser | Trp | Tyr | Phe | Thr | Glu  | Asn | Met | Glu | Arg | Asn  | Cys | Arg | Ala |
| 1385 |     |     |     |     |     | 1390 |     |     |     |     | 1395 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Cys | Asn | Ile | Gln | Met | Glu  | Asp | Pro | Thr | Phe | Lys  | Glu | Asn | Tyr |
| 1400 |     |     |     |     |     | 1405 |     |     |     |     | 1410 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Phe | His | Ala | Ile | Asn | Gly  | Tyr | Ile | Met | Asp | Thr  | Leu | Pro | Gly |
| 1415 |     |     |     |     |     | 1420 |     |     |     |     | 1425 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Val | Met | Ala | Gln | Asp | Gln  | Arg | Ile | Arg | Trp | Tyr  | Leu | Leu | Ser |
| 1430 |     |     |     |     |     | 1435 |     |     |     |     | 1440 |     |     |     |

|                                                             |      |      |
|-------------------------------------------------------------|------|------|
| Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe Ser Gly His |      |      |
| 1445                                                        | 1450 | 1455 |
|                                                             |      |      |
| Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met Ala Leu Tyr |      |      |
| 1460                                                        | 1465 | 1470 |
|                                                             |      |      |
| Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser |      |      |
| 1475                                                        | 1480 | 1485 |
|                                                             |      |      |
| Lys Ala Gly Ile Trp Arg Val Glu Cys Leu Ile Gly Glu His Leu |      |      |
| 1490                                                        | 1495 | 1500 |
|                                                             |      |      |
| His Ala Gly Met Ser Thr Leu Phe Leu Val Tyr Ser Asn Lys Cys |      |      |
| 1505                                                        | 1510 | 1515 |
|                                                             |      |      |
| Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile Arg Asp Phe Gln |      |      |
| 1520                                                        | 1525 | 1530 |
|                                                             |      |      |
| Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala |      |      |
| 1535                                                        | 1540 | 1545 |
|                                                             |      |      |
| Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys Glu |      |      |
| 1550                                                        | 1555 | 1560 |
|                                                             |      |      |
| Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile |      |      |
| 1565                                                        | 1570 | 1575 |
|                                                             |      |      |
| His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu |      |      |
| 1580                                                        | 1585 | 1590 |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Tyr  | Ile | Ser | Gln | Phe | Ile | Ile  | Met | Tyr | Ser | Leu | Asp  | Gly | Lys | Lys |
| 1595 |     |     |     |     |     | 1600 |     |     |     |     | 1605 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Gln | Thr | Tyr | Arg | Gly | Asn  | Ser | Thr | Gly | Thr | Leu  | Met | Val | Phe |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Gly | Asn | Val | Asp | Ser | Ser  | Gly | Ile | Lys | His | Asn  | Ile | Phe | Asn |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Pro | Ile | Ile | Ala | Arg | Tyr  | Ile | Arg | Leu | His | Pro  | Thr | His | Tyr |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Ile | Arg | Ser | Thr | Leu | Arg  | Met | Glu | Leu | Met | Gly  | Cys | Asp | Leu |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Ser | Cys | Ser | Met | Pro | Leu  | Gly | Met | Glu | Ser | Lys  | Ala | Ile | Ser |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Ala | Gln | Ile | Thr | Ala | Ser  | Ser | Tyr | Phe | Thr | Asn  | Met | Phe | Ala |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Trp | Ser | Pro | Ser | Lys | Ala  | Arg | Leu | His | Leu | Gln  | Gly | Arg | Ser |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Ala | Trp | Arg | Pro | Gln | Val  | Asn | Asn | Pro | Lys | Glu  | Trp | Leu | Gln |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     | 1725 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Asp | Phe | Gln | Lys | Thr | Met  | Lys | Val | Thr | Gly | Val  | Thr | Thr | Gln |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     | 1740 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Gly  | Val | Lys | Ser | Leu | Leu | Thr  | Ser | Met | Tyr | Val | Lys  | Glu | Phe | Leu |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     | 1755 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Ser | Ser | Ser | Gln | Asp | Gly  | His | Gln | Trp | Thr | Leu  | Phe | Phe | Gln |
| 1760 |     |     |     |     |     | 1765 |     |     |     |     | 1770 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Gly | Lys | Val | Lys | Val | Phe  | Gln | Gly | Asn | Gln | Asp  | Ser | Phe | Thr |
| 1775 |     |     |     |     |     | 1780 |     |     |     |     | 1785 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Val | Val | Asn | Ser | Leu | Asp  | Pro | Pro | Leu | Leu | Thr  | Arg | Tyr | Leu |
| 1790 |     |     |     |     |     | 1795 |     |     |     |     | 1800 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Ile | His | Pro | Gln | Ser | Trp  | Val | His | Gln | Ile | Ala  | Leu | Arg | Met |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     | 1815 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Val | Leu | Gly | Cys | Glu | Ala  | Gln | Asp | Leu | Tyr | Asp  | Lys | Thr | His |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     | 1830 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Cys | Pro | Pro | Cys | Pro | Ala  | Pro | Glu | Leu | Leu | Gly  | Gly | Pro | Ser |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     | 1845 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Phe | Leu | Phe | Pro | Pro | Lys  | Pro | Lys | Asp | Thr | Leu  | Met | Ile | Ser |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     | 1860 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Thr | Pro | Glu | Val | Thr | Cys  | Val | Val | Val | Asp | Val  | Ser | His | Glu |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     | 1875 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Pro | Glu | Val | Lys | Phe | Asn  | Trp | Tyr | Val | Asp | Gly  | Val | Glu | Val |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     | 1890 |     |     |     |



|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| His  | Asn | Ala | Lys | Thr | Lys | Pro  | Arg | Glu | Glu | Gln | Tyr  | Asn | Ser | Thr |
| 1895 |     |     |     |     |     | 1900 |     |     |     |     | 1905 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Arg | Val | Val | Ser | Val | Leu  | Thr | Val | Leu | His | Gln  | Asp | Trp | Leu |
| 1910 |     |     |     |     |     | 1915 |     |     |     |     | 1920 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Gly | Lys | Glu | Tyr | Lys | Cys  | Lys | Val | Ser | Asn | Lys  | Ala | Leu | Pro |
| 1925 |     |     |     |     |     | 1930 |     |     |     |     | 1935 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Pro | Ile | Glu | Lys | Thr | Ile  | Ser | Lys | Ala | Lys | Gly  | Gln | Pro | Arg |
| 1940 |     |     |     |     |     | 1945 |     |     |     |     | 1950 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Pro | Gln | Val | Tyr | Thr | Leu  | Pro | Pro | Ser | Arg | Asp  | Glu | Leu | Thr |
| 1955 |     |     |     |     |     | 1960 |     |     |     |     | 1965 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Asn | Gln | Val | Ser | Leu | Thr  | Cys | Leu | Val | Lys | Gly  | Phe | Tyr | Pro |
| 1970 |     |     |     |     |     | 1975 |     |     |     |     | 1980 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Asp | Ile | Ala | Val | Glu | Trp  | Glu | Ser | Asn | Gly | Gln  | Pro | Glu | Asn |
| 1985 |     |     |     |     |     | 1990 |     |     |     |     | 1995 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Tyr | Lys | Thr | Thr | Pro | Pro  | Val | Leu | Asp | Ser | Asp  | Gly | Ser | Phe |
| 2000 |     |     |     |     |     | 2005 |     |     |     |     | 2010 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Leu | Tyr | Ser | Lys | Leu | Thr  | Val | Asp | Lys | Ser | Arg  | Trp | Gln | Gln |
| 2015 |     |     |     |     |     | 2020 |     |     |     |     | 2025 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Asn | Val | Phe | Ser | Cys | Ser  | Val | Met | His | Glu | Ala  | Leu | His | Asn |
| 2030 |     |     |     |     |     | 2035 |     |     |     |     | 2040 |     |     |     |

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
 2045 2050 2055

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<211> 1834

<212> PRT

<213> Artificial Sequence

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Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser  
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Trp Asp Tyr Met Gln Gly Ala Pro Thr Ser Glu Ser Ala Thr Pro Glu  
 35 40 45

Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly  
 50 55 60

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala  
 65 70 75 80

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu  
 85 90 95

Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly

100

105

110

Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser  
115 120 125

Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser  
130 135 140

Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
145 150 155 160

Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala Gly  
165 170 175

Ser Pro Thr Ser Thr Glu Glu Gly Ala Ser Ser Ser Asp Leu Gly Glu  
180 185 190

Leu Pro Val Asp Ala Arg Phe Pro Pro Arg Val Pro Lys Ser Phe Pro  
195 200 205

Phe Asn Thr Ser Val Val Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr  
210 215 220

Asp His Leu Phe Asn Ile Ala Lys Pro Arg Pro Pro Trp Met Gly Leu  
225 230 235 240

Leu Gly Pro Thr Ile Gln Ala Glu Val Tyr Asp Thr Val Val Ile Thr  
245 250 255

Leu Lys Asn Met Ala Ser His Pro Val Ser Leu His Ala Val Gly Val

260

265

270

Ser Tyr Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser  
275 280 285

Gln Arg Glu Lys Glu Asp Asp Lys Val Phe Pro Gly Gly Ser His Thr  
290 295 300

Tyr Val Trp Gln Val Leu Lys Glu Asn Gly Pro Met Ala Ser Asp Pro  
305 310 315 320

Leu Cys Leu Thr Tyr Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp  
325 330 335

Leu Asn Ser Gly Leu Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser  
340 345 350

Leu Ala Lys Glu Lys Thr Gln Thr Leu His Lys Phe Ile Leu Leu Phe  
355 360 365

Ala Val Phe Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser  
370 375 380

Leu Met Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala Trp Pro Lys Met  
385 390 395 400

His Thr Val Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly  
405 410 415

Cys His Arg Lys Ser Val Tyr Trp His Val Ile Gly Met Gly Thr Thr

420

425

430

Pro Glu Val His Ser Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg

435

440

445

Asn His Arg Gln Ala Ser Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr

450

455

460

Ala Gln Thr Leu Leu Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His

465

470

475

480

Ile Ser Ser His Gln His Asp Gly Met Glu Ala Tyr Val Lys Val Asp

485

490

495

Ser Cys Pro Glu Glu Pro Gln Leu Arg Met Lys Asn Asn Glu Glu Ala

500

505

510

Glu Asp Tyr Asp Asp Asp Leu Thr Asp Ser Glu Met Asp Val Val Arg

515

520

525

Phe Asp Asp Asp Asn Ser Pro Ser Phe Ile Gln Ile Arg Ser Val Ala

530

535

540

Lys Lys His Pro Lys Thr Trp Val His Tyr Ile Ala Ala Glu Glu Glu

545

550

555

560

Asp Trp Asp Tyr Ala Pro Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr

565

570

575

Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr

580

585

590

Lys Lys Val Arg Phe Met Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg  
595 600 605

Glu Ala Ile Gln His Glu Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly  
610 615 620

Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg  
625 630 635 640

Pro Tyr Asn Ile Tyr Pro His Gly Ile Thr Asp Val Arg Pro Leu Tyr  
645 650 655

Ser Arg Arg Leu Pro Lys Gly Val Lys His Leu Lys Asp Phe Pro Ile  
660 665 670

Leu Pro Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val Thr Val Glu Asp  
675 680 685

Gly Pro Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser  
690 695 700

Phe Val Asn Met Glu Arg Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu  
705 710 715 720

Leu Ile Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly Asn Gln Ile Met  
725 730 735

Ser Asp Lys Arg Asn Val Ile Leu Phe Ser Val Phe Asp Glu Asn Arg

740

745

750

Ser Trp Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala  
 755 760 765

Gly Val Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser Asn Ile Met His  
 770 775 780

Ser Ile Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser Val Cys Leu  
 785 790 795 800

His Glu Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp  
 805 810 815

Phe Leu Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val  
 820 825 830

Tyr Glu Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe  
 835 840 845

Met Ser Met Glu Asn Pro Gly Leu Trp Ile Leu Gly Cys His Asn Ser  
 850 855 860

Asp Phe Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys  
 865 870 875 880

Asp Lys Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser  
 885 890 895

Ala Tyr Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser

900

905

910

Gln Asn Pro Pro Val Leu Lys Arg His Gln Ala Glu Ile Thr Arg Thr  
 915 920 925

Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser  
 930 935 940

Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn  
 945 950 955 960

Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala  
 965 970 975

Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val  
 980 985 990

Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val  
 995 1000 1005

Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr  
 1010 1015 1020

Arg Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile  
 1025 1030 1035

Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln  
 1040 1045 1050

Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Glu



1055

1060

1065

Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys  
 1070 1075 1080

Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His His Met  
 1085 1090 1095

Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe  
 1100 1105 1110

Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly  
 1115 1120 1125

Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly  
 1130 1135 1140

Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe  
 1145 1150 1155

Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn  
 1160 1165 1170

Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys  
 1175 1180 1185

Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr  
 1190 1195 1200

Leu Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr

1205

1210

1215

Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe  
 1220 1225 1230

Ser Gly His Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met  
 1235 1240 1245

Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met  
 1250 1255 1260

Leu Pro Ser Lys Ala Gly Ile Trp Arg Val Glu Cys Leu Ile Gly  
 1265 1270 1275

Glu His Leu His Ala Gly Met Ser Thr Leu Phe Leu Val Tyr Ser  
 1280 1285 1290

Asn Lys Cys Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile Arg  
 1295 1300 1305

Asp Phe Gln Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro  
 1310 1315 1320

Lys Leu Ala Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser  
 1325 1330 1335

Thr Lys Glu Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala Pro  
 1340 1345 1350

Met Ile Ile His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe

1355

1360

1365

Ser Ser Leu Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp  
 1370 1375 1380

Gly Lys Lys Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu  
 1385 1390 1395

Met Val Phe Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His Asn  
 1400 1405 1410

Ile Phe Asn Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro  
 1415 1420 1425

Thr His Tyr Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly  
 1430 1435 1440

Cys Asp Leu Asn Ser Cys Ser Met Pro Leu Gly Met Glu Ser Lys  
 1445 1450 1455

Ala Ile Ser Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn  
 1460 1465 1470

Met Phe Ala Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu Gln  
 1475 1480 1485

Gly Arg Ser Asn Ala Trp Arg Pro Gln Val Asn Asn Pro Lys Glu  
 1490 1495 1500

Trp Leu Gln Val Asp Phe Gln Lys Thr Met Lys Val Thr Gly Val

1505

1510

1515

Thr Thr Gln Gly Val Lys Ser Leu Leu Thr Ser Met Tyr Val Lys  
 1520 1525 1530

Glu Phe Leu Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr Leu  
 1535 1540 1545

Phe Phe Gln Asn Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp  
 1550 1555 1560

Ser Phe Thr Pro Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr  
 1565 1570 1575

Arg Tyr Leu Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala  
 1580 1585 1590

Leu Arg Met Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr Asp  
 1595 1600 1605

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly  
 1610 1615 1620

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu  
 1625 1630 1635

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val  
 1640 1645 1650

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly

1655

1660

1665

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr  
 1670 1675 1680

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
 1685 1690 1695

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys  
 1700 1705 1710

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly  
 1715 1720 1725

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp  
 1730 1735 1740

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly  
 1745 1750 1755

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln  
 1760 1765 1770

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
 1775 1780 1785

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg  
 1790 1795 1800

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala

1805

1810

1815

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly

1820

1825

1830

Lys

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<212> PRT

<213> Artificial Sequence

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<400> 115

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Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser

20

25

30

Trp Asp Tyr Met Gln Gly Ala Pro Thr Ser Glu Ser Ala Thr Pro Glu

35

40

45

Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly

50

55

60

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala

65

70

75

80

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Ser | Gly | Ser | Glu | Thr | Pro | Gly | Thr | Ser | Glu | Ser | Ala | Thr | Pro | Glu |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Gly | Pro | Gly | Thr | Ser | Thr | Glu | Pro | Ser | Glu | Gly | Ser | Ala | Pro | Gly |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Ser | Ser | Ser | Asp | Leu | Gly | Glu | Leu | Pro | Val | Asp | Ala | Arg | Phe | Pro |
|     |     |     | 115 |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Arg | Val | Pro | Lys | Ser | Phe | Pro | Phe | Asn | Thr | Ser | Val | Val | Tyr | Lys |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Lys | Thr | Leu | Phe | Val | Glu | Phe | Thr | Asp | His | Leu | Phe | Asn | Ile | Ala | Lys |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     | 160 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Arg | Pro | Pro | Trp | Met | Gly | Leu | Leu | Gly | Pro | Thr | Ile | Gln | Ala | Glu |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val | Tyr | Asp | Thr | Val | Val | Ile | Thr | Leu | Lys | Asn | Met | Ala | Ser | His | Pro |
|     |     | 180 |     |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val | Ser | Leu | His | Ala | Val | Gly | Val | Ser | Tyr | Trp | Lys | Ala | Ser | Glu | Gly |
|     | 195 |     |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Glu | Tyr | Asp | Asp | Gln | Thr | Ser | Gln | Arg | Glu | Lys | Glu | Asp | Asp | Lys |
| 210 |     |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val | Phe | Pro | Gly | Gly | Ser | His | Thr | Tyr | Val | Trp | Gln | Val | Leu | Lys | Glu |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     | 240 |     |

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Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu
      245              250              255

Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala
      260              265              270

Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr
      275              280              285

Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser
      290              295              300

Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala
305              310              315              320

Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn
      325              330              335

Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp
      340              345              350

His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu
      355              360              365

Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu
      370              375              380

Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu
385              390              395              400

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Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly

405

410

415

Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu

420

425

430

Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr

435

440

445

Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser

450

455

460

Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val

465

470

475

480

His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val

485

490

495

Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly

500

505

510

Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr

515

520

525

Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly

530

535

540

Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile

545

550

555

560

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Phe | Lys | Asn | Gln | Ala | Ser | Arg | Pro | Tyr | Asn | Ile | Tyr | Pro | His | Gly |
|     |     |     | 565 |     |     |     |     |     | 570 |     |     |     |     | 575 |     |
| Ile | Thr | Asp | Val | Arg | Pro | Leu | Tyr | Ser | Arg | Arg | Leu | Pro | Lys | Gly | Val |
|     |     |     | 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |
| Lys | His | Leu | Lys | Asp | Phe | Pro | Ile | Leu | Pro | Gly | Glu | Ile | Phe | Lys | Tyr |
|     |     | 595 |     |     |     |     | 600 |     |     |     |     | 605 |     |     |     |
| Lys | Trp | Thr | Val | Thr | Val | Glu | Asp | Gly | Pro | Thr | Lys | Ser | Asp | Pro | Arg |
|     | 610 |     |     |     |     | 615 |     |     |     |     | 620 |     |     |     |     |
| Cys | Leu | Thr | Arg | Tyr | Tyr | Ser | Ser | Phe | Val | Asn | Met | Glu | Arg | Asp | Leu |
| 625 |     |     |     |     | 630 |     |     |     |     | 635 |     |     |     |     | 640 |
| Ala | Ser | Gly | Leu | Ile | Gly | Pro | Leu | Leu | Ile | Cys | Tyr | Lys | Glu | Ser | Val |
|     |     |     | 645 |     |     |     |     | 650 |     |     |     |     | 655 |     |     |
| Asp | Gln | Arg | Gly | Asn | Gln | Ile | Met | Ser | Asp | Lys | Arg | Asn | Val | Ile | Leu |
|     |     | 660 |     |     |     |     |     | 665 |     |     |     |     | 670 |     |     |
| Phe | Ser | Val | Phe | Asp | Glu | Asn | Arg | Ser | Trp | Tyr | Leu | Thr | Glu | Asn | Ile |
|     |     | 675 |     |     |     |     |     | 680 |     |     |     |     | 685 |     |     |
| Gln | Arg | Phe | Leu | Pro | Asn | Pro | Ala | Gly | Val | Gln | Leu | Glu | Asp | Pro | Glu |
|     | 690 |     |     |     |     | 695 |     |     |     |     | 700 |     |     |     |     |
| Phe | Gln | Ala | Ser | Asn | Ile | Met | His | Ser | Ile | Asn | Gly | Tyr | Val | Phe | Asp |
| 705 |     |     |     |     | 710 |     |     |     |     | 715 |     |     |     |     | 720 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Leu | Gln | Leu | Ser | Val | Cys | Leu | His | Glu | Val | Ala | Tyr | Trp | Tyr | Ile |
|     |     |     |     | 725 |     |     |     |     | 730 |     |     |     |     | 735 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Ser | Ile | Gly | Ala | Gln | Thr | Asp | Phe | Leu | Ser | Val | Phe | Phe | Ser | Gly |
|     |     |     | 740 |     |     |     |     | 745 |     |     |     |     | 750 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Thr | Phe | Lys | His | Lys | Met | Val | Tyr | Glu | Asp | Thr | Leu | Thr | Leu | Phe |
|     |     | 755 |     |     |     |     | 760 |     |     |     |     | 765 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Phe | Ser | Gly | Glu | Thr | Val | Phe | Met | Ser | Met | Glu | Asn | Pro | Gly | Leu |
|     | 770 |     |     |     |     | 775 |     |     |     |     | 780 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Trp | Ile | Leu | Gly | Cys | His | Asn | Ser | Asp | Phe | Arg | Asn | Arg | Gly | Met | Thr |
| 785 |     |     |     |     | 790 |     |     |     |     | 795 |     |     |     |     | 800 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Leu | Leu | Lys | Val | Ser | Ser | Cys | Asp | Lys | Asn | Thr | Gly | Asp | Tyr | Tyr |
|     |     |     | 805 |     |     |     |     |     | 810 |     |     |     |     | 815 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Asp | Ser | Tyr | Glu | Asp | Ile | Ser | Ala | Tyr | Leu | Leu | Ser | Lys | Asn | Asn |
|     |     | 820 |     |     |     |     |     | 825 |     |     |     |     | 830 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Ile | Glu | Pro | Arg | Ser | Phe | Ser | Gln | Asn | Pro | Pro | Val | Leu | Lys | Arg |
|     | 835 |     |     |     |     |     | 840 |     |     |     |     | 845 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| His | Gln | Ala | Glu | Ile | Thr | Arg | Thr | Thr | Leu | Gln | Ser | Asp | Gln | Glu | Glu |
| 850 |     |     |     |     |     | 855 |     |     |     | 860 |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ile | Asp | Tyr | Asp | Asp | Thr | Ile | Ser | Val | Glu | Met | Lys | Lys | Glu | Asp | Phe |
| 865 |     |     |     |     | 870 |     |     |     |     | 875 |     |     |     | 880 |     |

Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys

885

890

895

Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr

900

905

910

Gly Met Ser Ser Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly

915

920

925

Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly

930

935

940

Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly

945

950

955

960

Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val

965

970

975

Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu

980

985

990

Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn

995

1000

1005

Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln

1010

1015

1020

His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp

1025

1030

1035

|                                                             |      |      |
|-------------------------------------------------------------|------|------|
| Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly |      |      |
| 1040                                                        | 1045 | 1050 |
|                                                             |      |      |
| Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro |      |      |
| 1055                                                        | 1060 | 1065 |
|                                                             |      |      |
| Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe |      |      |
| 1070                                                        | 1075 | 1080 |
|                                                             |      |      |
| Thr Ile Phe Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met |      |      |
| 1085                                                        | 1090 | 1095 |
|                                                             |      |      |
| Glu Arg Asn Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp Pro |      |      |
| 1100                                                        | 1105 | 1110 |
|                                                             |      |      |
| Thr Phe Lys Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile |      |      |
| 1115                                                        | 1120 | 1125 |
|                                                             |      |      |
| Met Asp Thr Leu Pro Gly Leu Val Met Ala Gln Asp Gln Arg Ile |      |      |
| 1130                                                        | 1135 | 1140 |
|                                                             |      |      |
| Arg Trp Tyr Leu Leu Ser Met Gly Ser Asn Glu Asn Ile His Ser |      |      |
| 1145                                                        | 1150 | 1155 |
|                                                             |      |      |
| Ile His Phe Ser Gly His Val Phe Thr Val Arg Lys Lys Glu Glu |      |      |
| 1160                                                        | 1165 | 1170 |
|                                                             |      |      |
| Tyr Lys Met Ala Leu Tyr Asn Leu Tyr Pro Gly Val Phe Glu Thr |      |      |
| 1175                                                        | 1180 | 1185 |

|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|
| Val  | Glu | Met | Leu | Pro | Ser | Lys  | Ala | Gly | Ile | Trp | Arg | Val  | Glu | Cys |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     |     | 1200 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Leu  | Ile | Gly | Glu | His | Leu | His  | Ala | Gly | Met | Ser | Thr | Leu  | Phe | Leu |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     |     | 1215 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Val  | Tyr | Ser | Asn | Lys | Cys | Gln  | Thr | Pro | Leu | Gly | Met | Ala  | Ser | Gly |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     |     | 1230 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| His  | Ile | Arg | Asp | Phe | Gln | Ile  | Thr | Ala | Ser | Gly | Gln | Tyr  | Gly | Gln |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     |     | 1245 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Trp  | Ala | Pro | Lys | Leu | Ala | Arg  | Leu | His | Tyr | Ser | Gly | Ser  | Ile | Asn |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     |     | 1260 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ala  | Trp | Ser | Thr | Lys | Glu | Pro  | Phe | Ser | Trp | Ile | Lys | Val  | Asp | Leu |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     |     | 1275 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Leu  | Ala | Pro | Met | Ile | Ile | His  | Gly | Ile | Lys | Thr | Gln | Gly  | Ala | Arg |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     |     | 1290 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gln  | Lys | Phe | Ser | Ser | Leu | Tyr  | Ile | Ser | Gln | Phe | Ile | Ile  | Met | Tyr |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     |     | 1305 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ser  | Leu | Asp | Gly | Lys | Lys | Trp  | Gln | Thr | Tyr | Arg | Gly | Asn  | Ser | Thr |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     |     | 1320 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Gly  | Thr | Leu | Met | Val | Phe | Phe  | Gly | Asn | Val | Asp | Ser | Ser  | Gly | Ile |
| 1325 |     |     |     |     |     | 1330 |     |     |     |     |     | 1335 |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Lys  | His | Asn | Ile | Phe | Asn | Pro  | Pro | Ile | Ile | Ala | Arg  | Tyr | Ile | Arg |
| 1340 |     |     |     |     |     | 1345 |     |     |     |     | 1350 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | His | Pro | Thr | His | Tyr | Ser  | Ile | Arg | Ser | Thr | Leu  | Arg | Met | Glu |
| 1355 |     |     |     |     |     | 1360 |     |     |     |     | 1365 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Met | Gly | Cys | Asp | Leu | Asn  | Ser | Cys | Ser | Met | Pro  | Leu | Gly | Met |
| 1370 |     |     |     |     |     | 1375 |     |     |     |     | 1380 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Ser | Lys | Ala | Ile | Ser | Asp  | Ala | Gln | Ile | Thr | Ala  | Ser | Ser | Tyr |
| 1385 |     |     |     |     |     | 1390 |     |     |     |     | 1395 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Thr | Asn | Met | Phe | Ala | Thr  | Trp | Ser | Pro | Ser | Lys  | Ala | Arg | Leu |
| 1400 |     |     |     |     |     | 1405 |     |     |     |     | 1410 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| His  | Leu | Gln | Gly | Arg | Ser | Asn  | Ala | Trp | Arg | Pro | Gln  | Val | Asn | Asn |
| 1415 |     |     |     |     |     | 1420 |     |     |     |     | 1425 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Lys | Glu | Trp | Leu | Gln | Val  | Asp | Phe | Gln | Lys | Thr  | Met | Lys | Val |
| 1430 |     |     |     |     |     | 1435 |     |     |     |     | 1440 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Gly | Val | Thr | Thr | Gln | Gly  | Val | Lys | Ser | Leu | Leu  | Thr | Ser | Met |
| 1445 |     |     |     |     |     | 1450 |     |     |     |     | 1455 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Val | Lys | Glu | Phe | Leu | Ile  | Ser | Ser | Ser | Gln | Asp  | Gly | His | Gln |
| 1460 |     |     |     |     |     | 1465 |     |     |     |     | 1470 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Thr | Leu | Phe | Phe | Gln | Asn  | Gly | Lys | Val | Lys | Val  | Phe | Gln | Gly |
| 1475 |     |     |     |     |     | 1480 |     |     |     |     | 1485 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Asn  | Gln | Asp | Ser | Phe | Thr | Pro  | Val | Val | Asn | Ser | Leu  | Asp | Pro | Pro |
| 1490 |     |     |     |     |     | 1495 |     |     |     |     | 1500 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Leu | Thr | Arg | Tyr | Leu | Arg  | Ile | His | Pro | Gln | Ser  | Trp | Val | His |
| 1505 |     |     |     |     |     | 1510 |     |     |     |     | 1515 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Ile | Ala | Leu | Arg | Met | Glu  | Val | Leu | Gly | Cys | Glu  | Ala | Gln | Asp |
| 1520 |     |     |     |     |     | 1525 |     |     |     |     | 1530 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Tyr | Asp | Lys | Thr | His | Thr  | Cys | Pro | Pro | Cys | Pro  | Ala | Pro | Glu |
| 1535 |     |     |     |     |     | 1540 |     |     |     |     | 1545 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Leu | Gly | Gly | Pro | Ser | Val  | Phe | Leu | Phe | Pro | Pro  | Lys | Pro | Lys |
| 1550 |     |     |     |     |     | 1555 |     |     |     |     | 1560 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Thr | Leu | Met | Ile | Ser | Arg  | Thr | Pro | Glu | Val | Thr  | Cys | Val | Val |
| 1565 |     |     |     |     |     | 1570 |     |     |     |     | 1575 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Asp | Val | Ser | His | Glu | Asp  | Pro | Glu | Val | Lys | Phe  | Asn | Trp | Tyr |
| 1580 |     |     |     |     |     | 1585 |     |     |     |     | 1590 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Asp | Gly | Val | Glu | Val | His  | Asn | Ala | Lys | Thr | Lys  | Pro | Arg | Glu |
| 1595 |     |     |     |     |     | 1600 |     |     |     |     | 1605 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Gln | Tyr | Asn | Ser | Thr | Tyr  | Arg | Val | Val | Ser | Val  | Leu | Thr | Val |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | His | Gln | Asp | Trp | Leu | Asn  | Gly | Lys | Glu | Tyr | Lys  | Cys | Lys | Val |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |



|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|
| Ser  | Asn | Lys | Ala | Leu | Pro | Ala  | Pro | Ile | Glu | Lys | Thr | Ile  | Ser | Lys |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     |     | 1650 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ala  | Lys | Gly | Gln | Pro | Arg | Glu  | Pro | Gln | Val | Tyr | Thr | Leu  | Pro | Pro |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     |     | 1665 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ser  | Arg | Asp | Glu | Leu | Thr | Lys  | Asn | Gln | Val | Ser | Leu | Thr  | Cys | Leu |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     |     | 1680 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Val  | Lys | Gly | Phe | Tyr | Pro | Ser  | Asp | Ile | Ala | Val | Glu | Trp  | Glu | Ser |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     |     | 1695 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Asn  | Gly | Gln | Pro | Glu | Asn | Asn  | Tyr | Lys | Thr | Thr | Pro | Pro  | Val | Leu |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     |     | 1710 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Asp  | Ser | Asp | Gly | Ser | Phe | Phe  | Leu | Tyr | Ser | Lys | Leu | Thr  | Val | Asp |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     |     | 1725 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Lys  | Ser | Arg | Trp | Gln | Gln | Gly  | Asn | Val | Phe | Ser | Cys | Ser  | Val | Met |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     |     | 1740 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| His  | Glu | Ala | Leu | His | Asn | His  | Tyr | Thr | Gln | Lys | Ser | Leu  | Ser | Leu |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     |     | 1755 |     |     |
|      |     |     |     |     |     |      |     |     |     |     |     |      |     |     |
| Ser  | Pro | Gly | Lys |     |     |      |     |     |     |     |     |      |     |     |
| 1760 |     |     |     |     |     |      |     |     |     |     |     |      |     |     |

&lt;210&gt; 116

&lt;211&gt; 1726

&lt;212&gt; PRT

<213> Artificial Sequence

<220>

<223> pSYNFVIII 271 protein sequence

<400> 116

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Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser  
20 25 30

Trp Asp Tyr Met Gln Gly Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr  
35 40 45

Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
50 55 60

Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Ala Ser Ser Ser  
65 70 75 80

Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro Arg Val Pro  
85 90 95

Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys Thr Leu Phe  
100 105 110

Val Glu Phe Thr Asp His Leu Phe Asn Ile Ala Lys Pro Arg Pro Pro  
115 120 125

Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val Tyr Asp Thr

130

135

140

Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val Ser Leu His  
145 150 155 160

Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp  
165 170 175

Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val Phe Pro Gly  
180 185 190

Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn Gly Pro Met  
195 200 205

Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser His Val Asp  
210 215 220

Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu Leu Val Cys  
225 230 235 240

Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu His Lys Phe  
245 250 255

Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp His Ser Glu  
260 265 270

Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala  
275 280 285

Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg Ser Leu Pro

290

295

300

Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His Val Ile Gly  
305 310 315 320

Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu Gly His Thr  
325 330 335

Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile Ser Pro Ile  
340 345 350

Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly Gln Phe Leu  
355 360 365

Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met Glu Ala Tyr  
370 375 380

Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg Met Lys Asn  
385 390 395 400

Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp Ser Glu Met  
405 410 415

Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe Ile Gln Ile  
420 425 430

Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His Tyr Ile Ala  
435 440 445

Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu Ala Pro Asp

450

455

460

Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro Gln Arg Ile  
465 470 475 480

Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr Asp Glu Thr  
485 490 495

Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile Leu Gly Pro  
500 505 510

Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile Phe Lys Asn  
515 520 525

Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile Thr Asp Val  
530 535 540

Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys His Leu Lys  
545 550 555 560

Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys Trp Thr Val  
565 570 575

Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys Leu Thr Arg  
580 585 590

Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala Ser Gly Leu  
595 600 605

Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly

610

615

620

Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe Ser Val Phe

625

630

635

640

Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu

645

650

655

Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser

660

665

670

Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu

675

680

685

Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly

690

695

700

Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys

705

710

715

720

His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly

725

730

735

Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp Ile Leu Gly

740

745

750

Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala Leu Leu Lys

755

760

765

Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr

770

775

780

Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro  
785 790 795 800

Arg Ser Phe Ser Gln Asn Pro Pro Val Leu Lys Arg His Gln Ala Glu  
805 810 815

Ile Thr Arg Thr Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp  
820 825 830

Asp Thr Ile Ser Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp  
835 840 845

Glu Asp Glu Asn Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His  
850 855 860

Tyr Phe Ile Ala Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser  
865 870 875 880

Ser Pro His Val Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln  
885 890 895

Phe Lys Lys Val Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr Gln  
900 905 910

Pro Leu Tyr Arg Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly Pro  
915 920 925

Tyr Ile Arg Ala Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn

930

935

940

Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Glu

945

950

955

960

Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys Pro

965

970

975

Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val Gln His His Met Ala Pro

980

985

990

Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val

995

1000

1005

Asp Leu Glu Lys Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu

1010

1015

1020

Val Cys His Thr Asn Thr Leu Asn Pro Ala His Gly Arg Gln Val

1025

1030

1035

Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe Asp Glu Thr

1040

1045

1050

Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn Cys Arg Ala

1055

1060

1065

Pro Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys Glu Asn Tyr

1070

1075

1080

Arg Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr Leu Pro Gly



1085

1090

1095

Leu Val Met Ala Gln Asp Gln Arg Ile Arg Trp Tyr Leu Leu Ser  
 1100 1105 1110

Met Gly Ser Asn Glu Asn Ile His Ser Ile His Phe Ser Gly His  
 1115 1120 1125

Val Phe Thr Val Arg Lys Lys Glu Glu Tyr Lys Met Ala Leu Tyr  
 1130 1135 1140

Asn Leu Tyr Pro Gly Val Phe Glu Thr Val Glu Met Leu Pro Ser  
 1145 1150 1155

Lys Ala Gly Ile Trp Arg Val Glu Cys Leu Ile Gly Glu His Leu  
 1160 1165 1170

His Ala Gly Met Ser Thr Leu Phe Leu Val Tyr Ser Asn Lys Cys  
 1175 1180 1185

Gln Thr Pro Leu Gly Met Ala Ser Gly His Ile Arg Asp Phe Gln  
 1190 1195 1200

Ile Thr Ala Ser Gly Gln Tyr Gly Gln Trp Ala Pro Lys Leu Ala  
 1205 1210 1215

Arg Leu His Tyr Ser Gly Ser Ile Asn Ala Trp Ser Thr Lys Glu  
 1220 1225 1230

Pro Phe Ser Trp Ile Lys Val Asp Leu Leu Ala Pro Met Ile Ile

1235

1240

1245

His Gly Ile Lys Thr Gln Gly Ala Arg Gln Lys Phe Ser Ser Leu  
 1250 1255 1260

Tyr Ile Ser Gln Phe Ile Ile Met Tyr Ser Leu Asp Gly Lys Lys  
 1265 1270 1275

Trp Gln Thr Tyr Arg Gly Asn Ser Thr Gly Thr Leu Met Val Phe  
 1280 1285 1290

Phe Gly Asn Val Asp Ser Ser Gly Ile Lys His Asn Ile Phe Asn  
 1295 1300 1305

Pro Pro Ile Ile Ala Arg Tyr Ile Arg Leu His Pro Thr His Tyr  
 1310 1315 1320

Ser Ile Arg Ser Thr Leu Arg Met Glu Leu Met Gly Cys Asp Leu  
 1325 1330 1335

Asn Ser Cys Ser Met Pro Leu Gly Met Glu Ser Lys Ala Ile Ser  
 1340 1345 1350

Asp Ala Gln Ile Thr Ala Ser Ser Tyr Phe Thr Asn Met Phe Ala  
 1355 1360 1365

Thr Trp Ser Pro Ser Lys Ala Arg Leu His Leu Gln Gly Arg Ser  
 1370 1375 1380

Asn Ala Trp Arg Pro Gln Val Asn Asn Pro Lys Glu Trp Leu Gln

1385

1390

1395

Val Asp Phe Gln Lys Thr Met Lys Val Thr Gly Val Thr Thr Gln  
 1400 1405 1410

Gly Val Lys Ser Leu Leu Thr Ser Met Tyr Val Lys Glu Phe Leu  
 1415 1420 1425

Ile Ser Ser Ser Gln Asp Gly His Gln Trp Thr Leu Phe Phe Gln  
 1430 1435 1440

Asn Gly Lys Val Lys Val Phe Gln Gly Asn Gln Asp Ser Phe Thr  
 1445 1450 1455

Pro Val Val Asn Ser Leu Asp Pro Pro Leu Leu Thr Arg Tyr Leu  
 1460 1465 1470

Arg Ile His Pro Gln Ser Trp Val His Gln Ile Ala Leu Arg Met  
 1475 1480 1485

Glu Val Leu Gly Cys Glu Ala Gln Asp Leu Tyr Asp Lys Thr His  
 1490 1495 1500

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser  
 1505 1510 1515

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser  
 1520 1525 1530

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu

1535

1540

1545

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
 1550 1555 1560

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr  
 1565 1570 1575

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu  
 1580 1585 1590

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 1595 1600 1605

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg  
 1610 1615 1620

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr  
 1625 1630 1635

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro  
 1640 1645 1650

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn  
 1655 1660 1665

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe  
 1670 1675 1680

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln

1685

1690

1695

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn

1700

1705

1710

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

1715

1720

1725

&lt;210&gt; 117

&lt;211&gt; 1901

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; pSYN FVIII protein sequence 272

&lt;400&gt; 117

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe

1

5

10

15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser

20

25

30

Trp Asp Tyr Met Gln Gly Ala Pro Thr Ser Glu Ser Ala Thr Pro Glu

35

40

45

Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly

50

55

60

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala

65

70

75

80

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Ser | Gly | Ser | Glu | Thr | Pro | Gly | Thr | Ser | Glu | Ser | Ala | Thr | Pro | Glu |
|     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Gly | Pro | Gly | Thr | Ser | Thr | Glu | Pro | Ser | Glu | Gly | Ser | Ala | Pro | Gly |
|     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Pro | Ala | Gly | Ser | Pro | Thr | Ser | Thr | Glu | Glu | Gly | Thr | Ser | Glu | Ser |
|     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Thr | Pro | Glu | Ser | Gly | Pro | Gly | Ser | Glu | Pro | Ala | Thr | Ser | Gly | Ser |
| 130 |     |     |     |     | 135 |     |     |     |     |     | 140 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Thr | Pro | Gly | Thr | Ser | Glu | Ser | Ala | Thr | Pro | Glu | Ser | Gly | Pro | Gly |
| 145 |     |     |     | 150 |     |     |     | 155 |     |     |     |     |     | 160 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Pro | Ala | Gly | Ser | Pro | Thr | Ser | Thr | Glu | Glu | Gly | Ser | Pro | Ala | Gly |
|     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Pro | Thr | Ser | Thr | Glu | Glu | Gly | Ala | Ser | Ser | Ser | Asp | Leu | Gly | Glu |
|     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Pro | Val | Asp | Ala | Arg | Phe | Pro | Pro | Arg | Val | Pro | Lys | Ser | Phe | Pro |
|     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Phe | Asn | Thr | Ser | Val | Val | Tyr | Lys | Lys | Thr | Leu | Phe | Val | Glu | Phe | Thr |
| 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | His | Leu | Phe | Asn | Ile | Ala | Lys | Pro | Arg | Pro | Pro | Trp | Met | Gly | Leu |
| 225 |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |     |

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Leu Gly Pro Thr Ile Gln Ala Glu Val Tyr Asp Thr Val Val Ile Thr
      245              250              255

Leu Lys Asn Met Ala Ser His Pro Val Ser Leu His Ala Val Gly Val
      260              265              270

Ser Tyr Trp Lys Ala Ser Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser
      275              280              285

Gln Arg Glu Lys Glu Asp Asp Lys Val Phe Pro Gly Gly Ser His Thr
      290              295              300

Tyr Val Trp Gln Val Leu Lys Glu Asn Gly Pro Met Ala Ser Asp Pro
305              310              315              320

Leu Cys Leu Thr Tyr Ser Tyr Leu Ser His Val Asp Leu Val Lys Asp
      325              330              335

Leu Asn Ser Gly Leu Ile Gly Ala Leu Leu Val Cys Arg Glu Gly Ser
      340              345              350

Leu Ala Lys Glu Lys Thr Gln Thr Leu His Lys Phe Ile Leu Leu Phe
      355              360              365

Ala Val Phe Asp Glu Gly Lys Ser Trp His Ser Glu Thr Lys Asn Ser
      370              375              380

Leu Met Gln Asp Arg Asp Ala Ala Ser Ala Arg Ala Trp Pro Lys Met
385              390              395              400

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His Thr Val Asn Gly Tyr Val Asn Arg Ser Leu Pro Gly Leu Ile Gly

405

410

415

Cys His Arg Lys Ser Val Tyr Trp His Val Ile Gly Met Gly Thr Thr

420

425

430

Pro Glu Val His Ser Ile Phe Leu Glu Gly His Thr Phe Leu Val Arg

435

440

445

Asn His Arg Gln Ala Ser Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr

450

455

460

Ala Gln Thr Leu Leu Met Asp Leu Gly Gln Phe Leu Leu Phe Cys His

465

470

475

480

Ile Ser Ser His Gln His Asp Gly Met Glu Ala Tyr Val Lys Val Asp

485

490

495

Ser Cys Pro Glu Glu Pro Gln Leu Arg Met Lys Asn Asn Glu Glu Ala

500

505

510

Glu Asp Tyr Asp Asp Asp Leu Thr Asp Ser Glu Met Asp Val Val Arg

515

520

525

Phe Asp Asp Asp Asn Ser Pro Ser Phe Ile Gln Ile Arg Ser Val Ala

530

535

540

Lys Lys His Pro Lys Thr Trp Val His Tyr Ile Ala Ala Glu Glu Glu

545

550

555

560





Leu Ile Cys Tyr Lys Glu Ser Val Asp Gln Arg Gly Asn Gln Ile Met

725

730

735

Ser Asp Lys Arg Asn Val Ile Leu Phe Ser Val Phe Asp Glu Asn Arg

740

745

750

Ser Trp Tyr Leu Thr Glu Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala

755

760

765

Gly Val Gln Leu Glu Asp Pro Glu Phe Gln Ala Ser Asn Ile Met His

770

775

780

Ser Ile Asn Gly Tyr Val Phe Asp Ser Leu Gln Leu Ser Val Cys Leu

785

790

795

800

His Glu Val Ala Tyr Trp Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp

805

810

815

Phe Leu Ser Val Phe Phe Ser Gly Tyr Thr Phe Lys His Lys Met Val

820

825

830

Tyr Glu Asp Thr Leu Thr Leu Phe Pro Phe Ser Gly Glu Thr Val Phe

835

840

845

Met Ser Met Glu Asn Pro Gly Leu Trp Ile Leu Gly Cys His Asn Ser

850

855

860

Asp Phe Arg Asn Arg Gly Met Thr Ala Leu Leu Lys Val Ser Ser Cys

865

870

875

880

Asp Lys Asn Thr Gly Asp Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser  
 885 890 895

Ala Tyr Leu Leu Ser Lys Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser  
 900 905 910

Gln Asn Gly Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
 915 920 925

Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser  
 930 935 940

Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser  
 945 950 955 960

Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly  
 965 970 975

Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro  
 980 985 990

Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr  
 995 1000 1005

Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu  
 1010 1015 1020

Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
 1025 1030 1035

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ser  | Pro | Ala | Gly | Ser | Pro | Thr  | Ser | Thr | Glu | Glu | Gly  | Ser | Pro | Ala |
| 1040 |     |     |     |     |     | 1045 |     |     |     |     | 1050 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ser | Pro | Thr | Ser | Thr | Glu  | Glu | Gly | Thr | Ser | Thr  | Glu | Pro | Ser |
| 1055 |     |     |     |     |     | 1060 |     |     |     |     | 1065 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Gly | Ser | Ala | Pro | Gly | Thr  | Ser | Glu | Ser | Ala | Thr  | Pro | Glu | Ser |
| 1070 |     |     |     |     |     | 1075 |     |     |     |     | 1080 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Pro | Gly | Thr | Ser | Glu | Ser  | Ala | Thr | Pro | Glu | Ser  | Gly | Pro | Gly |
| 1085 |     |     |     |     |     | 1090 |     |     |     |     | 1095 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Ser | Glu | Ser | Ala | Thr | Pro  | Glu | Ser | Gly | Pro | Gly  | Ser | Glu | Pro |
| 1100 |     |     |     |     |     | 1105 |     |     |     |     | 1110 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Thr | Ser | Gly | Ser | Glu | Thr  | Pro | Gly | Ser | Glu | Pro  | Ala | Thr | Ser |
| 1115 |     |     |     |     |     | 1120 |     |     |     |     | 1125 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ser | Glu | Thr | Pro | Gly | Ser  | Pro | Ala | Gly | Ser | Pro  | Thr | Ser | Thr |
| 1130 |     |     |     |     |     | 1135 |     |     |     |     | 1140 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Glu | Gly | Thr | Ser | Thr | Glu  | Pro | Ser | Glu | Gly | Ser  | Ala | Pro | Gly |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     | 1155 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Ser | Thr | Glu | Pro | Ser | Glu  | Gly | Ser | Ala | Pro | Gly  | Ser | Glu | Pro |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Thr | Ser | Gly | Ser | Glu | Thr  | Pro | Gly | Thr | Ser | Glu  | Ser | Ala | Thr |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Pro  | Glu | Ser | Gly | Pro | Gly | Thr  | Ser | Thr | Glu | Pro | Ser  | Glu | Gly | Ser |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     | 1200 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Pro | Ala | Ser | Ser | Pro | Pro  | Val | Leu | Lys | Arg | His  | Gln | Ala | Glu |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     | 1215 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Thr | Arg | Thr | Thr | Leu | Gln  | Ser | Asp | Gln | Glu | Glu  | Ile | Asp | Tyr |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Asp | Thr | Ile | Ser | Val | Glu  | Met | Lys | Lys | Glu | Asp  | Phe | Asp | Ile |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Asp | Glu | Asp | Glu | Asn | Gln  | Ser | Pro | Arg | Ser | Phe  | Gln | Lys | Lys |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Arg | His | Tyr | Phe | Ile | Ala  | Ala | Val | Glu | Arg | Leu  | Trp | Asp | Tyr |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     | 1275 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Met | Ser | Ser | Ser | Pro | His  | Val | Leu | Arg | Asn | Arg  | Ala | Gln | Ser |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     | 1290 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ser | Val | Pro | Gln | Phe | Lys  | Lys | Val | Val | Phe | Gln  | Glu | Phe | Thr |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     | 1305 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Gly | Ser | Phe | Thr | Gln | Pro  | Leu | Tyr | Arg | Gly | Glu  | Leu | Asn | Glu |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     | 1320 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| His  | Leu | Gly | Leu | Leu | Gly | Pro  | Tyr | Ile | Arg | Ala | Glu  | Val | Glu | Asp |
| 1325 |     |     |     |     |     | 1330 |     |     |     |     | 1335 |     |     |     |

|         |                     |                     |             |
|---------|---------------------|---------------------|-------------|
| Asn Ile | Met Val Thr Phe Arg | Asn Gln Ala Ser Arg | Pro Tyr Ser |
| 1340    | 1345                | 1350                |             |
|         |                     |                     |             |
| Phe Tyr | Ser Ser Leu Ile Ser | Tyr Glu Glu Asp Gln | Arg Gln Gly |
| 1355    | 1360                | 1365                |             |
|         |                     |                     |             |
| Ala Glu | Pro Arg Lys Asn Phe | Val Lys Pro Asn Glu | Thr Lys Thr |
| 1370    | 1375                | 1380                |             |
|         |                     |                     |             |
| Tyr Phe | Trp Lys Val Gln His | His Met Ala Pro Thr | Lys Asp Glu |
| 1385    | 1390                | 1395                |             |
|         |                     |                     |             |
| Phe Asp | Cys Lys Ala Trp Ala | Tyr Phe Ser Asp Val | Asp Leu Glu |
| 1400    | 1405                | 1410                |             |
|         |                     |                     |             |
| Lys Asp | Val His Ser Gly Leu | Ile Gly Pro Leu Leu | Val Cys His |
| 1415    | 1420                | 1425                |             |
|         |                     |                     |             |
| Thr Asn | Thr Leu Asn Pro Ala | His Gly Arg Gln Val | Thr Val Gln |
| 1430    | 1435                | 1440                |             |
|         |                     |                     |             |
| Glu Phe | Ala Leu Phe Phe Thr | Ile Phe Asp Glu Thr | Lys Ser Trp |
| 1445    | 1450                | 1455                |             |
|         |                     |                     |             |
| Tyr Phe | Thr Glu Asn Met Glu | Arg Asn Cys Arg Ala | Pro Cys Asn |
| 1460    | 1465                | 1470                |             |
|         |                     |                     |             |
| Ile Gln | Met Glu Asp Pro Thr | Phe Lys Glu Asn Tyr | Arg Phe His |
| 1475    | 1480                | 1485                |             |

|         |                     |                     |             |
|---------|---------------------|---------------------|-------------|
| Ala Ile | Asn Gly Tyr Ile Met | Asp Thr Leu Pro Gly | Leu Val Met |
| 1490    | 1495                | 1500                |             |
|         |                     |                     |             |
| Ala Gln | Asp Gln Arg Ile Arg | Trp Tyr Leu Leu Ser | Met Gly Ser |
| 1505    | 1510                | 1515                |             |
|         |                     |                     |             |
| Asn Glu | Asn Ile His Ser Ile | His Phe Ser Gly His | Val Phe Thr |
| 1520    | 1525                | 1530                |             |
|         |                     |                     |             |
| Val Arg | Lys Lys Glu Glu Tyr | Lys Met Ala Leu Tyr | Asn Leu Tyr |
| 1535    | 1540                | 1545                |             |
|         |                     |                     |             |
| Pro Gly | Val Phe Glu Thr Val | Glu Met Leu Pro Ser | Lys Ala Gly |
| 1550    | 1555                | 1560                |             |
|         |                     |                     |             |
| Ile Trp | Arg Val Glu Cys Leu | Ile Gly Glu His Leu | His Ala Gly |
| 1565    | 1570                | 1575                |             |
|         |                     |                     |             |
| Met Ser | Thr Leu Phe Leu Val | Tyr Ser Asn Lys Cys | Gln Thr Pro |
| 1580    | 1585                | 1590                |             |
|         |                     |                     |             |
| Leu Gly | Met Ala Ser Gly His | Ile Arg Asp Phe Gln | Ile Thr Ala |
| 1595    | 1600                | 1605                |             |
|         |                     |                     |             |
| Ser Gly | Gln Tyr Gly Gln Trp | Ala Pro Lys Leu Ala | Arg Leu His |
| 1610    | 1615                | 1620                |             |
|         |                     |                     |             |
| Tyr Ser | Gly Ser Ile Asn Ala | Trp Ser Thr Lys Glu | Pro Phe Ser |
| 1625    | 1630                | 1635                |             |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Trp  | Ile | Lys | Val | Asp | Leu | Leu  | Ala | Pro | Met | Ile | Ile  | His | Gly | Ile |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Thr | Gln | Gly | Ala | Arg | Gln  | Lys | Phe | Ser | Ser | Leu  | Tyr | Ile | Ser |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Phe | Ile | Ile | Met | Tyr | Ser  | Leu | Asp | Gly | Lys | Lys  | Trp | Gln | Thr |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Arg | Gly | Asn | Ser | Thr | Gly  | Thr | Leu | Met | Val | Phe  | Phe | Gly | Asn |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Asp | Ser | Ser | Gly | Ile | Lys  | His | Asn | Ile | Phe | Asn  | Pro | Pro | Ile |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Ala | Arg | Tyr | Ile | Arg | Leu  | His | Pro | Thr | His | Tyr  | Ser | Ile | Arg |
| 1715 |     |     |     |     |     | 1720 |     |     |     |     | 1725 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Thr | Leu | Arg | Met | Glu | Leu  | Met | Gly | Cys | Asp | Leu  | Asn | Ser | Cys |
| 1730 |     |     |     |     |     | 1735 |     |     |     |     | 1740 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Met | Pro | Leu | Gly | Met | Glu  | Ser | Lys | Ala | Ile | Ser  | Asp | Ala | Gln |
| 1745 |     |     |     |     |     | 1750 |     |     |     |     | 1755 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Thr | Ala | Ser | Ser | Tyr | Phe  | Thr | Asn | Met | Phe | Ala  | Thr | Trp | Ser |
| 1760 |     |     |     |     |     | 1765 |     |     |     |     | 1770 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Ser | Lys | Ala | Arg | Leu | His  | Leu | Gln | Gly | Arg | Ser  | Asn | Ala | Trp |
| 1775 |     |     |     |     |     | 1780 |     |     |     |     | 1785 |     |     |     |



|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Arg  | Pro | Gln | Val | Asn | Asn | Pro  | Lys | Glu | Trp | Leu | Gln  | Val | Asp | Phe |
| 1790 |     |     |     |     |     | 1795 |     |     |     |     | 1800 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Lys | Thr | Met | Lys | Val | Thr  | Gly | Val | Thr | Thr | Gln  | Gly | Val | Lys |
| 1805 |     |     |     |     |     | 1810 |     |     |     |     | 1815 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Leu | Leu | Thr | Ser | Met | Tyr  | Val | Lys | Glu | Phe | Leu  | Ile | Ser | Ser |
| 1820 |     |     |     |     |     | 1825 |     |     |     |     | 1830 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gln | Asp | Gly | His | Gln | Trp  | Thr | Leu | Phe | Phe | Gln  | Asn | Gly | Lys |
| 1835 |     |     |     |     |     | 1840 |     |     |     |     | 1845 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Lys | Val | Phe | Gln | Gly | Asn  | Gln | Asp | Ser | Phe | Thr  | Pro | Val | Val |
| 1850 |     |     |     |     |     | 1855 |     |     |     |     | 1860 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Ser | Leu | Asp | Pro | Pro | Leu  | Leu | Thr | Arg | Tyr | Leu  | Arg | Ile | His |
| 1865 |     |     |     |     |     | 1870 |     |     |     |     | 1875 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Gln | Ser | Trp | Val | His | Gln  | Ile | Ala | Leu | Arg | Met  | Glu | Val | Leu |
| 1880 |     |     |     |     |     | 1885 |     |     |     |     | 1890 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Cys | Glu | Ala | Gln | Asp | Leu  | Tyr |     |     |     |      |     |     |     |
| 1895 |     |     |     |     |     | 1900 |     |     |     |     |      |     |     |     |

&lt;210&gt; 118

&lt;211&gt; 1515

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; pSYN VWF 031 protein sequence

&lt;400&gt; 118

Met Ile Pro Ala Arg Phe Ala Gly Val Leu Leu Ala Leu Ala Leu Ile  
1 5 10 15

Leu Pro Gly Thr Leu Cys Ala Glu Gly Thr Arg Gly Arg Ser Ser Thr  
20 25 30

Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly  
35 40 45

Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly  
50 55 60

Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys  
65 70 75 80

Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu  
85 90 95

Phe Val Asn Gly Thr Val Thr Gln Gly Asp Gln Arg Val Ser Met Pro  
100 105 110

Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys  
115 120 125

Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly  
130 135 140

Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly

145 150 155 160

Leu Cys Gly Asn Phe Asn Ile Phe Ala Glu Asp Asp Phe Met Thr Gln  
165 170 175

Glu Gly Thr Leu Thr Ser Asp Pro Tyr Asp Phe Ala Asn Ser Trp Ala  
180 185 190

Leu Ser Ser Gly Glu Gln Trp Cys Glu Arg Ala Ser Pro Pro Ser Ser  
195 200 205

Ser Cys Asn Ile Ser Ser Gly Glu Met Gln Lys Gly Leu Trp Glu Gln  
210 215 220

Cys Gln Leu Leu Lys Ser Thr Ser Val Phe Ala Arg Cys His Pro Leu  
225 230 235 240

Val Asp Pro Glu Pro Phe Val Ala Leu Cys Glu Lys Thr Leu Cys Glu  
245 250 255

Cys Ala Gly Gly Leu Glu Cys Ala Cys Pro Ala Leu Leu Glu Tyr Ala  
260 265 270

Arg Thr Cys Ala Gln Glu Gly Met Val Leu Tyr Gly Trp Thr Asp His  
275 280 285

Ser Ala Cys Ser Pro Val Cys Pro Ala Gly Met Glu Tyr Arg Gln Cys  
290 295 300

Val Ser Pro Cys Ala Arg Thr Cys Gln Ser Leu His Ile Asn Glu Met

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 305 |     |     |     |     | 310 |     |     |     |     |     | 315 |     |     |     |     | 320 |
| Cys | Gln | Glu | Arg | Cys | Val | Asp | Gly | Cys | Ser | Cys | Pro | Glu | Gly | Gln | Leu |     |
|     |     |     |     | 325 |     |     |     | 330 |     |     |     |     |     | 335 |     |     |
| Leu | Asp | Glu | Gly | Leu | Cys | Val | Glu | Ser | Thr | Glu | Cys | Pro | Cys | Val | His |     |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     |     | 350 |     |     |
| Ser | Gly | Lys | Arg | Tyr | Pro | Pro | Gly | Thr | Ser | Leu | Ser | Arg | Asp | Cys | Asn |     |
|     |     | 355 |     |     |     |     |     | 360 |     |     |     |     |     | 365 |     |     |
| Thr | Cys | Ile | Cys | Arg | Asn | Ser | Gln | Trp | Ile | Cys | Ser | Asn | Glu | Glu | Cys |     |
|     | 370 |     |     |     |     |     |     | 375 |     |     |     |     |     | 380 |     |     |
| Pro | Gly | Glu | Cys | Leu | Val | Thr | Gly | Gln | Ser | His | Phe | Lys | Ser | Phe | Asp |     |
| 385 |     |     |     |     |     |     |     | 390 |     |     |     |     |     | 395 |     | 400 |
| Asn | Arg | Tyr | Phe | Thr | Phe | Ser | Gly | Ile | Cys | Gln | Tyr | Leu | Leu | Ala | Arg |     |
|     |     |     |     | 405 |     |     |     |     |     |     |     |     |     | 410 |     | 415 |
| Asp | Cys | Gln | Asp | His | Ser | Phe | Ser | Ile | Val | Ile | Glu | Thr | Val | Gln | Cys |     |
|     |     |     | 420 |     |     |     |     |     |     |     |     |     |     | 425 |     | 430 |
| Ala | Asp | Asp | Arg | Asp | Ala | Val | Cys | Thr | Arg | Ser | Val | Thr | Val | Arg | Leu |     |
|     |     | 435 |     |     |     |     |     |     |     |     |     |     |     | 440 |     | 445 |
| Pro | Gly | Leu | His | Asn | Ser | Leu | Val | Lys | Leu | Lys | His | Gly | Ala | Gly | Val |     |
|     | 450 |     |     |     |     |     |     |     |     |     |     |     |     | 455 |     | 460 |
| Ala | Met | Asp | Gly | Gln | Asp | Ile | Gln | Leu | Pro | Leu | Leu | Lys | Gly | Asp | Leu |     |

465 470 475 480

Arg Ile Gln His Thr Val Thr Ala Ser Val Arg Leu Ser Tyr Gly Glu  
485 490 495

Asp Leu Gln Met Asp Trp Asp Gly Arg Gly Arg Leu Leu Val Lys Leu  
500 505 510

Ser Pro Val Tyr Ala Gly Lys Thr Cys Gly Leu Cys Gly Asn Tyr Asn  
515 520 525

Gly Asn Gln Gly Asp Asp Phe Leu Thr Pro Ser Gly Leu Ala Glu Pro  
530 535 540

Arg Val Glu Asp Phe Gly Asn Ala Trp Lys Leu His Gly Asp Cys Gln  
545 550 555 560

Asp Leu Gln Lys Gln His Ser Asp Pro Cys Ala Leu Asn Pro Arg Met  
565 570 575

Thr Arg Phe Ser Glu Glu Ala Cys Ala Val Leu Thr Ser Pro Thr Phe  
580 585 590

Glu Ala Cys His Arg Ala Val Ser Pro Leu Pro Tyr Leu Arg Asn Cys  
595 600 605

Arg Tyr Asp Val Cys Ser Cys Ser Asp Gly Arg Glu Cys Leu Cys Gly  
610 615 620

Ala Leu Ala Ser Tyr Ala Ala Ala Cys Ala Gly Arg Gly Val Arg Val

625 630 635 640

Ala Trp Arg Glu Pro Gly Arg Cys Glu Leu Asn Cys Pro Lys Gly Gln  
645 650 655

Val Tyr Leu Gln Cys Gly Thr Pro Cys Asn Leu Thr Cys Arg Ser Leu  
660 665 670

Ser Tyr Pro Asp Glu Glu Cys Asn Glu Ala Cys Leu Glu Gly Cys Phe  
675 680 685

Cys Pro Pro Gly Leu Tyr Met Asp Glu Arg Gly Asp Cys Val Pro Lys  
690 695 700

Ala Gln Cys Pro Cys Tyr Tyr Asp Gly Glu Ile Phe Gln Pro Glu Asp  
705 710 715 720

Ile Phe Ser Asp His His Thr Met Cys Tyr Cys Glu Asp Gly Phe Met  
725 730 735

His Cys Thr Met Ser Gly Val Pro Gly Ser Leu Leu Pro Asp Ala Val  
740 745 750

Leu Ser Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg  
755 760 765

Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp Asn Leu Arg Ala Glu  
770 775 780

Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr Asp Leu Glu Cys Met

785 790 795 800

Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro Pro Gly Met Val Arg  
805 810 815

His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys Pro Cys Phe His Gln  
820 825 830

Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr  
835 840 845

Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp  
850 855 860

Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly  
865 870 875 880

Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp  
885 890 895

Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys  
900 905 910

Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu  
915 920 925

Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys  
930 935 940

Arg Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Arg

|     |     |     |      |     |     |     |     |     |      |     |     |     |     |     |      |
|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|
| 945 | 950 | 955 | 960  |     |     |     |     |     |      |     |     |     |     |     |      |
| Tyr | Ile | Ile | Leu  | Leu | Leu | Gly | Lys | Ala | Leu  | Ser | Val | Val | Trp | Asp | Arg  |
|     |     |     | 965  |     |     |     |     |     | 970  |     |     |     |     |     | 975  |
| His | Leu | Ser | Ile  | Ser | Val | Val | Leu | Lys | Gln  | Thr | Tyr | Gln | Glu | Lys | Val  |
|     |     |     | 980  |     |     |     |     |     | 985  |     |     |     |     |     | 990  |
| Cys | Gly | Leu | Cys  | Gly | Asn | Phe | Asp | Gly | Ile  | Gln | Asn | Asn | Asp | Leu | Thr  |
|     |     |     | 995  |     |     |     |     |     | 1000 |     |     |     |     |     | 1005 |
| Ser | Ser | Asn | Leu  | Gln | Val | Glu | Glu | Asp | Pro  | Val | Asp | Phe | Gly | Asn |      |
|     |     |     | 1010 |     |     |     |     |     | 1015 |     |     |     |     |     | 1020 |
| Ser | Trp | Lys | Val  | Ser | Ser | Gln | Cys | Ala | Asp  | Thr | Arg | Lys | Val | Pro |      |
|     |     |     | 1025 |     |     |     |     |     | 1030 |     |     |     |     |     | 1035 |
| Leu | Asp | Ser | Ser  | Pro | Ala | Thr | Cys | His | Asn  | Asn | Ile | Met | Lys | Gln |      |
|     |     |     | 1040 |     |     |     |     |     | 1045 |     |     |     |     |     | 1050 |
| Thr | Met | Val | Asp  | Ser | Ser | Cys | Arg | Ile | Leu  | Thr | Ser | Asp | Val | Phe |      |
|     |     |     | 1055 |     |     |     |     |     | 1060 |     |     |     |     |     | 1065 |
| Gln | Asp | Cys | Asn  | Lys | Leu | Val | Asp | Pro | Glu  | Pro | Tyr | Leu | Asp | Val |      |
|     |     |     | 1070 |     |     |     |     |     | 1075 |     |     |     |     |     | 1080 |
| Cys | Ile | Tyr | Asp  | Thr | Cys | Ser | Cys | Glu | Ser  | Ile | Gly | Asp | Cys | Ala |      |
|     |     |     | 1085 |     |     |     |     |     | 1090 |     |     |     |     |     | 1095 |
| Ala | Phe | Cys | Asp  | Thr | Ile | Ala | Ala | Tyr | Ala  | His | Val | Cys | Ala | Gln |      |



1100

1105

1110

His Gly Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln

1115

1120

1125

Ser Cys Glu Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Ala Glu

1130

1135

1140

Trp Arg Tyr Asn Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln

1145

1150

1155

His Pro Glu Pro Leu Ala Cys Pro Val Gln Cys Val Glu Gly Cys

1160

1165

1170

His Ala His Cys Pro Pro Gly Lys Ile Leu Asp Glu Leu Leu Gln

1175

1180

1185

Thr Cys Val Asp Pro Glu Asp Cys Pro Val Cys Glu Val Ala Gly

1190

1195

1200

Arg Arg Phe Ala Ser Gly Lys Lys Val Thr Leu Asn Pro Ser Asp

1205

1210

1215

Pro Glu His Cys Gln Ile Cys His Cys Asp Val Val Asn Leu Thr

1220

1225

1230

Cys Glu Ala Cys Gln Glu Pro Ile Ser Gly Gly Gly Gly Ser Gly

1235

1240

1245

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly

1250

1255

1260

Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Val Pro Arg Gly Ser  
 1265 1270 1275

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Lys Thr His Thr  
 1280 1285 1290

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val  
 1295 1300 1305

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg  
 1310 1315 1320

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp  
 1325 1330 1335

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 1340 1345 1350

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 1355 1360 1365

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn  
 1370 1375 1380

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala  
 1385 1390 1395

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu

1400

1405

1410

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys  
 1415 1420 1425

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser  
 1430 1435 1440

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn  
 1445 1450 1455

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe  
 1460 1465 1470

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly  
 1475 1480 1485

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His  
 1490 1495 1500

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
 1505 1510 1515

&lt;210&gt; 119

&lt;211&gt; 1778

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; pSYN VWF 034 protein sequence

&lt;400&gt; 119

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Met Ile Pro Ala Arg Phe Ala Gly Val Leu Leu Ala Leu Ala Leu Ile
1           5           10           15

Leu Pro Gly Thr Leu Cys Ala Glu Gly Thr Arg Gly Arg Ser Ser Thr
           20           25           30

Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly
           35           40           45

Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly
50           55           60

Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys
65           70           75           80

Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu
           85           90           95

Phe Val Asn Gly Thr Val Thr Gln Gly Asp Gln Arg Val Ser Met Pro
           100          105          110

Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys
           115          120          125

Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly
130          135          140

Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly
145          150          155          160

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Leu Cys Gly Asn Phe Asn Ile Phe Ala Glu Asp Asp Phe Met Thr Gln

165

170

175

Glu Gly Thr Leu Thr Ser Asp Pro Tyr Asp Phe Ala Asn Ser Trp Ala

180

185

190

Leu Ser Ser Gly Glu Gln Trp Cys Glu Arg Ala Ser Pro Pro Ser Ser

195

200

205

Ser Cys Asn Ile Ser Ser Gly Glu Met Gln Lys Gly Leu Trp Glu Gln

210

215

220

Cys Gln Leu Leu Lys Ser Thr Ser Val Phe Ala Arg Cys His Pro Leu

225

230

235

240

Val Asp Pro Glu Pro Phe Val Ala Leu Cys Glu Lys Thr Leu Cys Glu

245

250

255

Cys Ala Gly Gly Leu Glu Cys Ala Cys Pro Ala Leu Leu Glu Tyr Ala

260

265

270

Arg Thr Cys Ala Gln Glu Gly Met Val Leu Tyr Gly Trp Thr Asp His

275

280

285

Ser Ala Cys Ser Pro Val Cys Pro Ala Gly Met Glu Tyr Arg Gln Cys

290

295

300

Val Ser Pro Cys Ala Arg Thr Cys Gln Ser Leu His Ile Asn Glu Met

305

310

315

320



|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Ile | Gln | His | Thr | Val | Thr | Ala | Ser | Val | Arg | Leu | Ser | Tyr | Gly | Glu |
|     |     |     |     | 485 |     |     |     |     | 490 |     |     |     |     | 495 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Leu | Gln | Met | Asp | Trp | Asp | Gly | Arg | Gly | Arg | Leu | Leu | Val | Lys | Leu |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     |     | 510 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Pro | Val | Tyr | Ala | Gly | Lys | Thr | Cys | Gly | Leu | Cys | Gly | Asn | Tyr | Asn |
|     |     | 515 |     |     |     |     | 520 |     |     |     |     | 525 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Asn | Gln | Gly | Asp | Asp | Phe | Leu | Thr | Pro | Ser | Gly | Leu | Ala | Glu | Pro |
|     | 530 |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Val | Glu | Asp | Phe | Gly | Asn | Ala | Trp | Lys | Leu | His | Gly | Asp | Cys | Gln |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Leu | Gln | Lys | Gln | His | Ser | Asp | Pro | Cys | Ala | Leu | Asn | Pro | Arg | Met |
|     |     |     |     | 565 |     |     |     |     |     | 570 |     |     |     | 575 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr | Arg | Phe | Ser | Glu | Glu | Ala | Cys | Ala | Val | Leu | Thr | Ser | Pro | Thr | Phe |
|     |     |     | 580 |     |     |     |     |     | 585 |     |     |     | 590 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Ala | Cys | His | Arg | Ala | Val | Ser | Pro | Leu | Pro | Tyr | Leu | Arg | Asn | Cys |
|     |     | 595 |     |     |     |     |     | 600 |     |     |     | 605 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Tyr | Asp | Val | Cys | Ser | Cys | Ser | Asp | Gly | Arg | Glu | Cys | Leu | Cys | Gly |
|     | 610 |     |     |     |     | 615 |     |     |     |     | 620 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Leu | Ala | Ser | Tyr | Ala | Ala | Ala | Cys | Ala | Gly | Arg | Gly | Val | Arg | Val |
| 625 |     |     |     |     | 630 |     |     |     |     | 635 |     |     |     | 640 |     |





|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Met | Gly | Cys | Val | Ser | Gly | Cys | Leu | Cys | Pro | Pro | Gly | Met | Val | Arg |
|     |     |     |     | 805 |     |     |     |     | 810 |     |     |     |     | 815 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| His | Glu | Asn | Arg | Cys | Val | Ala | Leu | Glu | Arg | Cys | Pro | Cys | Phe | His | Gln |
|     |     |     |     | 820 |     |     |     |     | 825 |     |     |     |     | 830 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Lys | Glu | Tyr | Ala | Pro | Gly | Glu | Thr | Val | Lys | Ile | Gly | Cys | Asn | Thr |
|     |     |     |     | 835 |     |     |     |     | 840 |     |     |     |     | 845 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cys | Val | Cys | Arg | Asp | Arg | Lys | Trp | Asn | Cys | Thr | Asp | His | Val | Cys | Asp |
|     |     |     |     | 850 |     |     |     |     | 855 |     |     |     |     | 860 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Thr | Cys | Ser | Thr | Ile | Gly | Met | Ala | His | Tyr | Leu | Thr | Phe | Asp | Gly |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 865 |     |     |     |     | 870 |     |     |     |     | 875 |     |     |     |     | 880 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Lys | Tyr | Leu | Phe | Pro | Gly | Glu | Cys | Gln | Tyr | Val | Leu | Val | Gln | Asp |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 885 |     |     |     |     | 890 |     |     |     |     | 895 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Cys | Gly | Ser | Asn | Pro | Gly | Thr | Phe | Arg | Ile | Leu | Val | Gly | Asn | Lys |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 900 |     |     |     |     | 905 |     |     |     |     | 910 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gly | Cys | Ser | His | Pro | Ser | Val | Lys | Cys | Lys | Lys | Arg | Val | Thr | Ile | Leu |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 915 |     |     |     |     | 920 |     |     |     |     | 925 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val | Glu | Gly | Gly | Glu | Ile | Glu | Leu | Phe | Asp | Gly | Glu | Val | Asn | Val | Lys |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     | 930 |     |     |     |     | 935 |     |     |     |     | 940 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Pro | Met | Lys | Asp | Glu | Thr | His | Phe | Glu | Val | Val | Glu | Ser | Gly | Arg |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| 945 |     |     |     |     | 950 |     |     |     |     | 955 |     |     |     |     | 960 |

|     |     |     |      |     |     |     |     |     |      |     |     |     |     |      |     |
|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|
| Tyr | Ile | Ile | Leu  | Leu | Leu | Gly | Lys | Ala | Leu  | Ser | Val | Val | Trp | Asp  | Arg |
|     |     |     | 965  |     |     |     |     |     | 970  |     |     |     |     | 975  |     |
|     |     |     |      |     |     |     |     |     |      |     |     |     |     |      |     |
| His | Leu | Ser | Ile  | Ser | Val | Val | Leu | Lys | Gln  | Thr | Tyr | Gln | Glu | Lys  | Val |
|     |     |     | 980  |     |     |     |     |     | 985  |     |     |     |     | 990  |     |
|     |     |     |      |     |     |     |     |     |      |     |     |     |     |      |     |
| Cys | Gly | Leu | Cys  | Gly | Asn | Phe | Asp | Gly | Ile  | Gln | Asn | Asn | Asp | Leu  | Thr |
|     |     |     | 995  |     |     |     |     |     | 1000 |     |     |     |     | 1005 |     |
|     |     |     |      |     |     |     |     |     |      |     |     |     |     |      |     |
| Ser | Ser | Asn | Leu  | Gln | Val | Glu | Glu | Asp | Pro  | Val | Asp | Phe | Gly | Asn  |     |
|     |     |     | 1010 |     |     |     |     |     | 1015 |     |     |     |     | 1020 |     |
|     |     |     |      |     |     |     |     |     |      |     |     |     |     |      |     |
| Ser | Trp | Lys | Val  | Ser | Ser | Gln | Cys | Ala | Asp  | Thr | Arg | Lys | Val | Pro  |     |
|     |     |     | 1025 |     |     |     |     |     | 1030 |     |     |     |     | 1035 |     |
|     |     |     |      |     |     |     |     |     |      |     |     |     |     |      |     |
| Leu | Asp | Ser | Ser  | Pro | Ala | Thr | Cys | His | Asn  | Asn | Ile | Met | Lys | Gln  |     |
|     |     |     | 1040 |     |     |     |     |     | 1045 |     |     |     |     | 1050 |     |
|     |     |     |      |     |     |     |     |     |      |     |     |     |     |      |     |
| Thr | Met | Val | Asp  | Ser | Ser | Cys | Arg | Ile | Leu  | Thr | Ser | Asp | Val | Phe  |     |
|     |     |     | 1055 |     |     |     |     |     | 1060 |     |     |     |     | 1065 |     |
|     |     |     |      |     |     |     |     |     |      |     |     |     |     |      |     |
| Gln | Asp | Cys | Asn  | Lys | Leu | Val | Asp | Pro | Glu  | Pro | Tyr | Leu | Asp | Val  |     |
|     |     |     | 1070 |     |     |     |     |     | 1075 |     |     |     |     | 1080 |     |
|     |     |     |      |     |     |     |     |     |      |     |     |     |     |      |     |
| Cys | Ile | Tyr | Asp  | Thr | Cys | Ser | Cys | Glu | Ser  | Ile | Gly | Asp | Cys | Ala  |     |
|     |     |     | 1085 |     |     |     |     |     | 1090 |     |     |     |     | 1095 |     |
|     |     |     |      |     |     |     |     |     |      |     |     |     |     |      |     |
| Ala | Phe | Cys | Asp  | Thr | Ile | Ala | Ala | Tyr | Ala  | His | Val | Cys | Ala | Gln  |     |
|     |     |     | 1100 |     |     |     |     |     | 1105 |     |     |     |     | 1110 |     |

|         |                     |                     |             |
|---------|---------------------|---------------------|-------------|
| His Gly | Lys Val Val Thr Trp | Arg Thr Ala Thr Leu | Cys Pro Gln |
| 1115    | 1120                | 1125                |             |
|         |                     |                     |             |
| Ser Cys | Glu Glu Arg Asn Leu | Arg Glu Asn Gly Tyr | Glu Ala Glu |
| 1130    | 1135                | 1140                |             |
|         |                     |                     |             |
| Trp Arg | Tyr Asn Ser Cys Ala | Pro Ala Cys Gln Val | Thr Cys Gln |
| 1145    | 1150                | 1155                |             |
|         |                     |                     |             |
| His Pro | Glu Pro Leu Ala Cys | Pro Val Gln Cys Val | Glu Gly Cys |
| 1160    | 1165                | 1170                |             |
|         |                     |                     |             |
| His Ala | His Cys Pro Pro Gly | Lys Ile Leu Asp Glu | Leu Leu Gln |
| 1175    | 1180                | 1185                |             |
|         |                     |                     |             |
| Thr Cys | Val Asp Pro Glu Asp | Cys Pro Val Cys Glu | Val Ala Gly |
| 1190    | 1195                | 1200                |             |
|         |                     |                     |             |
| Arg Arg | Phe Ala Ser Gly Lys | Lys Val Thr Leu Asn | Pro Ser Asp |
| 1205    | 1210                | 1215                |             |
|         |                     |                     |             |
| Pro Glu | His Cys Gln Ile Cys | His Cys Asp Val Val | Asn Leu Thr |
| 1220    | 1225                | 1230                |             |
|         |                     |                     |             |
| Cys Glu | Ala Cys Gln Glu Pro | Ile Ser Gly Thr Ser | Glu Ser Ala |
| 1235    | 1240                | 1245                |             |
|         |                     |                     |             |
| Thr Pro | Glu Ser Gly Pro Gly | Ser Glu Pro Ala Thr | Ser Gly Ser |
| 1250    | 1255                | 1260                |             |

|         |                     |                         |             |
|---------|---------------------|-------------------------|-------------|
| Glu Thr | Pro Gly Thr Ser     | Glu Ser Ala Thr Pro Glu | Ser Gly Pro |
| 1265    |                     | 1270                    | 1275        |
|         |                     |                         |             |
| Gly Ser | Glu Pro Ala Thr Ser | Gly Ser Glu Thr Pro     | Gly Thr Ser |
| 1280    |                     | 1285                    | 1290        |
|         |                     |                         |             |
| Glu Ser | Ala Thr Pro Glu Ser | Gly Pro Gly Thr Ser     | Thr Glu Pro |
| 1295    |                     | 1300                    | 1305        |
|         |                     |                         |             |
| Ser Glu | Gly Ser Ala Pro Gly | Ser Pro Ala Gly Ser     | Pro Thr Ser |
| 1310    |                     | 1315                    | 1320        |
|         |                     |                         |             |
| Thr Glu | Glu Gly Thr Ser Glu | Ser Ala Thr Pro Glu     | Ser Gly Pro |
| 1325    |                     | 1330                    | 1335        |
|         |                     |                         |             |
| Gly Ser | Glu Pro Ala Thr Ser | Gly Ser Glu Thr Pro     | Gly Thr Ser |
| 1340    |                     | 1345                    | 1350        |
|         |                     |                         |             |
| Glu Ser | Ala Thr Pro Glu Ser | Gly Pro Gly Ser Pro     | Ala Gly Ser |
| 1355    |                     | 1360                    | 1365        |
|         |                     |                         |             |
| Pro Thr | Ser Thr Glu Glu Gly | Ser Pro Ala Gly Ser     | Pro Thr Ser |
| 1370    |                     | 1375                    | 1380        |
|         |                     |                         |             |
| Thr Glu | Glu Gly Thr Ser Thr | Glu Pro Ser Glu Gly     | Ser Ala Pro |
| 1385    |                     | 1390                    | 1395        |
|         |                     |                         |             |
| Gly Thr | Ser Glu Ser Ala Thr | Pro Glu Ser Gly Pro     | Gly Thr Ser |
| 1400    |                     | 1405                    | 1410        |

|         |         |         |         |         |         |         |         |
|---------|---------|---------|---------|---------|---------|---------|---------|
| Glu Ser | Ala Thr | Pro Glu | Ser Gly | Pro Gly | Thr Ser | Glu Ser | Ala     |
| 1415    |         |         | 1420    |         |         | 1425    |         |
|         |         |         |         |         |         |         |         |
| Thr Pro | Glu Ser | Gly Pro | Gly Ser | Glu Pro | Ala Thr | Ser Gly | Ser     |
| 1430    |         |         | 1435    |         |         | 1440    |         |
|         |         |         |         |         |         |         |         |
| Glu Thr | Pro Gly | Ser Glu | Pro Ala | Thr Ser | Gly Ser | Glu Thr | Pro     |
| 1445    |         |         | 1450    |         |         | 1455    |         |
|         |         |         |         |         |         |         |         |
| Gly Ser | Pro Ala | Gly Ser | Pro Thr | Ser Thr | Glu Glu | Gly Thr | Ser     |
| 1460    |         |         | 1465    |         |         | 1470    |         |
|         |         |         |         |         |         |         |         |
| Thr Glu | Pro Ser | Glu Gly | Ser Ala | Pro Gly | Thr Ser | Thr Glu | Pro     |
| 1475    |         |         | 1480    |         |         | 1485    |         |
|         |         |         |         |         |         |         |         |
| Ser Glu | Gly Ser | Ala Pro | Gly Ser | Glu Pro | Ala Thr | Ser Gly | Ser     |
| 1490    |         |         | 1495    |         |         | 1500    |         |
|         |         |         |         |         |         |         |         |
| Glu Thr | Pro Gly | Thr Ser | Glu Ser | Ala Thr | Pro Glu | Ser Gly | Pro     |
| 1505    |         |         | 1510    |         |         | 1515    |         |
|         |         |         |         |         |         |         |         |
| Gly Thr | Ser Thr | Glu Pro | Ser Glu | Gly Ser | Ala Pro | Asp Ile | Gly     |
| 1520    |         |         | 1525    |         |         | 1530    |         |
|         |         |         |         |         |         |         |         |
| Gly Gly | Gly Gly | Ser Gly | Gly Gly | Gly Gly | Ser Leu | Val Pro | Arg Gly |
| 1535    |         |         | 1540    |         |         | 1545    |         |
|         |         |         |         |         |         |         |         |
| Ser Gly | Gly Asp | Lys Thr | His Thr | Cys Pro | Pro Cys | Pro Ala | Pro     |
| 1550    |         |         | 1555    |         |         | 1560    |         |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Glu  | Leu | Leu | Gly | Gly | Pro | Ser  | Val | Phe | Leu | Phe | Pro  | Pro | Lys | Pro |
| 1565 |     |     |     |     |     | 1570 |     |     |     |     | 1575 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Asp | Thr | Leu | Met | Ile | Ser  | Arg | Thr | Pro | Glu | Val  | Thr | Cys | Val |
| 1580 |     |     |     |     |     | 1585 |     |     |     |     | 1590 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Val | Asp | Val | Ser | His | Glu  | Asp | Pro | Glu | Val | Lys  | Phe | Asn | Trp |
| 1595 |     |     |     |     |     | 1600 |     |     |     |     | 1605 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Tyr  | Val | Asp | Gly | Val | Glu | Val  | His | Asn | Ala | Lys | Thr  | Lys | Pro | Arg |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Glu | Gln | Tyr | Asn | Ser | Thr  | Tyr | Arg | Val | Val | Ser  | Val | Leu | Thr |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Leu | His | Gln | Asp | Trp | Leu  | Asn | Gly | Lys | Glu | Tyr  | Lys | Cys | Lys |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Ser | Asn | Lys | Ala | Leu | Pro  | Ala | Pro | Ile | Glu | Lys  | Thr | Ile | Ser |
| 1655 |     |     |     |     |     | 1660 |     |     |     |     | 1665 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Ala | Lys | Gly | Gln | Pro | Arg  | Glu | Pro | Gln | Val | Tyr  | Thr | Leu | Pro |
| 1670 |     |     |     |     |     | 1675 |     |     |     |     | 1680 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Ser | Arg | Asp | Glu | Leu | Thr  | Lys | Asn | Gln | Val | Ser  | Leu | Thr | Cys |
| 1685 |     |     |     |     |     | 1690 |     |     |     |     | 1695 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Val | Lys | Gly | Phe | Tyr | Pro  | Ser | Asp | Ile | Ala | Val  | Glu | Trp | Glu |
| 1700 |     |     |     |     |     | 1705 |     |     |     |     | 1710 |     |     |     |

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 1715 1720 1725

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
 1730 1735 1740

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val  
 1745 1750 1755

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser  
 1760 1765 1770

Leu Ser Pro Gly Lys  
 1775

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<211> 1565

<212> PRT

<213> Artificial Sequence

<220>

<223> pSYN VWF 036 protein sequence

<400> 120

Met Ile Pro Ala Arg Phe Ala Gly Val Leu Leu Ala Leu Ala Leu Ile  
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Leu Pro Gly Thr Leu Cys Ala Glu Gly Thr Arg Gly Arg Ser Ser Thr  
 20 25 30

Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly

35

40

45

Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly  
 50 55 60

Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys  
 65 70 75 80

Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu  
 85 90 95

Phe Val Asn Gly Thr Val Thr Gln Gly Asp Gln Arg Val Ser Met Pro  
 100 105 110

Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys  
 115 120 125

Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly  
 130 135 140

Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly  
 145 150 155 160

Leu Cys Gly Asn Phe Asn Ile Phe Ala Glu Asp Asp Phe Met Thr Gln  
 165 170 175

Glu Gly Thr Leu Thr Ser Asp Pro Tyr Asp Phe Ala Asn Ser Trp Ala  
 180 185 190

Leu Ser Ser Gly Glu Gln Trp Cys Glu Arg Ala Ser Pro Pro Ser Ser



195

200

205

Ser Cys Asn Ile Ser Ser Gly Glu Met Gln Lys Gly Leu Trp Glu Gln  
 210 215 220

Cys Gln Leu Leu Lys Ser Thr Ser Val Phe Ala Arg Cys His Pro Leu  
 225 230 235 240

Val Asp Pro Glu Pro Phe Val Ala Leu Cys Glu Lys Thr Leu Cys Glu  
 245 250 255

Cys Ala Gly Gly Leu Glu Cys Ala Cys Pro Ala Leu Leu Glu Tyr Ala  
 260 265 270

Arg Thr Cys Ala Gln Glu Gly Met Val Leu Tyr Gly Trp Thr Asp His  
 275 280 285

Ser Ala Cys Ser Pro Val Cys Pro Ala Gly Met Glu Tyr Arg Gln Cys  
 290 295 300

Val Ser Pro Cys Ala Arg Thr Cys Gln Ser Leu His Ile Asn Glu Met  
 305 310 315 320

Cys Gln Glu Arg Cys Val Asp Gly Cys Ser Cys Pro Glu Gly Gln Leu  
 325 330 335

Leu Asp Glu Gly Leu Cys Val Glu Ser Thr Glu Cys Pro Cys Val His  
 340 345 350

Ser Gly Lys Arg Tyr Pro Pro Gly Thr Ser Leu Ser Arg Asp Cys Asn

355

360

365

Thr Cys Ile Cys Arg Asn Ser Gln Trp Ile Cys Ser Asn Glu Glu Cys  
370 375 380

Pro Gly Glu Cys Leu Val Thr Gly Gln Ser His Phe Lys Ser Phe Asp  
385 390 395 400

Asn Arg Tyr Phe Thr Phe Ser Gly Ile Cys Gln Tyr Leu Leu Ala Arg  
405 410 415

Asp Cys Gln Asp His Ser Phe Ser Ile Val Ile Glu Thr Val Gln Cys  
420 425 430

Ala Asp Asp Arg Asp Ala Val Cys Thr Arg Ser Val Thr Val Arg Leu  
435 440 445

Pro Gly Leu His Asn Ser Leu Val Lys Leu Lys His Gly Ala Gly Val  
450 455 460

Ala Met Asp Gly Gln Asp Ile Gln Leu Pro Leu Leu Lys Gly Asp Leu  
465 470 475 480

Arg Ile Gln His Thr Val Thr Ala Ser Val Arg Leu Ser Tyr Gly Glu  
485 490 495

Asp Leu Gln Met Asp Trp Asp Gly Arg Gly Arg Leu Leu Val Lys Leu  
500 505 510

Ser Pro Val Tyr Ala Gly Lys Thr Cys Gly Leu Cys Gly Asn Tyr Asn

515

520

525

Gly Asn Gln Gly Asp Asp Phe Leu Thr Pro Ser Gly Leu Ala Glu Pro  
530 535 540

Arg Val Glu Asp Phe Gly Asn Ala Trp Lys Leu His Gly Asp Cys Gln  
545 550 555 560

Asp Leu Gln Lys Gln His Ser Asp Pro Cys Ala Leu Asn Pro Arg Met  
565 570 575

Thr Arg Phe Ser Glu Glu Ala Cys Ala Val Leu Thr Ser Pro Thr Phe  
580 585 590

Glu Ala Cys His Arg Ala Val Ser Pro Leu Pro Tyr Leu Arg Asn Cys  
595 600 605

Arg Tyr Asp Val Cys Ser Cys Ser Asp Gly Arg Glu Cys Leu Cys Gly  
610 615 620

Ala Leu Ala Ser Tyr Ala Ala Ala Cys Ala Gly Arg Gly Val Arg Val  
625 630 635 640

Ala Trp Arg Glu Pro Gly Arg Cys Glu Leu Asn Cys Pro Lys Gly Gln  
645 650 655

Val Tyr Leu Gln Cys Gly Thr Pro Cys Asn Leu Thr Cys Arg Ser Leu  
660 665 670

Ser Tyr Pro Asp Glu Glu Cys Asn Glu Ala Cys Leu Glu Gly Cys Phe

675

680

685

Cys Pro Pro Gly Leu Tyr Met Asp Glu Arg Gly Asp Cys Val Pro Lys  
 690 695 700

Ala Gln Cys Pro Cys Tyr Tyr Asp Gly Glu Ile Phe Gln Pro Glu Asp  
 705 710 715 720

Ile Phe Ser Asp His His Thr Met Cys Tyr Cys Glu Asp Gly Phe Met  
 725 730 735

His Cys Thr Met Ser Gly Val Pro Gly Ser Leu Leu Pro Asp Ala Val  
 740 745 750

Leu Ser Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg  
 755 760 765

Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp Asn Leu Arg Ala Glu  
 770 775 780

Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr Asp Leu Glu Cys Met  
 785 790 795 800

Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro Pro Gly Met Val Arg  
 805 810 815

His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys Pro Cys Phe His Gln  
 820 825 830

Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr

835

840

845

Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp  
 850 855 860

Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly  
 865 870 875 880

Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp  
 885 890 895

Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys  
 900 905 910

Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu  
 915 920 925

Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys  
 930 935 940

Arg Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Arg  
 945 950 955 960

Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg  
 965 970 975

His Leu Ser Ile Ser Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val  
 980 985 990

Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln Asn Asn Asp Leu Thr

995

1000

1005

Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val Asp Phe Gly Asn  
 1010 1015 1020

Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg Lys Val Pro  
 1025 1030 1035

Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met Lys Gln  
 1040 1045 1050

Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val Phe  
 1055 1060 1065

Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val  
 1070 1075 1080

Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala  
 1085 1090 1095

Ala Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln  
 1100 1105 1110

His Gly Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln  
 1115 1120 1125

Ser Cys Glu Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Ala Glu  
 1130 1135 1140

Trp Arg Tyr Asn Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln

1145

1150

1155

His Pro Glu Pro Leu Ala Cys Pro Val Gln Cys Val Glu Gly Cys

1160

1165

1170

His Ala His Cys Pro Pro Gly Lys Ile Leu Asp Glu Leu Leu Gln

1175

1180

1185

Thr Cys Val Asp Pro Glu Asp Cys Pro Val Cys Glu Val Ala Gly

1190

1195

1200

Arg Arg Phe Ala Ser Gly Lys Lys Val Thr Leu Asn Pro Ser Asp

1205

1210

1215

Pro Glu His Cys Gln Ile Cys His Cys Asp Val Val Asn Leu Thr

1220

1225

1230

Cys Glu Ala Cys Gln Glu Pro Ile Ser Gly Gly Gly Gly Ser Gly

1235

1240

1245

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly

1250

1255

1260

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly

1265

1270

1275

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly

1280

1285

1290

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly

1295

1300

1305

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu  
 1310 1315 1320

Val Pro Arg Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
 1325 1330 1335

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu  
 1340 1345 1350

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr  
 1355 1360 1365

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 1370 1375 1380

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp  
 1385 1390 1395

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
 1400 1405 1410

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His  
 1415 1420 1425

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn  
 1430 1435 1440

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys



1445

1450

1455

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg

1460

1465

1470

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys

1475

1480

1485

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly

1490

1495

1500

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser

1505

1510

1515

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser

1520

1525

1530

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu

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Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro

1550

1555

1560

Gly Lys

1565

<210> 121

<211> 247

<212> PRT

<213> Artificial Sequence

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&lt;223&gt; pSYN Fc-015 protein sequence

&lt;400&gt; 121

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro

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Gly Ser Thr Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro

20 25 30

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys

35 40 45

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val

50 55 60

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp

65 70 75 80

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr

85 90 95

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp

100 105 110

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu

115 120 125

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg

130 135 140

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys

145 150 155 160

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp

165 170 175

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys

180 185 190

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser

195 200 205

Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser

210 215 220

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser

225 230 235 240

Leu Ser Leu Ser Pro Gly Lys

245

<210> 122

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<212> PRT

<213> Artificial Sequence

<220>

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<400> 122

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly

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10

15

SequenceListing.txt

Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu Val Pro Arg Gly Ser Gly

202530

Gly

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<211> 1240

<212> PRT

<213> Artificial Sequence

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<223> VWF-D1D2D'D3

<400> 123

Met Ile Pro Ala Arg Phe Ala Gly Val Leu Leu Ala Leu Ala Leu Ile

151051

Leu Pro Gly Thr Leu Cys Ala Glu Gly Thr Arg Gly Arg Ser Ser Thr

202530

Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly

354045

Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly

505560

Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys

65707580

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Val | Ser | Leu | Ser | Val | Tyr | Leu | Gly | Glu | Phe | Phe | Asp | Ile | His | Leu |
|     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Phe | Val | Asn | Gly | Thr | Val | Thr | Gln | Gly | Asp | Gln | Arg | Val | Ser | Met | Pro |
|     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Tyr | Ala | Ser | Lys | Gly | Leu | Tyr | Leu | Glu | Thr | Glu | Ala | Gly | Tyr | Tyr | Lys |
|     | 115 |     |     |     |     | 120 |     |     |     | 125 |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Ser | Gly | Glu | Ala | Tyr | Gly | Phe | Val | Ala | Arg | Ile | Asp | Gly | Ser | Gly |
| 130 |     |     |     |     |     | 135 |     |     |     | 140 |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asn | Phe | Gln | Val | Leu | Leu | Ser | Asp | Arg | Tyr | Phe | Asn | Lys | Thr | Cys | Gly |
| 145 |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Cys | Gly | Asn | Phe | Asn | Ile | Phe | Ala | Glu | Asp | Asp | Phe | Met | Thr | Gln |
|     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Gly | Thr | Leu | Thr | Ser | Asp | Pro | Tyr | Asp | Phe | Ala | Asn | Ser | Trp | Ala |
|     |     | 180 |     |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Ser | Ser | Gly | Glu | Gln | Trp | Cys | Glu | Arg | Ala | Ser | Pro | Pro | Ser | Ser |
|     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Cys | Asn | Ile | Ser | Ser | Gly | Glu | Met | Gln | Lys | Gly | Leu | Trp | Glu | Gln |
| 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cys | Gln | Leu | Leu | Lys | Ser | Thr | Ser | Val | Phe | Ala | Arg | Cys | His | Pro | Leu |
| 225 |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Asp | Pro | Glu | Pro | Phe | Val | Ala | Leu | Cys | Glu | Lys | Thr | Leu | Cys | Glu |
|     |     |     | 245 |     |     |     |     | 250 |     |     |     |     |     | 255 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cys | Ala | Gly | Gly | Leu | Glu | Cys | Ala | Cys | Pro | Ala | Leu | Leu | Glu | Tyr | Ala |
|     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Thr | Cys | Ala | Gln | Glu | Gly | Met | Val | Leu | Tyr | Gly | Trp | Thr | Asp | His |
|     |     | 275 |     |     |     |     |     | 280 |     |     |     |     | 285 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Ala | Cys | Ser | Pro | Val | Cys | Pro | Ala | Gly | Met | Glu | Tyr | Arg | Gln | Cys |
|     | 290 |     |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val | Ser | Pro | Cys | Ala | Arg | Thr | Cys | Gln | Ser | Leu | His | Ile | Asn | Glu | Met |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cys | Gln | Glu | Arg | Cys | Val | Asp | Gly | Cys | Ser | Cys | Pro | Glu | Gly | Gln | Leu |
|     |     |     | 325 |     |     |     |     |     | 330 |     |     |     |     | 335 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Asp | Glu | Gly | Leu | Cys | Val | Glu | Ser | Thr | Glu | Cys | Pro | Cys | Val | His |
|     |     | 340 |     |     |     |     |     |     | 345 |     |     |     |     | 350 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Gly | Lys | Arg | Tyr | Pro | Pro | Gly | Thr | Ser | Leu | Ser | Arg | Asp | Cys | Asn |
|     |     | 355 |     |     |     |     |     | 360 |     |     |     |     | 365 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr | Cys | Ile | Cys | Arg | Asn | Ser | Gln | Trp | Ile | Cys | Ser | Asn | Glu | Glu | Cys |
|     | 370 |     |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Pro | Gly | Glu | Cys | Leu | Val | Thr | Gly | Gln | Ser | His | Phe | Lys | Ser | Phe | Asp |
| 385 |     |     |     |     |     | 390 |     |     |     | 395 |     |     |     |     | 400 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Arg | Tyr | Phe | Thr | Phe | Ser | Gly | Ile | Cys | Gln | Tyr | Leu | Leu | Ala | Arg |
|     |     |     | 405 |     |     |     |     | 410 |     |     |     |     |     | 415 |     |
| Asp | Cys | Gln | Asp | His | Ser | Phe | Ser | Ile | Val | Ile | Glu | Thr | Val | Gln | Cys |
|     |     | 420 |     |     |     |     |     | 425 |     |     |     |     | 430 |     |     |
| Ala | Asp | Asp | Arg | Asp | Ala | Val | Cys | Thr | Arg | Ser | Val | Thr | Val | Arg | Leu |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |
| Pro | Gly | Leu | His | Asn | Ser | Leu | Val | Lys | Leu | Lys | His | Gly | Ala | Gly | Val |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |
| Ala | Met | Asp | Gly | Gln | Asp | Ile | Gln | Leu | Pro | Leu | Leu | Lys | Gly | Asp | Leu |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |
| Arg | Ile | Gln | His | Thr | Val | Thr | Ala | Ser | Val | Arg | Leu | Ser | Tyr | Gly | Glu |
|     |     |     | 485 |     |     |     |     |     | 490 |     |     |     |     | 495 |     |
| Asp | Leu | Gln | Met | Asp | Trp | Asp | Gly | Arg | Gly | Arg | Leu | Leu | Val | Lys | Leu |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     |     |     | 510 |     |
| Ser | Pro | Val | Tyr | Ala | Gly | Lys | Thr | Cys | Gly | Leu | Cys | Gly | Asn | Tyr | Asn |
|     |     | 515 |     |     |     |     |     | 520 |     |     |     |     | 525 |     |     |
| Gly | Asn | Gln | Gly | Asp | Asp | Phe | Leu | Thr | Pro | Ser | Gly | Leu | Ala | Glu | Pro |
|     | 530 |     |     |     |     | 535 |     |     |     |     | 540 |     |     |     |     |
| Arg | Val | Glu | Asp | Phe | Gly | Asn | Ala | Trp | Lys | Leu | His | Gly | Asp | Cys | Gln |
| 545 |     |     |     |     | 550 |     |     |     |     | 555 |     |     |     |     | 560 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Leu | Gln | Lys | Gln | His | Ser | Asp | Pro | Cys | Ala | Leu | Asn | Pro | Arg | Met |
|     |     |     |     | 565 |     |     |     |     | 570 |     |     |     |     | 575 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Thr | Arg | Phe | Ser | Glu | Glu | Ala | Cys | Ala | Val | Leu | Thr | Ser | Pro | Thr | Phe |
|     |     |     | 580 |     |     |     |     | 585 |     |     |     |     | 590 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Ala | Cys | His | Arg | Ala | Val | Ser | Pro | Leu | Pro | Tyr | Leu | Arg | Asn | Cys |
|     |     | 595 |     |     |     |     |     | 600 |     |     |     |     | 605 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Arg | Tyr | Asp | Val | Cys | Ser | Cys | Ser | Asp | Gly | Arg | Glu | Cys | Leu | Cys | Gly |
|     | 610 |     |     |     |     |     | 615 |     |     |     | 620 |     |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Leu | Ala | Ser | Tyr | Ala | Ala | Ala | Cys | Ala | Gly | Arg | Gly | Val | Arg | Val |
| 625 |     |     |     |     |     | 630 |     |     |     | 635 |     |     |     |     | 640 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Trp | Arg | Glu | Pro | Gly | Arg | Cys | Glu | Leu | Asn | Cys | Pro | Lys | Gly | Gln |
|     |     |     |     |     | 645 |     |     |     | 650 |     |     |     |     | 655 |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Val | Tyr | Leu | Gln | Cys | Gly | Thr | Pro | Cys | Asn | Leu | Thr | Cys | Arg | Ser | Leu |
|     |     |     | 660 |     |     |     |     |     | 665 |     |     |     | 670 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Tyr | Pro | Asp | Glu | Glu | Cys | Asn | Glu | Ala | Cys | Leu | Glu | Gly | Cys | Phe |
|     |     |     | 675 |     |     |     |     |     | 680 |     |     |     | 685 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Cys | Pro | Pro | Gly | Leu | Tyr | Met | Asp | Glu | Arg | Gly | Asp | Cys | Val | Pro | Lys |
|     |     |     | 690 |     |     |     |     |     | 695 |     |     | 700 |     |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ala | Gln | Cys | Pro | Cys | Tyr | Tyr | Asp | Gly | Glu | Ile | Phe | Gln | Pro | Glu | Asp |
| 705 |     |     |     |     |     |     | 710 |     |     |     | 715 |     |     |     | 720 |



Ile Phe Ser Asp His His Thr Met Cys Tyr Cys Glu Asp Gly Phe Met

725

730

735

His Cys Thr Met Ser Gly Val Pro Gly Ser Leu Leu Pro Asp Ala Val

740

745

750

Leu Ser Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg

755

760

765

Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp Asn Leu Arg Ala Glu

770

775

780

Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr Asp Leu Glu Cys Met

785

790

795

800

Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro Pro Gly Met Val Arg

805

810

815

His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys Pro Cys Phe His Gln

820

825

830

Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr

835

840

845

Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp

850

855

860

Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly

865

870

875

880

Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp

885

890

895

Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys

900

905

910

Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu

915

920

925

Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys

930

935

940

Arg Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Arg

945

950

955

960

Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg

965

970

975

His Leu Ser Ile Ser Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val

980

985

990

Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln Asn Asn Asp Leu Thr

995

1000

1005

Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val Asp Phe Gly Asn

1010

1015

1020

Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg Lys Val Pro

1025

1030

1035

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Leu  | Asp | Ser | Ser | Pro | Ala | Thr  | Cys | His | Asn | Asn | Ile  | Met | Lys | Gln |
| 1040 |     |     |     |     |     | 1045 |     |     |     |     | 1050 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Met | Val | Asp | Ser | Ser | Cys  | Arg | Ile | Leu | Thr | Ser  | Asp | Val | Phe |
| 1055 |     |     |     |     |     | 1060 |     |     |     |     | 1065 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Asp | Cys | Asn | Lys | Leu | Val  | Asp | Pro | Glu | Pro | Tyr  | Leu | Asp | Val |
| 1070 |     |     |     |     |     | 1075 |     |     |     |     | 1080 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Ile | Tyr | Asp | Thr | Cys | Ser  | Cys | Glu | Ser | Ile | Gly  | Asp | Cys | Ala |
| 1085 |     |     |     |     |     | 1090 |     |     |     |     | 1095 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Phe | Cys | Asp | Thr | Ile | Ala  | Ala | Tyr | Ala | His | Val  | Cys | Ala | Gln |
| 1100 |     |     |     |     |     | 1105 |     |     |     |     | 1110 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| His  | Gly | Lys | Val | Val | Thr | Trp  | Arg | Thr | Ala | Thr | Leu  | Cys | Pro | Gln |
| 1115 |     |     |     |     |     | 1120 |     |     |     |     | 1125 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Cys | Glu | Glu | Arg | Asn | Leu  | Arg | Glu | Asn | Gly | Tyr  | Glu | Cys | Glu |
| 1130 |     |     |     |     |     | 1135 |     |     |     |     | 1140 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Trp  | Arg | Tyr | Asn | Ser | Cys | Ala  | Pro | Ala | Cys | Gln | Val  | Thr | Cys | Gln |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     | 1155 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| His  | Pro | Glu | Pro | Leu | Ala | Cys  | Pro | Val | Gln | Cys | Val  | Glu | Gly | Cys |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| His  | Ala | His | Cys | Pro | Pro | Gly  | Lys | Ile | Leu | Asp | Glu  | Leu | Leu | Gln |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |

Thr Cys Val Asp Pro Glu Asp Cys Pro Val Cys Glu Val Ala Gly  
 1190 1195 1200

Arg Arg Phe Ala Ser Gly Lys Lys Val Thr Leu Asn Pro Ser Asp  
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Pro Glu His Cys Gln Ile Cys His Cys Asp Val Val Asn Leu Thr  
 1220 1225 1230

Cys Glu Ala Cys Gln Glu Pro  
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<400> 124

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Asn Leu Arg Ala Glu Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr  
 20 25 30

Asp Leu Glu Cys Met Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro  
 35 40 45

Pro Gly Met Val Arg His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys

50

55

60

Pro Cys Phe His Gln Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys  
 65 70 75 80

Ile Gly Cys Asn Thr Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr  
 85 90 95

Asp His Val Cys Asp Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr  
 100 105 110

Leu Thr Phe Asp Gly Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr  
 115 120 125

Val Leu Val Gln Asp Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile  
 130 135 140

Leu Val Gly Asn Lys Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys  
 145 150 155 160

Arg Val Thr Ile Leu Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly  
 165 170 175

Glu Val Asn Val Lys Arg Pro Met Lys Asp Glu Thr His Phe Glu Val  
 180 185 190

Val Glu Ser Gly Arg Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser  
 195 200 205

Val Val Trp Asp Arg His Leu Ser Ile Ser Val Val Leu Lys Gln Thr

210

215

220

Tyr Gln Glu Lys Val Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln  
225 230 235 240

Asn Asn Asp Leu Thr Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val  
245 250 255

Asp Phe Gly Asn Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg  
260 265 270

Lys Val Pro Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met  
275 280 285

Lys Gln Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val  
290 295 300

Phe Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val  
305 310 315 320

Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala Cys  
325 330 335

Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln His Gly  
340 345 350

Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln Ser Cys Glu  
355 360 365

Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Cys Glu Trp Arg Tyr Asn

370

375

380

Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln His Pro Glu Pro Leu

385

390

395

400

Ala Cys Pro Val Gln Cys Val Glu Gly Cys His Ala His Cys Pro Pro

405

410

415

Gly Lys Ile Leu Asp Glu Leu Leu Gln Thr Cys Val Asp Pro Glu Asp

420

425

430

Cys Pro Val Cys Glu Val Ala Gly Arg Arg Phe Ala Ser Gly Lys Lys

435

440

445

Val Thr Leu Asn Pro Ser Asp Pro Glu His Cys Gln Ile Cys His Cys

450

455

460

Asp Val Val Asn Leu Thr Cys Glu Ala Cys Gln Glu Pro

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&lt;400&gt; 125

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| gatacagtg tcatctact taagaacatg gcttcccatc ctgtcagtct tcatgctgtt    | 360  |
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|                                                                    |      |
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| aagtacaaaa aagtccgatt tatggcatac acagatgaaa cctttaagac tcgtgaagct  | 1380 |
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| gatctagctt caggactcat tggccctctc ctcatctgct acaaagaatc tgtagatcaa  | 1740 |
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| ttctctcaa acccaccagt cttgaaacgc catcaacggg aaataactcg tactactctt   | 2340 |
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| caggatcaaa ggattcgatg gtatctgctc agcatgggca gcaatgaaaa catccattct  | 3240 |
| attcatttca gtggacatgt gttcactgta cgaaaaaaag aggagtataa aatggcactg  | 3300 |
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| gtgtacagca ataagtgtca gactcccctg ggaatggctt ctggacacat tagagatttt  | 3480 |
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| tccggatcaa tcaatgcctg gagcaccaag gagccctttt cttggatcaa ggtggatctg  | 3600 |
| ttggcaccaa tgattattca cggcatcaag acccagggtg cccgtcagaa gttctccagc  | 3660 |
| ctctacatct ctcagtttat catcatgtat agtcttgatg ggaagaagtg gcagacttat  | 3720 |

|                                                                    |      |
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| cagaatggca aagtaaaggt ttttcagggg aatcaagact ccttcacacc tgtggtgaac  | 4260 |
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| agcgtcctca ccgtcctgca ccaggactgg ctgaatggca aggagtacaa gtgcaaggtc  | 4680 |
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Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg  
35 40 45

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val  
50 55 60

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile  
65 70 75 80

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln  
85 90 95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser  
100 105 110

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser  
115 120 125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp  
130 135 140

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu  
145 150 155 160

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser  
165 170 175

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile  
180 185 190

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr  
195 200 205

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly  
210 215 220

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp  
225 230 235 240

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr  
245 250 255

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Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
      260              265              270

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
      275              280              285

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
      290              295              300

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
305              310              315              320

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
      325              330              335

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
      340              345              350

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
      355              360              365

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
      370              375              380

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
385              390              395              400

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
      405              410              415

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Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn

420

425

430

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met

435

440

445

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu

450

455

460

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu

465

470

475

480

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro

485

490

495

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys

500

505

510

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe

515

520

525

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp

530

535

540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg

545

550

555

560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu

565

570

575

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val

580

585

590

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu

595

600

605

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp

610

615

620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val

625

630

635

640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp

645

650

655

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe

660

665

670

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr

675

680

685

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro

690

695

700

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly

705

710

715

720

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp

725

730

735



|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Tyr | Glu | Asp | Ser | Tyr | Glu | Asp | Ile | Ser | Ala | Tyr | Leu | Leu | Ser | Lys |
|     |     | 740 |     |     |     |     |     | 745 |     |     |     |     | 750 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asn | Asn | Ala | Ile | Glu | Pro | Arg | Ser | Phe | Ser | Gln | Asn | Pro | Pro | Val | Leu |
|     |     | 755 |     |     |     |     |     | 760 |     |     |     |     | 765 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Lys | Arg | His | Gln | Arg | Glu | Ile | Thr | Arg | Thr | Thr | Leu | Gln | Ser | Asp | Gln |
|     |     | 770 |     |     |     |     |     | 775 |     |     |     |     | 780 |     |     |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Glu | Glu | Ile | Asp | Tyr | Asp | Asp | Thr | Ile | Ser | Val | Glu | Met | Lys | Lys | Glu |
| 785 |     |     |     |     |     | 790 |     |     |     |     | 795 |     |     |     | 800 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Phe | Asp | Ile | Tyr | Asp | Glu | Asp | Glu | Asn | Gln | Ser | Pro | Arg | Ser | Phe |
|     |     |     |     |     |     | 805 |     |     |     |     | 810 |     |     |     | 815 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Gln | Lys | Lys | Thr | Arg | His | Tyr | Phe | Ile | Ala | Ala | Val | Glu | Arg | Leu | Trp |
|     |     |     |     |     |     | 820 |     |     |     |     |     |     |     |     | 830 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Tyr | Gly | Met | Ser | Ser | Ser | Pro | His | Val | Leu | Arg | Asn | Arg | Ala | Gln |
|     |     |     |     |     |     | 835 |     |     |     |     |     |     |     |     | 845 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Ser | Gly | Ser | Val | Pro | Gln | Phe | Lys | Lys | Val | Val | Phe | Gln | Glu | Phe | Thr |
|     |     |     |     |     |     | 850 |     |     |     |     |     |     |     |     | 860 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Asp | Gly | Ser | Phe | Thr | Gln | Pro | Leu | Tyr | Arg | Gly | Glu | Leu | Asn | Glu | His |
| 865 |     |     |     |     |     | 870 |     |     |     |     |     |     |     |     | 880 |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Leu | Gly | Leu | Leu | Gly | Pro | Tyr | Ile | Arg | Ala | Glu | Val | Glu | Asp | Asn | Ile |
|     |     |     |     |     |     | 885 |     |     |     |     |     |     |     |     | 895 |

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Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser Phe Tyr Ser
      900              905              910

Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala Glu Pro Arg
      915              920              925

Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe Trp Lys Val
      930              935              940

Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys Lys Ala Trp
945              950              955              960

Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His Ser Gly Leu
      965              970              975

Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn Pro Ala His
      980              985              990

Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe Thr Ile Phe
      995              1000             1005

Asp Glu Thr Lys Ser Trp Tyr Phe Thr Glu Asn Met Glu Arg Asn
      1010             1015             1020

Cys Arg Ala Pro Cys Asn Ile Gln Met Glu Asp Pro Thr Phe Lys
      1025             1030             1035

Glu Asn Tyr Arg Phe His Ala Ile Asn Gly Tyr Ile Met Asp Thr
      1040             1045             1050

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|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Leu  | Pro | Gly | Leu | Val | Met | Ala  | Gln | Asp | Gln | Arg | Ile  | Arg | Trp | Tyr |
| 1055 |     |     |     |     |     | 1060 |     |     |     |     | 1065 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Leu | Ser | Met | Gly | Ser | Asn  | Glu | Asn | Ile | His | Ser  | Ile | His | Phe |
| 1070 |     |     |     |     |     | 1075 |     |     |     |     | 1080 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Gly | His | Val | Phe | Thr | Val  | Arg | Lys | Lys | Glu | Glu  | Tyr | Lys | Met |
| 1085 |     |     |     |     |     | 1090 |     |     |     |     | 1095 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Leu | Tyr | Asn | Leu | Tyr | Pro  | Gly | Val | Phe | Glu | Thr  | Val | Glu | Met |
| 1100 |     |     |     |     |     | 1105 |     |     |     |     | 1110 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Pro | Ser | Lys | Ala | Gly | Ile  | Trp | Arg | Val | Glu | Cys  | Leu | Ile | Gly |
| 1115 |     |     |     |     |     | 1120 |     |     |     |     | 1125 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | His | Leu | His | Ala | Gly | Met  | Ser | Thr | Leu | Phe | Leu  | Val | Tyr | Ser |
| 1130 |     |     |     |     |     | 1135 |     |     |     |     | 1140 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Lys | Cys | Gln | Thr | Pro | Leu  | Gly | Met | Ala | Ser | Gly  | His | Ile | Arg |
| 1145 |     |     |     |     |     | 1150 |     |     |     |     | 1155 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Phe | Gln | Ile | Thr | Ala | Ser  | Gly | Gln | Tyr | Gly | Gln  | Trp | Ala | Pro |
| 1160 |     |     |     |     |     | 1165 |     |     |     |     | 1170 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Leu | Ala | Arg | Leu | His | Tyr  | Ser | Gly | Ser | Ile | Asn  | Ala | Trp | Ser |
| 1175 |     |     |     |     |     | 1180 |     |     |     |     | 1185 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Lys | Glu | Pro | Phe | Ser | Trp  | Ile | Lys | Val | Asp | Leu  | Leu | Ala | Pro |
| 1190 |     |     |     |     |     | 1195 |     |     |     |     | 1200 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Met  | Ile | Ile | His | Gly | Ile | Lys  | Thr | Gln | Gly | Ala | Arg  | Gln | Lys | Phe |
| 1205 |     |     |     |     |     | 1210 |     |     |     |     | 1215 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Ser | Leu | Tyr | Ile | Ser | Gln  | Phe | Ile | Ile | Met | Tyr  | Ser | Leu | Asp |
| 1220 |     |     |     |     |     | 1225 |     |     |     |     | 1230 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Lys | Lys | Trp | Gln | Thr | Tyr  | Arg | Gly | Asn | Ser | Thr  | Gly | Thr | Leu |
| 1235 |     |     |     |     |     | 1240 |     |     |     |     | 1245 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Val | Phe | Phe | Gly | Asn | Val  | Asp | Ser | Ser | Gly | Ile  | Lys | His | Asn |
| 1250 |     |     |     |     |     | 1255 |     |     |     |     | 1260 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ile  | Phe | Asn | Pro | Pro | Ile | Ile  | Ala | Arg | Tyr | Ile | Arg  | Leu | His | Pro |
| 1265 |     |     |     |     |     | 1270 |     |     |     |     | 1275 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | His | Tyr | Ser | Ile | Arg | Ser  | Thr | Leu | Arg | Met | Glu  | Leu | Met | Gly |
| 1280 |     |     |     |     |     | 1285 |     |     |     |     | 1290 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Cys  | Asp | Leu | Asn | Ser | Cys | Ser  | Met | Pro | Leu | Gly | Met  | Glu | Ser | Lys |
| 1295 |     |     |     |     |     | 1300 |     |     |     |     | 1305 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Ile | Ser | Asp | Ala | Gln | Ile  | Thr | Ala | Ser | Ser | Tyr  | Phe | Thr | Asn |
| 1310 |     |     |     |     |     | 1315 |     |     |     |     | 1320 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Phe | Ala | Thr | Trp | Ser | Pro  | Ser | Lys | Ala | Arg | Leu  | His | Leu | Gln |
| 1325 |     |     |     |     |     | 1330 |     |     |     |     | 1335 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Arg | Ser | Asn | Ala | Trp | Arg  | Pro | Gln | Val | Asn | Asn  | Pro | Lys | Glu |
| 1340 |     |     |     |     |     | 1345 |     |     |     |     | 1350 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Trp  | Leu | Gln | Val | Asp | Phe | Gln  | Lys | Thr | Met | Lys | Val  | Thr | Gly | Val |
| 1355 |     |     |     |     |     | 1360 |     |     |     |     | 1365 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Thr  | Thr | Gln | Gly | Val | Lys | Ser  | Leu | Leu | Thr | Ser | Met  | Tyr | Val | Lys |
| 1370 |     |     |     |     |     | 1375 |     |     |     |     | 1380 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Phe | Leu | Ile | Ser | Ser | Ser  | Gln | Asp | Gly | His | Gln  | Trp | Thr | Leu |
| 1385 |     |     |     |     |     | 1390 |     |     |     |     | 1395 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Phe | Gln | Asn | Gly | Lys | Val  | Lys | Val | Phe | Gln | Gly  | Asn | Gln | Asp |
| 1400 |     |     |     |     |     | 1405 |     |     |     |     | 1410 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ser  | Phe | Thr | Pro | Val | Val | Asn  | Ser | Leu | Asp | Pro | Pro  | Leu | Leu | Thr |
| 1415 |     |     |     |     |     | 1420 |     |     |     |     | 1425 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Arg  | Tyr | Leu | Arg | Ile | His | Pro  | Gln | Ser | Trp | Val | His  | Gln | Ile | Ala |
| 1430 |     |     |     |     |     | 1435 |     |     |     |     | 1440 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Leu  | Arg | Met | Glu | Val | Leu | Gly  | Cys | Glu | Ala | Gln | Asp  | Leu | Tyr | Asp |
| 1445 |     |     |     |     |     | 1450 |     |     |     |     | 1455 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Lys  | Thr | His | Thr | Cys | Pro | Pro  | Cys | Pro | Ala | Pro | Glu  | Leu | Leu | Gly |
| 1460 |     |     |     |     |     | 1465 |     |     |     |     | 1470 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Pro | Ser | Val | Phe | Leu | Phe  | Pro | Pro | Lys | Pro | Lys  | Asp | Thr | Leu |
| 1475 |     |     |     |     |     | 1480 |     |     |     |     | 1485 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Met  | Ile | Ser | Arg | Thr | Pro | Glu  | Val | Thr | Cys | Val | Val  | Val | Asp | Val |
| 1490 |     |     |     |     |     | 1495 |     |     |     |     | 1500 |     |     |     |

|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|------|-----|-----|-----|
| Ser  | His | Glu | Asp | Pro | Glu | Val  | Lys | Phe | Asn | Trp | Tyr  | Val | Asp | Gly |
| 1505 |     |     |     |     |     | 1510 |     |     |     |     | 1515 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Val  | Glu | Val | His | Asn | Ala | Lys  | Thr | Lys | Pro | Arg | Glu  | Glu | Gln | Tyr |
| 1520 |     |     |     |     |     | 1525 |     |     |     |     | 1530 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asn  | Ser | Thr | Tyr | Arg | Val | Val  | Ser | Val | Leu | Thr | Val  | Leu | His | Gln |
| 1535 |     |     |     |     |     | 1540 |     |     |     |     | 1545 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Asp  | Trp | Leu | Asn | Gly | Lys | Glu  | Tyr | Lys | Cys | Lys | Val  | Ser | Asn | Lys |
| 1550 |     |     |     |     |     | 1555 |     |     |     |     | 1560 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Ala  | Leu | Pro | Ala | Pro | Ile | Glu  | Lys | Thr | Ile | Ser | Lys  | Ala | Lys | Gly |
| 1565 |     |     |     |     |     | 1570 |     |     |     |     | 1575 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gln  | Pro | Arg | Glu | Pro | Gln | Val  | Tyr | Thr | Leu | Pro | Pro  | Ser | Arg | Asp |
| 1580 |     |     |     |     |     | 1585 |     |     |     |     | 1590 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Glu  | Leu | Thr | Lys | Asn | Gln | Val  | Ser | Leu | Thr | Cys | Leu  | Val | Lys | Gly |
| 1595 |     |     |     |     |     | 1600 |     |     |     |     | 1605 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Phe  | Tyr | Pro | Ser | Asp | Ile | Ala  | Val | Glu | Trp | Glu | Ser  | Asn | Gly | Gln |
| 1610 |     |     |     |     |     | 1615 |     |     |     |     | 1620 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Pro  | Glu | Asn | Asn | Tyr | Lys | Thr  | Thr | Pro | Pro | Val | Leu  | Asp | Ser | Asp |
| 1625 |     |     |     |     |     | 1630 |     |     |     |     | 1635 |     |     |     |
|      |     |     |     |     |     |      |     |     |     |     |      |     |     |     |
| Gly  | Ser | Phe | Phe | Leu | Tyr | Ser  | Lys | Leu | Thr | Val | Asp  | Lys | Ser | Arg |
| 1640 |     |     |     |     |     | 1645 |     |     |     |     | 1650 |     |     |     |

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
1655 1660 1665

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly  
1670 1675 1680

Lys

<210> 127

<211> 78

<212> PRT

<213> Artificial Sequence

<220>

<223> AE72 XTEN

<400> 127

Gly Ala Pro Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser  
1 5 10 15

Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala  
20 25 30

Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu  
35 40 45

Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr  
50 55 60

Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ala Ser Ser

65

70

75

&lt;210&gt; 128

&lt;211&gt; 144

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; AE144\_2A

&lt;400&gt; 128

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala Gly

1 5 10 15

Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly

20 25 30

Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly

35 40 45

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu

50 55 60

Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu

65 70 75 80

Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly

85 90 95

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr Glu

100 105 110



Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu  
115 120 125

Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
130 135 140

<210> 129

<211> 144

<212> PRT

<213> Artificial Sequence

<220>

<223> AE144\_3B

<400> 129

Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser  
1 5 10 15

Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser  
20 25 30

Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
35 40 45

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr Glu  
50 55 60

Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly  
65 70 75 80

Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr Glu  
100 105 110

Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser  
115 120 125

Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly  
130 135 140

<210> 130

<211> 144

<212> PRT

<213> Artificial Sequence

<220>

<223> AE144\_4A

<400> 130

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala  
1 5 10 15

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu  
20 25 30

Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly  
35 40 45

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu  
50 55 60

Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu  
65 70 75 80

Ser Gly Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly  
85 90 95

Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala Gly  
100 105 110

Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro Glu  
115 120 125

Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly  
130 135 140

<210> 131

<211> 144

<212> PRT

<213> Artificial Sequence

<220>

<223> AE144\_5A

<400> 131

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala  
1 5 10 15

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu  
20 25 30

Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly

35

40

45

Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr Glu  
50 55 60

Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser  
65 70 75 80

Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
85 90 95

Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser  
100 105 110

Ala Thr Pro Glu Ser Gly Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser  
115 120 125

Thr Glu Glu Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly  
130 135 140

&lt;210&gt; 132

&lt;211&gt; 144

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; AE144\_6B

&lt;400&gt; 132

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser  
1 5 10 15

Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu  
20 25 30

Ser Gly Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly  
35 40 45

Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro Gly Ser Glu Pro Ala  
50 55 60

Thr Ser Gly Ser Glu Thr Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser  
65 70 75 80

Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly  
85 90 95

Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Glu Pro Ala  
100 105 110

Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu  
115 120 125

Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly  
130 135 140

<210> 133

<211> 144

<212> PRT

<213> Artificial Sequence

<220>

<223> AG144\_A

&lt;400&gt; 133

Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser Pro  
1 5 10 15

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser Pro Ser Ala Ser Thr  
20 25 30

Gly Thr Gly Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro  
35 40 45

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro  
50 55 60

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser  
65 70 75 80

Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro  
85 90 95

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Thr Pro Gly  
100 105 110

Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
115 120 125

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
130 135 140

&lt;210&gt; 134

&lt;211&gt; 144

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; AG144\_B

&lt;400&gt; 134

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr  
1 5 10 15

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
20 25 30

Thr Gly Ser Pro Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro  
35 40 45

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro  
50 55 60

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser Pro Ser Ala Ser Thr  
65 70 75 80

Gly Thr Gly Pro Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro  
85 90 95

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ala Ser Pro  
100 105 110

Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
115 120 125

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 130 135 140

<210> 135

<211> 144

<212> PRT

<213> Artificial Sequence

<220>

<223> AG144\_C

<400> 135

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser Pro  
 1 5 10 15

Gly Thr Ser Ser Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 20 25 30

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 35 40 45

Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Thr Pro Gly  
 50 55 60

Ser Gly Thr Ala Ser Ser Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser  
 65 70 75 80

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
 85 90 95

Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro Gly Ser Ser Thr



100

105

110

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala  
115 120 125

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
130 135 140

&lt;210&gt; 136

&lt;211&gt; 144

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; AG144\_F

&lt;400&gt; 136

Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro Gly Ser Ser Pro  
1 5 10 15

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser  
20 25 30

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
35 40 45

Gly Ser Ser Thr Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Pro  
50 55 60

Ser Ala Ser Thr Gly Thr Gly Pro Gly Ala Ser Pro Gly Thr Ser Ser  
65 70 75 80

Thr Gly Ser Pro Gly Ser Ser Pro Ser Ala Ser Thr Gly Thr Gly Pro  
85 90 95

Gly Thr Pro Gly Ser Gly Thr Ala Ser Ser Ser Pro Gly Ser Ser Thr  
100 105 110

Pro Ser Gly Ala Thr Gly Ser Pro Gly Ser Ser Thr Pro Ser Gly Ala  
115 120 125

Thr Gly Ser Pro Gly Ala Ser Pro Gly Thr Ser Ser Thr Gly Ser Pro  
130 135 140

<210> 137

<211> 288

<212> PRT

<213> Artificial Sequence

<220>

<223> AE288\_2

<400> 137

Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu  
1 5 10 15

Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly  
20 25 30

Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro  
35 40 45

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr

50

55

60

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu  
65 70 75 80

Gly Ser Ala Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
85 90 95

Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Thr  
100 105 110

Glu Pro Ser Glu Gly Ser Ala Pro Gly Ser Pro Ala Gly Ser Pro Thr  
115 120 125

Ser Thr Glu Glu Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
130 135 140

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Ser Glu Pro  
145 150 155 160

Ala Thr Ser Gly Ser Glu Thr Pro Gly Thr Ser Glu Ser Ala Thr Pro  
165 170 175

Glu Ser Gly Pro Gly Ser Glu Pro Ala Thr Ser Gly Ser Glu Thr Pro  
180 185 190

Gly Thr Ser Glu Ser Ala Thr Pro Glu Ser Gly Pro Gly Thr Ser Thr  
195 200 205

Glu Pro Ser Glu Gly Ser Ala Pro Gly Thr Ser Glu Ser Ala Thr Pro

210

215

Glu Ser Gly Pro Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu  
225 230 235 240

Gly Ser Pro Ala Gly Ser Pro Thr Ser Thr Glu Glu Gly Ser Pro Ala  
245 250 255

Gly Ser Pro Thr Ser Thr Glu Glu Gly Thr Ser Glu Ser Ala Thr Pro  
260 265 270

Glu Ser Gly Pro Gly Thr Ser Thr Glu Pro Ser Glu Gly Ser Ala Pro  
275 280 285

<210> 138  
<211> 20  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> linker

<400> 138

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly  
1 5 10 15

Gly Gly Gly Ser  
20

<210> 139  
<211> 5  
<212> PRT

<213> Artificial Sequence

<220>

<223> linker

<220>

<221> REPEAT

<222> (1)..(5)

<223> May be repeated 1 to 10 times

<400> 139

Gly Gly Gly Gly Ser

1 5

<210> 140

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> linker

<220>

<221> REPEAT

<222> (2)..(6)

<223> May be repeated 1 to 10 times

<400> 140

Ser Gly Gly Gly Gly Ser

1 5