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- before the expiration $g$ the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h)) bogenicity, have enhanced activity, or have more than one of these characteristics are described as are methods for making chimeric clotting factors and methods for improving hemostasia using these clotting factors.


## CHIMERIC CLOTTING FACTORS

## RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application Serial No. 61/363,183 filed July 9, 2010: U.S. Provisional Patent Application No. 61/363,186 filed July 9, 2010; U.S. Provisional Patent Application No. 61/442,029 filed February 11, 201 1; U.S. Provisional Patent Application No, 61/442,150 filed February 11, 201 1: U.S. Provisional Patent Application No. 61/442,055 filed February 11, 2011 : U.S. Provisional Patent Application No. 61/467,880 filed March 25, 201 1; and U.S. Provisional Patent Application No. 61/491,762 filed May 31, 201 1. The entire contents of the above-referenced provisional patent applications are incorporated herein by reference.

## BACKGROUND OF THE INVENTION

[0002] Initiation of the extrinsic clotting pathway is mediated by the formation of a complex between tissue factor, which is exposed as a result of injury to a vessel wall, and Factor Vila. This complex then converts Factors IX and X to their active forms. Factor Xa converts limited amounts of prothrombin to thrombin on the tissue factorbearing cell. This resulting thrombin is then able to diffuse away from the tissuefactor bearing cell and activate platelets, and Factors V and VIII, making Factors Va and Villa. During the propagation phase of coagulation, Factor Xa is generated by Factor IXa (in complex with factor Villa) on the surface of activated platelets. Factor Xa , in complex with the cefaclor Factor Va, activates prothrombin into thrombin, generating a thrombin burst. The cascade culminates in the conversion of fibrinogen to fibrin by thrombin, which results in the formation of a fibrin clot. Factor VII and tissue factor are key players in the initiation of blood coagulation.
[0003] Factor VII is a plasma glycoprotein that circulates in blood as a single-chain zymogen. The zymogen is catalytically inactive. Although single-chain Factor VII may be converted to two-chain Factor Vila by a variety of factors in vitro, Factor Xa is an important physiological activator of Factor VII. The conversion of zymogen

Factor VII into the activated two-chain molecule occurs by cleavage of the peptide bond linking the Arginine residue at amino acid position 152 and the lie residue at amino acid position 153. In the presence of tissue factor, phospholipids and calcium ions, the two-chain Factor VMa activates Factor X or Factor IX. Factor VIIa is thought to be the physiologic initiator of the clotting cascade by acting at the surface of a TFbearing cell, typically a damaged endothelial cell, and generating the initial amount of thrombin that then diffuses to platelets to activate them and prime them for the propagation phase of thrombin generation. Therapeutically, recombinant FV IIa acts by activating Factor X on the surface of activated platelets, bypassing the need for FIXa or FVIIIa to generate a thrombin burst during the propagation phase of coagulation. Since FVIIa has relatively low affinity for platelets, recombinant FVIIa is dosed at supra-physiological levels. This process is thought be tissue factorindependent
[0004] Human factor IX circulates as a single-chain glycoprotein (mol wt 57,000). It is present in plasma as a zymogen and is converted to a serine protease, Factor

XXaß (more commonly referred to as FIXa\}, by Factor XIa (activated plasma thromboplastin antecedent) in the presence of calcium ions. In the activation reaction, two internal peptide bonds are hydrolyzed in Factor IX. These cleavages occur at a specific arginyl-alanine peptide bond and a specific arginyl-valine peptide bond. This results in the release of an activation peptide (mol wt approximately equal to 11,000 ) from the internal region of the precursor molecule and the generation of Factor $] \mathrm{Xa} \beta$ (mol wt approximately equal to 46,000 ). Factor IX aff is composed of a light chain (mol wt approximately equal to 18,000$\}$ and a heavy chain (mol wt approximately equal to 28,000 ), and these chains are held together by a disulfide bond.
[0005] Factor X is also synthesized as a single-chain polypeptide containing the light and heavy chains connected by an Arg-Lys-Arg tripeptide. The single-chain molecule is then converted to the light and heavy chains by cleavage of two (or more) internal peptide bonds. In plasma, these two chains are linked together by a disulfide bond, forming Factor X. Activated Factor X, Factor Xa, participates in the final common pathway whereby prothrombin is converted to thrombin, which in turn converts fibrinogen to fibrin.
[0006] Clotting factors have been administered to patients to improve hemostasis for some time. The advent of recombinant DNA technology has significantly improved
treatment for patients with clotting disorders, allowing for the development of safe and consistent protein therapeutics. For example, recombinant activated factor VII has become widely used for the treatment of major bleeding, such as that which occurs in patients having haemophilia A or B, deficiency of coagulation Factors XI or VII, defective platelet function, thrombocytopenia, or von Wiliebrand's disease. Recombinant factor IX is therapeutically useful as well.
[0007] Although such recombinant molecules are effective, there is a need for improved versions which localize the therapeutic to sites of coagulation, have improved pharmacokinetic properties, have reduced clearance rates, have improved manufacture bi!ity, have reduced thrombogeniciry, or have enhanced activity, or more than one of these characteristics.

## SUMMARY OF THE INVENTION

[0008] The instant invention relates to chimeric clotting factors which have enhanced activity. The present invention features inter alia methods for making chimeric clotting factors, the chimeric clotting factors made using these methods, and methods for improving hemostasis using these clotting factors. The chimeric clotting factors of the invention possess enhanced pharmacokinetic properties, have reduced clearance rates, have improved manufaccurability, have reduced thrombogenicity, have enhanced activity, or more than one of these characteristics. In one embodiment, improved clotting factors of the invention have increased activity where needed, e.g., by targeting the clotting factor to platelets or by being present in a subject in an activatable form (a non-naturally occurring activatable form) that is activated at the site of clot formation.
[0009] In one aspect, the invention pertains to a chimeric clotting factor which comprises a clotting factor selected from the group consisting of FVII, FIX and FX and a targeting moiety which binds to platelets and optionally a spacer moiety between the clotting factor and the targeting moiety.
[0010] In one embodiment, the clotting factor comprises a structure represented by the formula A B C, wherem A is the clotting factor; wherem B is a spacer moiety; and wherein C is at least one targeting moiety which binds to platelets.
[0011] In one embodiment, the clotting factor comprises a structure from amino terminus to carboxy terminus represented by a formula selected from the group consisting of: A B C; C B A
[0012] In one embodiment, the clotting factor exhibits increased generation of thrombin in the presence of platelets as compared to an appropriate control lacking the at least one targeting moiety.
[0013] In one embodiment, the clotting factor comprises a scaffold moiety and, optionally, a second spacer moiety.
[0014] In one embodiment, the clotting factor further comprises D and E, wherein D is a spacer moiety; and E is a scaffold moiety and wherein the chimeric clotting factor comprises a structure from amino terminus to carboxy terminus represented by a formula selected from the group consisting of: A B C D E; A D E B C; ED A B C; C B ADE; EDCBA; and CBEDA.
[0015] In one embodiment, E is a dimeric Fc region comprising a first Fc moiety, F1 and a second Fc moiety, F2.
[0016] In one embodiment, the clotting factor is expressed as a polypeptide comprising a cleavable $\mathrm{scFc}(\mathrm{cscFc})$ linker interposed between two Fc moieties, wherein the cscFc linker is adjacent to at least one enzymatic cleavage site which results in cleavage of the cscFc polypeptide linker.
[0017] In one embodiment, the cscFc linker is adjacent to at least one enzymatic cleavage site which results in cleavage of the cscFc linker,
[0018] In one embodiment, the chimeric clotting factor of claim 9, wherein the at least one enzymatic cleavage site is an intracellular processing site.
[0019] In one embodiment, wherein the polypeptide linker is flanked by two enzymatic cleavage sites which are recognized by the same or by different enzymes. [0020] In one embodiment, the polypeptide linker has a length of about 10 to about 50 amino acids.
[0021] In one embodiment, the polypeptide linker has a length of about 20 to about 30 amino acids.
[0022] In one embodiment, the polypeptide linker comprises a giy/ser peptide.
[0023] In one embodiment, the gly/ser peptide is of the formula $\left(\mathrm{Gly}_{4} \mathrm{Ser}_{\mathrm{r}}\right) \mathfrak{n}$, or $\operatorname{Ser}\left(\mathrm{Gly}_{4} \mathrm{Ser}\right) \mathrm{n}$ wherein n is a positive integer selected from the group consisting of 1 , $2,3,4,5,6,7,8,9$ and 10 . In one embodiment, the $\left(\mathrm{Gly}_{4} \mathrm{Ser}\right) \mathrm{n}$ linker is selected
from the group consisting of $\left(\right.$ Gly $\left._{4} \operatorname{Ser}\right) 6, \operatorname{Ser}\left(\mathrm{Gly}_{4} \operatorname{Ser}\right) 6,\left(\mathrm{Gly}_{4} \operatorname{Ser}\right) 4$ and $\operatorname{Ser}\left(\mathrm{Gly}_{4}\right.$ Ser)4.
[0024] In one embodiment, the clotting factor comprises two polypeptide chains. [0025] In one embodiment, the chimeric clotting factor has a strurure selected from the group consisting of: A linked to F1 via a spacer moiety and C linked to F2; A linked to $\overline{1} 1$ via a spacer moiety and $C$ linked to $F 2$ via a spacer moiety; A linked to F1 and C is Jinked to F2 via a spacer moiety; A finked to Fi via a spacer moiety and C is linked to F2 via a spacer moiety.
[0026] In one embodiment, a chimeric clotttig factor comprises two polypeptides wherein the first polypeptide comprises the moieties A B F1; A B Fl; A B F1; or A B F1 D C and the second polypeptide comprises the moieties C F2; C D F2; F2 D C; or F2 D C, wherein the two polypeptide chains form an Fc region.
[0027] In one embodiment, the targeting moiety is fused to at least one of the polypeptide chains of the Fc region. In one embodiment, the targeting moiety is fused to at least one of F1 and F2 directly. In one embodiment, the targeting moiety is fused to at least one of F1 and F2 via a spacer moiety. In one embodiment, the targeting moiety is fused to at least one of F1 and F2 via a cleavable linker. In one embodiment, the targeting moiety is selected from the group consisting of: an antibody molecule, an antigen binding fragment of an antibody molecule, an scFv molecule, a receptor binding portion of a receptor, a peptide. I\{\} one embodiment, wherein the targeting moiety binds to resting platelets. In one embodiment, the targeting moiety selectively binds to activated platelets. In one embodiment, the targeting moiety selectively binds to a target selected from the group consisting of: GPIba, GPVI, and the nonactive form of GPIIb/IIIa. In one embodiment, the targeting moiety selectively binds to a target selected from the group consisting of: the active form of GPIIb/IIIa, P selectin, GMP--33, LAMP-1, LAMP-2, CD40L, and LOX-1. In one embodiment, the targeting moiety binds to the GPIb complex In one embodiment, the targeting moiety is a peptide selected from the group consisting of: PS4, OSL, and OS2. In one embodiment, the targeting moiety comprises an antibody variable regions from an antibody selected from the group consisting of: SCE5, MB9, and AP3.
[0028] $\mathrm{I}_{\mathfrak{D}}$ one embodiment, wherein the clotting factor is Factor VII.
[0029] In one embodiment, the clotting factor is a high specific activity variant of Factor VII. In one embodiment, the clotting factor is Factor IX. In one embodiment,
the clotting factor is a high specific activity variant of Factor IX. In one embodiment, the clotting factor is Factor X . In one embodiment, clotting factor is a high specific activity variant of Factor X.
[0030] In one embodiment, the clotting factor is secreted by a cell in active form. In one embodiment, the clotting factor is activated in vivo.
[0031] In one embodiment, the chimeric clotting factor comprises a heterologous enzymatic cleavage site not naturally present in the dotting factor.
[0032] In one embodiment, the enzymatic cleavage site is genetically fused to the amino terminus of the heavy chain moiety of the clotting factor.
[0033] In one embodiment, the clotting factor comprises a scaffold moiety is a protein molecule which increases the hydrodvnamic radius of the chimeric clotting factor. In one embodiment, the scaffold moiety, if present, is selected from the group consisting of albumin and XTEN*
[0034] In another aspect, the invention pertains to a polypeptide comprising FVII, which FVII comprises a heterologous enzymatic cleavage site activatable by a component of the clotting cascade.
[0035] In one embodiment, the polypeptide comprises a scaffold moiety and, optionally, a spacer moiety.
[0036] In one embodiment, the scaffold moiety is a dimeric Fc region comprising a first Fc moiety, F1 and a second Fc moiety, F2.
[0037] In one embodiment, the clotting factor comprises two polypeptide chains.
[0038] In one embodiment, the chimeric clotting factor has a struture selected from the group consisting of: the clotting factor linked to the first Fc moiety via a spacer moiety; the clotting factor linked to the second Fc moiety via a spacer moiety; the clotting factor is directly linked to Fl ; and the clotting factor is directly linked to F2. [0039] In one embodiment, the chimeric clotting factor further comprises a targeting moiety.
[0040] In one embodiment, the chimeric clotting factor is synthesized as a single polypeptide chain comprising a cscFc linker. In one embodiment, the $\operatorname{cscFc}$ linker is linked to (e.g., directly linked or adjacent to) at least one enzymatic cleavage site which results in cleavage of the linker.
[0041] In one embodiment, the at least one enzymatic cleavage site is an intracellular processing site. In one embodiment, the cscFc linker is flanked by two enzymatic cleavage sites which are recognized by the same or by different enzymes. In one
embodiment, the $\operatorname{cscFc}$ linker has a length of about 10 to about 50 amino acids. In one embodiment, the cscFc linker has a length of about 20 to about 30 amino acids.
[0042] In one embodiment, the cscFc linker comprises a gly/ser peptide.
[0043] In one embodiment, wherein the gly/ser peptide is of the formula ( $\mathrm{Gly}_{4} \mathrm{Ser}$ )n, or $\operatorname{Ser}\left(\mathrm{Gly}_{4} \mathrm{Sef}\right) \mathrm{n}$ wherein n is a positive integer selected from the group consisting of $1,2,3,4,5,6,7,8,9$ and 10 . In one embodiment, the $\left(\mathrm{Gly}_{4}\right.$ Ser) n linker is selected from the group consisting of $\left(\mathrm{Gly}_{4} \operatorname{Ser}\right) 6, \operatorname{Ser}\left(\mathrm{Gly}_{4} \operatorname{Ser}\right) 6,\left(\mathrm{Gly}_{4} \operatorname{Ser}\right) 4$ and $\operatorname{Ser}\left(\mathrm{Gly}_{4}\right.$ Ser)4.
[0044] In one embodiment, the clotting factor is a high specific activity variant of Factor VII. In one embodiment, the heterologous enzymatic cleavage site present in the chimeric clotting factor is cleaved at the site of clot formation. In one embodiment, the cleavage site is selected from the group consisting of: a factor XIa cleaveage site, a factor Xa cleavage site, and a thrombin cleavage site. In one embodiment, the enzymatic cleavage site is genetically fused to the amino terminus of the heavy chain moiety of the clotting factor.
[0045] In one embodiment, the targeting moiety binds to resting platelets. In one embodiment, the targeting moiety selectively binds to activated platelets.
[0046] In one embodiment, wherein the targeting moiety selectively binds to a target selected from the group consisting of: GPIba, GPVI, and the nonactive form of GPIIb/IIIa. In one embodiment, the targeting moiety selectively binds to a target selected from the group consisting of: the active form of GPIIb/IIIa, P selectin, GMP33, LAMP - 1, LAMP-2, CD40L, and LOX-1.
[0047] In one embodiment, the scaffold moiety is a protein molecule which increases the hydrodynamic radius of the chimeric clotting factor. In one embodiment, the scaffold moiety, if present, is selected from the group consisting of albumin and X'IE N ${ }^{\circledR}$
[0048] In one aspect the invention pertains to a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: A B C; C B A; A B CDE; A DEBC, EDABC, CBADE, EDCBA, CBEDA, wherein A an activatable clotting factor, B is absent or is a linker, C is a targeting moiety, D is absent or is a linker, and E is a scaffold moiety.
[0049] $\mathrm{I}_{\mathrm{n}}$ one embodiment, the clotting factor comprises a light and heavy chain of a clotting factor and each of the light and heavy chains are expressed as separate polypeptide chains.
[0050] In one embodiment, the invention pertains to a nucleic acid molecule encoding a chimeric clotting factor of the invention. In one embodiment, the nucleic acid molecule is present in a vector. In one embodiment, the vector further comprises a nucleotide sequence encoding an enzyme which cleaves at least one of the enzymatic cleavage sites.
[0051] In one embodiment, the invention pertains to a host cell comprising the expression vector of the invention. In one embodiment, the host cell expresses an enzyme capable of intracellular processing. In one embodiment, the enzyme is endogenous to the cell In one embodiment, the enzyme is heterologous to the cell. [0052] In another embodiment, the invention pertams to a method for producing a chimeric clotting factor comprising culturing the host cell in culture and recovering the chimeric clotting factor from the medium.
[0053] In another embodiment, the invention pertams to a processed, heterodimeric polypeptide comprising two polypeptide chains, wherein said processed, heterodimeric polypeptide is made by expressing the vector in a cell cultured in cell culture medium and isolating the mature, heterodimeric polypeptide from the culture medium.
[0054] In one embodiment, the invention pertains to a composition comprising a chimeric clotting factor and a pharmaceutically acceptable carrier.
[0055] In another embodiment, the invention pertams to a composition comprising the nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. [0056] In another embodiment, the invention pertains to a method for improving hemostasis in a subject, comprising administering the composition of the invention. [0057] in one aspect, the invention pertains to an chimeric clotting factor which comprises a fight chain moiety and a heavy chain moiety of a clotting factor, and at least one targeting moiety, wherein said targeting moiety (i) specifically binds to platelets, (ii) is not interposed between the light and heavy chains of the clotting factor, and wherein said chimeric clotting factor exhibits increased generation of thrombin in the presence of platelets as compared to an appropriate control lacking the at least one targeting moiety. [0058] In another aspect, the invention pertains to an chimeric clotting factor, which comprises the moieties A-B-C-D-E in linear sequence wherein A is a clotting factor, an activatable clotting factor, or an activated clotting factor: B
is absent or is a linker; C is a targeting moiet $\}^{\prime} ; \mathrm{D}$ is absent or is a linker; and E is absent or is a scaffold moiety.
[0059] In still another aspect, the invention pertains to an chimeric clotting factor, which comprises a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: $\mathrm{ABC} ; \mathrm{ABCDE}$;
$\mathrm{ADEBC}, \mathrm{EDABC}, \mathrm{CBADE}, \mathrm{EDCBA}, \mathrm{CBEDA}$, wherein A is a clotting factor, an activatable clotting factor or an activated clotting factor, B is absent or is a linker, C is a targeting moiet $\}^{\prime}$. D is absent or is a linker, and E is a scaffold moiety.
[0060] In yet another aspect, the invention pertains to an chimeric clotting factor, which comprises a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: ABF1:F2;
$\mathrm{ABF} 1: \mathrm{CDF} 2$; $\mathrm{ABF} 1: \mathrm{F} 2 \mathrm{DC}, \mathrm{ABF} 1 \mathrm{DC}: F 2 \mathrm{DC}$, wherein A is a clothng factor, an activatable clotting factor or an activated clotting factor, B is absent or is a linker, C is a targeting moiet $\}^{\prime}$. D is absent or is a linker, and $\mathrm{F}\{$ and F 2 are each an Fc moiety, and : represents dimerization mediated by the F1 and F2 chains of two polypeptide chains.
[0061] In still another aspect, the invention pertains to an chimeric clotting factor which comprises a light chain moiety and a heavy chain moiety of a clotting factor, and at least one targeting moiety, wherein said targeting moiety specifically binds to platelets, wherein the chimeric clotting factor comprises a disulfide linked Fc region which comprises two polypeptide chains.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0062] Figure 1 illustrates exemplary chimeric clotting factor constracts comprising a targeting domain. These exemplary constructs comprise an Fc region.
[0063] Figure 2 illustrates exemplary chimeric clotting factor constructs comprising a targeting domain. These exemplary constructs comprise a cieavable single chain Fc ( cscFc ) in which the scFc linker is processed by a cell in which it is expressed to form a two chain Fc region.
[0064] Figure 3 illustrates exemplary chimeric clotting factor constracts which comprise a targeting domain and wherein the clotting factor moiety lacks a Gla domain. These exemplary constructs comprise an Fc region.
[0065] Figure 4 illustrates exemplary chimeric clotting factor constructs which comprise a targeting domain and wherein the clotting factor moiety lacks a G1a domain. These exemplary constructs comprise a cleavable single chain $\mathrm{Fc}(\mathrm{cscFc})$ in which the scFc linker is processed by a cell in which it is expressed to form a two chain Fc region.
[0066] Figure 5 illustrates exemplary chimeric clotting factor constmcts which are activated (e.g., after activation in vitro or by separate expression of the light and heavy chains of the clotting factor) or which are activatable, i.e., comprise a moiety which is cleavable in vivo at the site of a clot (see panels D and E). These exemplary constructs comprise an Fc region. Constructs A, B, and C did not express well in early experiments.
[0067] Figure 6 illustrates exemplary chimeric clotting factor constructs which are activated (e.g., after activation in vitro or by separate expression of the light and heavy chains of the clotting factor) or which are activatable, i.e., comprise a moiety which is cleavable in vivo at the site of a clot. These exemplary constmcts comprise a cleavable single chain $\mathrm{Fc}(\mathrm{cscFc})$ in which the scFc linker is processed by a cell in which it is expressed to form a two chain Fc region,
[0068] Figure 7 illustrates exemplary chimeric clotting factor constmcts comprising a targeting domain.
[0069] Figure 8 illustrates exemplary chimeric clotting factor constmcts which comprise a targeting domain and wherein the clotting factor moiety lacks a Gla domain.
[0070] Figure 9 illustrates exemplary chimeric clotting factor constructs which are activated (e.g., after activation in vitro or by separate expression of the light and. heavy chains of the clotting factor) or which are activatable, i.e., comprise a moiety which is cleavable in vivo at the site of a clot.
[0071] Figure $\{0$ shows SDS PAGE for purification and activation of FV XX0 0 !.
[0072] Figure 11 shows SDS PAGE for purification of active FVII-053.
[0073] Figure 12 shows schematics of FVII-011 and FVII-102 and shows binding of FVIII-011 and FVH-027 to activated platelets determined by FACS.
[0074] Figure 13 shows thrombin generation assay to measure activity of FVII-027, FVII-01I and Novoseven in the presence of activated platelets.
[0075] Figure 14 shows that PAC-1 eliminates increased binding to platelets and increased rates of thrombin generation associated with FVII-027.
[0076] Figure 15 shows constructs used in a thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets.
[0077] Figure 16 shows a thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets.
[0078] Figure 17 shows the constructs used in the thrombin generation assay to measure activity of FVII-044, FVTT-045, FVII-046, FVII-0 11 and Novoseven in the presence of activated platelets shown in Figure 18.
[0079] Figure 18 show's a thrombin generation assay to measure activity of FVII-044, FVII-Q45, FVII-046, FVII-0 11 and Novoseven in the presence of activated platelets. [0080] Figure 19 shows the constructs used in the thrombin generation assay to measure activity of FVII-047, FVII-048, FVII-049, FVII-0 11 and Novoseven in the presence of activated platelets shown in Figure 20.
[0081] Figure 20 shows a thrombin generation assay to measure activity of FVII-047, FVII-048, FVII-049, FVII-01 1 and Novoseven in the presence of activated platelets. [0082] Figure 21 show's the construct used in the thrombin generation assay to measure activity of FVH-053 and FVII-0 11 in the presence of activated platelets shown in Figure 22,
[0083] Figure 22 shows a thrombin generation assay to measure activity of FVII- 053 and FVII-01 1 in the presence of activated platelets
[0084] Figure 23 shows that PAC-1 eliminates increased rate of thrombin generation associated with FVII-053
[0085] Figure 24 shows the constructs used in the Western blot analysis of FVIIFc species following transient transfection of HEK 293 cells and protein A pulldown shown in Figure 25.
[0086] Figure 25 shows Western blot analysis of FVOFc species following transient transfection of HEK 293 cells and protein A pulldown.
[0087] Figure 26 shows Western blot of protein A immunoprecipitation following transient transfection of $\mathrm{pSYN}-\mathrm{FW}-024$ with or without $\mathrm{pSYN}-\mathrm{PC} 5-003$. Lane 1 , pSYN-FVII-024, non reducing; lane $2, \mathrm{pSYN}-\mathrm{FVII}-024$, non reducing; lane $3, \mathrm{pSYN}-$ FVII-024, reducing; lane 4, pSYN-FVH-024, reducing.
[0088] Figure 27 shows Western blot analysis (Fc western) of FVIIFc species following transient transfection of HEK 293 cells and protein A pulldown .
[0089] Figure 28 shows FVII-039 and FVfl-040 treatment by FXIa.
[0090] Figure 29 shows that an FVIIaFc variant targeted to active form of GPIIbma shows an increased rate of thrombin generation.
[0091] Figure 30 shows a Rotation Thromoboelastometry (ROTEM) assay to measure the activity of FVXI-088 and wild type recombinant FVIIaFc in hemophilia A human blood. Clotting Time, Clot Forming Time and Alpha Angle parameters are shown. [0092] Figure 31 shows exemplary cleavage sites and illustrative positions of such cleavage sites in activatable clotting factor constructs. In this Figure FVII is used as an example.
[0093] Figure 32 shows cleavage of the constructs illustrated in Figure 31.
[0094] Figure 33 shows additional activatable constructs and a Western blot illustrating their cleavage.
[0095] Figure 34 shows the results of a thrombin generation assay using the FVII-062 and -090 constructs. FVII-062 is a negative control which lacks a thrombin cleavage site, so the contstract cannot be activated. FVII-090 contains the ALRPR cleavage site and so is activatable by thrombin.
[0096] Figure 35 illustrates the cleavage of high specific activity FVII variants. FVII heavy chain-Fc and light chain Fc collapse in 1 band because the heavy chain loses a glycosylation site after insertion of the trypsin 170 loop and becomes smaller.
[0097] Figure 36 illustrates the results of a thrombin generation assay using FVII-090 and FVII- 100.
[0098] Figure 37 illustrates the results of a thrombin generation assay using FVII-090 and FVII-115.
[0099] Figure 38 illustrates amidolytic activity of activatable FVIIFC activated with thrombin. Amidolytic activity of the activatable variants can be measured following thrombin activation and there is increased amidolytic activity for the high specific activity variants as compared to FVII-090. In these assays, after activation of the activatable molecule by thrombin, hirudin is added to inhibit thrombin cleavage of the chromogenic substrate. In this manner, the thrombin does not interfere with the ability to detect FV lia activity.
[00100] Figure 39 illustrates the results of an assay measuring activation of FX by FVIIa using substrate S2765, which is not cleaved by FVIIa. In this assay, 10 uM of FX was incubated with FVIIaFc for 15 minutes at $37^{\circ} \mathrm{C}$. The reaction was quenched with EDTA and substrate was added. Figure 39 shows the results of the
control experiment which demonstrates that FX activation by FVIIaFe can be detected.
[00101] Figure 40 shows FXa generation activity by "activatable FVTJFc." The experiment shown in Figure 40 shows that there is an increase in FX activation activity for the high specific activity variants. In this experiment. FVIIFc ( 100 nM ) was activated with thrombin ( 100 nM ) Hirudin was added to inhibit the thrombin. FX (lOuM) was added, followed by EDTA to inhibit the reaction. The activity of FX was measured by detecting the FXa substrate.
[00102] Figure 41 illustrates exemplary activatable construct formats, including an activatable monomer structure used in FVII- 1 18, FVII- 119, and FVII- 127.
[00103] Figure 42 illustrates the efficiency of thrombin cleavage of activatable constructs, specifically monomelic (FVI-118 and -119) as compared to the heterodimeric (FVXI-090).
[00104] Figure 43 illustrates the results of a thrombin generation assay to compare wild type activatable FVIIFc (FVII-118) to the high specific activity variant (FVII-127).
[00105] Figure 44A illustrates several targeted constructs. In this instance, an SCE5 scFv which binds to the active conformation of GPIIbIIla was included at various sites in the contruct. Figure 44B illustrates the results of thrombin generation assays in platelet-rich FVII-deficient plasma using these constructs. N7 is the Novoseven control. Figure 44C illustrates the binding of recombinant FVIIaFe variants to platelets by FACs.
[00106] Figure 45A illustrates several targeted FVIIa constructs which include AP3, an scFv against GPIIbllla present on resting and activated platelets. Figure 45B shows the results of thrombin generation assays in platelet-rich FVIII-deficient. plasma. Figure 45 C shows the results of binding of rFVTIaFc variants to platelets by FACS
[00107] Figure 46A shows several targeted FVIIa constructs that target GPIbaipha using peptides that bind to that molecule, specifically, the PS4, OS1, and OS2 peptides. Figure 46B shows the results of thrombin generation assays in platelet-rich FVIII-deficient plasma using the C terminal peptide constructs shown in Figure 46A. [00108] Figure 47A shows the results of thrombin generation assays in plateletrich FVIII-deficient plasma using the N terminal peptide constructs shown in Figure 46A. Figure 47B shows a direct comparison of FVII-045 and FVII-048.
[001091 Figure 48 shows the binding of FV11-045 and FVII-048 and wild type FVIIaFc to platelets as determined by FACS. The figure also shows the affinity of the targeting peptides as reported in Bernard et al. Biochemistry 2008, 47:4674-4682.
[00110] Figure 49A shows an exemplary targeted FV111 construct. Figure 49B shows the results of a thrombin generation assay in FV XI deficient platelet-rich plasma. In this experiment, the assay was activated with tissue factor (top panel) or by platelet activation (bottom panel).
[00111] Figure 50 shows results of an experiment measuring half life of a targeted FVII construct comprising a gla domain (FVII-011) and lacking a gla domain (FV [\}-028).
[00112] Figure 51A shows several FlX construct comprising targeting moieties, in this case SCE5 scFv. Figure 5]B shows the results of thrombin generation assays in platelet-rich FIX-deficient plasma using the constmcts of Figure 5\} A. Figure 51C illustrates that both FIX-068 and FIX-088 have at least 4 times more activity than FiX-042 as measured by Thrombin generation.
[00113] Figure 52A shows the results of a thrombin generation assaycomparing FTX-090 and Benefix. Figure 52B shows that the activity of FXX-090 is almost 4 times that of Benefix.
[00114] Figure 53A shows a targeted FIX construct comprising a peptide that binds to GPIb, present on resting and activated platelets. Figure 53 B shows the results of thrombin generation assays in platelet-rich FIX deficient plasma. Figure 53C demonstrates that F1X--089 is roughly 4-times stronger than FIX-042 as measured by thrombin generation, while having a lower specific activity.

## DETAILED DESCRIPTION OF THE INVENTION

[00115] The present invention relates to chimeric clotting factors. The present invention is based, at least in part, on the development of novel ways to enhance the efficacy, pharmacokinetic properties, and/or manufacturability of clotting factors. In one embodiment, improved clotting factors of the invention have increased activity where needed, e.g., by targeting the clotting factor to platelets or by being present in a subject in an activatable form (a non-naturally occurring activatable form) that is activated at the site of clot formation. This can be accomplished, e.g., by targeting the clotting factors or by making them in an activatable form.
[00116] In one embodiment, the subject clotting factors are targeted to the site of coagulation. By incorporating a targeting moiety which targets the clotting factor to resting or activated platelets, the activity of a dotting factor can be enhanced. For example, in the case of factor VII, unlike endogenous FVI that is likely activated by tissue factor (TF) at endothelial cell surfaces to generate activated factor $\mathrm{X}(\mathrm{FXa})$, exogenous FVTIa likely generates $\mathrm{FXa} / \mathrm{FIXa}$ in a TF independent manner, most effective at the surface of activated platelets where other cloiting factors are localized. However, physiologically FVIla acts at the surface of a TF-bearing ceil, such as an endothelial cell, and has low affinity for platelets. It has been hypothesized that therapeutic recombinant FVIla acts by converting Factor X into Factor Xa on the surface of activated platelets. To overcome low platelet affinity and be effective at treating bleeds, recombinant FVIla is dosed at supra-physiological levels. Therefore, in the case of FVTIa, targeting to platelet surfaces could significantly increase the efficacy of this molecule. Although other clotting factors (e.g. FIX, FVIII, FX) have higher affinity to platelets, these too may exhibit enhanced activity by incorporating platelet targeting moieties. In addition, FVIla has a relatively short halt-life (-2.3 hours) in humans. This short half-life likely contributes to the need to dose recombinant FVTIa multiple times to control a bleed. Thus, targeting clotting factors, and in particular FVTIa, to platelets improves efficiency.
[00117] The targeting moiety can be positioned at a number of places in a chimeric doting factor. Exemplary structures of targeted chimeric clotting factors are set forth, e.g., in Figures 1-4, 7, 8, 17, 19, 21, 44, 46, 49, 51, and 53.
[00118] In another embodiment, a chimeric clotting factor of the invention is made in a form that is activatable at the cite of coagulation. For use in bypass therapy exogenous clotting factors are only efficacious when given in the activated form. However, such activated dotting factors are rapidly inactivated by endogenous pathways (e.g. antithrombin III, TFPI), leading to clearance of the active form and a short effective half life. Giving higher doses does not solve this problem as it can result in thrombogenic effects. Thus, in one embodiment, the invention pertains to an "activatable" chimeric clotting factor constructs which comprise a heterologous enzymatic cleavage site not normally present in the clotting factor. These molecules circulate as enhanced zymogens and have a longer half life due to the lack of inactivation upon dosing, but can readily be activated at the site of clotting by cleavage by an enzyme. In one embodiment, such a heterologous enzymatic cleavage
site is one for an enzyme produced during the clotting cascade. For example, in one embodiment, the heterologous cleavage site of an activatable construct comprises a Factor XIa, Xa, or thrombin cleavage site. Exemplary FXIa cleavage sites include, e.g.,: TQSFNDFTR and SVSQTSKLTR. Exemplary thrombin cleavage sites include, e.g.,: DFLAEGGGVR, TTK1KPR, and ALRPR. In one embodiment, a heterologous cleavage site is interposed between the light and heavy chains of the clotting factor. In another embodiment, a heterologous cleavage site is not interposed between the two chains of the clotting factor. In one embodiment, the heterologous cleavage site is amino terminal to the heavy chain of the clotting factor.
[00139] The heterologous cleavage site is present in a cleavable linker can be positioned at a number of places in a chimeric cloting factor. Exemplary structures of activatable chimeric clotting factors are set forth, e.g., in Figures 5, 6, 9, 29, 27, 31, and 41. Exemplary such constructs are activated in the presence of clot formation and are described in more detail below.
[00120] In one embodiment, a chimeric clotting factor of the invention comprises a scaffold, e.g., to enhance the hydrodynamic radius of the molecule. For example, a chimeric clotting factor of the invention may be a fusion protein.

Exemplary scaffolds include, e.g.. FcRn binding moieties (e.g., complete Fc regions or portions thereof which bind to FcRn ), single chain Fc regions ( ScFc regions, e.g., as described in US 2008/0260738, WO 2008/012543, or WO 2008/1439545), cleaveable scFc regions (comprising a cscFc regions as described herein), less complicated proteins or portions thereof, e.g., XTen polypeptides*", or albumin. [00121] In one embodiment, a chimeric clotting factor of the invention employs an Fc region or an FcRn binding portion thereof as a scaffold moiety. In one embodiment, the Fc moiety to which the chimeric clotting factor is fused is a naturally occurring (or wild type (WT)) Fc moiety. In another embodiment, the Fc moiety comprises one or more variations in sequence.
[00122] In another embodiment, the Fc moiety is a scFc moiety (e.g., comprising a non-cleavable or a cscFc linker). In a construct comprising a cscFc linker an unprocessed molecule comprises a cleavable single chain Fc region in which the component Fc moieties are genetically-fused in a single polypeptide chain forming a functional, single chain, dimeric Fc region. The cscFc linker can link the Fc moieties that will comprise the dimeric Fc region of the polypeptide in tandem or may link one Fc moiety to a non-Fc moiety of the construct, e.g., a clotting factor or
targeting moiety, which is, in turn, linked to a second Fc moiety. The cscFc linker is interposed between the Fc moieties that comprise the scFc region and is flanked by at least one enzymatic cleavage site, e.g., an intracellular enzymatic processing sites. In one embodiment, the scFc linker is flanked by two enzymatic cleavage sites resulting in the excision of the linker (e.g., all or substantially all of the linker) when the protein encoded by the nucleic acid molecule is processed in a cell, In another embodiment, the scFc linker is adjacent to at least one enzymatic cleaveage site that allows for excision of the linker in vitro after the polypeptide has been secreted by a cell or comprises at least one enzymatic cleaveage site that allows for cleavage of the linker in vivo after the construct is administered to a subject. Thus, in one embodiment, although the such a polypeptide comprises scFc region(s) encoded in a single open reading frame $(\mathrm{OKF})$ as part of one contiguous nucleotide sequence in unprocessed form, the cscFc linker is enzymatically cleaved (e.g., prior to administration or in vivo after administration), resulting in a polypeptide which is a heterodimeric molecule comprising an Fc region which is not fused in a single amino acid chain, i.e., the resulting processed construct has a Fc region which comprises two polypeptide chains. In such embodiments, all or substantially all of the linker is excised, while in some embodiments, a portion of the cleavage site may remain, e.g., four arginines of the RRRR cleavage site.
[00123] In one embodiment, the scFc linker is flanked by two processing sites for cleavage. The two processing sites can be the same or different. In one embodiment, at least one processing site is a cluster of basic amino acid residues as recognized by arginine kex2/furin enzymes. Such enzymes cleave immediately Cterminal to an arginine residue. In another embodiment, at least one cleavage site is one that may be cleaved in vivo, for example a cleavage site recognized by thrombin. [00124] In one embodiment, a chimeric clotting factor of the invention is manufactured in an activated form in the context of an scFc molecule comprising a csFc linker. For example, Factor VII, is generally produced recombinantly as a zymogen, and requires activation during manufacturing to produce the active form for administration. In one embodiment, a chimeric clotting factor of the invention is secreted from the cell in which it is expressed in active form to improve manufacture bility. As is set forth in more detail below, such clotting factors can be produced by incorporating a single chain Fc region into the molecule. Single chain Fc regions are formed by dimerization of Fc moieties which are present in a single
polypeptide chain. In one embodiment, such a construct comprises an scFc polypeptide linker linking the two Fc moieties of the scFc which is adjacent to at least one intracellular processing site. Cleavage of such a construct is delayed until late in the secretory pathway, e.g., when the protein colocalizes with active processing enzymes in the rrans-Golgi apparatus.
[00125] In one embodiment, a cell expressing a construct encoding a polypeptide of the invention endogenous!\}' expresses an enzyme which cleaves the scFc linker at one or more processing sites resulting in a dimeric molecule comprising two polypeptide chains. In another embodiment, a cell expressing a construct encoding a polypeptide of the invention exogenously expresses an enzyme which cleaves the scFc linker at one or more processing sites.
[00126] In one embodiment, a chimeric clotting factor of the invention can combine two or more of these features to create an optimized construct e.g. targeting an activatable fusion protein constract to resting platelets, such that it can be activated efficiently as well as at a higher local concentration at the site of active coagulation. Exemplary such combination constructs include chimeric clotting factors that are both targeted and comprise an scFc linker for enhanced processing. In another embodiment, a constract of the invention is targeted and activatable.
[00127] Exemplary constructs of the invention are illustrated in the accompanying Figures and sequence listing. In one embodiment, the invention pertains to a polypeptide having the structure as set forth in the Figures. In another embodiment, the invention pertains to a polypeptide having the sequence set forth in the accompanying sequence listing or the the nucleic acid molecule encoding such polypeptides. In one embodiment, the invention pertains to a mature form of a polypeptide having the sequence set forth in the accompanying sequence listing. It will be understood that these constructs and nucleic acid molecules encoding them can be used to improve heraostasis in a subject.
[00128] In order to provide a clear understanding of the specification and claims, the following definitions are provided below.

## I. Definitions

[00129] As used herein, the term "protein" or "polypeptide" refers to a polymer of two or more of the natural amino acids or non-natural amino acids.
[00130] The term "amino acid" includes alanine (Ala or A); arginine (Arg or R ); asparagine (Asn or N ); aspartic acid (Asp or D ); cysteine (Cys or C); glutamine (Gin or Q); glutamic acid (Glu or E); glycine (Gly or G); histidine (His or H); isoleucine (lie or 1): leucine (Leu or L); lysine (Lys or K); methionine (Met or M); phenylalanine (Phe or F); proline (Pro or P); serine (Ser or S\}; threonine (Thr or T); tryptophan (Tip or W); tyrosine (Tyr or Y); and valine (Val or V). Non-traditional amino acids are also within the scope of the invention and include norleucine, ornithine, norvaline, homoserine, and other amino acid residue analogues such as those described in Elknan ei al. Meth. Enzym. 202:301-336 (1991). To generate such non-naturally occurring amino acid residues, the procedures of Noren et at. Science 244:182 (1989) and Ellman et al, supra, can be used. Briefly, these procedures involve chemically activating a suppressor tRNA with a non-naturally occurring amino acid residue followed by in vitro transcription and translation of the RNA. Introduction of the non-traditional amino acid can also be achieved using peptide chemistries known in the art. As used herein, the term "polar amino acid' " includes amino acids that have net zero charge, but have non-zero partial charges in different portions of their side chains (e.g. M, F, W, S, Y, N, Q, C). These amino acids can participate in hydrophobic interactions and electrostatic interactions. As used herein, the term "charged amino acid" include amino acids that can have non-zero net charge on their side chains (e.g. R, K, H, E, D). These amino acids can participate in hydrophobic interactions and electrostatic interactions.
[00131] An "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a predetermined amino acid sequence (an amino acid sequence of a starting polypeptide) with a second, different "replacement" amino acid residue. An "amino acid insertion" refers to the incorporation of at least one additional amino acid into a predetermined amino acid sequence. While the insertion will usually consist of the insertion of one or two amino acid residues, the present larger "peptide insertions", can be made, e.g. insertion of about three to about five or even up to about ten, fifteen, or twenty amino acid residues. The inserted residue(s) may be naturally occurring or non-naturally occurring as disclosed above. An "amino acid deletion" refers to the removal of at least one amino acid residue from a predetermined amino acid sequence.

Polypeptides may be either monomers or multimers. For example, in one embodiment, a protein of the invention is a dimer. A dimeric polypeptide of the
invention may comprise two polypeptide drains or may consist of one polypeptide chain (e.g., in the case of an scFc molecule). In one embodiment, the dimers of the invention are homodimers, comprising two identical monomelic subunits or polypeptides (e.g., two identical Fc moieties or two identical biologically active moieties). in another embodiment, the dimers of the in vention are heterodimers, comprising two non-identical monomelic subunits or polypeptides (e.g., comprising two different clotting factors or portions thereof or one clotting factor only). See, e.g., U.S. patent 7404956, incorporated herein by reference.
[00133] As used herein, the term "polypeptide linkers" refers to a peptide or polypeptide sequence (e.g., a synthetic peptide or polypeptide sequence) which connects two domains in a linear amino acid sequence of a polypeptide chain. In one embodiment, the the polypeptides of invention are encoded by nucleic acid molecules that encode polypeptide linkers which either directly or indirectly connect the two Fc moieties which make up the construct. These linkers are referred to herein as 'scFc linkers" and the scFc linker is interposed between the two Fc moieties of a polypeptide which comprises it. If the scFc linker connects two Fc moieties contiguously in the linear polypeptide sequence, it is a "direct" linkage. In contract, the scFc linkers may link the first Fc moiety to a binding moiety which is, in turn, linked to the second Fc moiety, thereby forming an indirect linkage. These scFc linkers permit the formation of a single chain genetic construct. In one embodiment, the polypeptides also comprise enzymatic cleavage sites which result in the scFe linker being eleavabie (a cscFc linker) and, in one embodiment, substantially excised (e.g., during processing by a cell). Thus, the resulting processed polypeptide is a dimeric molecule comprising at least two amino acid chains and substantially lacking extraneous linker amino acid sequences. In some embodiments, all or substantially all of the linker is excised, while in some embodiments, a portion of the cleavage site may remain, e.g., four arginines of the RRRR cleavage site.
[00134] In another embodiment, another type of polypeptide linker, herein referred to as a "spacer" may be used to connect different moieties, e.g., a clotting factor or targeting moiety to an Fc moiety on the polypeptide. This type of linker may provide flexibility to the polypeptide molecule. Spacers are not typically cleaved; however in certain embodiments, such cleavage may be desirable. Exemplary positions of spacers are shown in the accompanying drawings. Spacers can be located between the clotting factors, targeting moieties, and/or scaffolds, e.g., at the N or C
terminus of these moieties. In one embodiment, these linkers are not removed during processing.
[00135] A third type of linker which may be present in a chimeric clotting factor of the invention is herein referred to as a "cleavable linker" which comprises a heterologous cleavage site (e.g., a factor XIa, Xa , or thrombin cleavage site) and which may include additional spacer linkers on either the N terminal of C terminal or both sides of the cleavage site. Exemplary locations for such sites are shown in the accompanying drawings and include, e.g., placement adjacent to targeting moieties. In another embodiment, such linkers may be adjacent to a clotting factor or portion thereof. For example, in one embodiment, a cleavable linker may be fused to the N terminus of the heavy chain of a clotting factor to make an activatable form of the clotting factor. In such cases, the cleavable linker may include additional spacer linkers at the N terminus of the cleavage site, but requires direct fusion at the C terminus of the cleavage site to the amino terminus of the heavy chain of the clotting factor.
[00136] As used herein, the term "gly-ser polypeptide linker" refers to a peptide that consists of glycine and serine residues. An exemplary gly/ser polypeptide linker comprises the amino acid sequence $\left(\mathrm{Gly}_{4} \mathrm{Ser}_{\mathrm{n}_{\mathbf{n}}}\right.$ (SEQ ID NO;4) Another exemplary gly/ser polypeptide linker comprises the amino acid sequence S(Gly ${ }_{4}$ Ser) ${ }_{n}$.
[00137] In one embodiment, $\mathrm{n}=1$. In one embodiment, $\mathrm{n}=2$. In another embodiment, $\mathrm{n}=3$, i.e., $\left(\mathrm{Gly}_{4} \mathrm{Ser}\right)_{3}$. In another embodiment, $\mathrm{n}=4$, i.e., $\left(\mathrm{Gly}_{4} \mathrm{Ser}\right)_{4}$ (SEQ ID NO:6). In another embodiment, $\mathrm{n}=5$. In yet another embodiment, $\mathrm{n}=6$. In another embodiment, $n=\Rightarrow$. In yet another embodiment, $n=8$. In another embodiment, $\mathrm{n}=9$. In yet another embodiment, $\mathrm{n}=10$. Another exemplary gly/ser polypeptide linker comprises the amino acid sequence $\operatorname{Ser}\left(\mathrm{Gly}_{4} \operatorname{Ser}_{\mathrm{n}}\right.$ (SEQ ID NO:26). In one embodiment, $\mathrm{n}=$ - . In one embodiment, $\mathrm{n}=2$. In a preferred embodiment, $\mathrm{n}=3$. In another embodiment, $\mathrm{n}=4$. In another embodiment, $\mathrm{n}=5$. In yet another embodiment, $\mathrm{n}=6$.
[00138] A polypeptide or amino acid sequence "derived from" a designated polypeptide or protein refers to the origin of the polypeptide. Preferably, the polypeptide or amino acid sequence which is derived from a particular sequence has an amino acid sequence that is essentially identical to that sequence or a portion thereof, wherein the portion consists of at least 10-20 amino acids, preferably at least

20-30 amino acids, more preferably at least 30-50 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the sequence. [00139] Polypeptides derived from another peptide may have one or more mutations relative to the starting polypeptide, e.g.. one or more amino acid residues which have been substituted with another amino acid residue or which has one or more amino acid residue insertions or deletions. Preferably, the polypeptide comprises an amino acid sequence which is not naturally occurring. Such variants necessarily have less than $100 \%$ sequence identity or similarity with the starting antibody. In a preferred embodiment, the variant will have an amino acid sequence from about $75 \%$ to less than $100 \%$ amino acid sequence identity or similarity with the amino acid sequence of the starting polypeptide, more preferably from about $80 \%$ to less than $100 \%$, more preferably from about $85 \%$ to less than $100 \%$, more preferably from about $90 \%$ to less than $100 \%$ (e.g., $91 \%, 92 \%, 93 \%, 94 \%, 95 \%, 96 \%, 97 \%$, $98 \%, 99 \%$ ) and most preferably from about $95 \%$ to less than $100 \%$, e.g., over the length of the variant molecule. In one embodiment, there is one amino acid difference between a starting polypeptide sequence and the sequence derived therefrom. Identity or similarity with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical (i.e. same residue) with the starting amino acid residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.
[00140] Preferred polypeptides of the invention comprise an amino acid sequence (e.g., at least one clotting factor or Fc moiety or domain) derived from a human protein sequence. However, polypeptides may comprise one or more amino acids from another mammalian species. For example, a clotting factor, Fc domain, or targeting moiety may be derived from a non-human species and included in the subject polypeptides. Alternatively, one or more amino acids may be present in a polypeptide which are derived from a non-human species. Preferred polypeptides of the invention are not immunogenic.
[00141] It will also be understood by one of ordinary skill in the art that the polypeptides of the invention may be altered such that they vary in amino acid sequence from the naturally occurring or native polypeptides from which they were derived, while retaining the desirable activity of the native polypeptides. For example, nucleotide or amino acid substitutions leading to conservative substitutions or changes at "non-essential" amino acid residues may be made. An isolated nucleic
acid molecule encoding a non-natural variant of a polypeptide derived from an immunoglobulin (e.g., an Fc domain, moiety, or antigen binding site) can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of the immunoglobulin such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations may be introduced by standard techniques, such as site-directed mutagenesis andPCR-mediated mutagenesis.
[00142] The polypeptides of the invention may comprise conservative amino acid substitutions at one or more amino acid residues, e.g., at essential or nonessential amino acid residues. 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, a nonessential amino acid residue in a polypeptide may be replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members. Alternatively, in another embodiment, mutations may be introduced randomly along all or part of a coding sequence, such as by saturation mutagenesis, and the resultant mutants can be incorporated into polypeptides of the invention and screened for their ability to bind to the desired target.
[00143] In the context of polypeptides, a "linear sequence" or a "sequence" is the order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide.
[00144] As used herein, the terms "linked," "fused", or "fusion" refer to linkage via a peptide bonds (e.g., genetic fusion), chemical conjugation or other means. For example, one way in which molecules or moieties can be linked employs polypeptide linkers which link the molecules or moieties via peptide bonds. The terms
"genetically fused," "genetically linked" or "genetic fusion" are used interchangeably and refer to the co-linear, covaient linkage or attachment of two or more proteins, polypeptides, or fragments thereof via their individual peptide backbones, through genetic expression of a single polynucleotide molecule encoding those proteins, polypeptides, or fragments. Such genetic fusion results in the expression of a single contiguous genetic sequence. Preferred genetic fusions are in frame, i.e., two or more open reading frames (ORFs) are fused to form a continuous longer ORF, in a manner that maintains the correct reading frame of the original ORFs. Thus, the resulting recombinant fusion protein is a single polypeptide containing two or more protein segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally sojoined in nature). In this case, the single polypeptide is cleaved during processing to yield dimeric molecules comprising two polypeptide chains.
[00145] As used herein, the term "Fc region" is defined as the portion of a polypeptide which corresponds to the Fc region of native immunoglobulin, i.e., as formed by the dimeric association of the respective Fc domains of its two heavy chains. A native Fc region is homodimeric and comprises two polypeptide chains. 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).
[00146] As used herein, the term "Fc domain" refers to the portion of a single immunoglobulin heavy chain beginning in the hinge region just upstream of the papain cleavage site (i.e. residue 216 in $\operatorname{IgG}$, 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 CH 2 domain, and a CH 3 domain.
[00147] As used herein, the term " Fc domain portion" or " Fc moiety" includes an amino acid sequence of an Fc domain or derived from an Fc domain. In certain embodiments, an Fc moiety comprises at least one of: a hinge (e.g., upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, a CH 4 domain, or a variant, portion, or fragment thereof. In other embodiments, an Fc moiety comprises a complete Fc domain (i.e., a hinge domain, a CH 2 domain, and a CH 3 domain). In one embodiment, a Fc moiety comprises a hinge domain (or portion
thereof) fused to a CH3 domain (or portion thereof). In another embodiment, an Fc moiety comprises a CH 2 domain (or portion thereof) fused to a CH 3 domain (or portion thereof). In another embodiment, an Fc moiety consists of a CH3 domain or portion thereof. In another embodiment, an Fc moiety consists of a hinge domain (or portion thereof) and a CH 3 domain (or portion thereof). In another embodiment, a Fc moiety consists of a CH 2 domain (or portion thereof) and a CH 3 domain. In another embodiment, a Fc moiety consists of a hinge domain (or portion thereof) and a CH2 domain (or portion thereof). In one embodiment, an Fc moiety lacks at least a portion of a CH 2 domain (e.g., all or part of a CH 2 domain).
[00148] 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 a-phase and longer $\beta$-phase. The a-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. Therefore, in a preferred embodiment, 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.
[00149] As used herein the term "moiety" refers to a component part or constituent of a chimeric polypeptide.
[00150] As used herein, the term "targeting moiety" refers to a molecule, fragment thereof or a component of a polypeptide which localizes or directs the polypeptides of the invention to a desired site or cell. If one embodiment, a construct of the invention comprises a "targeting moiety" which enhances the activity of the polypeptide, e.g., by localizing the molecule to a desired site. Such a moiety may be, e.g., an antibody or variant thereof (e.g., and scFv) or a peptide. In another embodiment, such a targeting moiety may be a polypeptide, a receptor binding portion of a ligand, or a ligand binding portion of a receptor which is linked to a polypeptide of the invention and binds to the desired target, e.g., on a cell or tissue. The targeting moiety may be genetically fused to a construct, chemically conjugated to the construct
or linked to the construct via a spacer. For example, targeting moieties may be attached to a construct of the invention by formation of a bond between the targeting moiety and an Fc moiety of a construct, where the targeting moiety comprises a first functional group and the Fc moiety comprises a second functional group, and where the first and second functional groups are capable of reacting with each other to form a chemical bond (see, e.g., U.S. patent 7381408). In one embodiment, a targeting moiety binds to platelets. Exemplary targeting moieties are described in more detail below.
[00 51 1] In one embodiment a targeting moiety for use in a construct of the invention comprises an antibody variant. The term "antibody variant" or "modified antibody" includes an antibody which does not occur in nature and which has an amino acid sequence or amino acid side chain chemistry which differs from that of a naturally-derived antibody by at least one amino acid or amino acid modification as described herein. As used herein, the term "antibody variant" includes synthetic forms of antibodies which are altered such that they are not naturally occurring, e.g.. antibodies that comprise at least two heavy chain portions but not two complete heavy chains (such as, domain deleted antibodies or minibodies); multispecific forms of antibodies (e.g., bispecific, trispecific, etc.) altered to bind to two or more different antigens or to different epitopes on a single antigen); heavy chain molecules joined to scFv molecules; single-chain antibodies; diabodies; triabodies; and antibodies with altered effector function and the like.
[00152] As used herein the term "scFv molecule" includes binding molecules which consist of one light chain variable domain (VL) or portion thereof, and one heavy chain variable domain ( VH ) or portion thereof wherein each variable domain (or portion thereof) is derived from the same or different antibodies. scFv molecules preferably comprise an scFv linker interposed between the VH domain and the VL domain. ScFv molecules are known in the art and are described, e.g., in US patent 5,892,019, Ho et al, 1989. Gene 77:51; Bird et al. 1988 Science 242:423; Pantoliano et al. 1991. Biochemistry 30:10117; Milenic et al. 1991. Cancer Research 51:6363; Takkinen et al. 1991. Protein Engineering 4:837.
[00153] A "scFv linker" as used herein refers to a moiety interposed between the VL and VH domains of the scFv . scFv linkers preferably maintain the scFv molecule in a antigen binding conformation. In one embodiment, a scFv linker comprises or consists of an scFv linker peptide. In certain embodiments, a scFv linker
peptide comprises or consists of a gly-ser polypeptide linker. In other embodiments, a scFv linker comprises a disulfide bond.
[00154] The term "giycosylation" refers to the eovalent linking of one or more carbohydrates to a polypeptide. Typically, giycosylation is a posttranslational event which can occur within the intracellular milieu of a cell or extract therefrom. The term giycosylation includes, for example, N-linked giycosylation (where one or more sugars are linked to an asparagine residue) and/or O-linked giycosylation (where one or more sugars are linked to an amino acid residue having a hydroxy! group (e.g., serine or threonine). In one embodiment, a molecule of the invention is glycosylated. In another embodiment, a molecule of the invention is aglycosylated. In yet another embodiment, a molecule of the invention has reduced giycosylation as compared to that in a wild type Fc region.
[00155] As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CHI and CL regions are linked by native disulfide bonds and the two heavy chains are linked by two native disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system)
[00156] The term "vector' ' or "expression vector" is used herein to mean vectors used in accordance with the present invention as a vehicle for introducing into and expressing a desired polynucleotide in a cell. As known to those skilled in the art, such vectors may easily be selected from the group consisting of plasmids, phages, viruses and retroviruses. In geiieral, vectors compatible with the instant invention will comprise a selection marker, appropriate restriction sites to facilitate cloning of the desired gene and the ability to enter and/or replicate in eukaryotic or prokaryotic cells. [00157] Numerous expression vector systems may be employed to produce the chimeric clotting factors of the invention. For example, one class of vector utilizes DMA elements which are derived from animal viruses such as bovine papilloma viras, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV) or SV40 virus. Additionally, cells which have integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow selection of transfected host cells. The marker may provide for prototrophy to an auxotrophic host, biocide resistance (e.g., antibiotics) or resistance to heavy metals
such as copper. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. In one embodiment, an inducible expression system can be employed. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include signal sequences, splice signals, as well as transcriptional promoters, enhancers, and termination signals. In one embodiment, a secretion signal, e.g., any one of several well characterized bacteria! leader peptides (e.g., pelB, phoA, or ompA), can be fused in-frame to the N terminus of a polypeptide of the invention to obtain optimal secretion of the polypeptide. (Lei et ai. (1988), Nature, 331:543; Better et al. (1988) Science, 240:1041; Mullinax etal, (1990). PNAS, 87:8095).
[00158] The term "host ceil' ' refers to a cell that has been transformed with a vector constructed using recombinant DNA techniques and encoding at least one heterologous gene. In descriptions of processes for isolation of proteins from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of protein unless it is clearly specified otherwise. In other words, reco very of protein from the "cells" may mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells. The host cell line used for protein expression is most preferably of mammalian origin; those skilled in the art are credited with ability to preferentially determine particular host cell lines which are best suited for the desired gene product to be expressed therein. Exemplary host cell lines include, but are not limited to, DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative ofCVI with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), PerC6 cells), HAK (hamster kidney line), SP2/0 (mouse myeloma), P3x63-Ag3.653 (mouse myeloma), BFA-lclBPT (bovine endothelial cells), RAJI (human lymphocyte) and 293 (human kidney). Host cell lines are typically available from commercial services, the American Tissue Culture Collection or from published literature. The polypeptides of the invention can also be expressed in non-mammalian cells such as bacteria or yeast or plant cells. In this regard it will be appreciated that various unicellular nonmammalian microorganisms such as bacteria can also be transformed; i.e. those capable of being grown in cultures or fermentation. Bacteria, which are susceptible to transformation, include members of the enterobacteriaceae, such as strains of Escherichia coli or Salmonella; Bacillaceae, such as Bacillus subtilis; Pneumococcus;

Streptococcus, and Haemophilus influenzae. It will further be appreciated that, when expressed in bacteria, the polypeptides typically become part of inclusion bodies. The polypeptides must be isolated, purified and then assembled into functional molecules. [00159] In addition to prokaryotes, eukaryotie microbes may also be used. Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used among eukaryotie microorganisms although a number of other strains are commonly available including Pichia pastoris. For expression in Saccharomyces, the plasmid YRp7, for example, (Stinchcomb et a!., (1979), Nature, 282:39; Kingsman et ah, (1979), Gene, 7:141; Tschemper et al., (1980), Gene, 10:157\} is commonly used. This plasmid already contains the TRP I gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, (1977), Genetics, 85:12\}. The presence of the trpi iesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.
[00160] As used herein the term "endogenous" refers to molecules (e.g. nucleic acid and/or protein molecules) that are naturally present in a cell. In contrast, the term "exogenous'Or "heterologous" refers to such molecules that are not normally found in a given context, e.g., in a cell or in a polypeptide. For example, an exogenous or heterologous molecule may be introduced into a cell and are only present after manipulation of the cell, e.g., by transfection or other forms of genetic engineering or a heterologous amino acid sequence may be present in a protein in which it is not naturally found.
[00161] 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 and SVSQTSKLTR. Exemplary thrombin cleavage sites include, e.g, DFLAEGGGVR, TTKIKPR, LVPRG SEQ ID NO:35) and ALRPR. Other enzymatic cleavage sites are known in the art. [00162] As used herein, the term "processing site" or "intracellular processing site" refers to a type of enzymatic cleavage site in a polypeptide which is the 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-Goigi 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., furin, $\mathrm{PC} 2, \mathrm{PCl} / \mathrm{Pc} 3, \mathrm{PC} 4, \mathrm{PACE} 4, \mathrm{PC} 5 / \mathrm{PC}_{6}$; and

LPC/PC7/PC8/SPC7. Other processing sites are known in the art.
[00163] In constructs that include more than one processing or cleavage site, it will be understood that such sites may be the same or different.
[00164] 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.
[00165] As used herein, the phrase "subject that would benefit from administration of a polypeptide" includes subjects, such as mammalian subjects, that would benefit from administration of polypeptides of the invention, e.g., to improve hemostasis.
[00166] A "chimeric protein" or "fusion protein", as used herein, refers to any protein comprised of a first amino acid sequence derived from a first source, bonded, covalently or non-covalently, to a second amino acid sequence derived from a second source, wherein the first and second source are not the same. A first source and a second source that are not the same can include two different biological entities, or two different proteins from the same biological entity, or a biological entity and a non-biological entity. A chimeric protein can include for example, a protein derived from at least 2 different biological sources, A biological source can include any nonsynthetically produced nucleic acid or amino acid sequence (e.g. a genomic or cDNA sequence, a plasmid or viral vector, a native virion or a mutant or analog, as further
described herein, of any of the above). A synthetic source can include a protein or nucleic acid sequence produced chemically and not by a biological system (e.g. solid phase synthesis of amino acid sequences). A chimeric protein can also include a protein derived from at least 2 different synthetic sources or a protein derived from at least one biological source and at least one synthetic source. A chimeric protein may also comprise a first amino acid sequence derived from a first source, covalently or non-eovalently linked to a nucleic acid, derived from any source or a small organic or inorganic molecule derived from any source. The chimeric protein may comprise a linker molecule between the first and second amino acid sequence or between the first amino acid sequence and the nucleic acid, or between the first amino acid sequence and the small organic or inorganic molecule.
[00167] As used herein, the term "clotting factor," refers to molecules, or analogs thereof naturally occurring or recombinant!\}' produced which prevent or decrease the duration of a bleeding episode in a subject. In other words, it means molecules having pro-clotting activity, i.e., are responsible for the conversion of fibrinogen into a mesh of insoluble fibrin causing the blood to coagulate or clot. [00168] Clotting activity, as used herein, means the ability to participate in a cascade of biochemical reactions that culminates in the formation of a fibrin clot and/or reduces the severity, duration or frequency of hemorrhage or bleeding episode.
[00169] Hemostasia, as used herein, means the stopping or slowing of bleeding or hemorrhage; or the stopping or slowing of blood flow through a blood vessel or body part.
[00170] 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), 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 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 Naegeii (Glanzmann thrombasthenia\}. In liver
failure (acute and chronic forms), there is insufficient production of coagulation factors by the liver; this may increase bleeding risk.
[00171] The chimeric molecules of the invention can be used prophylacticly. 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, or stem cell transplantation
[00172] On-demand treatment 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 illiopsoas 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.
[001731 As used herein the term "acute bleeding " refers to a bleeding episode regardless of the underlying cause. For exmaple, 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.
[00174] 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, the prophylaxis of one or more symptoms associated with a disease or condition.

## H. Clofting Eactors

[00175] In particular, the invention pertains to improved versions of factors VII, IX, and X. These factors are all structurally related in that in each the amino terminal end of the light chain is not amenable to the incorporation of additional moieties. Similarly, the amino terminal end of the heavy chain of these three clotting factors is not amenable to the incorporation of additional moieties, with the exception of cleaveable moieties, i.e., moieties linked via a cleavage site or moieties which consist of a cleaveage site. The chimeric clotting factor constructs of the invention were designed based on these shared properties and it will be understood that although factor VII is often shown to illustrate exemplary embodiments of the invention, the subject constructs may be made using factor VII, IX, or X. For example, one of skill in the art would understand that the FVI portion of a construct of the invention could be substituted with a FVIII, FIX or FX portion to make an enhanced version of one of these clotting factors.
[00176] Exemplary chimeric clotting factor constructs of the invention are set forth in the accompanying Figures. Although the Figures generally illustrate the clotting Factor as a single chain (in its zymogen form) it will be understood that the clotting factor may also be present in its active form in a construct of the invention, e.g. as a two chain, disulfide bonded form.
[00177] In one embodiment, a chimeric clotting factor of the invention is expressed by a cell in active form. In another embodiment, a chimeric clotting factor is expressed in inactive form and is subsequently activated under appropriate conditions in vitro such that the active form of the clotting factor is present in the construct. In another embodiment, a chimeric clotting factor of the invention comprises a clotting factor in inactive form and the clotting factor is activated in vivo after administration.
[00178] In one embodiment, an scFc scaffold can be used to produce an active form of a molecule. Certain clotting factors are produced recombinant! \}' as zymogens and, therefore, require activation during manufacturing. Active forms of Factors VII, IX, and $X$ are comprised of dimeric molecules in which the heavy and light chain are linked only by a disulfide bond.
[001 79] In one embodiment, a chimeric clotting factor is activated prior to administration to a subject to improve hemostasis. Methods for activating clotting factors are known in the art. For example, in one embodiment, a chimeric clotting
factor of the invention is contacted with media containing $\mathrm{CaCl}_{2}$ at a concentration of approximately 5 mM .
[00180] In another embodiment, a chimeric clotting factor of the invention is secreted in active form by a cell in which it is expressed. In one embodiment, an active chimeric clotting factor is made by expressing the heavy and light chain of a clotting factor as separate polypeptides.
[00181] In another embodiment, the N-terminus of the heavy chain of the clotting factor is modified to comprise an intracellular processing site which delays the activation of the clotting factor during synthesis until later in the secretory pathway, (i.e. until protein colocafizes with active processing enzymes in the transGoigi network), leading to greater productivity. Exemplary such intracellular processing sites include those recognized by furin. Exemplary cleavage sites for this family of enzymes include an amino acid sequence comprising the motif Arg-Xaa-Lys/Arg-Arg.
[00182] In a preferred embodiment, an active construct of the invention is made in the context of an Fc fusion protein, e.g., using an scFc linker (e.g., a cscfc linker\}.
[00183] Exemplary constructs are shown in the accompanying figures.
[00184] In one embodiment, the invention pertains to processed (e.g., mature) polypeptides in which the at least one cleavage site adjacent to an scFc polypeptide linker has been cleaved such that the molecule is no longer a single polypeptide chain such that the polypeptide is comprised of at least two polypeptide chains (owing to cleavage at the enzymatic cleavage site(s) P1 and/or P2).
[00185] In one embodiment, such processed polypeptides comprise a clotting factor or portion thereof linked to the second Fc moiety (i.e., the second Fc moiety when counting from the amino terminus to the carboxy terminus prior to cleavage of the polypeptide linker) which has a free amino terminus after cleavage of the polypeptide linker.
[00186] In one embodiment, a clotting factor attached to the N-terminus of the second Fc moiety is catalytically active, e.g., has enzymatic activity. In another embodiment, a clotting factor attached to the N -terminus of the second Fc moiety is secreted by a cell as a zymogen requiring further enzymatic processing of the clotting factor in order to be fully activated.

In one embodiment, the invention pertains to clotting factors which are secreted from cells in active or activated form without the need for further activation
during processing. For example, Factor VII is generally produced recombinantly as a zymogen and requires activation during manufacturing to produce the active form for administration. In one embodiment, a polypeptide of the invention is secreted from the cell in which it is expressed in active form to improve manufacturability. As is set forth in more detail below, such clotting factors can be produced by expressing the light chain of a clotting factor and the heavy chain of a clotting factor separately in the context of an scFc molecule comprising a cscFc linker. Activation of such a construct is delayed until late in the secretory pathway during processing, e.g., when the protein colocalizes with active processing enzymes in the trans-Golgi apparatus.
[00188] In one embodiment, a clotting factor of the invention is a mature form of Factor VII or a variant thereof. Factor VII (FVII, F7: also referred to as Factor 7, coagulation factor VII, serum factor VII, serum prothrombin conversion accelerator, SPCA, proconvertin and eptacog alpha) is a serine protease that is part of the coagulation cascade. FVII includes a G1a domain, two EGF domains (EGF-1 and EGF-2), and a serine protease domain (or peptidase SI domain) that is highly conserved among all members of the peptidase SI family of serine proteases, such as for example with chymotrypsin. FVII occurs as a single chain zymogen, an activated zymogen -like two- chain polypeptide and a fully activated two-chain form. As used herein, a "zymogen-like" protein or polypeptide refers to a protein that has been activated by proteolytic cleavage, but still exhibits properties that are associated with a zymogen, such as, for example, low or no activity, or a conformation that resembles the conformation of the zymogen form of the protein. For example, when it is not bound to tissue factor, the two-chain activated form of FVII is a zymogen-like protein; it retains a conformation similar to the undeaved FVII zymogen, and, thus, exhibits very low activity. Upon binding to tissue factor, the two-chain activated form of FVII undergoes conformational change and acquires its full activity as a coagulation factor.
[00189] Exemplary FVII variants include those with increased specific activity, e.g., mutations that increase the activity of FVII by increasing its enzymatic activity (Kcat or Km ). Such variants have been described in the art and include, e.g., mutant forms of the molecule as described for example in Persson et al. 2001. PNAS 98:13583; Petrovan and Ruf. 2001, J. Biol. Chem. 276:6616; Persson et al. 2001 J. Biol. Chem. 276:29195; Soejima et al. 2001. I Biol Chem. 276:17229; Soejima et al. 2002. J. Biol Chem. 247:49027. In one embodiment, a variant form of FVII
includes the mutations Exemplar\}' mutations include V158D-E296V-M298Q. In another embodiment, a variant form of FVI includes a replacement of amino acids 608-619 (LQQSRKVGDSPN, corresponding to the 170- loop) from the FVII mature sequence with amino acids EASYFGK from the 170 -loop of trypsin. High specific activity variants of FIX are also known in the art. Fir example, Simioni et al. (2009 N.E. Journal of Medicine 361:1671) describe an R338L mutation. Chang et al. (1988 JBC 273:12089) and Pierri et al. (2009 Human Gene Therapy 20:479) describe an R338A mutation. Other mutations are known in the art and include those described, e.g., in Zogg and Brandstetter. 2009 Structure 17:1669: Sichler etl al. 2003. J. Biol. Chem. 278:4121; and Sturzebecher et al. 1997. FEBS Lett 412:295. The contents of these references are incorporated herein by reference.
[00190] Full activation, which occurs upon conformational change from a zymogen-like form, occurs upon binding to is co-factor tissue factor. Also, mutations can be introduced that result in the conformation change in the absence of tissue factor. Hence, reference to FVIIa includes both two-chain forms thereof, the zymogen-like form and the fully activated two-chain form.
[00191] In one embodiment, a clotting factor of the invention is a mature form of Factor VIII or a variant thereof. FVIII functions in the intrinsic pathway of blood coagulation as a cofactor to accelerate the activation of factor X by factor IXa, a reaction that occurs on a negatively charged phospholipid surface in the presence of calcium ions. FVIII is synthesized as a 2351 amino acid single-chain polypeptide having the domain structure A 1-A2-B-A3-C1-C2. Wehar, G.A.et al, Nature 312:337-342 (1984) and Toole, J. J. et al, Nature 312:342-347 (1984). The domain structure of FVIII is identical to that of the homologous coagulation factor, factor V (FV). Kane, W.H. et al., PNAS (USA) 83:6800-6804 (1986) and Jenny, R, J. et al., PNAS (USA) 84:4846-4850 (1987). The FVIII A-domains are 330 amino acids and have $40 \%$ amino acid identity with each other and to the A-domain of FV and the plasma copper-binding protein ceruloplasmin. Takahashi, N. et al, PNAS (USA) 81:390-394 (1984). Each C-domain is 150 amino acids and exhibits $40 \%$ identity to the C-domains of FV , and to proteins that bind glycoconjugates and negati vely charged phospholipids. Stubbs, J. D. et al., PNAS (USA) 87:8417-8421 (1990). The FVIII B-domain is encoded by a single exon and exhibits little homology to any known protein including FV B-domain. Gitschier, J. et al., Nature 312:326-330 (1984) and Cripe, L. D. et al, Biochemistry 31:3777-3785 (1992).
[00192] FVIII is secreted into plasma as a heterodimer of a heavy chain (domains A1-A2-B) and a light chain (domains A3-C1-C2) associated through a noncovalent divalent metal ion linkage between the Al- and A3-domains. In plasma, FVII is stabilized by binding to von Wiliebrand factor. More specifically, the FVII light chain is bound by noncovalent interactions to a primary binding site in the amino terminus of von Wiliebrand factor. Upon proteolytic activation by thrombin, FVIII is activated to a heterotrimer of 2 heavy chain fragments (Al, a 50 kDa fragment, and A2, a 43 kDa fragment) and the light chain (A3-C1-C2, a 73 kDa chain). The active form of FVIII (FVIIIa) thus consists of an Al-subunit associated through the divalent metal ion linkage to a thrombin-cleaved A3-C1-C2 light chain and a free A2 subunit associated with the Al domain through an ion association. Eaton, D. et al, Biochemistry 25: 505 (1986); Loilar, P. et al, J. Biol Chem. 266: 12481 (1991); and Fay, P. J. et al., J. Biol. Chem. 266: 8957 (1991). This FVIIIa heterotrimer is unstable and subject to rapid inactivation through dissociation of the A 2 subunit under physiological conditions.
[00193] In one embodiment, a clotting factor comprises a B-domain deleted version of factor VIII, "B-domain" of Factor VIII, 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 Factor VIII. The other human Factor VIII domains are defined by the following amino acid residues: Al, residues Alal-Arg372; A2, residues Ser373-Arg740; A3, residues Serl690-Asn2019; Cl, residues Lys2020-Asn2172: C2, residues Ser2173Tyr2332. The A3-C1-C2 sequence includes residues Serl690-Tyr2332. The remaining sequence, residues Glul649-Argl 689, is usually referred to as the a3 acidic region. The locations of the boundaries for all of the domains, including the Bdomains, for porcine, mouse and canine Factor VIII are also known in the art. In one embodiment, the B domain of Factor VIII is deleted ("B-domain-deleted factor VIII" or "HDD FVIII"). An example of a BDD FVIII is REFACTO ${ }^{\circledR}$ (recombinant BDD FVIII with S743/Q1638 fusion), which is known in the art.
[00194] A "B-domain-deleted Factor VIII" 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 $6,458,563$, each of which is incorporated herein by reference in its entirety. In some embodiments, a B-domain-deleted Factor VIII
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 version Factor VIII) (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: 6, i.e., SEQ ID NO: 2). In some embodiments, a B-domain-deleted Factor VIII 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, 1 12 ; 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 Factor VIII 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, which is incorporated herein by reference in its entirety. In some embodiments, a B-domain-deleted Factor VIII 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), incorporated herein by reference in its entirety. A B-domain-deleted Factor VIII may also contain a deletion of amino acids 771-1666 or amino acids 868-1562 of Factor VII. Meuiien P., et al. Protein Eng. 2(4): 301-6 (1988), incorporated herein by reference in its entirety. 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, UNA (1987) 6:553-564)), 741 though 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\}), each of
which is incorporated herein by reference in its entirety. Each of the foregoing deletions may be made in any Factor VIII sequence. In one embodiment, the invention pertains to a targeted version of FVIII, wherein the targeting (i) specifically binds to platelets, (ii) is not interposed between the light and heavy chains of the clotting factor, and wherein said chimeric clotting factor exhibits increased generation of thrombin in the presence of platelets as compared to an appropriate control lacking the at least one targeting moiety.
[00195] In one embodiment, a clotting factor of the invention is a mature form of Factor IX or a variant thereof Factor IX circulates as a 415 amino acid, single chain plasma zymogen (A, Vysotchin et al., J. Biol. Chem. 268, 8436 (1993)). The zymogen of FIX is activated by FXla or by the tissue factor/FVIIa complex. Specific cleavages between arginine-alanine 145-146 and arginine-valine 180-181 result in a light chain and a heavy chain linked by a single disulfide bond between cysteine 132 and cysteine 289 (S. Bajaj et al., Biochemistry 22, 4047 (1983)). The structural organization of FIX is similar to that of the vitamin K-dependent blood clotting proteins FVI, FX and protein C (B. Furie and B. Furie, supra). The approximately 45 amino acids of the amino terminus comprise the gamma-carboxyglutamic acid, or gla, domain. This is followed by two epidermal growth factor homology domains (EGF), an activation peptide and the catalytic "heavy chain" which is a member of the serine protease family (A. Vysotchin et al., J. Biol. Chem. 268, 8436 (1993); S. Spitzer et al, Biochemical Journal 265, 219 (1990); H, Brandstetter et al., Proc. Natl. Acad Sci. USA 92, 9796 (1995)).
[00196] In one embodiment, a clotting factor of the invention is a mature form of Factor X. Factor X is a vitamin-K dependent glycoprotein of a molecular weight of 58.5 kDa , which is secreted from liver cells into the plasma as a zymogen. Initially factor X is produced as a prepropeptide with a signal peptide consisting in total of 488 amino acids. The signal peptide is cleaved off by signal peptidase during export into the endoplasmatic reticulum, the propeptide sequence is cleaved off after gamma carboxylation took place at the first \& glutamic acid residues at the N -terminus of the mature N -terminal chain. A further processing step occurs by cleavage between Arg 182 and Serl 83. This processing step also leads concomitantly to the deletion of the tripeptide Arg180-Lysl81-Arg182. The resulting secreted factor X zymogen consists of an N -terminal light chain of 139 amino acids (M, 16,200) and a C-terminal heavy chain of 306 amino acids (M, 42,000) which are covalentiy linked via a
disulfide bridge between Cys172 and Cys342. Further posttranslational processing steps include the .beta.-hydroxy! ahon of Asp 103 as well as N - and O-type glycosylation.
[00197] It will be understood that in addition to wild type (WT) versions of these clotting factors or biologically active portions thereof, the present invention may also employ precursor truncated forms thereof that have activity, allelic variants and species variants, variants encoded by splice variants, and other variants, including polypeptides that have at least $40 \%, 45 \%, 50 \%, 55 \%, 65 \%, 70 \%, 75 \%, 80 \%, 85 \%$, $90 \%, 95 \%, 96 \%, 97 \%, 98 \%, 99 \%$ or more sequence identity to the mature form of the clottmg factor and which retain the ability to promote clot formation. For example, modified FVII polypeptides and variants thereof which retain at least one activity of a FVII, such as TF binding, factor X binding, phospholipid binding, and/or coagulant activity of a FVII may be employed. By retaining activity, the activity can be altered, such as reduced or increased, as compared to a wild-type clotting factor so long as the level of activity retained is sufficient to yield a detectable effect. Exemplary sequences of clotting factors that can be used in the constructs of the invention are found in the accompanying sequence listing.
[00198] Exemplary modified polypeptides include, but are not limited to, tissue-specific isoforms and allelic variants thereof, synthetic molecules prepared by translation of nucleic acids, proteins generated by chemical synthesis, such as syntheses that include ligation of shorter polypeptides, through recombinant methods, proteins isolated from human and non-human tissue and cells, chimeric polypeptides and modified forms thereof. The instant clotting factors may also consist of fragments or portions of WT molecules that are of sufficient length or include appropriate regions to retain at least one activity (upon activation if needed) of a full-length mature polypeptide. Exemplary clotting factor variants are known in the art. [00199] As used herein, the term "Gla domain" refers to the conserved membrane binding motif which is pesent In vitamin K-dependent proteins, such as as prothrombin, coagulation factors VII, IX and X, proteins $\mathrm{C}, \mathrm{S}$, and Z. These proteins require vitamin K for the posttranslational synthesis of g-carboxyglutamic acid, an amino acid clustered in the N -terminal Gla domain of these proteins. All glutamic residues present in the domain are potential carboxylation sites and many of them are therefore modified by carboxylation. In the presence of calcium ions, the Gla domain interacts with phospholipid membranes that include phosphatidylserine. The Gla
domain also plays a role in binding to the FVIIa cofactor, tissue factor (TF). Cornplexed with TF, the Gla domain of FVIIa is loaded with seven Ca2+ ions, projects three hydrophobic side chains in the direction of the cell membrane for interaction with phospholipids on the cell surface, and has significant contact with the C-terminal domain of TF.
[00200] The Gla domain of factor VII comprises the uncommon amino acid _carboxyglutamic acid (Gla), which plays a vital role in the binding of clotting factors to negatively charged phospholipid surfaces.
[00201] The GLA domain is responsible for the high-affinity binding of calcium ions. It starts at the N -terminal extremity of the mature form of proteins and ends with a conserved aromatic residue. A conserved Gia-x(3)-Gla-x-Cys motif is found in the middle of the domain which seems to be important for substrate recognition by the carboxylase.
[00202] Using stopped-flow fluorescence kinetic measurements in combination with surface plasmon resonance analysis, the Gla domain has been found to be important in the sequence of events whereby the protease domain of FVIIa initiates contact with sTF (Biochemical and Biophysical Research Communications. 2005. 337:1276). In addition, clearance of clotting factors may be significantly mediated through Gla interactions, e.g., on liver cells and clearance receptors, e.g., EPCR.
[00203] In one embodiment, targeted clotting factors are modified to lack a Gla domain. The Gla domain is responsible for mediating clearance of clotting factors via multiple pathways, such as binding to liver cells, clearance receptors such as EPCR. etc. Thus, eliminating the Gla domain has beneficial effects on half life of clotting factors. Though Gla domain is also generally required for activity by localizing clotting factors to sites of coagulation, the inclusion of a platelet targeting domain moiety targets the Gla deleted clotting factor to platelets. In one embodiment, a clotting factor of the invention comprises a targeting moiety and lacks a Gla domain. For example, in the case of Factor VII, the Gla domain is present at the amino terminus of the light chain and consists of amino acids 1-35. The Gia domains of exemplary clotting factors are indicated in the accompanying sequence listing. This domain can be removed using standard molecular biology techniques, replaced with a targeting domain, and the modified fight chain incorporated into a construct of the invention. In one embodiment, a cleavage site may be introduced into constructs lacking a Gla domain to facilitate activation of the molecule. For example, in one
embodiment, such a cleavage site may be introduced between the amino acids that are cleaved when the clotting factor is activated (e.g., between amino acids 152 and 153 in the case of Factor VII). Exemplar\}' clotting factors lacking a Gla domain are shown in the accompanying figures
[061月204] In one embodiment, a cleavage site may be introduced into constructs lacking a Gla domain to facilitate activation of the molecule. For example, in one embodiment, such a cleavage site may be introduced between the amino acids that are cleaved when the clotting factor is activated (e.g., between amino acids 152 and 153 in the case of Factor VII). Exemplary clotting factors lacking a Gla domain are shown in the accompanying f gures,
[00205] Exemplary clotting factors are those of mammalian, e.g., human, origin. The sequences of exemplary clotting factors are presented in the accompanying sequence listing, e.g., alone or in the context of a chimeric clotting factor construct.

## III. Targeting Moieties

[00206] In one embodiment, a clotting factor of the invention is targeted to platelets to enhance its efficacy by localizing the clotting factor to the site of coagulation using a "targeting moiety" which binds to a target molecule expressed on platelets. Preferably the targeted molecules are not expressed on cells or tissues other than platelets, i.e., the targeting moieties specifically bind to platelets.
[00207] In one embodiment, receptors/conformations found on resting platelets are targeted. By doing so, sites for coagulation could be primed for enhanced efficacy. Targeting such molecule may also extend half life of the clotting factor and/or prevent clearance. Examples of such targets include Gplb of the Gplb/V/'\}X complex, and GpVI and nonactive form of GPחB/[ITa.
[00208] In one embodiment, receptors/conformations only found on activated platelets are targeted in order to localize the clotting factor to site of active coagulation. Examples of such targets include, e.g., the active form of GpIßb/ma as well as CD62P.
[00209] In one embodiment, a polypeptide of the invention comprises a "targeting moiety" which has affinity for and binds to platelets. For example, in one embodiment, a targeting moiety binds to the GPIb complex, e.g. GPIb-alpha.

Examples of such targeting moieties include the peptides PS4, 0 S1, and OS2 which
bind to both active and nonactive platelets (Benard et al. 2008 Biochemistry 47:4674); In another embodiment, a targeting moiety binds to the active conformation of GPHbllla. Examples of such targeting moieties inlcude SCE5 and MB9 variable regions which bind active platelets only (Schwarz et al. 2004 FASEB Journal express article 10.1096/fj.04-15 13fje; Schwarz et al. 2006 Circulation Research. 99:25-33; U.S. Patent publication 20070218067). In another embodiment, a targeting moiety binds to both the active/nonactive conformation of GPIIbllla. An example of such a targeting moiety is the variable region of the AP3 antibody (Peterson et al. 2003. Hemostasis, Thrombosis, and Vascular Biology 101:937; WO 20101 15866). Other targets and targeting moieties are known in the art. Another version of factor IX (the triple mutatnt V86A/E277A/R338A) with augmented clotting activities has been described by Lin et al. 2010. Journal of Thrombosis and Haemostasis 8: 1773). The contents of these references are incorporated herein by this reference.
[00210] The chimeric clotting factors of the invention can comprise one or more than one targeting moiety. Exemplary configurations are set forth in the accompanying Figures. Additionally, two or more targeting moieties may be linked to each other (e.g., via a spacer) in series, and the tandem array operably linked to a construct of the in vention. When two or more targeting moieties are present in a chimeric clotting factor of the invention, the moieties may be the same or different. [00211] In one embodiment, a targeting moiety is fused to a chimeric clotting factor of the invention by a cleaveable linker which may be cleaved to remove the targeting moiety at the site of a clot. In another embodiment, a targeting moiety is not attached via a cleaveable linker and, therefore, is not cleaved at the site of a clot. [00212] In one embodiment, the targeting moiety is located on the N - or C terminus of factor VIII. In another embodiment, a targeting moiety is located on the C-terminus of FVII, FIX, FX, or the C-terminus of either or both chains of FVIIa, FIXa, of FXa. In embodiments in which an Fc region or portion thereof is employed, the targeting moiety may be positioned at the N or C terminus of the second Fc chain, or the C-terminus of either or both Fc chains.
[00213] In one embodiment, a targeting moiety is not genetically fused directly to a construct, but rather is finked via a spacer or a chemical bond to the construct. For example, targeting moieties may be attached to a construct of the invention by formation of a bond between the targeting moiety and an Fc moiety of a construct,
where the targeting moiety comprises a first functional group and the Fc moietycomprises a second functional group, and where the first and second functional groups are capable of reacting with each other to form a chemical bond (see, e.g., U.S. patent 7381408).
[00214] In one embodiment, a polypeptide of the invention comprises at least one of an antigen binding site (e.g., an antigen binding site of an antibody, antibody variant, or antibody fragment), a polypeptide, a receptor binding portion of ligand, or a ligand binding portion of a receptor which specifically binds to platelets, e.g., resting or activated platelets. Exemplary targeting moieties include scFv molecules or peptides which bind to molecules to be targeted. Examples of targeting moieties are found in the instant examples and Figures. Other molecules useful as targeting moieties can readily be selected by one of skill in the art based upon the teaching herein.

## A. Antigen Binding Sites Which Bind to Platelets

[00215] In certain embodiments, a polypeptide of the invention comprises at least one antigen binding portion (e.g., binding site) of an antibody. In one embodiment, the antigen binding portion targets the polypeptide to platelets
[00216] In other embodiments, a a polypeptide of the invention may comprise an antigen binding portion. The term "antigen-binding portion" refers to a polypeptide fragment of an immunoglobulin, antibody, or antibody variant which binds antigen or competes with intact antibody (i.e., with the intact antibody from which they were derived) for antigen binding (i.e., specific binding \}. For example, said antigen binding portions can be derived from any of the antibodies or antibody variants described supra. Antigen binding portions caß be produced by recombinant or biochemical methods that are well known in the art. Exemplary antigen-binding portions include Fv, Fab, Fab', and (Fab')? as well as scFv molecules.
[00217] In other embodiments, a chimeric dotting factor of the invention may comprise a binding site from single chain binding molecule (e.g., a single chain variable region or scFv ). Techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, Science 242:423-442 (1988); Huston el al, Proc. Natl Acad. Sci. USA 55:5879-5883 (1988); and Ward et al, Nature 334:544554 (1989)) can be adapted to produce single chain binding molecules. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region
via an amino acid bridge, resulting in a single chain antibody. Techniques for the assembly of functional Fv fragments in E coli may also be used (Skerra et a!., Science .242:1038-104! (1988)).
[00218] In certain embodiments, a polypeptide of the invention comprises one or more binding sites or regions comprising or consisting of a single chain variable region sequence ( scFv ). Single chain variable region sequences comprise a single polypeptide having one or more antigen binding sites, e.g., a VLdomain linked by a flexible linker to a $\mathrm{V}_{\mathrm{H}}$ domain. The VL and/or VH domains may be derived from any of the antibodies or antibody variants described supra. ScFv molecules can be constructed in a $\mathbf{V}_{\mathrm{H}}$-lmker-VL orientation or $\mathbf{V} \mathbf{L}$-linker-Vn orientation. The flexible linker that links the $V_{\mathrm{L}}$ and $\mathrm{V}^{3} / 4$ domains that make up the antigen binding site preferably comprises from about 10 to about 50 amino acid residues. In one embodiment, the polypeptide linker is a gly-ser polypeptide linker. An exemplary gly/ser polypeptide linker is of the formula (Gly4Ser)n, wherein n is a positive integer (e.g., 1, 2, 3, 4, 5, or 6). Other polypeptide linkers are known in the art. Antibodies having single chain variable region sequences (e.g. single chain Fv antibodies) and methods of making said single chain antibodies are well-known in the art (see e.g., Ho et al. 1989. Gene 77:51; Bird et al. 1988 Science 242:423; Pantoiiano et al. 1991. Biochemistry 30:101 17; Milenic et al. 1991. Cancer Research 51:6363; Takkinen et al. 1991. Protein Engineering 4:837).
[00219] In certain embodiments, a scFv molecule employed in a polypeptide of the invention is a stabilized scFv molecule. In one embodiment, the stabilized cFv molecule may comprise a scFv linker interposed between a $\mathrm{V}_{\mathrm{H}}$ domain and a $\mathrm{V}_{\mathrm{L}}$ domain, wherein the $\mathrm{V}_{\mathrm{H}}$ and VLdomains are linked by a disulfide bond between an amino acid in the $\mathrm{V}_{\mathrm{H}}$ and an amino acid in the VLdomain. In other embodiments, the stabilized scFv molecule may comprise a scFv linker having an optimized length or composition. In yet other embodiments, the stabilized scFv molecule may comprise a $\mathrm{V}_{\mathrm{H}}$ or VL domain having at least one stabilizing amino acid substitution(s). In yet another embodiment, a stabilized scFv molecule may have at least two of the above listed stabilizing features.
[00220] Stabilized scFv molecules have improved protein stability or impart improved protein stability to the polypeptide to which it is operably linked. Preferred scFv linkers of the invention improve the thermal stability of a polypeptide of the invention by at least about $2^{\circ} \mathrm{C}$ or $3^{\circ} \mathrm{C}$ as compared to a conventional polypeptide

Comparisons can be made, for example, between the scFv molecules of the invention. In certain preferred embodiments, the stabilized scFv molecule comprises a $\left(\mathrm{Gly}_{4} \mathrm{Ser}\right)_{4} \mathrm{scFv}$ linker and a disulfide bond which Jinks $\mathrm{V}_{\mathrm{H}}$ amino acid 44 and VL amino acid 100. Other exemplary stabilized scFv molecules which may be employed in the polypeptides of the invention are described in US Pro visional Patent

Application No. 60/873,996, filed on December 8, 2006 or US Patent Application No. 11/725,970, filed on March 19, 2007, each of which is incorporated herein by reference in its entirety.
[00221] Polypeptides of the invention may comprise a variable region or portion thereof (e.g. a VL and/or VH domain) der ved from an antibody using art recognized protocols. For example, the variable domain may be derived from antibody produced in a non-human mammal, e.g., murine, guinea pig, primate, rabbit or rat, by immunizing the mammal with the antigen or a fragment thereof. See Harlow \& Lane, supra, incorporated by reference for all purposes. The immunoglobulin may be generated by multiple subcutaneous or intraperitoneal injections of the relevant antigen (e.g., purified tumor associated antigens or cells or cellular extracts comprising such antigens) and an adjuvant. This immunization typically elicits an immune response that comprises production of antigen-reactive antibodies from activated spienocytes or lymphocytes. [00222] While the variable region may be derived from polyclonal antibodies harvested from the serum of an immunized mammal, it is often desirable to isolate individual lymphocytes from the spleen, lymph nodes or peripheral blood to provide homogenous preparations of monoclonal antibodies (MAbs) from which the desired variable region is derived. Rabbits or guinea pigs are typically used for making polyclonal antibodies. Mice are typically used for making monoclonal antibodies. Monoclonal antibodies can be prepared against a fragment by injecting an antigen fragment into a mouse, preparing "hybridomas" and screening the hybridomas for an antibody that specifically binds to the antigen. In this well known process (Kohler el al, (1975), Nature, 256:495) the relatively short-lived, or mortal, lymphocytes from the mouse which has been injected with the antigen are fused with an immortal tumor cell line (e.g. a myeloma cell line), thus, producing hybrid cells or "hybridomas" which are both immortal and capable of producing the antibody genetically encoded by the B cell. The resulting hybrids are segregated into single genetic strains by selection, dilution, and regrowth with each individual strain comprising specific genes for the formation of a
single antibody. They produce antibodies which are homogeneous against a desired antigen and, in reference to their pure genetic parentage, are termed "monoclonal". [00223] Hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. Those skilled in the art will appreciate that reagents, cell lines and media for the formation, selection and growth of hybridomas are commercially available from a number of sources and standardized protocols are well established. Generally, culture medium in which the hybridoma cells are growing is assayed for production of monoclonal antibodies against the desired antigen.

Preferably, the binding specificity of the monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro assay, such as a radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (EL1SA). After hybridoma cells are identified that produce antibodies of the desired specificity, affinity and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp 59103 (Academic Press, 1986)\}. It will further be appreciated that the monoclonal antibodies secreted by the subclones may be separated from culture medium, ascites fluid or serum by conventional purification procedures such as. for example, affinity chromatography (e.g., proteiii-A, protein-G, or protein-L affinity chromatography), hydroxylapatite chromatography, gel electrophoresis, or dialysis.
[00224] Optionally, antibodies may be screened for binding to platelets of a specific activation state or to a specific region or desired fragment of the antigen without binding to other nonoverlapping fragments of the antigen. The latter screening can be accomplished by determining binding of an antibody to a collection of deletion mutants of the antigen and determining which deletion mutants bind to the antibody. Binding can be assessed, for example, by Western blot or ELISA. The smallest fragment to show specific binding to the antibody defines the epitope of the antibody. Alternatively, epitope specificity can be determined by a competition assayis which a test and reference antibody compete for binding to the antigen. If the test and reference antibodies compete, then they bind to the same epitope or epitopes sufficiently proximal such that binding of one antibody interferes with binding of the other.
[00225] DNA encoding the desired monoclonal antibodyor binding site thereof may be readily isolated and sequenced using any of the conventional procedures
described supra for the isolation of constant region domain sequences (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The isolated and subcloned hybridoma cells serve as a preferred source of such DNA. More particularly, the isolated DNA (which may be synthetic as described herein) may be used to clone the desired variable region sequences for incorporation in the polypeptides of the invention.
[00226] In other embodiments, the binding site is derived from a fully human antibody. Human or substantially human antibodies may be generated in transgenic animals (e.g., mice) that are incapable of endogenous immunoglobulin production (see e.g., U.S. Pat. Nos. 6,075,181, 5,939,598, 5,591,669 and 5,589,369, each of which is incorporated herein by reference). For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of a human immunoglobulin gene array to such germ line mutant mice will result in the production of human antibodies upon antigen challenge. Another preferred means of generating human antibodies using SCTD mice is disclosed in U.S. Pat. No. $5,811,524$ which is incorporated herein by reference. It will be appreciated that the genetic material associated with these human antibodies may also be isolated and manipulated as described herein.
[00227] In other aspects, the polypeptides of the invention may comprise antigen binding sites, or portions thereof, derived from modified forms of antibodies. Exemplary such forms include, e.g., minibodies, diabodies, triabodies, nanobodies, camelids, Dabs, tetravalent antibodies, intradiabodies (e.g., Jendreyko et al. 2003. J. Biol. Chem. 278:47813), fusion proteins (e.g., antibody cytokine fusion proteins, proteins fused to at least a portion of an Fc receptor), and bispecific antibodies. Other modified antibodies are described, for example in U.S. Pat. No. 4,745,055; EP 256,654; Faulkner et al, Nature 298:286 (1982); EP 120,694; EP 125,023; Morrison, j. Imniun. 123:793 (1979); Kohler et al., Proc. Natl. Acad, Sci. USA 77:2197 (1980); Raso et al., Cancer Res. 41:2073 (1981); Morrison et al., Ann. Rev. Immunol. 2:239 (1984); Morrison, Science 229:1202 (1985); Morrison et al, Proc. Natl. Acad. Sci. USA 8 :6851 (1984); EP 255,694; EP 266,663; and WO 88/03559. Reasserted immunoglobulin chains also are known. See, for example, U.S. Pat. No. 4,444,878; WO 88/03565; and EP 68,763 and references cited therein.
[00228] In another embodiment, a chimeric clotting factor of the invention comprises an antigen binding site or region which is a diabody or an antigen binding site derived therefrom. Diabodies are dimeric, tetravalent molecules each having a polypeptide similar to scFv molecules, but usually having a short (e.g., less than 10 and preferably 1-5) amino acid residue linker connecting both variable domains, such that the VL and VHdomains on the same polypeptide chain cannot interact. Instead, the $\mathrm{V}_{\mathrm{L}}$ and $\mathrm{V}_{\mathrm{H}}$ domain of one polypeptide chain interact with the $\mathrm{V}_{\mathrm{H}}$ and V L domain (respectively) on a second polypeptide chain (see, for example, WO 02/02781). In one embodiment, a polypeptide of the invention comprises a diabody which is operably linked to the N -terminus and/or C-terminus of at least one genetically-fused Fc region (i.e., scFc region).
[6] 2229] In certain embodiments, a polypeptide of the invention comprises a single domain binding molecule (e.g. a single domain antibody) as a targeting moiety. Exemplary single domain molecules mclude an isolated heavy chain variable domain (VH) of an antibody, i.e., a heavy chain variable domain, without a light chain variable domain, and an isolated light chain variable domain (VL) of an antibody, i.e., a light chain variable domain, without a heavy chain variable domain,. Exemplary singledomain antibodies employed in the binding molecules of the invention include, for example, the Camelid heavy chain variable domain (about 118 to 136 amino acid residues) as described in Hamers-Casterman, et al, Nature 363:446-448 (1993), and Dumoulin, et al., Protein Science 11:500-515 (2002). Other exemplary single domain antibodies include single VH or VL domains, also known as Dabs® (Domantis Ltd.. Cambridge, UK). Yet other single domain antibodies include shark antibodies (e.g., shark Ig-NARs). Shark Ig-NARs comprise a homodimer of one variable domain (VNAR) and five C-like constant domains (C-NAR), wherein diversity is concentrated in an elongated CDR3 region varying from 5 to 23 residues in length. In camelid species (e.g., llamas), the heavy chain variable region, referred to as VHH, forms the entire antigen-binding domain. The main differences between camelid VHH variable regions and those derived from conventional antibodies (VH) include (a) more hydrophobic amino acids in the light chain contact surface of VH as compared to the corresponding region in VHH , (b) a longer CDR3 in VHH , and (c) the frequent occurrence of a disulfide bond between CDR1 and CDR3 in VHH. Methods for making single domain binding molecules are described in US Patent Nos 6.005,079 and $6,765,087$, both of which are incorporated herein by reference. Exemplary single
domain antibodies comprising VHH domains include Nanobodies® (Abiynx NV, Ghent, Belgium).
[00230] Exemplary antibodies from which binding sites can be derived for use in the binding molecules of the invention are known in the art. Examples of such targeting moieties inlcude SCE5 and MB9 variable regions which bind active platelets only (Schwarz et al. 2004 FASEB Journal express article 10.1096/fj.041513fje; Schwarz et al. 2006 Circulation Research. 99:25-33; U.S. Patent publication 20070218067). In another embodiment, a targeting moiety binds to both the active/nonactive conformation of GPHbllla. An example of such a targeting moiety is the variable region of the AP3 antibody (Peterson et al, 2003. Hemostasis, Thrombosis, and Vascular Biology 101:937; WO 20 10115866).

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[00231] In certain other embodiments, the polypeptides of the invention comprise one or more platelet binding sites derived from a non--immunoglobulin binding molecule. As used herein, the term "non-immunoglobulin bindingmolecules" are binding molecules whose binding sites comprise a portion (e.g., a scaffold or framework) which is derived from a polypeptide other than an immunoglobulin, but which may be engineered (e.g., mutagenized) to confer a desired binding specificity to a platelet target Other examples of binding molecules comprising binding sites not derived from antibody molecules include receptor binding sites and ligand binding sites which bind to platelets,
[00232] Non-immunoglobulin binding molecules may be identified by selection or isolation of a target-binding variant from a library of binding molecules having artificially diversified binding sites. Diversified libraries can be generated using completely random approaches (e.g., error-prone PGR, exon shuffling, or directed evolution) or aided by art-recognized design strategies. For example, amino acid positions that are usually involved when the binding site interacts with its cognate target molecule can be randomized by insertion of degenerate codons, trinucleotides, random peptides, or entire loops at corresponding positions within the nucleic acid which encodes the binding site (see e.g., U.S. Pub. No. 20040132028). The location of the amino acid positions can be identified by investigation of the crystal structure of the binding site in complex with the target molecule. Candidate positions for randomization include loops, flat surfaces, helices, and binding cavities
of the binding site. In certain embodiments, amino acids within the binding site that are likely candidates for diversification can be identified using techniques known in the art. Following randomization, the diversified library may then be subjected to a selection or screening procedure to obtain binding molecules with the desired binding characteristics, e.g. specific binding platelets using methods known in the art. Selection can be achieved by art-recognized methods such as phage display, yeast display, or ribosome display. In one embodiment, molecules known in the art to bind to platelets may be employed in the constructs of the invention. For example, peptides which bind to GPIba as described in the art (e.g., PS4, 0S1, or 0S2) may be used (Benard et a\}. 2008. Biochemistry 47:4674-4682).

## IV. Activatable Clotting Factors

[00233] Clotting factors given for bypass therapy are efficacious when given in the activated form, since exogenous clotting factors are often not activated with sufficient kinetics to be effective. However, they are also rapidly inactivated by endogenous pathways (e.g., by anti thrombin III or TFPI), leading to clearance of the active form and a short effective half life. In one embodiment, a chimeric clotting factor of the invention is "activatable." Such activatable constructs circulate as an enhanced zymogen with a longer half life, but can be readily cleaved at the site of clotting when necessary.
[00234] In one embodiment, an activatable construct of the invention comprises a cieavable linker comprising, e.g., a factor XIa, Xa, or thrombin cleavage site (which is cleaved by factor XIa, Xa, or thrombin, respectively) leading to formation of the active form of the clotting factor at the site of a clot. Exemplar $\}^{\prime}$ factor FXIa cleavage sites include, e.g., TQSFNDFTR and SVSQTSKLTR. Exemplar \}' thrombin cleavage sites include, e.g., DFLAEGGGVR, TTKIKPR, and a sequence comprising or consisting of ALRPR (e.g. ALRPRWGGA)).
[00235] In one embodiment, the cieavable linker may be flanked on one or more sides (upstream, downstream or both) by a spacer moiety.
[00236] In one embodiment, the cieavable linker is interposed between the light chain and heavy chain of the clotting factor. In another embodiment, the cieavable linker is not interposed between the light chain and heavy chain of the clotting factor. In one embodiment, the cieavable linker is located amino terminal to the heavy chain.
[00237] Exemplary activatable constructs are shown in the accompanying Figures and following Examples.

## V. Scaffold Moieties

Some embodiments of the invention comprise a scaffold moiety, which can be selected from, e.g., a protein moiety, cscFc region, a Fc moiety, albumin, XTEN, etc.
A. Protein Moieties

In one embodiment, the scaffold is a protein moiety. Such a moiety may comprise a complete protein or a portion therof, or a synthetic molecule. Preferred protein moieties are of a sufficient molecular size that they improve the half life of a chimeric clotting factor of the invention when incorporated into a construct. For example, in one embodiment, an artificial protein, XTEN, may be included in a construct as a scaffold (Schellenberger et al. 2009. 27:1186). In another embodiment, albumin (e.g., human serum albumin) may be included in a construct of the invention. For example as known in the art, serum albumin (for example, HSA) can be used as a protein scaffold. In particular various domains and sub-domains of HSA, have a structure that is quite amenable to mutation or randomization for the generation of serum albumin scaffold-based protein libraries. Examples of albumin, e.g., fragments thereof, that may be used in the present invention are known. e.g., U.S. Patent No. 7,592,010; U.S. Patent No. 6,686,179; and Schulte, Thrombosis Res. 124 Suppl. 2;S6-S8 (2009), each of which is incorporated herein by reference in its entirety.

## B . scFc Regions

[00238] In one embodiment, the invention provides for polypeptides comprising at least one genetically fused Fc region or portion thereof within a single polypeptide chain (i.e., polypeptides comprising a single-chain $\mathrm{Fc}(\mathrm{scFc})$ region) in one embodiment, comprising a cscFc.
[00239] In one embodiment, a chimeric clotting factor which comprises a clotting factor selected from the group consisting of FVII, FIX and FX and a targeting moiety which binds to platelets and optionally a spacer moiety between the clotting factor and the targeting moiety. In another embodiment, polypeptide comprising FVII, which FVII comprises a heterologous enzymatic cleavage site activatable by a component of the clotting cascade.
[002401 In one embodiment, the invention provides unprocessed polypeptides in which at least two Fc moieties or domains (e.g., 2, 3, 4, 5, 6, or more Fc moieties or domains) within the same linear polypeptide chain that are capable of folding (e.g., intramolecularly or intermolecularly folding) to form one functional scFc region which is linked by an Fc polypeptide linker. For example, in one preferred embodiment, a polypeptide of the invention is capable of binding, via its scFc region, to at least one Fc receptor (e.g. an FcRn , an $\mathrm{Fc}_{\mathrm{y}} \mathrm{cyR}$ receptor (e.g., FeyR1I), or a complement protein (e.g. Clq)) in order to improve half life or trigger an immune effector function (e.g., antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cytotoxicity (CDCC) and/or to improve manufacturability). [00241 A variety of polypeptides of alternative designs are within the scope of the invention. For example, in one embodiment, a polypeptide comprises the moieties:

A-F1--P1-- L-P2-B-F2 (I)
[00242] in linear sequence from the amino to carboxy terminus wherein $A$, if present, is a clotting factor or portion thereof, Fl is a first Fc moiety or domain, Pl is an enzymatic cleavage site, L is an ScFc linker, P 2 is an enzymatic cleavage site B , if present, is a clotting factor or portion thereof, F2 is a second Fc moiety or domain and "-" represents a peptide bond. Formula (I) comprises at least an A or B and optionally both. A and B , if both present, can be the corresponding heavy and light chains of a clotting factor. Formula (I) comprises at least a P1 or P2 and optionally both. P 1 and P2, if both present, can be the same or different. Formula (I) comprises at least a F1 and F2. F1 and F2, if both present, can be the same or different.
[002431 Exemplary polypeptides according to formula 1 include: A-F1-P1- L-P2-F2; Fl-Pl - L-P2-B-F2: A-F1-P1- L-F2; Fl-Pl - L- B-F2; A-Fl- L-P2-F2; and Fl-L-P2-B-F2.
[00244] In one embodiment, F1 and F2 each comprise a CH 2 and CH 3 moiety. [00245] In one embodiment, after cleavage and substantial excision of the $\mathbf{c s c F c}$ linker (L), a polypeptide of the invention comprises two polypeptide chains where the first polypeptide chain comprises A linked to a first Fc moiety and where the second polypeptide chain comprises B linked to a second Fc moiety, where F! and F 2 dimerize to form an Fc region. In one embodiment, A and B are optionally present and are clotting factors or portions thereof.
[00246] In one embodiment, A is the light chain of a clotting factor and B is the heavy chain of a clotting factor. In one embodiment, B is the light chain of a clotting factor and A is the heavy chain of a clotting factor. In one embodiment, when A and B associate in the polypeptide, the polypeptide then forms a functional clotting factor, e.g., FVII, FIX or FX. . In one embodiment, such a polypeptide is enzymatically active upon secretion from a cell.

## i) Fc Moieties or Domains

[002471 Fc moieties useful as F1 and F2 for producing the polypeptides of the present invention may be obtained from a number of different sources. In preferred embodiments, an Fc moiety of the polypeptide is derived from a human immunoglobulin. It is understood, however, that the Fc moiety may be derived from an immunoglobulin 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 polypeptide Fc domain or portion thereof may be derived from any immunoglobulin class, including $\operatorname{IgM}, \operatorname{IgG}, \operatorname{Ig}, \operatorname{IgA}$ and $\operatorname{IgE}$, and any immunoglobulin isotype, including $\operatorname{IgGl}, \operatorname{IgG} 2, \operatorname{IgG} 3$ and $\operatorname{IgG} 4$, In a preferred embodiment, the human isotype IgGl is used.
|00248 A variety of Fc moiety gene sequences (e.g. human constant region gene sequences) are available in the form of publicly accessible deposits. Constant region domains comprising an Fc moiety sequence caß 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 antibodyencoding genes have been published and suitable Fc moiety sequences (e.g. hinge, CH 2 , and/or CH 3 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.
[00249] Fc moiety sequences can be cloned, e.g., using the polymerase chain reaction and primers which are selected to amplify the domain of interest. To done $\mathrm{an}_{\mathrm{n}} \mathrm{Fc}$ moiety sequence from an antibody, mRNA can be isolated from hybridoma, spleen, or lymph cells, reverse transcribed into DNA, and antibody genes amplified by PGR. 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., "PGR Protocols: A Guide to Methods and Applications" Xtmis et al. eels., Academic Press, San Diego, CA (1990); Ho et al. 1989. Gene 77:51; Horton et al. 1993. Methods Enzymol. 2 17:270), PGR 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, PGR 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.
[00250] The polypeptides of the invention may comprise two or more Fc moieties \{e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more Fc moieties). These two or more Fc moieties can form a Fc region. In one embodiment, the Fc moieties may be of different types. In one embodiment, at least one Fc moiety present in the polypeptide comprises a hinge domain or portion thereof. In another embodiment, the polypeptide of the invention comprises at feast one Fc moiety which comprises at least one CH2 domain or portion thereof. In another embodiment, the polypeptide of the invention comprises at least one Fc moiety which comprises at least one CH 3 domain or portion thereof. In another embodiment, the polypeptide of the invention comprises at least one Fc moiety which comprises at least one CH 4 domain or portion thereof. In another embodiment, the polypeptide of the invention comprises at least one Fc moiety which comprises at least one hinge domain or portion thereof and at least one CFG domain or portion thereof (e.g, in the hinge-CH2 orientation). In another embodiment, the polypeptide of the invention comprises at least one Fc moiety which comprises at least one CH 2 domain or portion thereof and at least one CH 3 domain or portion thereof (e.g, in the $\mathrm{CH} 2-\mathrm{CH} 3$ orientation). In another embodiment, the polypeptide of the invention comprises at least one Fc moiety comprising at least one hinge domain or portion thereof, at least one CH2 domain or portion thereof, and least
one CH 3 domain or portion thereof, for example in the orientation hinge- $\mathrm{CH} 2-\mathrm{CH} 3$, hinge- $\mathrm{CH} 3-\mathrm{CH} 2$, or $\mathrm{CH} 2-\mathrm{CH} 3-h i n g e$.
[00251] In certain embodiments, the polypeptide comprises at least one complete Fc region derived from one or more immunoglobulin heavy chains (e.g., an Fc domain including hinge, CH 2 , and CH 3 domains, although these need not be derived from the same antibody). In other embodiments, the polypeptide comprises at least two complete Fc regions derived from one or more immunoglobulin heavy chains. In preferred embodiments, the complete Fc moiety is derived from a human IgG immunoglobulin heavy chain (e.g., human IgGl ).
[00252] [n another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising a complete CH3 domain (about amino acids 341-438 of an antibody Fc region according to EU numbering). In another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising a complete CH2 domain (about amino acids 231-340 of an antibody Fc region according to EU numbering). In another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising at least a CH 3 domain, and at least one of a hinge region (about amino acids 216-230 of an antibody Fc region according to EU numbering), and a CH2 domain. In one embodiment, a polypeptid e of the invention comprises at least one Fc moiety comprising a hinge and a CH 3 domain. In another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising a hinge, a $\mathrm{CH}_{2}$, and a $\mathrm{CH}_{3}$ domain. In preferred embodiments, the Fc moiety is derived from a human IgG immunoglobulin heavy chain (e.g., human $\operatorname{IgGl}$ ). In one embodiment, an Fc moiety comprises or consists of amino acids corresponding to EU numbers 221 to 447.
[00253] In another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising an FcRn binding partner. An FcRn binding partner is a molecule or portion thereof that can be specifically bound by the FcRn receptor with consequent active transport by the FcRn receptor of the FcRn binding partner. 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 $10^{6} \mathrm{M}^{-1}$. or more preferably higher than $10^{8} \mathrm{M}^{-1}$. 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.
[00254] 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 ai. 1994, J. Exp. Med. 180:2377). The FcRn receptor binds $\operatorname{IgG}$ (but not other immunoglobulin classes such as $\operatorname{IgA}, \operatorname{IgM}, \operatorname{IgD}$, and $\operatorname{IgE}$ ) at relatively low pH , actively transports the IgG transcellulariy 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.
[00255] FcRn binding partners of 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, an d 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 $3\} 4$ 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 immunoglobulins or immunoglobulin 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.
[00256] The Fc region 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 $\operatorname{IgGl} \mathrm{Fc}(\mathrm{Fc} \gamma \ddot{\mathrm{I}})$ can be substituted without significant loss of Fc binding affinity for FcRn: P238A, S239A, K246A, K248A, D249A, M252A, T256A, E258A, T260A, D265A, S267.A, 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, L309.A, Q311 A, D312A, N315A, K317.A, 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 wildtype proline substituted by alanine at position number 238. As an example, one 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 wildtype amino acids at the positions specified above. Mutations may be introduced singly into Fc giving rise to more than one hundred FcRn binding partners distinct from native Fc. Additionally, combinations of two, three, or more of these individual mutations may be introduced together, giving rise to hundreds more FcRn binding partners. Moreover, one of the FcRn binding partners of a construct of the invention may be mutated and the other FcRn binding partner not mutated at all, or they both may be mutated but with different mutations. Any of the mutations described herein, including N297A, may be used to modify Fc, regardless of the biologically active molecule (e.g., EPO, IFN, Factor VII, Factor IX, T20). [00257] Certain of the above mutations may confer new functionality upon the 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 FcRn binding partner, and to render the FcRn binding partner incapable of binding to $\mathrm{Fc} \gamma \mathrm{R}$, FeyRILA, FcyRIIB, and FcyRIIIA, 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 affmity 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. Mutations believed to impart an increased affinity for FcRn include T256A, T307A, E380A, andN434A (Shields et ah 2001, J. Biol. Chem. 276:6591).
[00258] Additionally, at feast 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 IgGl "ELLG" to the corresponding sequence from IgG2 "PVA" (with one amino acid deletion). It has been shown that FeүR\}, FcyRIL and FcyRIII, which mediate various effector functions will not bind to IgGl when such mutations have been introduced. Ward and Ghetie 1995, Therapeutic Immunology 2:77 and Armour et al. 1999, Eur. J. Immunol. 29:2613.
[00259] In one embodiment, the FcRn binding partner is a polypeptide including the sequence PKNSSMISNTP (SEQ ID NO: 12) and optionally further including a sequence selected from HQSLGTQ (SEQ ID NO: 13), HQNLSDGK (SEQ ID NO: 14), HQNISDCK (SEQ ID NO: 24), or VISSHLGQ (SEQ ID NO: 25) (U.S. Pat. No. 5,739,277).
[00260] Two FcRn receptors can bind a single Fc molecule. Crystailographic 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, sublmgually, rectaily, 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.
[00261] The constant region domains or portions thereof making up an Fc moiety of a polypeptide of the invention may be derived from different immunoglobulin molecules. For example, a polypeptide of the invention may comprise a CH2 domain or portion thereof derived from an IgGl molecule and a CH3 region or portion thereof derived from an IgG3 molecule. In another example, a
polypeptide can comprise an Fc moiety comprising a hinge domain derived, in part, from an IgGl molecule and, in part, from an $\operatorname{IgG3}$ molecule. As set forth herein, it will be understood by one of ordinary skill ins the art that an Fc moiety may be altered such that it varies in amino acid sequence from a naturally occurring antibody molecule.
[00262] In another embodiment, a polypeptide of the invention comprises an scFc region comprising one or more truncated Fc moieties that are nonetheless sufficient to confer Fc receptor ( FcR ) binding properties to the Fc region. For example, the portion of an Fc domain that binds to FcRn (i.e., the FcRn binding portion) comprises from about amino acids $282-438$ of IgGl , EU numbering (with the primary contact sites being amino acids 248, 250-257, 272, 285, 288, 290-291, 308311 , and 314 of the CH 2 domain and amino acid residues $385-387$, 428, and 433-436 of the CH 3 domain. Thus, an Fc moiety of a polypeptide 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 $\operatorname{IgGl}, \mathrm{IgG} 2, \operatorname{IgG} 3$ and $\operatorname{IgG} 4$. In one embodiment, an FcRn binding portion from an antibody of the human isotype IgGl is used. In another embodiment, an FcRn binding portion from an antibody of the human isotype IgG4 is used.
[00263] In one embodiment, a polypeptide of the invention lacks one or more constant region domains of a complete Fc region, i.e., they are partially or entirely deleted.. In a certain embodiments polypeptides of the invention will lack an entire CH2 domain (ACH2 constructs). Those skilled in the art will appreciate that such constructs may be preferred due to the regulatory properties of the CH 2 domain on the catabolic rate of the antibody. In certain embodiments, polypeptides of the invention comprise CH 2 domain-deleted Fc regions derived from a vector (e.g., from IDEC Pharmaceuticals, San Diego) encoding an IgGi human constant region domain (see, e.g., WO 02/060955A2 and WG02/096948A2), This exemplary vector is engineered to delete the CH 2 domain and provide a synthetic vector expressing a domain-deleted. $\operatorname{lgG}_{\mathrm{i}}$ constant region. It will be noted that these exemplary constructs are preferably engineered to fuse a binding CH 3 domain directly to a hinge region of the respective Fc domain.
[00264] In other constructs it may be desirable to provide a spacer moiety between one or more constituent Fc moieties. For example, a spacer moiety may be
placed between a hinge region and a CH 2 domain and/or between a CH 2 and a CH 3 domams. For example, compatible constructs could be expressed wherein the CH 2 domain has been deleted and the remaining CH3 domain (synthetic or unsynthetic) is joined to the hinge region with a $5-20$ amino acid spacer moiety. Such a spacer moiety may be added, for instance, to ensure that the regulatory elements of the constant region domain remain free and accessible or that the hinge region remains flexible. Preferably, any linker peptide compatible with the instant invention will be relatively non-immunogenic and not prevent proper folding of the scFc region.
[00265] In certain embodiments, the polypeptides of the invention maycomprise a dimeric Fc region comprising Fc moieties of the same, or substantially the same, sequence composition (herein termed a "homodimeric Fc region"). In other embodiments, the polypeptides of the invention may comprise a dimeric Fc region comprising at least two Fc moieties which are of different sequence composition (i.e., herein termed a "heterodimeric Fc region"). In one exemplary embodiment, the heterodimeric Fc region comprises an amino acid substitution in a first Fc moiety (e.g., an amino acid substitution of Asparagine at EU position 297\}, but not in a second Fc moiety.
[00266] In certain embodiments, the Fc region is hemi-giycosylated. For example, the heteromeric scFc region may comprise a first, glycosylated. Fc moiety (e.g., a glycosylated CH 2 region) and a second, aglycosylated, Fc moiety (e.g., an aglycosylated CH2 region), wherein a linker is interposed between the glycosylated and aglycosylated Fc moieties. In other embodiments, the Fc region is fully glycosylated, i.e., all of the Fc moieties are glycosylated. In still further embodiments, the Fc region may be aglycosylated, i.e., none of the Fc moieties are glycosylated.
[00267] In certain embodiments, an Fc moiety employed in a polypeptide of the invention is altered, e.g., by amino acid mutation (e.g., addition, deletion, or substitution). For example, in one embodiment, an Fc moiety has at least one amino acid substitution as compared to the wild-type Fc from which the Fc moiety is derived. For example, wherein the Fc moiety is derived from a human IgGl antibody, a variant comprises at least one amino acid mutation (e.g., substitution) as compared to a wild type amino acid at the corresponding position of the human IgGl Fc region. [00268] The amino acid substitution(s) of an Fc variant may be located at a position within the Fc moiety referred to as corresponding to the position number that
that residue would be given in an Fc region in an antibody (as set forth using the EU numbering convention). One of skill in the art can readily generate alignments to determine what the EU number corresponding to a position in an Fc moiety would be. [00269] In one embodiment, the Fc variant comprises a substitution at an amino acid position located in a hinge domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH 2 domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH 3 domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH4 domain or portion thereof.
[00270] In certain embodiments, the polypeptides of the invention comprise an Fc variant comprising more than one amino acid substitution. The polypeptides of the invention may comprise, for example, $2,3,4,5,6,7,8,9,10$ or more amino acid substitutions. Preferably, the amino acid substitutions are spatially positioned from each other by an interval of at least 1 amino acid position or more, for example, at least $2,3,4,5,6,7,8,9$, or 10 amino acid positions or more. More preferably, the engineered amino acids are spatially positioned apart from each other by an interval of at least $5,10,15,20$, or 25 amino acid positions or more.
[00271] 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. $\mathrm{Fc} \mathrm{c}_{\mathrm{F}} \mathrm{RI}$, FcyRII, or FcyRIII) or complement proteins (e.g. Clq), or to trigger antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cvtotoxicit \}' (CDCC)). In other embodiments, the Fc variant provides an engineered cysteine residue
[00272] The polypeptides of the invention may employ art-recognized Fc variants which is 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, W096/14339A1, WO98/05787A1, W098/23289A1, W099/51642A1, W099/58572A1, WO00/09560A2, WO00/32767A1, WO00/42072A2, WO02/44215A2, WO02/060919A2, WO03/074569A2, WO04/016750A2,

WO04/029207A2, W004/03 5752A2, WO04/06335 1A2, WO04/074455A2, WO04/099249A2, WO05/040217A2, WO04/044859, WO05/070963A1, WO05/077981 A2, WO05/092925A2, WO05/123780A2, WO06/019447A1, WO06/047350A2, and WO06/085967A2; US Patent Publication Nos. US2007/023 1329, US2007/023 1329, US2007/0237765, US2007/0237766, US2007/0237767, US2007/0243 i 88, 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; $6,737,056 ; 6,821,505 ; 6,998,253 ; 7,083,784$; 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 di sclosed 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. [00273] In certain embodiments, a polypeptide of the invention comprises an amino acid substitution to an Fc moiety which alters the antigen-independent effector functions of the antibody, in particular the circulating half-life of the antibody.
[00274] Such polypeptides exhibit either increased or decreased binding to FcRn when compared to polypeptides 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 polypeptides of the invention exhibit reduced transport across the
epithelium of kidney glomeruli from the vasculature. In another embodiment, the polypeptides of the invention exhibit reduced transport across the blood brain barrier (BBB) from the brain, into the vascular space. In one embodiment, a polypeptide with altered FcRn binding comprises at least one Fc moiety (e.g, one or two Fc moieties) having one or more amino acid substitutions within the "FcRn binding loop' ' of an Fc moiety. The FcRn binding loop is comprised of amino acid residues 280-299 (according to EU numbering) of a wild-type, full-length, Fc moiety. In other embodiments, a polypeptide of the invention having altered FcRn binding affinity comprises at least one Fc moiety (e.g, one or two Fc moieties) having one or more amino acid substitutions within the 15 AFcRn "contact zone." As used herein, the temi 15 ÁFcRn "contact zone" includes residues at the following positions of a wildtype, 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 preferred embodiments, a polypeptide of the invention having altered FcRn binding affinity comprises at least one Fc moiet - (e.g, one or two Fc moieties) 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.
[00275] A polypeptide of the invention may also comprise an art recognized amino acid substitution which alters the glycosylation of the polypeptide. For example, the scFc region of the binding polypeptide may comprise an Fc moiety 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).
[00276] In other embodiments, a polypeptide of the invention comprises at least one Fc moiety having engineered cysteine residue or analog thereof which is located at the solvent-exposed surface. Preferably the engineered cysteine residue or analog thereof does not interfere with an effector function conferred by the scFc region. More preferably, the alteration does not interfere with the ability of the scFc region to bind to Fc receptors (e.g. FçRI, FcyRII, or FcyRIII) or complement
proteins (e.g. Clq), or to trigger immune effector function (e.g., antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cytotoxicity (CDCC)). [00277] In one embodiment, an unprocessed polypeptide of the invention may comprise a genetically fused Fc region (i.e., scFc region) having two or more of its constituent Fc moieties independently selected from the Fc moieties described herein. In one embodiment, the Fc moieties of a dimeric Fc region are the same. In another embodiment, at least two of the Fc moieties are different. For example, the Fc moieties of the polypeptides 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., I, 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 moieties of the polypeptides of the invention may differ in sequence at one or more amino acid positions. For example, at least two of the Fc moieties 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).

## VI. Polypeptide Linkers

[00278] As used herein, the term "polypeptide linkers" refers to a peptide or polypeptide sequence (e.g., a synthetic peptide or polypeptide sequence) which connects two domains in a linear amino acid sequence of a polypeptide chain. The polypeptides of invention are encoded by nucleic acid molecules that encode polypeptide linkers which either directly or indirectly connect the two Fc moieties which make up the construct,. These linkers are referred to herein as "scFc linkers". If the scFc linker connects two Fc moieties contiguously in the linear polypeptide sequence, it is a "direct" linkage. In contract, the scFc linkers may link the first Fc moiety to a binding moiety which is, in turn, linked to the second Fc moiety, thereby forming an indirect linkage. These scFc linkers $(\mathrm{L})$ result in the formation of a single chain genetic construct. However, in one embodiment, the scFc polypeptides also comprise enzymatic cleavage sites which result in the scFc linker being cleavable (an $\operatorname{cscFc}$ linker) and, in one embodiment, substantially excised (e.g., during processing by a cell). Thus, the processed molecule is a dimeric molecule comprising at least two amino acid chains and substantially lacking extraneous linker amino acid sequences. In some embodiments, all or substantially all of the linker is excised,
while in some embodiments, a portion of the cleavage site may remain, e.g., four argmines of the RERR cleavage site.
[00279] In another embodiment, another type of polypeptide linker, herein referred to as a "spacer" may be used to connect different moieties, e.g., a clotting factor or a targeting moiety to an Fc moiety. This type of polypeptide linker may provide flexibility to the polypeptide molecule. Spacers are not typically cleaved, however such cleavage may be desirable. Exemplary positions of spacers are shown in the accompanying drawings. Spacers can be located between the clotting factors, targeting moieties, and/or scaffolds, e.g., at the N or C terminus of these moieties. In one embodiment, these linkers are not removed during processing.
[00280] A third type of linker which may be present in a chimeric clotting factor of the invention is a cleavable linker which comprises a cleavage site (e.g., a factor XIa, Xa, or thrombin cleavage site) and which may include additional spacer linkers on either the N terminal of C terminal or both sides of the cleavage site. These cleavable linkers when incorporated into a clotting factor result in a chimeric molecule having a heterologous cleavage site. Exemplary locations for such sites are shown in the accompanying drawings and include, e.g., adjacent to targeting moieties, In another embodiment, such linkers may be adjacent to a clotting factor or portion thereof. For example, in one embodiment, a cleavable linker may be fused to the N terminus of the heavy chain of a clotting factor to make an activatable form of the clotting factor. In such cases, the cleavable linker may include additional spacer linkers at the N terminus of the cleavage site, but require direct fusion at the C terminus of the cleavage site to the amino terminus of the heavy chain of the clotting factor.
[00281] In one embodiment, an unprocessed polypeptide of the instant invention comprises two or more Fc domains or moieties linked via a cscFc linker to form an Fc region comprised in a single polypeptide chain. The cscFc linker is flanked by at least one enzymatic cleavage site, e.g., a site for processing by an intracellular enzyme. Cleavage of the polypeptide at the at least one enzymatic cleavage site results in a polypeptide which comprises at least two polypeptide chains. In one embodiment, an cscFc linker links F\{ or F2 to , e.g., a clotting factor, optionally via a cleavage site.
[00282] As is set forth above, other polypeptide linkers may optionally be used in a construct of the invention, e.g., to connect a clotting factor or targeting moiety to
an Fc moiety. One type of pollypeptide linker is referred to here as spacers. Some exemplar)' locations of spacers that can be used in connection with the invention include, e.g., polypeptides comprising GlySer amino acids such as those set forth in the accompanying figures and described in more detail below. In one embodiment, a spacer may be adjacent to one or more moieties each independently selected from clotting factor, scaffold moiety, e.g., Fc, cleavage site, and a targeting moiety. [00283] In one embodiment, the polypeptide linker is synthetic, i.e., nonnaturally occurring. In one embodiment, a polypeptide linker includes peptides (or polypeptides) (which may or may not be naturally occurring\} which comprise an amino acid sequence that links or genetically fuses a first linear sequence of amino acids to a second linear sequence of amino acids to which it is not naturally linked or genetically fused in nature. For example, in one embodiment the polypeptide linker may comprise non-naturally occurring polypeptides which are modified forms of naturally occurring polypeptides (e.g., comprising a mutation such as an addition, substitution or deletion). In another embodiment, the polypeptide linker may comprise non-naturally occurring amino acids. In another embodiment, the polypeptide linker may comprise naturally occurring amino acids occurring in a linear sequence that does not occur in nature. In still another embodiment, the polypeptide linker may comprise a naturally occurring polypeptide sequence.
[00284] For example, in certain embodiments, a polypeptide linker can be used to fuse identical Fc moieties, thereby forming a homomeric scFc region. In other embodiments, a polypeptide linker can be used to fuse different Fc moieties (e.g. a wild-type Fc moiety and an Fc moiety variant), thereby forming a heteronieric scFc region.
[00285] In another embodiment, a polypeptide linker comprises or consists of a gly-ser linker. In one embodiment, an scFc or cscFc linker comprises at least a portion of an immunoglobulin hinge and a gly-ser linker. As used herein, the term "gly-ser linker" refers to a peptide that consists of glycine and serine residues. An exemplary giy/ser linker comprises an amino acid sequence of the formula (Gly $\left.{ }_{4} \mathrm{Ser}\right) \mathrm{n}$ (SEQ ID NO: 4), wherein is a positive integer (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10). A preferred giy/ser linker is $\left(\mathrm{Gly}_{4} \mathrm{Ser}\right)_{2}$ (SEQ ID NO:29), (Gly $\left.{ }_{4} \mathrm{Ser}\right)_{4}$ (SEQ ID NO:6), or $\left(\mathrm{Gly}_{4} \mathrm{Ser}\right)_{6}$. (SEQ ID NO: 5) Another exemplary gly-ser linker is GGGSSGGGSG (SEQ ID NO: 30). In certain embodiments, said gly-ser linker may be inserted between two other sequences of the polypeptide linker (e.g., any of the polypeptide
linker sequences described herein). In other embodiments, a gly-ser linker is attached at one or both ends of another sequence of the polypeptide linker (e.g., any of the polypeptide linker sequences described herein). In yet other embodiments, two or more gly-ser linker are incorporated in series in a polypeptide linker. In one embodiment, a polypeptide linker of the invention comprises at least a portion of an upper hinge region (e.g., derived from an $\operatorname{IgG}\{, \operatorname{IgG} 2, \operatorname{IgG} 3$, or $\operatorname{IgG} 4$ molecule), at least a portion of a middle hinge region (e.g., derived from an $\operatorname{IgGl}, \operatorname{IgG} 2, \operatorname{IgG} 3$, or IgG4 molecule) and a series of gly/ser amino acid residues (e.g., a gly/ser linker such as ( $\left.\mathrm{Gly}_{4} \mathrm{Ser}\right) \mathrm{n}$ ) (SEQ ID NO:4)).
[00286] Polypeptide linkers of the invention are at least one amino acid in length and can be of varying lengths. In one embodiment, a polypeptide linker of the invention is from about 1 to about 50 amino acids in length. As used in this context, the term "about" indicates +/- two amino acid residues. Since linker length must be a positive interger, the length of from about 1 to about 50 amino acids in length, means a length of from 1-3 to 48-52 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about $10-20$ amino acids in length. In another embodiment, a polypeptide linker of the invention is from about 15 to about 50 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about 20 to about 45 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about $\$ 5$ to about 35 or about 20 to about 30 amino acids in length. In another embodiment, a polypeptide linker of the invention is from about $1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19$, $20,21,22,23,24,25,26,27,28,29,30,40,50$, or 60 amino acids in length. In one embodiment, a peptide linker of the invention is 20 or 30 amino acids in length. [00287] Polypeptide linkers can be introduced into polypeptide sequences using techniques known in the art. Modifications can be confirmed by DNA sequence analysis. Plasmid DNA can be used to transform host cells for stable production of the polypeptides produced,

## VM, Enzymatic Cleavage Sites

[00288] In one embodiment, one or more enzymatic cleavage site(s) is linked to e.g., flanks or is adjacent to, a cscFc linker (L) of an unprocessed polypeptide of the invention. Such cleavage sites can be upstream or downstream of the cscFc liner or both. For example, in one embodiment of a construct encoding a polypeptide of the
invention, a cleavage site is linked (e.g., directly or indirectly) to one or both ends of a cscFc linker (L).
[00289] For example, in one embodiment, a nucleic acid molecule of the invention specifies a polypeptide represented by the formula:

A-F1-P1-L-P2-B-F2 (I)
[00290] in linear sequence from the amino to carboxy termmus wherein A , if present, is a clotting factor or portion thereof, F 1 is a first Fc moiety or domain, P 1 is an enzymatic cleavage site, L is a $\operatorname{cscFc}$ linker, P 2 is an enzymatic cleavage site B , if present, is a clotting factor or portion thereof, F2 is a second Fc moiety or domain and "-" represents a peptide bond. Formula (I) comprises at least an A or B and optionally both. A and B , if both present, can be the corresponding heaw and light chains of a clotting factor. Formula (I) comprises at least a PI or P2 and optionally both. P1 and P2, if both present, can be the same or different. Formula (I) comprises at least a F1 and F2. F1 and F2, if both present, can be the same or different.
[00291] In another embodiment, a Factor X Ia or Xa cleavage site may be incorporated into a construct of the invention, e.g., in a cieavable linker. Exemplary FXIa cleavage sites include, e.g, TQSFNDFTR and SVSQTSKLTR. Exemplary thrombin cleavage sites include, e.g, DFLAEGGGVR, TTK1KPR, LVPRG SEQ ID $\mathrm{NO}: 35$ ) and ALRPRVVGGA Other useful cleavage sites are known in the art. [00292] In one embodiment, some portion of the linker may remain after cleavage at the at least one enzymatic cleavage site. In order to minimize the presence of extraneous amino acid sequences, two cleavage sites may be included in a polypeptide of the invention. In some embodiments, all or substantially all of the linker is excised, while in some embodiments, a portion of the cleavage site may remain, e.g., four arginines of the RRRR cleavage site.

Preparation of Polypeptides
[00293] A variety of methods are available for recombinantly producing a chimeric clotting factor of the invention. In one embodiment, the invention relates to a nucleic acid construct comprising a nucleic acid sequence encoding the chimeric proteins of the invention. It will be understood that because of the degeneracy of the code, a variety of nucleic acid sequences will encode the amino acid sequence of the polypeptide. The desired polynucleotide can be produced by de novo solid-phase DNA synthesis or by PCR mutagenesis of an earlier prepared polynucleotide.
[00294] Oligonucieotide-mediated mutagenesis is one method for preparing a substitution, in-frame insertion, or alteration (e.g., altered codon) to introduce a codon encoding an amino acid substitution (e.g., into an Fc variant moiety). For example, the starting polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a single-stranded DNA template. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that incorporates the oligonucleotide primer. In one embodiment, genetic engineering, e.g., primer-based PGR mutagenesis, is sufficient to incorporate an alteration, as defined herein, for producing a polynucleotide encoding a polypeptide of the invention.
[00295] For recombinant production, a polynucleotide sequence encoding the chimeric protein is inserted into an appropriate expression vehicle, i. e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation.
[00296] The nucleic acid encoding the chimeric protein is inserted into the vector in proper reading frame. The expression vector is then transfected into a suitable target cell which will express the polypeptide. Transfection techniques known in the art include, but are not limited to, calcium phosphate precipitation (Wigler et al. 1978, Cell 14 : 725) and electroporation (Neumann et al. 1982, EMBO, J. $1: 841$ ). A variety of host-expression vector systems may be utilized to express the chimeric proteins described herein in eukaryotic cells. In one embodiment, the eukaryotic cell is an animal cell, including mammalian cells (e. g. 293 cells, PerC6, CHO, BHK, Cos, HeLa cells). When the chimeric protein is expressed in a eukaryotic cell the DNA encoding the chimeric protein may also code for a signal sequence that will permit the chimeric protein to be secreted. One skilled in the art will understand that while the protein is translated the signal sequence is cleaved by the cell to form the mature chimeric protein. Various signal sequences are known in the art e.g., native factor VIl signal sequence, native factor IX signal sequence and the mouse IgK light chain signal sequence. Alternatively, where a signal sequence is not included the chimeric protein can be recovered by lysmg the cells.
[00297] The chimeric protein of the invention can be synthesized in a transgenic animal, such as a rodent, goat, sheep, pig, or cow. The term "transgenic animals"refers to non-human animals that have incorporated a foreign gene into their
genome. Because this gene is present in germiine tissues, it is passed from parent to offspring. Exogenous genes are introduced into single-celled embryos (Brinster et al 1985, Proc, Natl. Acad.Sci. USA 82 : 4438). Methods of producing transgenic animals are known in the art. including transgenics that produce immunoglobulin molecules (Wagner et al. 1981, Proc. Natl. Acad. Sci. USA 78: 6376: McKnight et al. 1983, Cell 34 : 335; Brinster et al. 1983, Nature 306: 332; Ritchie et al. 1984, Nature 312: 517; Bakiassarre et al. 2003, Theriogenology $59: 831$; Robl et al. 2003, Theriogenoiogy 59: 107; Malassagne et al. 2003, Xenotransplantation 10 (3): 267). [00298] The expression vectors can encode for tags that permit for easy purification or identification of the recombinantly produced protein. Examples include, but are not limited to, vector pUR278 (Ruther et al. 1983, EMBO j. 2: 1791) in which the chimeric protein described herein coding sequence may be ligated into the vector in frame with the lac z coding region so that a hybrid protein is produced; pGEX vectors may be used to express proteins 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 (e. g. PreCission Protease (Pharmacia, Peapack, N. J. )) for easy removal of the tag after purification.
[6] 299] For the purposes of this invention, numerous expression vector systems may be employed. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Expression vectors may include expression control sequences including, but not limited to, promoters (e.g., naturally-associated or heterologous promoters), enhancers, signal sequences, splice signals, enhancer elements, and transcription termination sequences. Preferably, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Expression vectors may also utilize DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV), cytomegalovirus (CMV), or SV40 virus. Others involve the use of polycistronic systems with internal ribosome binding sites.
[00300] Commonly, expression vectors contain selection markers (e.g., ampiciliin-resistance, hygromycin-resistance, tetracycline resistance or neomycin resistance $\}$ to permit detection of those cells transformed with the desired DNA
sequences (see, e.g.. Itakura et al., US Patent 4,704,362). Cells which have integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow selection of transfected host cells. The marker may provide for prototrophy to an auxotrophic host, biocide resistance (e.g., antibiotics) or resistance to heavy metals such as copper. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation.
[00301] A preferred expression vector is NEOSPLA (U.S. Patent No. $6,159,730)$. This vector contains the cytomegalovirus promoter/enhancer, the mouse beta gfobin major promoter, the SV40 origin of replication, the bovine growth hormone poiyadenylation sequence, neomycin phosphotransferase exon 1 and exon 2, the dihydrofolate reductase gene and leader sequence. This vector has been found to result in very high level expression of antibodies upon incorporation of variable and constant region genes, transfection in cells, followed by selection in G4 18 containing medium and methotrexate amplification. Vector systems are also taught in U.S. Fat. Nos. $5,736,137$ and $5,658,570$, each of which is incorporated by reference in its entirety herein. This system provides for high expression levels, e.g., > 30 pg/cell/day. Other exemplary vector systems are disclosed e.g., in U.S. Patent No. 6,413,777.
[00302] In other embodiments the polypeptides of the invention of the instant invention may be expressed using polycistronic constructs. In these expression systems, multiple gene products of interest such as multiple polypeptides of multimer binding protein may be produced from a single polycistronic construct. These systems advantageously use an internal ribosome entry site (IRES) to provide relatively high levels of polypeptides of the invention in eukaryotic host cells. Compatible IRES sequences are disclosed in U.S. Pat. No. 6,193,980 which is also incorporated herein. Those skilled in the art will appreciate that such expression systems may be used to effectively produce the full range of polypeptides disclosed in the instant application.
[00303] More generally, once the vector or DNA sequence encoding a polypeptide has been prepared, the expression vector may be introduced into an appropriate host cell. That is, the host cells may be transformed. Introduction of the plasmid into the host cell can be accomplished by various techniques well known to those of skill in the art. These include, but are not limited to, transfection (including
electrophoresis and electroporation), protoplast fusion, calcium phosphate precipitation, cell fusion with enveloped DNA, microinjection, and infection with intact virus. See, Ridgway, A. A. G. "Mammalian Expression Vectors" Chapter 24,2, pp. 470-472 Vectors, Rodriguez and Denhardt, Eds. (Butterworths, Boston, Mass. 1988). Most preferably, plasmid introduction into the host is via electroporation. The transformed cells are grown under conditions appropriate to the production of the light chains and heavy chains, and assayed for heavy and/or light chain protein synthesis. Exemplary assay techniques include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), or fiourescence-activated cell sorter analysis (FACS), immunohistochemistry and the like.
[00304] As used herein, the term "transformation" shall be used in a broad sense to refer to the introduction of DNA into a recipient host cell that changes the genotype and consequently results in a change in the recipient cell.
[00305] Along those same lines, "host cells" refers to cells that have been transformed with vectors constructed using recombinant DNA techniques and encoding at least one heterologous gene. In descriptions of processes for isolation of polypeptides from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of polypeptide unless it is clearly specified otherwise. In other words, recovery of polypeptide from the "cells" may mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells.
[00306] The host cell line used for protein expression is most preferably of mammalian origin; those skilled in the art are credited with ability to preferentially determine particular host cell lines which are best suited for the desired gene product to be expressed therein. Exemplary host cell lines include, but are not limited to, DG44 and DUXB1 1 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative of CVI with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), HAK (hamster kidney line), SF2/0 (mouse myeloma), F3.times.63-Ag3.653 (mouse myeloma), BFA-lclBPT (bovine endothelial cells), RAJI (human lymphocyte\}, PerC6, and 293 (human kidney). Host cell lines are typically available from commercial services, the American Tissue Culture Collection or from published literature.
[00307] In one embodiment, a host cell endogenously expresses an enzyme (or the enzymes) necessary to cleave an scFc linker (e.g., if such a linker is present and contains intracellular processing site(s)) during processing to form the mature polyptide. During this processing, the scFc linker may be substantially removed to reduce the presence of extraneous amino acids. In another embodiment of the invention, a host cell is transformed to express one or more enzymes which are exogenous to the cell such that processing of an scFc linker occurs or is improved.
[00308] In one embodiment an enzyme which may be endogenously or exogenously expressed by a cell is a member of the furin family of enzymes. Complete cDNA and amino acid sequences of human furin (i.e., PACE) were published in 1990. Van den Ouweland A M et al. (1990) Nucleic Acids Res. 18:664: Erratum in: Nucleic Acids Res. 18:1332 (1990).
[00309] U.S. Pat. No. 5,460,950, issued to Barr et al, describes recombinant PACE and the coexpression of PACE with a substrate precursor polypeptide of a heterologous protein to improve expression of active, mature heterologous protein.
[00310] U.S. Pat. No. 5,935,815, issued to van de Ven et al., likewise describes recombinant human furin (i.e., PACE; ) and the coexpression of furin with a substrate precursor polypeptide of a heterologous protein to improve expression of active, mature heterologous protein. Possible substrate precursors disclosed in this patent include a precursor of Factor IX. Other family members in the mammalian furin/ subtil isin/Kex2p~like proprotein convertase (PC) family in addition to PACE are reported to include PC1/PC3, PC2, PC4, PC5/6 (hereinafter referred to simply as PC5), PACE4, and LPC/PC7/PC8/SPC7. While these various members share certain conserved overall structural features, they differ in their tissue distribution, subcellular localization, cleavage specificities, and preferred substrates. For a review, see Nakayama K (1997) Biochem J. 327:625-35. Similar to PACE, these proprotein convertases generally include, beginning from the amino terminus, a signal peptide, a propeptide (that may be autocatalytically cleaved), a subtilisin-like catalytic domain characterized by Asp, His, Ser, and Asn/Asp residues, and a Homo B domain that is also essential for catalytic activity and characterized by an Arg-Gly-Asp (RGD) sequence, PACE, PACE4, and PCS also include a Cys-rich domain, the function of which is unknown. In addition, PCS has isoforms with and without a transmembrane domain; these different isoforms are known as PC5B and PC5A, respectively. Comparison between the amino acid sequence of the catalytic domain of PACE and
the amino acid sequences of the catalytic domains of other members of this family of proprotein convertases reveals the following degrees of identity: 70 percent for PC4; 65 percent for PACE4 and PCS; 61 percent for PC1/PC3; 54 percent for PC2; and 51 percent for LPC/PC7/PC8/SPC7. Nakayama K (1997) Biochem J. 327:625-35.
[00311] PACE and PACE4 have been reported to have partially overlapping but distinct substrates. In particular, PACE4, in striking contrast to PACE, has been reported to be incapable of processing the precursor polypeptide of FIX. Wasley L C et al. (1993) J Biol Chem. 268:8458-65; Rehemtulla A et al. (1993) Biochemistry. 32:11586-90.
[00312]
U.S. Pat. No. 5,840,529, issued to Seidah et al, discloses nucleotide and amino acid sequences for human PC7 and the notable ability of PC7, as compared to other PC family members, to cleave HIV gpl 60 to gpl20 and gp41.
[00313] Nucleotide and amino acid sequences of rodent PCS were first described as PCS by Lusson J et al. (1993) Proc Natl Acad Sci USA 90:6691-5 and as PC6 by Nakagawa T et al. (1993) J Biochem (Tokyo) 113: 132-5. U.S. Pat. No. 6,380,171, issued to Day et al, discloses nucleotide and amino acid sequences for human PC5A, the isoform without the transmembrane domain. The sequences of these enzymes and method of cloning them are known in the art.
[00314] Genes encoding the polypeptides of the invention can also be expressed in non-mammalian cells such as bacteria or yeast or plant cells. In this regard it will be appreciated that various unicellular non-mammalian microorganisms such as bacteria can also be transformed; i.e., those capable of being grown in cultures or fermentation. Bacteria, which are susceptible to transformation, include members of the enterobacteriaceae, such as strains of Escherichia coli or Salmonella; Bacillaceae, such as Bacillus subtilis; Pneumococcus; Streptococcus, and Haemophilus influenzae. It will further be appreciated that, when expressed in bacteria, the polypeptides typically become part of inclusion bodies. The polypeptides must be isolated, purified and then assembled into functional molecules.
[00315] In addition to prokaryates, eukaryotic microbes may also be used. Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used among eukaryotic microorganisms although a number of other strains are commonly available.

For expression in Saccharomyces, the plasmid YRp7, for example, (Stinchcomb et al., Nature, 282:39 (1979); Kingsman et al, Gene, 7:141 (1979); Tschemper et al, Gene,

10:157 (1980) \} is commonly used. This plasmid already contains the TRP1 gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, Genetics, 85:12 (1977)). The presence of the trpl lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Other yeast hosts such Pichia may also be employed. Yeast expression vectors having expression control sequences (e.g., promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C , and enzymes responsible for methanol, maltose, and galactose utilization.
[00316] Alternatively, polypeptide-coding nucleotide sequences can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal (see, e.g., Deboer et al., US 5,741,957, Rosen, US 5,304,489, and Meade et al, US 5,849,992). Suitable transgenes include coding sequences for polypeptides in operable linkage with a promoter and enhancer from a mammary gland specific gene, such as casein or beta lactoglobulin.
[00317] In vitro production allows scale-up to give large amounts of the desired polypeptides. 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, chromatography over DEAE-cellulose or (immuno-)affmity chromatography, e.g., after preferential biosynthesis of a synthetic hinge region polypeptide or prior to or subsequent to the HIC chromatography step described herein. An affinity tag sequence (e.g. a His(6) tag) may optionally be attached or included within the polypeptide sequence to facilitate downstream purification. [00318] In one embodiment, a host cell of the invention comprises a genetic construct encoding a polypeptide comprising an scFe scaffold and one or more
enzymes that can process a cscFc linker. The construct and the enzyme(s) can be expressed using a single vector or two vectors.
[00319] In one embodiment, the invention pertains to nucleic acid molecules which encode a polypeptide of the invention. In one embodiment, the nucleic acid molecule encodes a chimeric clotting factor selected from the group consisting of FVII, FIX and FX and which comprises a targeting moiety which binds to platelets and optionally a spacer moiety between the clotting factor and the targeting moiety. In another embodiment, the invention pertains to a nucleic acid molecule encoding a polypeptide comprising FVII, which FVII which comprises a heterologous enzymatic cleavage site activatable by a component of the clotting cascade.
[00320] Once expressed, the chimeric clotting factor can be purified according to standard procedures of the art, including ammonium sulfate precipitatioii, affinity column chromatography, KPL.C purification, gel electrophoresis and the like (see generally Scopes, Protein Purification (Springer-Verlag, N.Y.. (1982)) and see specifically the methods used in the instant Examples. Substantially pure proteins of at least about 90 to $95 \%$ homogeneity are preferred, and 98 to $99 \%$ or more homogeneity most preferred, for pharmaceutical uses.

## IX. Methods of Administering Polypeptides of the Invention

[00321] In another embodiment, the invention relates to a method of treating a subject with a hemostatic disorder comprising administering a therapeutically effective amount of an enhanced clottng factor of the Invention.
[00322] Compositions for administration to a subject include nucleic acid molecules which comprise a nucleotide sequence encoding a chimeric clotting factor of the invention (for gene therapy applications) as well as polypeptide molecules. [00323] In one embodiment, an enhanced cloting factor composition of the invention is administered in combination with at least one other agent that promotes hemostasis. Said other agent that promotes hemostasis Is a therapeutic with demonstrated clotting activity. As an example, but not as a limitation, hemostatic agent can include Factor V, Factor VII, Factor VIII, Factor IX, Factor X, Factor XI, Factor XII, Factor XIII, prothrombin, or fibrinogen 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.
[00324] In one embodiment of the invention, the composition (e.g., the polypeptide or nucleic acid molecule encoding the polypeptide) is one $m$ which the clotting factor is present in active form when administered to a subject. Such an activated molecule may be expressed by a cell in active form or may be activated in vitro prior to administration to a subject. In another embodiment, the composition is one in which the clotting factor is present in activatable form and the clotting factor is activated in vivo at the site of clotting after administration to a subject.
[00325] The chimeric clotting factor of the invention can be administered intravenously, subcutaneously, intramuscularly, or via any mucosal surface, e.g., orally, sublingual !\}, buccal \}y: sublingually, nasally, rectally, vaginally or via pulmonary route. The chimeric protein can be implanted within or linked to a biopolvmer solid support that allows for the slow release of the chimeric protein to the desired site.
[00326] 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. [00327] For buccal and sublingual administration the composition may take the form of tablets, lozenges or fast dissolving films according to conventional protocols. [00328] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray from a pressurized pack or nebulizer (e.g. in PBS), with a suitable propeliant, e.g., dichlorodifluoromethane, triehlorofluoromethane, 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.
[00329] In one embodiment, the route of administration of the polypeptides of the invention is parenteral. The term parenteral as used herein includes intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, recta! or vaginal administration. The intravenous form of parenteral administration is preferred. While all these forms of administration are clearly contemplated as being within the scope of the invention, a form for administration would be a solution for injection, in particular for intravenous or intraarterial injection or drip. Usually, a suitable pharmaceutical composition for injection may comprise a buffer (e.g. acetate, phosphate or citrate buffer), a surfactant (e.g. polysorbate), optionally a stabilizer agent (e.g. human albumin), etc. However, in other methods compatible with the teachings herein, the polypeptides can be delivered directly to the site of the adverse cellular population thereby increasing the exposure of the diseased tissue to the therapeutic agent.
[00330] Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. In the subject invention, pharmaceutically acceptable carriers include, but are not limited to, $0.01-0.1 \mathrm{M}$ and preferably 0.05 M phosphate buffer or $0.8 \%$ saline. Other common parenteral vehicles include sodium phosphate solutions, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishes, electrolyte replenishes, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present such as for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like.
[00331] More particularly, pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In such cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and will preferably be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propyiene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures
thereof. The proper fluidity 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.
[00332] Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols, such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin,
[00333] In any case, sterile injectable solutions can be prepared by incorporating an active compound (e.g., a polypeptide by itself or in combination with other active agents) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which 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, which yields a powder of an active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparations for injections are processed, filled into containers such as ampoules, bags, bottles, syringes or vials, and sealed under aseptic conditions according to methods known in the art. Further, the preparations may be packaged and sold in the form of a kit. Such articles of manufacture will preferably have labels or package inserts indicating that the associated compositions are useful for treating a subject suffering from, or predisposed to clotting disorders.
[00334] 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.
[00335] Effective doses of the compositions of the present invention, for the treatment of conditions vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophyiactic or therapeutic. Usually, the patient is a human but non-human mammals
including transgenic mammals can also be treated. Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.
[00336] In one embodiment, the dose of a biologically active moiety (e.g.. comprising FIX) can range from about 25 to $100 \mathrm{lU} / \mathrm{kg}$, e.g., $0.417 \mathrm{mg} / \mathrm{kg}$ to 1.67 $\mathrm{mg} / \mathrm{kg}$. In another embodiment, the dose of a biologically active moiety (e.g., comprising FVIII) can range from about 25 to $65 \mathrm{Hi} / \mathrm{kg}$, e.g., $0,003125 \mathrm{mg} / \mathrm{kg}$ to $0.008125 \mathrm{mg} / \mathrm{kg}$. In another embodiment, the dose of a biologically active moiety (e.g., comprising FVII), can range from about 90 to $270 \mathrm{ug} / \mathrm{kg}$ or 0.090 to 0.270 $\mathrm{mg} / \mathrm{kg}$.
[00337] Dosages can range from $1000 \mathrm{ug} / \mathrm{kg}$ to $0.1 \mathrm{ng} / \mathrm{kg}$ body weight. In one embodiment, the dosing range is $1 \mathrm{ug} / \mathrm{kg}$ to $100 \mathrm{ug} / \mathrm{kg}$. 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 at, e.g., STACLOT Vlla-rTF 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).
[00338] Doses intermediate in the above ranges are also intended to be within the scope of the invention. Subjects can be administered such doses daily, on alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. In some methods, two or more polypeptides may be administered simultaneously, in which case the dosage of each polypeptide administered falls within the ranges indicated.
[00339] Polypeptides of the invention can be administered on multiple occasions. Intervals between single dosages can be daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of modified polypeptide or antigen in the patient. Alternatively, polypeptides can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the polypeptide in the patient.
[00340] The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications,
compositions containing the polypeptides 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 minimize effects of disease. Such an amount is defined to be a "prophylactic effective dose." A relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to recei ve treatment for the rest of their lives.
[00341] Polypeptides of the invention can optionally be administered in combination with other agents that are effective in treating the disorder or condition in need of treatment (e.g., prophylactic or therapeutic).
[00342] As used herein, the administration of polypeptides of the invention in conjunction or combination with an adjunct therapy means the sequential, simultaneous, coextensive, concurrent, concomitant or contemporaneous administration or application of the therapy and the disclosed polypeptides. Those skilled in the art will appreciate that the administration or application of the various components of the combined therapeutic regimen may be timed to enhance the overall effectiveness of the treatment.

A skilled artisan (e.g. a physician) would be readily be able to discern effective combined therapeutic regimens without undue experimentation based on the selected adjunct therapy and the teachings of the instant specification.
[00343] it will further be appreciated that the polypeptides of the instant invention may be used in conjunction or combination with an agent or agents (e.g. to provide a combined therapeutic regimen). Exemplary agents with which a polypeptide of the invention may be combined include agents that represent the current standard of care for a particular disorder being treated. Such agents may be chemical or biologic in nature. The term "biologic" or "biologic agent" refers to any pharmaceutically active agent made from living organisms and/or their products which is intended for use as a therapeutic.
[00344] The amount of agent to be used in combination with the polypeptides of the instant invention may vary by subject or may be administered according to what is known in the art. See for example, Bruce A Chabner et al., Antineoplastic Agents, in GOODMAN \& OILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS 1233-1287 ((Joel G. Hardman et a!., eds., $9^{\text {th }}$ ed. 1996). In another embodiment, an amount of such an agent consistent with the standard of care is administered.
[00345] As previously discussed, the polypeptides of the present invention, may be administered in a pharmaceutically effective amount for the in vivo treatment
of clotting disorders. In this regard, it will be appreciated that the polypeptides of the invention can be formulated to facilitate administration and promote stability of the active agent. Preferably, pharmaceutical compositions in accordance with the present invention comprise a pharmaceutically acceptable, non-toxic, sterile carrier such as physiological saline, non-toxic buffers, preservatives and the like. Of course, the pharmaceutical compositions of the present invention may be administered in single or multiple doses to provide for a pharmaceutically effective amount of the polypeptide.
[00346] In one embodiment, a chimeric clotting factor of the invention can be administered as a nucleic acid molecule. Nucleic acid molecules can be administered using techniques known in the art, including via vector, plasmid. liposome, DNA injection, electroporation, gene gun, intravenously injection or hepatic artery infusion. Vectors for use in gene therapy embodiments are known in the art.
[00347] In keeping with the scope of the present disclosure, the chimeric clotting factors of the invention may be administered to a human or other animal in accordance with the aforementioned methods of treatment in an amount sufficient to produce a therapeutic or prophylactic effect.
[00348] The chimeric proteins of the invention have many uses as will be recognized by one skilled in the art, including, but not limited to methods of treating a subject with a disease or condition. The disease or condition can include, but is are not limited to, hemostatic disorders.
[00349] In one embodiment, the invention relates to a method of treating a subject having a hemostatic disorder comprising administering a therapeutically effective amount of at least one chimeric clotting factor of the invention.
[00350] The chimeric clotting factors of the invention treat or prevent a hemostatic disorder by promoting the formation of a fibrin clot. The chimeric clotting factor of the invention can activate any member of a coagulation cascade. The clotting factor can be a participant in the extrinsic pathway, the intrinsic pathway or both.
[00351] A chimeric clotting factor of the invention can be used to treat hemostatic disorders, e.g., those known to be treatable with the particular clotting factor present in the chimeric dotting factor. The hemostatic disorders that may be treated by administration of the chimeric protein of the invention include, but are not limited to. hemophilia A, hemophilia B, von Willebrand's disease, Factor XI deficiency (PTA deficiency), Factor XII deficiency, as well as deficiencies or
structural abnormalities in fibrinogen, prothrombin, Factor V, Factor VII, Factor X, or Factor XIII.
[00352] In one embodiment, the hemostatic disorder is an inher ted disorder. In one embodiment, the subject has hemophilia A , and the chimeric protein comprises Factor VII or Factor Villa. In another embodiment, the subject has hemophilia A and the chimeric clotting factor comprises Factor VII or Factor VIIa. In another embodiment, the subject has hemophilia B and the chimeric clotting factor comprises Factor IX or Factor IXa. In another embodiment, the subject has hemophilia B and the chimeric protein comprises Factor VII or Factor VIIa. In another embodiment, the subject has inhibitory antibodies to Factor VII or Factor Villa and the chimeric clotting factor comprises Factor VII or Factor VIa. In yet another embodiment, the subject has inhibitory antibodies against Factor IX or Factor IXa and the chimeric protein comprises Factor VII or Factor Vila.
[00353] The chimeric clotting factor of the invention can be used to prophyiacticaliy treat a subject with a hemostatic disorder. The chimeric clotting factor of the invention can be used to treat an acute bleeding episode in a subject with a hemostatic disorder.
[00354] In one embodiment, the hemostatic disorder is the result of a deficiency in a clotting factor, e.g., Factor IX, Factor VIII. In another embodiment, the hemostatic disorder can be the result of a defective clotting factor.
[00355] In another embodiment, the hemostatic disorder can be 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 autoimmune disease, or pregnancy. The acquired disorder can result from old age or from medication to treat an underlying secondary disorder (e.g. cancer chemotherapy).
[00356] The invention also relates to methods of treating a subject that does not have a hemostatic disorder or a secondary disease or condition resulting in acquisition of a hemostatic disorder. 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 at least one chimeric clotting factor of the invention. For example, in one embodiment, the subject in need of a general hemostatic agent is undergoing, or is about to undergo, surgery. The chimeric clotting factor of the invention can be administered prior to or after surgery as a prophylactic. The chimeric
clotting factor 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, or stem cell transplantation.
[00357] In another embodiment, the chimeric clotting factor 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.
[00358]
This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

## EXAMPLES

[00359] Throughout the examples, the following materials and methods were used unless otherwise stated.

## General Materials and Methods

[00360] In general, the practice of the present mvention 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 Engmeering: A Practical Approach (Practical Approach Series, 169), McCafferty, Ed, $\operatorname{Ir} 1 \operatorname{Pr}(1996)$; Antibodies: A Laboratory Manual, Harlow et al., CS.H.L. Press, Pub. (1999); and Current Protocols in Molecular Biology, eds. Ausubei et a!., John Wiley \& Sons (1992).

Example L Heterodimeric Constructs comprising FVIr-Fc and MB9-Fc at the amino terminus of the second Fc chain

Cloning of pSYN-FVII-027
[003611
The FV11-027 construct comprises cscFc for cleavage when processed during manufacture in a cell. The construct comprises a targeting moiety, a scFv moiety that binds to GPIIbllia, MB9.
[00362] Plasmid (pSYN-FVH-027) was generated for the expression FVIl-Fc and MB9-Fc heterodimer, where MB9 is a scFv previously shown to bind to receptor GPIIb/IIIa on activated platelets. Protein from p8YN-FVII~027 is expressed in the cell as a single polypeptide where the C-terminus of the FVII-Fc subunit is Jinked to the N -terminus of the MB9-Fc subunit by a (GGGGS)e $\mathrm{e}_{\mathrm{x}}$ polypeptide linker. Furthermore, RRRRS and RKRRKR sequences were inserted at the $5^{\prime}$ and 3 ' end of the polypeptide linker, respectively, for intracellular cleavage by proprotein convertases following the last Arg at each sequence. Consequently, ceils will express a 2 chain FVII-Fc/MB9-Fe heterodimer where the FVII-Fc chain has a RRRRS sequence at the C-terminus, but the remainder of the linker and the RKRRKR sequence have otherwise been removed.
[00363] As a first step a series of intermediate plasmid were generated using
the following primers:

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HindIII-SalI-BpsEI-Fc-F
AGTCAAGCTTGTCGACTCCGGAACTCCTGGGCGGACC
BamHI-I inker-Fc-R
CATCGGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCCGCCACCTTTAC
CCGGAGACA. GGGAGAGG
Be]I-FC-F
CAGTCTTGATCAGACAAAACTCACACATGCCCACC
SCFC-ECORI-R
AC TGAC GAAT TCTCATTTACCC GGAGAC AGGGAG
HindIII-Kozak-FVII-F:
CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG
FVII-HC-BspEI-R:
AGGAGTTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTCGGATCCCCCGCCACCGGAACCTCCA
CCGCCTGATCCACCCCCACCTGATCCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCGGGA
AATGGGGC TCGCAGGAGG
A 50 u ] PGR reaction was carried out with 25 pmol of Hindlll-Sall-BpEI-Fc-F and BamHI-linker-Fc-R and template pSYN-Fc-001 using the following cycle: \(95^{\circ} \mathrm{C} 2\) minutes; 30 cycles of \(\left(95^{\circ} \mathrm{C} 30\right.\) seconds. \(54^{\circ} \mathrm{C} 30\) seconds \(72^{\circ} \mathrm{C} 1\) minute \()\). The expected sized band ( -700 bp ) was gel purified with a Gel Extraction kit (Qiagen, Valencia, Calif.) and cloned into the Hindll and BamHI restriction sites of
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pBUDCE4 (Invitrogen, Carlsbad, Calif.) to generate intermediate pSYN-FVU-007. Primers Hindlll-Sail-BpEI-Fc-F and BamHI-linker-Fc-R amplify the Fc region starting at amino acid 22 (EU numbering) and add a Hind!! [ and a Sail restriction enzyme site immediately upstream of site Fc region, as well as a DNA fragment encoding a (GGGGS) $4_{x}$ linker followed by a BamHI site immediately downstream of the Fc coding region. Next, a 50 ul reaction was earned out with 25 prnol of $\mathrm{Bcll}-\mathrm{Fc}-\mathrm{F}$ and scFc-EcoRI-R, and template pSYN-Fc-01 1 using the same cycles as above. The expected sized band ( -700 bp ) was gel purified as above, cut with restriction enzymes BamHI and EcoRI, and cloned in the BcU/EcoRI restriction sites of pSYN-FVU-007 to generate the intermediate plasmid pSYN-FVTI-008. The primer pair Bell-Fe-F and scFc-EcoRI-R amplifies the Fc region while adding a Bell and EcoRI restriction sites immediately upstream and downstream of the Fc coding region, respectively. To generate the last intermediate plasmid, a 50 ul PGR reaction was carried out with 25 prnol of Hindm-Kozak-FVII-F and FVII-HC-BspEI-R and template pSYN-FVII-001 using the following cycle: $95^{\circ} \mathrm{C} 2$ minutes; 30 cycles of $\left(95^{\circ} \mathrm{C} 30\right.$ seconds, $55^{\circ} \mathrm{C} 30$ seconds, $72^{\circ}$ C 90 seconds). The primer pair amplifies the FVII coding region while adding a DNA fragment at the 3 ' end of the molecule encoding a (GGGGS)e ${ }_{x}$ polypeptide linker followed by a fragment of the Fc region ending at amino acid 221 (EU numbering). Primer HindIII-Kozak-FVII-F generates a Hind!! ! restriction site at the $5^{\prime}$ of the molecule followed by a Kozak sequence directly upstream of the FVII coding region. The FVII-HC-BspEI-R primer introduces DNA encoding the polypeptide linker as well as the Fc portion. The expected sized band ( $\sim 1500 \mathrm{bp}$ ) was gel purified as above and cloned into the $H$ indXXX Bp \& sites of pSYN -FVII-008 to generate pSYN-FVII-011.
[00364] Next, 2 DNA fragments were synthesized: Genescripi-FVXX-027-! and Genscript-FVII-026-2. Genescript-FVII-027-1 consists of a DNA fragment encoding a portion of the Fc region (starting at nucleotide 1306, EU numbering) followed by the sequence RRRRS-(GGGGS) ${ }_{5 x}$-RKRRKR followed by a portion of the MB9 scFy (residues 1-142). An EcoRI site was introduced in the coding sequence of MB9 using the degeneracy of the genetic code to preserve the proper amino acid sequence and overlaps the last 6 bases of Geneseript-FVIi-027-1. In addition, the first 6 bases at the 5' include a Sapl site found within the Fc region. Genscript-FVII-026-2 consists of a DN A fragment encoding a portion of the MB9 (residues 143-273) followed by a
(GGGGS ) ${ }_{6} \mathrm{x}$ polypeptide linker followed by the Fc region and an EcoRI site. An EcoRI site was introduced in the coding sequence of MB9 using the degerseracy of the genetic code to preserve the proper amino acid sequence and overlaps the first 6 bases of Genescript-FVII-026-2 .
[00365] Genescript-FVII -027-1 was cloned into the SapI and EcoRI sites of pSYN-FVTT-0l 1 to generate pSYN-FW-036. Next, Genscript-F\II-026-2 was cloned into the EcoRI site of pSYN-FVn-036 to generate pSYN-F VII-027. Correct orientation of the last cloning step was confirmed by restriction enzyme analysis and DNA sequencing.

Example 2. Heterodimeric Constructs comprising FV) the carboxy terminus of the second Fc chain

Cloning ofFVH-037
[00366] The FVII-037 construct is made using an scFc scaffold which is not cleaved during processing. In this construct the targeting moiety, again the MB 9 scFv which binds to GPIIbIII is attached to the c-terminus of the second Fc moiety.
[003671 Synthesis of DNA fragment Genseript-F VII-037 was outsourced (Genscript) This fragment comprises a portion of the Fc region (residues 434 to 447, EU numbering) followed by a (GGGGS)4x polypeptide linker and the MB9 scFv. A SapI/EcoRI fragment of Genscript-FVEI-037 was subcloned into the SapI/EcoRI of pSYN-FVTI-0 11 (refer to P0830) to generate and intermediate construct. A Sapl fragment from pSYN-F VII-O! 1 was subcloned into the Sap! sites of the intermediate construct to generate pSYNFVII-037.

Example 3. Heterodimeric Constructs comprising FVJI-Fc and a peptide against GPIb at the carboxy terminus of the second Fc chain Cloning of THE pSYN-FV 11-041 intermediate construct. [00368] In order to make this construct, the FVT]-041 construct was first made as an intermediate. Synthesis of DNA molecule Genscript-FVII-041 was outsourced (Genscript). This fragment was digested with Sapl and cloned into Sapl sites of pSYN-F VII-011 to generate pSYN-FVTI-041. This process introduces a unique Sall site (residues 412-413 EU numbering. GTG GAC to GTC GAC) in the second Fc. Cloning of pSYN-FVII-044-, -045 and -046.
[00369] The FVII-041 construct was used as the starting material to generate several constructs that comprise targeting moieties which are peptides that bind to GPIb. The PS4 peptide is used in the -044 construct, the OS1 peptide in the -045
construct, ad the OS2 peptide in the -046 construct. In these constructs an scFc scaffold is used and the peptides are attached via a linker $o$ the $C$-terminus of the second Fc moiety.
[00370] Synthesis of Genscript-FVII-044, -045 and -046 was outsourced (Genscript). These DNA fragments were cleaved with Sall/EcoRI and subcloned into the Sall/EcoRI sites of pSYN-FVTI-04 1 to generate pSYN-FVII-044, -045 and -046. Example 4, Heterodimeric Constructs comprising FVil-Fc and a peptide against GPIb at the amino terminus of the second Fc chain

Cloning of the pSYN-FVII-043 intermediate.
[00371] In order to make this construct, the FVII-043 construct was first made as an intermediate. Synthesis of DNA fragment Genscript-FVH-043 was outsourced (Genscript). This fragment comprises a DNA molecule encoding a portion of the Fc region (residues 232 to 447, EU numbering) followed by a (GGGGS)4x polypeptide linker and another portion of the Fc region (residues 221 to 238, EU numbering). This DNA fragment was digested with BspEI and Rsrll and subcloned into the BspEI/RsrII sites of $\mathrm{pSYN}-\mathrm{FVIG}$-042 to generate pSYN -FVII-050. This process introduces a unique Sail site (residues 412-413 EU numbering, GTG GAC to GTC GAC) in the first Fc. A Hind $11 / E c o R I$ fragment of pSYNFVII-050 was subcloned into the Hindin/EcoRI sites of pSYN-FVII-0 11 to generate pSYN-FVII-043.
Cloning of pSYN-FVII-047, -048 and -049.
[00372] The FVII-043 construct was used as the starting material to generate several constructs that comprise targeting moieties which are peptides that bind to GPIb. The PS4 peptide is used in the -047 construct, the 0 S 1 peptide in the -048 construct, and the OS2 peptide in the -049 construct. In these constructs an scFc scaffold is used and the peptides are interposed between the scFc linker and a linker which is attached to to the N -terminus of the second Fc moiety.
[00373] Synthesis of DNA molecules Genscript-FVII-047, -048 and -049 was outsourced (Genscript), A $\mathrm{Sa} 3 / \mathrm{RsrII}$ fragment from Genscript-FVII-047, -048 and 049 was subcloned into Sail/RsrII sites of pSYN-FVTI-043 to generate pSYN-FVH047, -048 and 049, respectively.

Example 5. Heterodimeric Constructs comprising a Gla-de\}eted FVII-Fc and a targeting molecule

Clonine of the FVII-028 intermediate
[00374] In order to make this construct, the FV11-028 construct was first made as an intermediate. Synthesis of DNA fragment Genscript-FVTT-028 was outsourced (Genscript). This fragment was cut with Hindmu/Xbal and subcloned into pSYN -FVn-01 1 to generate $\mathrm{pSYN}-\mathrm{FVH}-028$. Cloning ofFVH-053
[00375] The FVII-028 construct was used as the starting material to generate a construct that comprises a targeting moiety and employs a clotting factor that lacks a Gla domain. For this construct, amino acids 1-35 were removed from FV1I and an RKRRKR insertion was added after residue R152 (WT FVII numbering) to facilitate intracellular activation. The MB9 scFv served as the targeting moiety.
[00376] DNA molecule Genscript-FVH-025 was outsourced and an Xbal/BsiWI fragment from this molecule was subcloned into Xbal/BsiWI sites of pSYN-FVn-028 to generate $\mathrm{pSYN}-\mathrm{FVJJ}-053$

Example 6, Heterodimeric Constructs comprising a Factor Vli heavy and light chains as two separate polypeptides,

Cloning of pSYN-F VII-024 intermediate construct
[00377] The FVII-024 construct is one in which the heavy and light chains of factor FVII are not contiguous in a single chain molecule. The construct employs $\operatorname{cscFc}$ such that the cscFc linker is cleaved by proteases in the trans-Golgi network This cleavage results in linker removal as well as activation of FVII, resulting in the expression of activated FVIIaFc.
[00378] The coding sequence of FVII was obtained by reverse transcription coupled to polymerase chain reaction from a human liver mRNA library (Ambion, Austin, Texas) using the following primers:

```
FVII-Fl
```

GGGAATGTCAACAGGCAGGG
FVII-R1

CTTGGCTTTCTCICCACAGGC
[00379] A $50 \mu$ reaction was carried out with 10 pmol of each primer using the Superscript One-step RT-PCR with Platinum Taq system (Invitrogen, Carlsbad, Calif.) according to the manufacturer's standard protocol in a MJ thermocycler. The cycle used was $50^{\circ} \mathrm{C}$ for 30 minutes for the reverse transcription followed by
denaturing at $94^{\circ} \mathrm{C}$ for 2 minutes and 30 cycles of $\left(94^{\circ} \mathrm{C} 30\right.$ seconds, $53^{\circ} \mathrm{C} 30$ seconds, $72^{\circ} \mathrm{C} 90$ seconds) followed by 10 minutes at $72^{\circ} \mathrm{C}$ The expected sized band (-1400 bp) was gel-purified with a Gel Extraction kit (Qiagen, Valencia, Calif.) and cloned in pCR2.1 TOPO using the TOPO TA Cloning kit (Invitrogen, Carlsbad, Calif.) to produce the intermediate plasmid pSYN-FVII-001. To construct a plasmid for the expression of a two-chain FVח-Fc and Fc heterodimer, the FVI coding sequence was PCR-amplified using the following primers:

```
HindiII-Kozak-FVII--F
CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG
Bspel-Fc-FVII-R
CGACTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTCGGGAAATGGGGCTCGCAGG
```

[00380] The forward primer Hindlll-Kozak-FVII-F adds a Hind II restriction site followed by a Kozak sequence immediately upstream of the FVII coding region. The reverse primer Bspel-Fc-FVXX-K adds a fragment of the constant region of gGl (the Fc region) comprising amino acids 221-233 (EU numbering). This process also incorporates a BspEI restriction site at amino acids 231-233 using the degeneracy of the genetic code to preserve the correct amino acid sequence (EU numbering), A 50 u$\}$ reaction was earned out with 15 pmol of each primer and template pSYN-FVXX-001 using Platinum Pfx DNA Polymerase system according to manufacturer's protocol in a MJ Thermocycler using the following cycles: $95^{\circ} \mathrm{C} 2$ minutes; 30 cycles of $\left(95^{\circ} \mathrm{C}\right.$ 15 seconds, $49^{\circ} \mathrm{C} 30$ seconds, $68^{\circ} \mathrm{C} 90$ seconds): $68^{\circ} \mathrm{C} 10$ minutes, Plasmid pSYN-FIX-027 (pBUD FIXFe/Fc) was digested with Hindlll and BspEI and the expected sized band for the vector (approximately 5800 bp ) was purified away from the FIX insert (expected size band approximately 1480 bp) with a Gel Extraction kit (Qiagen, Valencia, Calif.). Next, the PCR-amplified FVII sequence was subcloned into Hindlll and EcoRI sites of the vector derived from pSYN-FIX-027 after removing the FIX insert. This generated pSYN-FVII-002 (pBUD FVIIFc/Fc). Next, A (GGGGS) 6x polypeptide linker was added between FVII and the Fc region coding sequences in pSYN-FVII-002 using the following primers:

```
FVII-linker-F:
CATCCCCAGCACGTACGTCC
FVII-Linker-R:
GGGCATGTGTGAGTTTTGTCTGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGAT
CCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCGGGAAATGGGGCTCGCAGGAGG
Fc-l inker-E:
```

GACAAAACTCACACATGCCCACC
Fc-linker--R:
GCAGAATTCTCATTTACCCGGAG
[00381] Two $12 \mu$ ï PGR reactions were carried out with either 12 pmol of FVTI-linker-F and FVIT-Linker-R (reaction 1) or Fc-linker-F and Fc-linker-R (reaction 2) using Expand High Fidelity System (Boehringer Mannheim. Indianapolis. Ind.) according to manufacturer's standard protocol in a MJ Thermocycler. The first and second reactions were carried out with i $u g$ of $\mathrm{pSYN}-\mathrm{FVII}-002$ as template using the following cycle: $94{ }^{\circ} \mathrm{C} .2$ minutes: 14 cycles of $\left(94^{\circ} \mathrm{C} .30\right.$ seconds, $55^{\circ} \mathrm{C} .30$ seconds, $72^{\circ} \mathrm{C} .2$ minutes); $72^{\circ} \mathrm{C} .10$ minutes. The expected sized bands ( 532 bp for reaction 1 and 670 bp for reaction 2) were gel purified with a Gel Extraction kit (Qiagen, Valencia, Calif.), then combined in a PCR reaction with 25 pmol of FVIT-linker-F and Fc-linker-R as before, but with 30 rounds of amplification. The expected sized band (1200 bp) was gel purified with a Gel Extraction kit (Qiagen, Valencia. Calif.) and digested with restriction enzymes KpnI and EcoRI. The expected sized band (920 bp) was gel purified as before and cloned into the Kpnl/EcoRi sites of pSYN-FVU-002 to generate pSYN-FVH-003 (pBUD FVIIFc/6x(GGGGS)/Fc).

Cloning of pSYN-FVH-024 to express a two-chain heterodimer
[00382] Plasmid (pSYN-FVII-024) was generated for the expression of a twochain heterodimer where one chain consists of the FVII light chain (residues 1-152) followed by a (GGGGS) $)_{6 \times}$ linker followed by the Fc region, while the other chain contains a FVII heavy chain (residues 153 to 406) followed by a (GGGGS $)_{6} \mathbf{x}$ linker followed by the Fc region. The plasmid is designed to express the heterodimer as a single polypeptide where the C-temiinus of the FVII heavy chain-linker-Fc chain is connected to the N -terminus of the heavy chain-linker-Fc chain by the following polypeptide sequence: RRRRS-(GGGGS) $6_{6_{x}}-$ RKRRKR, where the RRRRS and RKRRKR sequences are proprotein convertase cleavage sites. Intracellular cleavage by proprotein convertases following the last Arg at each cleavage site can result in removal of the polypeptide linker. Consequently, cells will express a 2 chain heterodimer where the FVII light chain-linker-Fc chain has a RRRRS sequence at the C-terminus, but the remainder of the linker and the RKRRKR sequence have otherwise been removed. Construction of the pSYN-FVH-024 and several intermediate plasrnids required the use of the following primers:

```
HindIII-Sail-BpEI--FC-F
```

AGTCAAGCTTGTCGACTCCGGAACTCCTGGGCGGACC
BamHI- linker (PACE1) -Fc-R
CATCGGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCCGCCACCGCTCC GGCGGCGCCGTTTACCCGGAGACAGGGAGAGG

HindiII-Kozak-FVII--F
CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG
BepEI-Fc-1 inker-FvJJLC-R
GAGTTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTCTGATCCCCCGCCACCGGAACCTCCACC GCCTGATCCACCCCCACCTGATCCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCTCGGCC TTGGGGTTTGCTGG

BamPI-2:1ink-pace - HC-E
CAGTCTGGATCCGGCGGTGGAGGTTCCGGTGGGGGTGGATCAAGGAAGAGGAGGAAGAGGATTGTGGGG GGCAAGGTGTGCC

Fc-EcoRI-R
ATGTCTGAA.TTCTCATTTACCCGGA.GACAGGGAGAGG
[00383] To generate the first intermediate plasmid, a PGR reaction was performed with 25 pmol of primers HindIII-SalI-BpEI-Fc-F and BamHI-linker(PACEl)-Fc-R and template pSYN-Fc-001 using Expand High Fidelity System (Boehringer Mannheim, Indianapolis, Ind.) according to manufacturer's standard protocol in a MJ Thermocycler. The following cycles were used: $95^{\circ} \mathrm{C} 2$ minutes; 30 cycles of $\left(95^{\circ} \mathrm{C} 30\right.$ seconds, $58^{\circ} \mathrm{C} 30$ seconds, and $72^{\circ} \mathrm{C} 1$ minute $) ; 72^{\circ} \mathrm{C}\{0$ minutes. The correct sized band (approximately 730 bp ) was gel purified as above and cloned into the HindII/B amHI sites of pBUDCE4 vector (Invitrogen, Carlsbad, Calif), generating pSYN-FVTi-014. PGR amplification with primers HindIII-SalI-BpEI-Fc-F and BaniHI-linker(PACE 1)-Fc-R generated a DNA fragment encoding a portion of the Fc region (Amino A X-Y) followed by an RRRRS sequence and (GGGGS) ${ }_{2 x}$ polypeptide linker. Primer HindIII-SalI-BpEI-Fc-F introduces a HindIII and Sall restriction site at the 5 ' end of the molecule, while primer BamHI-linker(PACEl)-Fe-R introduces a BamHI at the 3' end that overlaps the codons encoding the last 2 residues of the GGGGS linker (residues GS with codons GGA TCC) . Next, another PGR reaction was performed as above with primers Hindm-Kozak-FVII-F and BspEI-Fc-linker-FVULC-R and template pSYN-FVII-002 using the same conditions described for cloning of $\mathrm{pSYN}-\mathrm{FVII}-014$, but with an annealing temperature of $57^{\circ} \mathrm{C}$ The expected sized band (approximately 700 bp ) was gel purified and cloned into the HindII and BspEI sites of pSYN-FVII-014 to generate pSYN-FVII-023. Primers Hindlll-Kozak-FVII-F and BspEI-Fc-linker-FVIILC-R
amplified a DNA fragment encoding the FVII light chain followed by a (GGGGS) $\mathrm{f}_{3}$ : polypeptide linker and a portion of the Fc region up to amino acid 232 (EU numbering). Primer Hindlll-Kozak-FVII-F introduces a Hindi!! restriction site at the 5 ' end of the molecule followed by a Kozak sequence while primer BspEI-Fc-iinker-FVULC-R adds a BspeI site at the $3^{\prime}$ end of the molecule.

In the final step a PGR reaction was carried out as above with primers BamHI-2xlink-pace-HC-F and Fc-EcoRI-R and template pSYN-FVII-003 with the following cycles: $95^{\circ} \mathrm{C} 2$ minutes; 30 cycles of $\left(95^{\circ} \mathrm{C} 30\right.$ seconds, $55^{\circ} \mathrm{C} 30$ seconds, and $72^{\circ} \mathrm{C}$ 2 minute); $12^{\circ}$ C I minutes. This PCR reaction generated a DNA molecule encoding a (GGGGS) $3_{3_{4}}$ polypeptide linker followed by a RKRRKR sequence followed by the FVII heavy chain. Primers Bamffl-2xlink-pace-HC-F and Fc-EeoRI-R introduce a BamHI site and an EcoRI site at the 5' and 3'end of the molecule, respectively. The expected sized band (approximately 1600 bp ) was cloned into the BamHI and EcoRI sites ofpSYN-FVn-023 to generate pSYN-FVII-024.

## Cloning of intermediate BSY-FVIT-073

A silent mutation was introduced in the first Fc moiety of FVII-024 by PCR-based site-directed mutagenesis methods, resulting in the generation of a Sall site at DNA region encoding amino acids in position 412 and 413 (EU numbering). This generated the intermediate construct FVII-073

## Cloning of pSYN-FVII-057

The synthesis of the DNA sequence comprising nucleotides from the Sall to BsiWI sites of $\mathrm{pSYN}-\mathrm{FVII}-057$ was outsourced. This DNA was subcloned into the

Sall/BsiWI sites of pSYN-FVII-073 to generate pSYN-FVIl-057
 and pSYN-FVI最-062

These constructs were cloned as described for pSYN-FVH-057 (outsourced synthesis of DNA from Sail to BsiWI and subcloned into pSYN-FVII-073)
Cloning of pSYN-FVII-066
The synthesis of the DNA sequence comprising nucleotides from the Sall to RsrII sites of $\mathrm{pSYN}-\mathrm{FVH}$-Oóo was outsourced. This DNA was subcloned into the Sall/RsrII sites of $\mathrm{pSYN}-\mathrm{FVn}-043$ to generate $\mathrm{pSYN}-\mathrm{FVII}-066$
Cloning of pSYN-FVII-067

The synthesis of the DNA sequence comprising nucleotides from the Sall to EcoRI sites of pSYN-FV[I-067 was outsourced. This DNA was subcloned into the Sali/EcoRI sites of pSYN-FVn-041 to generate pSYN-FVH-067

Cloning of $\mathrm{pSYN}-\mathrm{FVU}-090$
The synthesis of the DNA sequence comprising nucleotides from the BamHI to BsiWI sites of $\mathrm{pSYN}-\mathrm{FVTi}-090$ was outsourced. This DNA was subcloned into pSYN-FVII-061 by 3-way ligation (where the outsourced DNA was cut with BamHI/BsiWI and pSYN-FVII-061 with BamHI/BsiWI/Notl) to generate pSYN-FVII-090

## Cloning of PSYN-FVII-100

A portion (amino acids 311 to 322 of the FVII mature sequence) of the 170 loop of FVII was replaced with the 170 loop of trypsin (amino acids EASYPGK). This mutation was introduced by standard overlapping PGR methods using the pSYN-FVTI-090 as template and backbone structure to generate pSYN-FVII-100 Cloiiiiig of pSYN-FVII-115

A triple point mutation (V158D, E296V and M298Q: mature FVII sequence numbering) was introduced into the FVII coding region of $\mathrm{pSYN}-\mathrm{FVII}-090$ by PGRbased site-directed mutagenesis to generate pSYN-FVTI-1 15

Cloning of pSYN-FVII-118
The synthesis of the DN A sequence comprising nucleotides from the Xbal to BsiWI sites of pSYN-FVII-1 18 was outsourced. This DNA was subcloned into the Xbal/BsiWl sites of pSYN-FVII-01 1 to generate $\mathrm{pSYN}-\mathrm{FVU}-118$

## Cloning of pSYN-FVII-119

The synthesis of the DNA sequence comprising nucleotides from the Xbal to BsiWI sites of pSYN-FVII-1 19 was outsourced. This DNA was subcloned into the Xbal/BsiWI sites of pSYN-FVH-01 1 to generate pSYN-FVII-1 19

## Cloning of pSYN-FVII-127

A DNA fragment comprising the 170 loop of trypsin was generated by PGR using pSYN-FVIUOO as template. This PGR reaction generated BsiWI and BspEI restriction sites at the $5^{\prime}$ and $3^{\prime}$, respectively. The DNA fragment was subcloned into the BsiWI/BspEI sites of pSYN-FVII-1 18 to generate pSYN-FVTI-1 27.

Cloning of pSYN-FIX-042

A Hindlll/BspEl fragment from pSYN-FIX-030 (as described in US Patent 7566565) was subcloned into the Find[I/B spEI sites of pSYN-FVII-0 11 to generate pSYN -FIX-042

Cloning of pSYN-FIX-068
A HmdIII/BspEI fragment from pSYN-FIX-030 (plasmid described in full in US7566565) was subcloned into the Fiindlll/BspEI sites of pSYN -FVII-066 to generate pSYN -FTX-068

## Cloning of pSYN-FIX-088

A BspEI-EcoRI fragment from pSYN-FIX-067 was subcioned into BspEI-EcoRI sites of pSYN-FIX-053 to generate pSYN-FfX-088

Cloning of pSYN-FIX-089
A BspEI-EcoRI fragment from pSYN-FIX-048 was subcloned into BspEI-EcoRI sites of pSYN -FIX-053 to generate pSYN -FIX-089

Cloning of pSYN-FIX-090
A DNA fragment comprising the FIX coding region from the Xbal site to the Cterminus of the protein followed by a $6 x$ (GGGGGS) linker, the SCE5 codingsequence and an EcoRI site was outsourced for synthesis and subcloned into the $\mathrm{Xbal} / E c o R I$ sites of pSYN-FIX-053 to generate pSYN-FIX-090. The SCE5 sequence is set forth below:

AQVQLQESGGaLVQPGGSLRLSCAASGFMFSRYAMSWVRQAPai^PEWV SGISGSGGSTYYADSVKGRF TVSRDNSKNTLYLQMNSLPAEDTAVY'YCA,RGATYTSRSDVPDOTSFDYWGQGTLVTVSSGSA,SAPKLEE GEFSEARVSELTQDPAVSVALGQTVRI TCQGDSIRNFYASWyQQKPGQAPTLVIYGSSKRPSGI PDRES ASSSGNTASLTITGAQAEDEADYYCLLYYGGGQQGVFGGGTKLTVLRQPKAAPSVTLFPPSSAA

## Cloning of pSYN-FVII-094

A DNA fragment comprising a sequence encoding a $6 x$ (GGGGS) linker followed by the SCE5 coding sequence was synthesized (outsourced) and cloned into the EcoRV/EcoRl sites of a pSYN-FVll-01 1 variant that had been previously modified to generate an EcoRV site at the C-terminus of the FVII coding region

Cloning of pSYN~FVJI~088
The synthesis of the DNA sequence comprising nucleotides from the Sail to Rsrll sites of $\mathrm{pSYN}-\mathrm{FVII}-088$ was outsourced. This DNA was subcloned into the Sal]/RsrI sites of pSYN-FVII-066 to generate pSYN-FVII-088

Cloning of pSYN-FVII-125
A DNA fragment was PGR amplified from pSYN-FVTi-088, comprising the AP3 region and part of the linker. This PCR reaction generated BamHI and EcoRI sites at
the $5^{\prime}$ and $3^{\prime}$ of the DNA fragment, respectively. This DNA fragment was subcloned into the BamHI/EcoRI sites of pSYN-FVII-0! 1 to generate pSYN-FVII-125

## Cloning of pSYN-FVin-041

The coding sequence of human recombinant B-domain deleted FVIII was obtained by reverse transcription-polymerase chain reaction (RT-PCR) from human liver poly A RNA (Clontech) using FVII-specifız primers. The FVIT sequence includes the native signal sequence for FVIII, The B-domain deletion starts after serine 743 (S743; 2287 bp ) and ends before glutamine 1638 (Q1638; 4969 bp ) for a total deletion of 2682 bp (SQ version).

The coding sequence for human recombinant Fc was obtained by RT-PCR from a human leukocyte cDNA library (Clontech) using Fc specific primers. Primers were designed such that the B-domain deleted FVIII sequence was fused directly to the N -terminus of the Fc sequence with no intervening linker. The FVIIIFc DNA sequence was cloned into the mammalian dual expression vector pBUDCE4. $\}$ (Invitrogen) under control of the CMV promoter.

A second identical Fc sequence including the mouse Igk signal sequence was obtained by RT-PCR and cloned downstream of the second promoter, FT' 1 a , in the expression vector pBUDCE 4 . 1. This final construct was designated $\mathrm{pSYN}-\mathrm{F}$ VII F 013.

A second plasmid was created from similar constructs using PGR and standard molecular biology techniques, in order to express rFVIIIBDD-Fc-Fc in which the rFVIIIBDDFc coding sequence was fused to the second Fc sequence with a (GGGGS) 4 linker, allowing for production of only the rFVIIIBDD-Fc monomerdimer hybrid in transient transfection. This construct was designated pSYN-FVIII041.

## Cloning of pSYN-FVIII-049

Generated intermediate pSYN-FVIII-048 by cloning NheI/Xhol fragment from pBUD-CE4.1 into pSYN-FVIII-013. The synthesis of a DNA fragment comprising the region from RsrII to Xbal sites of pSYN-F VIII-049 was outsourced. This fragment was subcloned into the $\mathrm{RsrII} / \mathrm{XbaI}$ sites of $\mathrm{pSYN}-\mathrm{FVIII}-048$ to generate pSYN-FVm-049

Cloning of $\mathrm{pSYN}-\mathrm{FVI} 1 \mathrm{I}-108$
A Sall/Rsrll fragment from pSYN-FVII-066 was subcloned into pSYN-FVIII-049 to generate pSYN-FVffl-108

Example 7. Additional Attempts at Expression of Activated Constructs [00384] Several other constructs were made with the goal of expressing activated FVII. However, these constructs did not successfully express activated molecules. By Western blot it was demonstrated that that the FVII heavy chain cannot be expressed with a free N terminus using a common method of fusing a heterologous signal peptide to the N -terminus of the heavy chain.

Cloning of pSYN-FVH-0 10
[00385] The FVII-G10 construct is one in which the heavy chain of factor VII was expressed in the context of an scFc scaffold and the light chain was expressed separately.
[00386] PCR-amplify with primer pairs FVII-HC-Hind3-IggKss-F/FVII-HC-BspEI-R, using pSYN-FVII-001 (see supra.\}. Clone in $\mathrm{BspEl} /$ Hindlll sites ofpSYN-FVII-008 (see supra), generating pSYN-FVII-009.
[00387] PGR amplify FVII light chain from pSYN-FVTI-003 (refer to P0830) with primers FVH-LC-Notl-F/ FVII-LC-XhoI-R and clone in pSYN-FVII-009 to generate pSYN-FVU-010

## Primers

FVIl-HC-BspEI-R
AGGAGTTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTCGGATCC CCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCCGCC ACCGGACCCACCTCCGCCGGAGCCACCGCCACCGGGAAATGGGGCTCGCA GGAGG

FVH-HC-Hind3-IggKss-f
ACTGACAAGCTTGCCGCCACCATGGAGACAGACACACTCCTGCTATGGGT ACTGCTGCTCTGGGTTCCAGGTTCCACTGGTATTGTGGGGGGCAAGGTGTG C

FVTI-LC-Notl-F
ACTGACGCGGCCGCGCCGCCACCATGGTCTCCCAGG

FVn-LC-XhoI-R
ACTGACCTCGAGTTATCGGCCTTGGGGTTTGCTGG
$\frac{\text { Cloning of pSYN-FVn-013 }}{[00388]}$ in the context of an scFc scaffold and the heavy chain was expressed separately.
[00389] PCR-amplify with primer pair FVTT-LC-linker-BamHI-R/KindI]. Kozak-FVU-F from pSYN-FVn-001 (refer to P0830) and clone in BamHI/Hindiil
sites of $\mathrm{pSYN}-\mathrm{FVll}-011$, generating pSYN-FVII-012. PCR-amplify FV11-HC from pSYN-FVn-009 using primer pair FVn-HC-Notl-F/FVII-HC-XhoI-R ad subclone in pSYN-FVXI-012 to generate $\mathrm{pSYN}-\mathrm{FVII}-013$

Primers
FVU-LC-6xlinker-BamHI-
RACTGACGGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCA CCTGATCCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCTCG GCCTTG GGGTTTG CTGGC
Hindlll-Kozak-FV![-F
CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG
FVH-HC-Notl-F
ACTGACGCGGCCGCGCCGCCACCATGGAGACAGAC
FVII-HC-Xhol-R
ACTGACCTCGAGTTAGGGAAATGGGGCTCGCAGGAG

Cloning of pSYN-FVII-0 18
[003901 For the FVI-018 construct, the heavy chain of FVII was epressed as an Fc fusion protein and the light chain of FVII was separately expressed as a separate Fc fusion protein.

100391] Primers FVII-HC-Hind3-IggKss-F/scFc-EcoRI-R were used to PCR amplify HCFVII-linker-Fc, using pSYN-FVII-OiO as template. Subclone in Hindll1/EcoRI sites of pBUDCE4. This makes pSYN-FVI!-017. Next, PCR-amplify from pSYN-FVH-013 with primers FVII-LC-Notl-F/FC-XHOI-R and subclone in Xhol/Notl sites of FVII-017. This makes PSYN-FVII-018

Primers
scFc-EcoRI-R
ACTGACGAATTCTCATTTACCCGGAGACAGGGAG

Fc-Xhol-R
AGCTCTCGAGTCATTTACCCGGAGACAGGG

Example 8, Attempts at Expression of Activatable Constructs

Cloning of FVII-039, -040
[00392] Several constructs were made in an attempt to generate constructs in which Factor VII can be activated in vivo at the site of clotting using an appropriate cleavage site, in this case the DFTR Factor Xla cleavage site.
[00393] The 039 construct was made in the context of an scFc scaffold. The construct included the FVII light chain, the FXIa cleavage site, and the FVII heavy chain with a 1153 V mutation in linear sequence attached to the N -terminus of the first Fc moiety.

The 040 construct was also made in the context of an scFc scaffold. The construct included the FVII light chain with an R152 deletion, the FXIa cleavage site, and the FVII heavy chain with an 1153 V mutation in linear sequence attached to the N temiinus of the first Fc moiety. The DFTR cleavage sequence is a natural FXIa sequence found in FIX. In FIX, the DFTR sequence is followed by a valine, so an I 152 V mutation was introduced in pSYN-FVTi-039, -040 to increase FXIa cleavage efficiency
[00394] Synthesis of DNA molecule Genseript-FVH-039 and -040 was outsourced (Genscript). An Xbal/BsiWI fragment from Genscript-FVII-039 and -040 was subcloned into $\mathrm{Xbal} / \mathrm{BsiWI}$ sites of $\mathrm{pSYN}-\mathrm{FVII}-011$ to generate pSYN -FVII-039 and -040 , respectively

## Example 9. Transient Transfection of Constructs

[00395] For expression of constructs, HEK-293-F cells were grown in suspension in Freestyle media (Invitrogen) supplemented with vitamin K3 (For FVII and FIX transfections only) (Sigma Aldrich, St. Louis, MO) to 2 , ig/liter (growth media) as suspension cells at $37^{\circ} \mathrm{C} / 10 \% \mathbf{C} \mathbf{0}_{2}$. Cells we subcultured every three to four days by seeding at cell density of $5 \times 10^{5}$ cells $/ \mathrm{ml}$.
[00396] Twenty-four hours prior to transfection cells were seeded at a density of $7 \mathrm{xl0}{ }^{5}$ cells $/ \mathrm{ml}$ in growth media supplemented with LONG ${ }^{\text {TM }}$ R3IGF- 1 (Sigma Aldrich, St. Louis, MO) to $20 \mu \mathrm{~g} / \mathrm{liter}$ (transfection media). On the day of transfection, a transfection solution was made with a volume equal to $5 \%$ of the total volume of the cell culture to be transfected. In the transfection solution DNA was added (final concentration $20 \mathrm{mg} / \mathrm{L}$ ) to a freshly made solution of PEI ( $60 \mathrm{mg} / \mathrm{L}$ ) in transfection media. The solution was swirled for 30 seconds and incubated for five minutes at room temperature before adding directly to the cell culture. Four hours later a volume equal to the cell culture volume of OptiCHO (Invitrogen) supplemented with vitamin K3, LONG ${ }^{\text {TM }}$ R3IGF-1 and $200 \mathrm{n}_{\mathrm{Z}} \mathrm{M}$ L-glutamine was added to the cells. The cell culture was allowed to grow as shown above and daily media samples were taken to assess protein expression. On the day of harvest, the cells were spun down and the media filtered in preparation for protein purification or protein analysis by protein A pulldown/western blot.

Example 16. Protein Purification of FVIIFc Molecules (except FVII-028 and FVII-053) and FIXFc molecules
[00397] FVIIFc molecules were purified from conditioned media using the following columns: 1) Anion exchange chromatography with pseudo-affinity elution (e.g. Q sepharose 4FF (GE Healthcare) followed by elution with varying levels of $\mathrm{CaCl}_{2}$ to selectively elute the most active species), followed by 2 ) shFcRn (soluble human FoRn) affinity (NHS- coupled shFcRn with sepharose 4FF beads) chromatography, binding Fc-containing proteins at low pH (e.g. pH 6.2) and eluting at neutral pH (e.g. pH 8.0). In some cases, an additional step was included utilizing cation exchange chromatography with NaCl elution. These purification steps utilized standard methods known to those in the art to generate purified proteins of $>95 \%$ purity by SEC analysis and SDS-PAGE. FIXFc proteins were purified as previously described in US Patent 7,566,565.

Example 11. Protein Purification of FV I-028 and FVli-053
[00398] FVII-028 and -053 were purified from conditioned media using the following columns: 1) Hydrophobic interaction chromatography (e.g. Phenyl FF (high sub) (GE Healthcare)), followed by 2) Anion/cation exchange chromatography with salt elution. These purification steps utilized standard methods known to those in the art to generate purified proteins of $>95 \%$ purity by SEC analysis and SDS-PAGE.

Example 12. Purification of FIX-090
[00399] FIX-090 was purified through a 2-step chromatography process, first using an immunoaffmit $\}^{\prime}$ chromatography step with an anti-GLA domain antibody, followed by anion exchange chromatography using pseudoaffinity elution similar to FIXFc proteins described above. These purification steps utilized standard methods known to those in the art to generate purified proteins of $>95 \%$ purity by SEC analysis and SDS-PAGE.

Example 13: Purification of FVIIIFc proteins
[00400] FVIIIFc proteins were purified from clarified and chemically defined harvest media using a two or three column purification process, including a FVIIspecific affinity purification step (McCue 2009) followed by a combination of anion exchange with standard NaCl elution and/or shFcRn (soluble human FcRn ) affinity (NHS- coupled shFcRn with sepharose 4FF beads) chromatography, binding Fccontaining proteins at low pH (e.g. pH 6.2 ) and eluting at neutral pH (e.g. pH 8.0 ).

These purification steps utilized standard methods known to those in the art to generate purified proteins of $>95 \%$ purity by SEC analysis and SDS-PAGE.
 pooled,, and total protein was concentrated to $4 \mathrm{mg} / \mathrm{ml}$. The $\mathrm{CaCl}_{2}$ concentration was raised to 5 mM and the sample was incubated at $4^{\circ} \mathrm{C}$ for 24 to 48 hours until at least $80 \%$ of FVIIFc was activated. The extent of activation was assessed by SDS PAGE (Figure 10\}

Example 15. FVIIa activity assays, soluble tissue factor method [00402] Specific activity of the FVIIaFc variants was determined by the soluble tissue factor method. Unlike lipidated full length tissue factor, soluble tissue factor (extracellular portion of tissue factor) can't activate FVII into FVIIa, but it acts as an activator of the conversion of factor X into factor Xa by FVIIa. To determine the specific activity of FVIIaFc variants, A STACLOT^ FVII-rTF kit (Diagnostica Stago, Asnieres, France) was used following manufacturer's recommendations. Table 1 summarizes the data and shows comparable specific activity for all variants.

Table 1. Specific activity of FVIIaFc variants based on the soluble tissue factor method

| FVhaFe | ¢3/n解 |
| :---: | :---: |
| FVilols | 991 |
| FVIL-024 | 929 |
| FVIl-027 | 790 |
| FV1.037 | 1131 |
| FVIT-044 | 1300 |
| FV1-045 | 906 |
| FVIM046 | 1145 |
| FVIl-047 | 924 |
| FVIH048 | 973 |
| FVII-049 | 1130 |
| FV1-053 | 929 |

[00403] In this example, the following reagents and methods were used: Reagents

ADP: Sigma Aldrich, cat\# A2754, stock 1 mM , working concentration 10 uM
SFLLRN peptide: in-house synthesis, stock concentration $5 \mathrm{mg} / \mathrm{ml}(6.7 \mathrm{mM})$, working coiicentration $50 \mathrm{ug} / \mathrm{ml}(67 \mathrm{uM})$

FVU antibody-FITC- labeled: Affinity Biologicals SAFVII-APFTC
Platelet buffer: 15 mM HEPES, $138 \mathrm{mM} \mathrm{NaCl}, 2.7 \mathrm{mM} \mathrm{KC1}, 1 \mathrm{mM} \mathrm{MgC12}, 5 \mathrm{mM}$
CaC12, 5.5 mM dextrose and $\mathrm{mg} / \mathrm{ml}$ BSA, pH 7.4
Method
${ }^{\circledR}$ Count platelets

- Add 20 ul of $-2-4 \times 10^{8}$ cells $/ \mathrm{ml}$ gel-purified platelets to 1 ml of platelet buffer
* Make 100 ul aliquot for each sample
- Add agonist and FVIiaFc (to desired concentration) as needed Incubate at 37C for 15 minutes
* Add equal volume of $\mathrm{HBS} / 5 \mathrm{mM} \mathrm{CaCl} 2 / \mathrm{I} .5 \%$ formaldehyde, incubate 20 at RT
* Spin $15^{\prime}$ at 3000 g
- Wash in HBS/ $5 \mathrm{mMCaC} 12 / \mathrm{mg} / \mathrm{ml} \mathrm{BSA}$, spin again and resuspend in 100 ul of platelet buffer.

Add 2.5 ul of FVII antibody-FITC-labeled and incubate for $30^{\prime}$ at room temperature.

* Analyze by FACs

Example 17. Thrombin Generation assay
[00404] In this example, the following reagents and methods were used:

Reagents
FV: F!?, cat\#HCV-0!00, lot\#Z0413, $5.1 \mathrm{mg} / \mathrm{ml}$
Prothrombin: HTI, cat\#HCP-0010, lot\# Z0128, $4.8 \mathrm{mg} / \mathrm{ml}$
FX: HTI, cat\# HCX-0050, iot\#X0401, $5.4 \mathrm{mg} / \mathrm{ml}$
ATHi: HTI, cat\# HCATII- 0120 , lot\#Y040 $1,8.2 \mathrm{mg} / \mathrm{ml}$

TFPI: American Diagnostica, cat\# 4900PC, lot\# $081031,100 \mathrm{ug} / \mathrm{ml}$
Reader: Fluorskan, Thermo Electron Fluorometer
Thrombin Calibrator: Thrombinoscope, cat\# TS20.00
Fluca: Thrombinoscope, cat\# TS50.00
Platelet buffer: 15 mM Hepes pH 7.4, $138 \mathrm{mM} \mathrm{NaCl}, 2.7 \mathrm{mM} \mathrm{KC} 1,1 \mathrm{mM} \mathrm{MgC} 32,5.5$
niM Dextrose, supplemented with $1 \mathrm{mg} / \mathrm{ml}$ BSA before using
ADP: Sigma Aldrieh, cał\# A2754, stock 1 mM , working concentration 10 uM
SFLLR $\mathbb{N}$ peptide: in-house synthesis, stock concentration $5 \mathrm{mg} / \mathrm{ml}(6.7 \mathrm{mM})$, working concentration $50 \mathrm{ug} / \mathrm{ml}(67 \mathrm{uM})$

|  | Primary slock $(\mathrm{mg} / \mathrm{ml})$ | Working <br> solution <br> $(\mathrm{ug} / \mathrm{ml})$ | $[\mathrm{final]}$ <br> $\mathrm{ug} / \mathrm{ml}$ |
| :--- | :--- | :--- | :--- |
| FV | 5.1 | 105.6 | 4.4 |
| FII | 4.8 | 1200 | 54 |
| FX | 5.4 | 120 | 5 |
| ATIII | 8.2 | 1800 | 75 |
| TFPI | 0.1 | 1.44 | 0.06 |
| Platelet | $2-10 \mathrm{E} 8$ | 1.74 E 8 | 0.6 E 8 |
| FVITaFc | $1 \mathrm{mg} / \mathrm{ml}(10 \mathrm{uM})$ | 1200 nM | 200 nM |
| FVIIaFc | $1 \mathrm{mg} / \mathrm{ml}(10 \mathrm{uM})$ | 200 nM | 62.5 nM |
| FVilaFc | $1 \mathrm{mg} / \mathrm{ml}(10 \mathrm{uM})$ | 62.5 nM | 12.5 nM |

$\frac{\text { Method }}{[00405]}$-Set up software according to manufacturer's recommendations
[00406] -Prewarm water and Fluca buffer
[00407] -Make clotting factor mix. Dilute stock concentration of FV, FIT, FX,
ATIII and TFPI to make working solution. Need $5 \mathrm{ul} / \mathrm{well}$, so for a 30 well assay prepare 180 ul of each Mix all the clotting factor solutions in a single bulk solution
[00408] -Premake FVITaFc dilutions. Make 1200 nM solution ( 12 ul into 88 ul of buffer) in 100 ul and dilute 4-fold twice ( 25 ul into 75 ul of buffer) to obtain 200 nM and 62.5 nM solutions
[00409] -Make calibrator solution ( 1 ml of warm water in calibrator vial
[00410] -Add 20 ul of buffer or calibrator to the wells
[00411] -Add 25 ul of clotting factor mix to the wells (or 25 ul of buffer to calibrator wells)
[00412] -Add 20 ul of FVIIaFc to the wells (or buffer to calibrator wells)
[00413] -Add 35 ul of platelets (previously add ADP and SFLLRN). Add platelets to calibrator well
[00414] -Put plate in instrument, prepare Fluca buffer and start reaction (add 20 ul Fluca/wefl) 5 minutes after putting plate into instrument.

Example 18. Analysis of protein generated from transient transfections
[00415] For analysis of protein from transient transfections, conditioned media from transfections of pSYN-FVTY-010, 011, -013, -018, -003, -019-020 and -024 were subjected to protein A immunoprecipitation. Briefly, cell culture supernatant was mixed with approximately 50 ul of protein A-Sepharose $50 \%$ slurry and incubated at $4^{\circ} \mathrm{C}$ with rocking for 1 hour, then centrifuged to pellet the protein A beads. Beads were washed twice by resuspending in 1 ml of PBS, spinning and aspirating. The beads were resuspended with sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing or nonreducing conditions, heated for 5 minutes at $10 Q^{\circ} \mathrm{C}$, spun down and loaded on SDS-PAGE gels and run according to standard protocols. Gels were transferred to nitrocellulose membranes and Western blots were performed to detect the Fc region or the FVII light chain. For Fc detection, the antibody used was a goat anti-human $\operatorname{IgG}$ (Fc specific)-horseradish peroxidase conjugate (Pierce ImmunoPure antibody, catalog \#31413). For FVII light chain detection an anti light chain monoclonal antibody was used (Green Mountain, clone 6MA-219). The antibodies were diluted 1:15,000 (for Fc detection) or 1:200 (for light chain detection) in PBST (PBS with $0.1 \%$ Tween-20) with $5 \%$ nonfat dry milk and incubated with the membrane for I hour at room temperature. The membrane was then washed in PBST 3 times for 10 minutes and signal was detected by a chemiluminescent method for Fc detection. For FVII light chain detection, the membrane was further incubated for one hour in a solution containing HRP-labeled goat anti-mouse antibody (Southern Biotech, \# 1010-05) diluted 1:5000 in PBST. The membrane was also washed in PBST 3 times for 10 minutes and the signal was detected by a chemiluminescent method. Chemiluminescent detection was performed
using ECL Plus Western Blotting Detection System (Amersham Biosciences catalog \#RPN2132) according to manufacturer's protocol Signal was visualized in a Storm 840 Phosphorimager (Molecular Devices).
[00416] The effect of PCS on the processing of the proprotein convertase cleavage sites in the FVii-024 linker was tested as shown in Figure 26. Under nonreducing conditions the effect of PCS on cleavage site processing can not be detected because the FVII light ehain-Fe and FVI heavy chain-Fc subunits remain linked via 2 disulfide bonds in the Fc region (lanes 2 and 3). Under reducing conditions we observed partial processing of FVII-024 generated from cells not cotransfected with PC5 (lane 4), but full processing when the cells were cotransfected with PCS (lane 5). Full processing of the linker results in secretion of activated FVII (FVIIa), since a free N -terminus of the heavy chain is required and sufficient to activate the protein.

Example 19. Cleavage of FVi-039 and FVII-040 by FXIa
[00417] The activation FVII-039 and FVII-040 by FXIa, as a result of the FXIa cleavage site inserted immediately upstream of the FVII light chain in these proteins, was characterized in vitro. A $1.5 \mu \mathrm{M}$ solution of FV!\}-039, FVII-040 or FVII-011 (non activated) containing 15 nM FXIa in 50 mM Tris-HC3, $100 \mathrm{mM} \mathrm{NaCl}, 10 \mathrm{mM}$ $\mathrm{CaCl}_{2}, \mathrm{pH} 7.4$ was incubated for 5 or 20 minutes, and cleavage of FVIIFc was determined by SDS PAGE under reducing conditions, followed by SYPRO Ruby staining (Invitrogen). FXIa failed to cleave FVII-039, FVTI-040 and nonactivated FVII-01 1, as shown in Figure 28.

## Example 20, Alternative Activatable FVIIFc constructs

Failure of FXIa to cleave FVII-039 and FVII-040 may have been caused by inaccessibility of the protease to the cleavage site by steric hindrance. To improve FXIa or thrombin cleavage site accessibility, the sites will be placed upstream of the heavy chain in a structure where the heavy chain is not preceded by the light chain (Light chain-1 mker-Fc-scFcfinker-FXIa/thrombin cleavage site-heavy chain-lmkerFc ). In some embodiment, the heavy chain will comprise the I 152 V mutation. Once the best cleavage site is determined, a $\operatorname{cscFc}$ will be introduced so that the cell secretes
a heterodimeric protein with the following structure: light chain-linker- Fc which is disulfide bonded to a second chain: FXIa/thrombin cleavage site-heavy chain-linker Fc.

In order to improve the level of activation observed with the constructs shown in Figure 28, a second generation of activatable variants illustrated in Figure 31 (similar in structure to those shown in Figure 6E) was used to increase accessibility of the cleavage site. In this example, FXIa and thrombin cleavage sites were used for these constracts (See Figure 31). Constracts were transiently transfected as previously described. FVIIFc was captured from media with protein A. FVIIFc bound to the beads was put in buffer and FXIa or thrombin was added and incubated. FVIIFc was eluted from beads with SDS PAGE loading buffer at 100 C for 5 minutes. The gel was loaded and western blot performed to detect Fc as previously described and the results are shown in Figure 32. As shown in Figure 32, both the thrombin and factor X I a cleavage sites could be cleaved to yield FVII heavy and light chain molecules in the presence of the appropriate enzyme. Best cleavage was observed for constructs FVII-060 and FVII-061, while no cleavage was observed for the negative control (FVII-062) in the presence of thrombin.

Example 21. A Factor VII Activatable Construct
[00419] The constructs depicted in Figure 33 were (FVII-090, FVII-089 and FVII-062) were cloned, expressed and purificatied as previously described (these proteins do not require activation). Due to a cloning error a "VVGGA" sequence was inserted after the ALRPR thrombin cleavage sequence of FVII-060 and FVII-061, but while this insertion would be expected to affect the activity, it would not be predicted to affect the assessment of cleavage by thrombin in SDS-PAGE based assays. This sequence was removed in FVII-089 and FVII-090. To 125 nM of FVII-090, FVTI089, FVII-062, or plasma-derived FVII (FVII) increasing concentrations of thrombin were added and incubated for 10 minutes at $37^{\circ} \mathrm{C}$. The mixture was run on SDSPAGE gel to determine cleavage by thrombin (Figure 33). Generation of FVII light chain-Fc and FVII heavy chain-Fc was observed for FVII-089 and FVII-090 after incubation with thrombin. The fact that there was no cleavage of plasma-derived FVII or the FVII-062 negative control by thrombin shows specificity. No significant difference in cleavage efficiency was observed for FVII-089 and FVII-090.
[00420] Thrombin generation assays were used to measure activity of activatable variant FVII-090. A thrombin generation assays in FVIII-deficient platelet-rich plasma was performed as previously described, but replacing clotting factors and platelets with FVII[-deficient platelet-rich plasma. The results depicted in Figure 34 are from an assay in which thrombin generation was activated with 50 nM of FVIIaFc. As shown in Figure 34, thrombin is generated by 50 nM of FVIIaFc. The addition of 200 nM FVII-090 (not FVIX-062, the negative control) to 50 nM of FVIIaFc results in a significant increase in thrombin generation, suggesting that FVI-090 becomes activated by thrombin generated by FVIIaFc. FVII-090 in the absence of any FVIIaFc activation also shows increased thrombin generation relative to FVII-062 in the absence of activation. This could be caused by activation of FVII-090 from small amounts of thrombin generated by residual levels of tissue factor or contact pathway activation.

Example 22. A High Specific Activity Factor VII Activatable Construct
[00421] To make the high specific activity version of Factor VII, FVII-100, amino acids 311 to 322 of the FVII mature sequence (LQQSRKVGDSPM, corresponding to the 170- loop) from FVII-090, were replaced with amino acids EASYPGK from the 170-loop of trypsin. This substitution has been shown to confer high specific activity.
[00422] An additional high specific activity version of Factor VII, FVII- 1 15, was constructed. In this version, the 170 loop is wild type, but there are three point mutations in the heavy chain of FVII, V158D, E296V and M298Q. FVII- 100 and FVII-1 15 are illustrated in Figure 41
[00423] In a soluble tissue factor assay, the specific activity of FVII-011(wild type FVIIaFc) is $10,000 \mathrm{IU} / \mathrm{mg}$. FVII-090 has a specific activity of $0.32 \mathrm{lU} / \mathrm{rag}$, FVII100 has a specific activity of $0.25 \mathrm{IU} / \mathrm{mg}$, and FVII- 115 has a specific activity of 14 $\mathrm{IU} / \mathrm{mg}$. Thus, each of the activatable forms (prior to activation by the appropriate enzyme) is essentially inactive in this assay.
[00424] In the context of activated FVII, such high specific activity variants have the potential to be more efficacious, but also to be more susceptible to inhibition by proteins such as antithrombin. This inhibition depends on FVila being active; therefore, high specific activity variants which are activatable (dosed as nonactive
proteins) should be more resistant to antithrombin inhibition while having the potential to have high specific activity once activated at the site of injury.
[00425] The cleavage of purified FVII-090 and FVII- 100 and 115 (high specific activity variants) by thrombin was tested as previously described. The results are shown in Figure 35. SDS PAGE analysis showed how the 3 proteins were cleaved by thrombin with comparable efficiency. For FVII-100, FVII heavy ehain-Fe and light chain Fc collapse in 1 band because a glycosylation site is removed from the heavy chain after insertion of the trypsin 170 loop, reducing the mass of the FVII HCFc band which therefore migrates faster on the gel and comigrates with the FVII LCFc band.. Thrombin generation assays were used to measure activity of acdvatable variant FVII-090 and high specific activity variant FVII-100. As set forth previously herein, thrombin generation was tested in a reconstituted system with human platelets, Factor X, Factor V, prothrombin, antithrombin and tissue-factor pathway inhibitor. Activity was measured with or without 5 in M thrombin activation.
[00426] As shown in Figure 36, activity of FVII-090 is enhanced in the presence of thrombin, suggesting activation of FVII-090 by thrombin. However, activity is significantly increased in the context of the high specific activity variant FVII-100 with thrombin activation. High activity with longer initiation time for FVII100 in the absence of thrombin, suggests that residual levels of tissue factor, thrombin or contact pathway activation can generate enough thrombin to activate FVII-100 without exogenous addition of thrombin. Figure 37 shows that similar results were obtained for the other high specific activity variant, FVII-115.

Example 23. Confirmation of activity of activatable variants using various assays
[010427] In this example, chromogenic assays were used to measure FVII activity. One of the assays used measures the amidolytic activity of FVIIaFc by measuring the cleavage of a chromogenic substrate by FVIa. Another of these measures the FX activation activity by measuring the ability of FVIIa to activate FX, as determined by measuring levels of a chromogenic substrate that is cleaved by activated FX (FXa).
[00428] In amidolytic assays, the chromogenic substrate Chromozyme t-PA was used. FVIIa cleaves this substrate in a dose dependent manner. The substrate is
also cleaved by thrombin, but the cleavage by thrombin can be inhibited by hirudin (data not shown).
[00429] As shown in Figure 38, amidolytic activity of the activatable variants can be measured following thrombin activation and there is increased amidolytic activity for the high specific activity variants as compared to FVH-090. In these assays, after activation of the activatable molecule by thrombin, hirudin is added to inhibit thrombin cleavage of the chromogenic substrate. In this manner, the thrombin does not interfere with the ability to detect FV II a activity.
[00430] The activation of FX by FVIIa is also enhanced in the high specific activity FVII activatable variants. To measure activation of FX by FVIIa, substrate S2765 was used. This chromogenic substrate is also recognized by FX. In the assay, 10 uM of FX was incubated with FVIIaFc for 15 minutes and the reaction was quenched with EDTA. Figure 39 shows the results of the control experiment which demonstrates that FX activation by FVIIaFc can be detected.
[00431] The experiment shown in Figure 40 shows that there is an increase in FX activation activity for the high specific activity activatable variant FVII- 100. In this experiment, FVIIFc ( 100 nM ) was activated with thrombin ( 100 nM ) for 20 minutes at 37C. Hirudin was added to inhibit the thrombin. FX (1OuM) was added and incubated for 15 minutes at 37 C , followed by EDTA to inhibit the reaction. S2765 substrate was added and FXa generation was detected by monitoring substrate cleavage

Example 24. Monomeric Fc molecules ears also be synthesized in activatable form
[00432] Three monomeric constructs were made as shown in Figure 41. In FVII- 118, an ALRPR cleavage site was in serted between the light chain and heavy chain. In FVII-1 19, the sequence GGGGS-ALRPR was inserted between the light chain and heavy chain. For FVII-127, the construct was made like FVII-1 18, but with the same high specific activity mutation used in FVII- 100. The specific activity of the non-activated purified forms of these constructs was tested in a soluble tissue factor assay and compared to FVII-01 ! (wild type FVIIaFc which had an activity of 10,000 RJ/mg). FVII-1 18 had an activity of $4.5 \mathrm{lU} / \mathrm{mg}$ and FVII- 127 had an activity of 1.8 $\mathrm{U} / \mathrm{mg}$, demonstrating that these molecules had essentially no activity in their activatable form.
[004331 Thrombin cleavage reactions of FVII- 118, FVII- 119 and FVII--09G
followed by SDS PAGE analysis were performed as previously described. As shown in Figure 42, the cleavage site in an activatable construct can be cleaved in the context of both the monomer and heterodimer Fc molecules. In the figure, the decrease in the intensity of the nonactivated FVIIFc band with increasing thrombin concentration is similar for the FVII-118, FVII-119, and FVII-090 constructs.
[00434] Another activatable monomelic construet,FVTI-127, was made and tested. FVII- 127 has the backbone of FVII- 118, but the same 170 loop substitution used in FVII- 100 to confer high specific activity. As shown in Figure 43, the activity of FVII- 127 is significantly increased as compared to FV[I-1] 8 lacking the high specific activity amino acid substitution. High activity with longer initiation time for F VII- 127 in the absence of thrombin, suggests that residual levels of tissue factor, thrombin or contact pathway activation can generate enough thrombin to activate FVII- 127 without exogenous addition of thrombin. FVII- 127 activity is accelerated by thrombin.

Example 25. FVIIaFc variants targeted to the active form of GPIbyla In this example the constructs illustrated in Figure 44A were cloned, transiently expressed, purified and activated as previously described. FVH-066 was cotrasfected with PCS to fully process the cscFc linker, described in the protein sequence, connecting the first Fc moiety to the platelet targeting moiety. These constructs employed the targeting moiety SCE5. a scFv against the active conformation of GPIIbllla. SCE5 has been shown to crossreact with mouse and human receptor. The SCE5 targeting moiety was placed at the N-terminus (FVII-066) or C terminus (FVII067) of the second Fc moiety of FVIIaFc, In addition, the SCE5 was placed at the Cterminus of FVIIa (FVII-094). FVIIaFc (FVII-011) and Novoseven were used as controls. As shown in Figure 44B, these proteins were tested by thrombin generation assays in FVIII-deficient human plasma as previously described. These experiments revealed increased rates of thrombin generation for all the proteins containing the SCE5 targeting moiety relative to the controls. The highest rates of thrombin generation were observed for FVII-066, followed by FVII-094 and FVII-067, suggesting that the placement of the SCE5 targeting moiety can have a significant effect on the activity of the protein. Binding of these proteins to activated human platelets was determined by FACS assays as previously described (Figure 44C). All
the FVIIa proteins containing the SCE5 targeting moiety showed increased binding to platelets relative to the FVIIaFc control. This shows that attaching the SCE5 targeting moiety to FVIIa can increase its affininity from platelets. Since the SCE5 targeting moiety has been shown to interact with the mouse GPIIbllia receptor, FVII-G66 was tested in thrombin generation assays using mouse FVHI-deficient platelet rich pasma, as well as in a reconstituted system using human purified components and platelets, as previously described (Figure 29). We observed increased rates of thrombin generation for FVH-Óo relative to the controls in both systems.

Example 26. Additional FVIIaFc variants targeted to the active form of GPIibilla

In this example, construct FVII-027 illustrated in Figure $12 A$ was cloned, expressed (with PCS cotransfection to fully process the cscFc linker, described in the protein sequence, connecting the first Fc moiety to the platelet targeting moiety), purified and activated as previously described. This construct employs the targeting moiety MB9, a scFv that has been shown to bind to the active conformation of GPIIblla. FACS assays were performed as previously described to assess binding to activated platelets, and FV11-027 was shown to bind to activated platelets with higher affinity than the FVII-01 1 control (Figure 12B). Thrombin generation assays were performed with reconstituted purified human proteins and platelets as previously decribed (Figures 13A, 13B and 13C). FVII-027 showed increased rates of thrombin generation relative to the controls. Figure 13D illustrates that FVII-027 has four times more activity than FVII-01 1 or Novoseven, based on thrombin generation assays. Figure 14 illustrates that the enhanced platelet binding and thrombin generation activity of FVII-027 were abrogated by PAC1, an antibody that competes with MB9 for binding to the activated form of GPIIblla, demonstrating the effects are mediated by the interaction of MB9 with the activated form of GPIIbllla. The MB9 targeting moiety was also placed at the C -terminus of the second Fc moiety of FVIIaFc to generate FVTI-037 illustrated in Figure 15. Thombin generation assays in a FVIIdeficient reconstituted system with platelets revelaed increased rates of thrombin generation for FVII-037 relative to the Novoseven control (Figure 16)

Example 27. Factor VII constructs targeted to both activated and nonactivated platelets
[00435] In this example, the constructs illustrated in Figure 45A were cloned, expressed, purified and activated as previously described. FVII-088 was cotransfected with PC5 to fully process the cscFc linker, described in the protein sequence, connecting the first Fc moiety to the platelet targeting moiety. FVII-088 and FVII-125 employed the AP3 targeting moiety, a scFv that binds to both active and nonactive conformations of human GPIIbllla. The results in Figure 45B show thrombin generation assays in FVIII-deficient platelet-rich plasma, and both FVII-088 and FVII-125 showed increased rates of thrombin generation relative to the controls, demonstrating that targeting FVIlaFc or FVIIa to the active and nonactive conformation of GPIbJIIa results in increased activity. Binding of FVII-088 and FVIlaFc to activated human platelets was tested by FACS (Figure 45C). These data reveal that FVII-088 binds to platelets with higher affinity than FVIlaFc (FVII-0! 1), showing thai the AP3 targeting moiety can increase the affinity of FVIlaFc for platelets.

Rotation Thromboelastometry (ROTEM®. Pentapharm GmbH, Munich, Germany) is another method to evaluate platelet-targeted FVIIa constructs, since it allows for the characterization of several coagulation parameters in whole blood (in the presence of platelets). The ability of FVII-088 and wild type recombinant FVIlaFc ( rFVIIaFc ) to form firm and stable clots was evaluated by ROTEM with Calcium Chloride as activator (NATEM) following manufacturer's recommendations. Hemophilia A blood from a human donor was spiked with FVIIFc to a final concentration of 100,30 or 10 n . The NATEM reaction was initiated by the addition of $\mathrm{CaC}^{3} 4$. Coagulation parameters, including Clotting Time (relates to coagulation initiation time), Clot Formation Time (relates to rates of coagulation) and Alpha Angle (relates to rates of coagulation) were assessed as shown in Figure 30, FVII-088 showed a significant reduction in the Clotting Time and Clot Forming Time and an increase in the alpha angle relative to wild type rFVIIaFc, consistent with enhanced coagulation kinetics for FVII-088. These data demonstrate that FVII-088 has enhanced activity relative to wild type FVIlaFc in agreement with the thrombin generation assay data

Example 28. Use of peptides for targeting FVIIa to platelets
[00436] The constructs illustrated in Figure 46A were cloned, expressed, purified and activated as previously described. These proteins were made using
peptides that bind to platelet receptor GPIb-alpha (found in both activated and nonactivated platelets), specifically PS4, OSi, and OS2 as platelet targeting moieties. In making these molecules the peptide was attached to either the N or the C terminus of the second Fc moiety of the construct. The FV11-044 construct employed the PS4 peptide attached to the C terminus of the second Fc moiety of the construct; FVII-045 employed the OS1 peptide attached to the C terminus of the second Fc moiety of the construct; and the FVii-046 construct employed the OS2 molecule attached to the C terminus of the second Fc moiety of the constract. In contrast, the FVH-047 constract employed the PS4 peptide attached to the N terminus of the second Fc moiety of the construct; the FVII-048 molecule employed the OSl peptide attached to the N terminus of the second Fc moiety of the construct; and the FVII- 049 molecule employed the OS2 peptide attached to the N -terminus of the second Fc moiety of the construct. Thrombin generation assays were performed using FVIII-deficient platelet rich plasma as previously described. As shown in Figure 46B, when the assay was performed with limiting concentrations of FVHa, each of the FVII-044, FVII-045, and FVII-046 C-terminal fusion constructs exhibited enhanced thrombin generation as compared to the Novoseven control. A similar result is shown in Figure 47A for the N-terminai fusion constructs. Figure 47B shows that the FVII-045 constract may be marginally better than the FVII-048 construct in this assay, but that again both the N and C terminal fusions are better than the Novoseven control.In addition, there is a correlation between the published GPIb-alpha affinity for each peptide (Figure 48) and the increase in the activity associated with that peptide when recombinantly fused to FVIIaFc. Figure 48 shows the binding of FVII-049, FVII-048 and wild type FVIIaFc (FVII-01 i control) to activated platelets as determined by FACS as well as the affinity for the targeting peptides reported in Bernard et al. Biochemistry 2008. 47:4674-4682. FACS data revealed increased affinity of FVII-045 and FVII-048 for platelets relative to the FVII-01 i control

Example 29. An FVIIIFc variant targeted to the active form of GPIibi ma [00437] The constructs illustrated in Figure 49A were made as previously described. FVIII-041 is wild type FVIIIFc, while FVIII-108 has a SCE5 platelet targeting moiety at the N-terminus of the second Fc moiety. For expression, FVIII108 was cotransfected with PCS to fully process the cscFc linker, described in the protein sequence, connecting the first Fc moiety to the platelet targeting moiety.

These proteins were tested in thrombin generation assays using FVJll deficient platelet rich plasma as previously described, but in addition the thrombin generation assay was also activated with tissue factor As shown in Figure 49B, no significant improvement was seen using the targeted version of FVIIIFc. It is noteworthy that the thrombin generation assays described herein measure thrombin generation on the surface of platelets and, therefore, are an accurate measure of activity.

Example 30. Making and testing $a^{a}$ version of FVil targeted to platelets that lacks a Gla domain
[00438] In this example a version of FVIIaFc illustrated in Figure 2] was generated. This protein has the MB9 scFv at the N -terminus and a deletion that removes the Gla domain. FVII-053 contains an RKRRKR sequence inserted between the light and heavy chain for intracellular activation. FVII-053 was transiently expressed (cotrasnfected with PCS for processing of the RKRRKR sequence which results in activation of the protein) and purified as previously described. Thrombin generation assays with purified components and platelets reveal that FVII-053 has some activity (Figure 22), even though this activity is compromised relative to the FVII-011 control (Figure 22D). Data in Figure 23 show how the PAC1 antibody, which competes with MB9 for GPIIbllla binding, inhibits thrombin generation activity associated with FVII-053, suggesting that platelet targeting is important for activity. Another construct identical to FVII-053, but without the RKRRKR insertion was generated (FVII-028) and tested in a Pk study in the nonactivated together with nonactivated FVII-01 1. As shown in Figure 50, the terminal half-life of the targeted, Gla-less FVII-028 molecule was nearly three times longer (20.3 hours) than that of the FVII-01 $\{$ control ( 7.1 hours), suggesting that removing the Gla domain increases the terminal half-life of FVIIFc

Example 31. Platelet Targeted FIX Molecules
[00439] In this example, the FIX constructs illustrated in Figure 51A were made and tested. FIX-068 was cotransfected with PCS to fully process the cscFc linker, described in the protein sequence, connecting the first Fc moiety to the platelet targeting moiety. FIX-068 has the SCE5 platelet targeting moiety at the N-terminus of the second Fc moiety, while FIX-088 has SCE5 at the C-terminus of the second Fc moiety. FIX-090 is a FIX construct without an Fc domain and has the SCE5 moiety
attached at the C-terminus of the FIX protein. FIX-042 is a FIXFc as a single chain Fc without a targeting moiety and was made as a control BeneFIX (Pfizer) was also used as a control. To remove trace amounts of activated FIX (FIXa) from the BeneFIX sample that cause misleading results in thrombin generation assays, the BeneFIX sample was treated with the irreversible active site inhibitor glutamyl-glyeyl-arginylchloromethylketone (Hematologic Technologies). BeneFIX was incubated with an excess amount of the inhibitor for 180 minutes at room temperature. The sample was then dialyzed to remove unbound mhibitor. The treated BeneFIX is hereafter referred to as BeneFIX. The specific activities of the molecules made were FIX-042, 6 IU/nmol; FIX068, 5.1 IU/nmol; FIX-OSS, 3.5 IU/nmol; FIX-090, $13.8 \mathrm{IU} / \mathrm{nmol}$, and BeneFIX, $12 \mathrm{IU} / \mathrm{nmol}$. When these constructs (FIX-068, FIX-088 and FIX-042) were tested in a thrombin generation assay in platelet-rich FIX-deficient plasma as shown in Figure 51B, each of the targeted molecules had a higher activity than the FIX-042 control. Figure 51C illustrates that both FIX-068 and FIX-088 have at least 4 times more activity than FIX-042 as measured by thrombin generation. Since the specific activity of FIX-042 is higher than FIX-068 and FIX-088, the increased activity observed in the thrombin generation assays may be underestimated, and therefore the increased activity by platelet targeting may be greater than 4-fold.
[00440] As shown in Figure 52A, FIX-090 (which lacks an Fc) also shows increased activity relative to BeneFIX, suggesting that targeting FIX to platelets in the absence of Fc also increases activity. Figure 52B shows that the activity of FIX-090 is at least 4 times that of BeneFIX. Since both FIX-090 and BeneFIX have similar specific activities, the 4 -fold increase in activity in thrombin generations assays must be caused by the platelet targeting effect

Example 32. Use of peptides for targeting FIX to platelets
[00441] In this example, the FIX-089 construct illustrated in Figure 53A was cloned, transiently expressed and purified as previously described. The molecule comprises the OSI peptide, which binds to GPIb-alpha receptor, attached to the N terminus of the second Fc moiety of the construct. The specific activity of the FIX089 construct was $2.4 \mathrm{IU} / \mathrm{nmol}$ as compared to $6 \mathrm{IU} / \mathrm{nmol}$ for the control FIX-042 molecule,
[00442] As shown in Figure 53B, the FIX-089 molecule is more active than the FIX-042 control in thrombin generation assays with FIX-platelet rich plasma; this is
particularly evident at limiting concentrations of FIX. Figure 53C demonstrates that FIX-089 is roughly 4-times stronger than FIX-042 as measured by thrombin generation, while having a lower specific activity. This further suggests that targeting to GPIb increases the activity of FIXFc.

## DRAFT SEQUENCE LISTING

FVII-027 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVT or MB9 to Fc region is underlined, and linker with proprotein convextase processing sites is shown in bold MVSSALj3 3 LC. . LLLGLyGCLA SFEE ARE IFK DAE RTKLENI THKDDQLICV NENGGCEQYC KRNASKPQGR IVGGKVCPKG NlJAvlge hd lsehdgdeqs LpErTfeserd LafvrfsLvs If'éymfeagy sdgskdsckg yIEWLQKLMR SEPRPGVLLR ppCPAPELLG GPSVFLFPPK AKTKPREEQY NSTYRVVSVL
QVYtLPpsRD ELTKNQVSLT

YSKLTVDK3R WQQGNVFSCS SGGGGSGGG SGGGGSR\&RR RQAPGQGLEW MGWINPNSGG RGRALYNRND RSPNWfong AVFVT2EEAJL
$\begin{aligned} & \text { SYSDGDQCAS } \\ & \text { SDHTGTKRSC }\end{aligned}$ $\frac{\text { GVLHRRRRAN }}{\text { SPCQNGGSCK }}$ afleelrpgs DQLQSYICFC lereckeeqc UFAFEGRNCE
 RRVAQVIIPS TYVPGTtMHD 1ALLRLKQP V VLTDHVVPLC GWGQLLDRGA TALELMvLNV DSGGPHAFHY RGTMYYTGTY APFPGGGGSG GGGSGGGG3G GGGSGGGGS GGGSDKTHTC EKDTLMISRI PEVTCVVVDV 3HEDPEVKFK WYVDGVEVHN tVLKQDWLNG KEYKCKVSNK ALPAFIEKTI SKAKGQPREF Clvkgfypsd IAvelesigg pelinykttpp vidsdgsffl vmheal hliy tekslslupg Krrrrsggeg scegcssgege KRAEVQLVQS GAEVNKPGAS vKVSCKASGY TFTGYYMHWV TNYAQKFQGW VTMTRDT3 IS TAYMEL3RLR SDDTAVYYCA QGTLVTVSSG $\begin{array}{ll}\text { VAPGQTARIT } & \text { CGGNNIG3KS } \\ \text { LTISRVEA.GD } & \text { EADYYCQVWD }\end{array}$ GGGGSGGGGS GGGGSGGGGS TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH GKEYKCKVSN KALPAPIEKT ISKAKGQPRE DIAve wiesng epennykttp YTQKSLSLSP GK

DNA sequerce of $\mathrm{FVn}-027$
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Genscript -FVn-027-1 DNA sequence
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Genscript- $\mathrm{F}^{\wedge} /$ 11-026-2 DNA sequence gaattctcag aagcacgcgt acaggctgtg ccaggacaga eggecaggat tacctgtggg tggtaccagc agaagecagg ccctcaggga tccctgagcg atcagcagqg tegaageegg $\mathrm{a}_{\mathrm{g}}$ tgatcatg gccecctcgg tcactctgtt ggtgggtccg gtggcggcgg gggggatcag acaaaactca ccgtcagtct tcctcttccc gaggtcacat gcgtggtggt taegtggaeg gcgtggaggt agcacgtacc gtgtggtcag gagtacaagt gcaaggtctc
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FVll-037 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or MB9 to Fc region is underlined, and linker connecting both Fes sites is shown in bold

|  |  | AvEVTOEEAH | N | AFLEELRPGS | KEEQC |
| :---: | :---: | :---: | :---: | :---: | :---: |
| SFEEAREIFK | DAERTKLFWI | SYSDGDQCAS | SPCQNGGSCK | DQLQSYICFC | LPAFEGRNCE |
| HKDDQLICV | NENGGCEQYC | SDHTGTKRSC R | RCHEGYSLL A | DGVSCTPTVE | YPCGKIPILE |
| KRNASKPQGR | IVGGKVCPKG | ECPWQVLLLV | NGAQLCGGTL | INTIWVVSAA | R |
| NLIAVIGEHD | LSEHDGDEQS | RRVAQVIIPS | TYVPGTTNHD | IALLRLHQPV | VLTDHVVPLC |
| LPERTFSERT | LAFVRFSLVS | GWGQLLDRGA | TALE LVIVLiNV | PRLMTQDCLQ | QSRKVGDSPN |
| InEYMFCA.GY | SDGSKDSCKG | DSGGPHATH X | RG?WYLTGIV | SKGQGCATVG | HFGVYTRVSQ |
| YJEWLQRLMR | SEPRPGVLLR | APFPGGGGSG | GGGSGGGGSG | GGGSGGGGSG | G |
| PPCPAPELLG | GPSVFLFPPK | PKDTLMISRT | PEVTCVVVDV | SHEDPEVKFN | WYVDGVEV HN |
| AKTKPREEQY | NSTYRWSVL | TVLHQDWLNG | KEYKCEV SNK | ALPAPIEKTI | SKAKGQPREP |
| QVYTLPP3RD | ELTKNQVSLT | CLVKGFYPSD | IAVEWESNGQ | PENNYKTTPP | VLDSDGSFFL |
| YSKLTVDKSR | WQQGNVFSCS | VMHEALHNHY | TOKSLSLSPG | KGGGGSGGGG | SGGGGSGGGG |
| SDKTHTCPPC | PAPELLGGPS | VFLFPPKPKD | TLMISRTPEV | TCWVDVSHE | DPEVKFNWYV |
| DGVEVHNAKT | KPREEQYNST | YRVVSVLTVL | HQDWLNGKEY | KCKVSNKALP | APTEK'ISKA |
| KGQPREPQVY | TLPPSRDELT | KNQVSITCEV | KGFYPSDIAV | EWESNGQPEN | NYKTTPPVLD |
| SDGSFFLY3K | LTVDKSRWQQ | GNVF SC SVIVH | EALHNHYTQK | SLSLSPGK GG | GG3GGGGSGG |
| GG3GGGGSGG | GGSGGGG3AE | VQLVQSGAEV | NKPGASVKVS | CKASGYTITIG | YYMRGVRQAP |
| GQGLEWMGNI | KPNSGGTNYA | QKFQGWVTMT | RDI'SISTAYM | ELSRLRSDDT | AVYYCARGRA |
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| QTARITCGGN | NIG SK SVQWY | QQKPGQAPVL | VVYDDSDRPS | GIPERFSG3N | SGNMATLTIS |
| RVEAGDEADY | YCQVWDSSSD | HVVFGGGTKL | ! VLGQPKAAP | SVTLFPPSAA | A |

## FIX-037 DNA sequence

40 atggtctc.ee aggccctcag gcagtcttcg taacccagga gcgttcctgg aggagctgeg tccttcgagg aggeceggga tcttacagtg atggggacca gaccagctcc agtcctatat aegcacaagg atgaccagct agtgaccaca cgggcaccaa gacggggtgt cotgcacacc aaaagaaatg ccagcaaacc ag9tcct atcaacacca tctgggtggt aacctgatcg cggtgctggg cggcgggtgg cgcaggtcat atcgcgctgc tccgcctgca ctgcccgaac ggacgttctc ggctgg ggcc agetgetgg a cccoggctga tgacccagga ateaeggagt acatgetctg gacagtggag gcccacatgc agetggggee agggctgege tacatcgagt ggctgcaaaa gccccai:ttc. ccggtggcgg gggggtggat caggcggtgg ccaccgtgcc cagctcogga
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| tgcgcttctc | attggtcagc |
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FVII－053 amino acid sequence．Signal sequence is shown in dotted underline，linker region connecting FVII to Fc region is underlined，linker connecting both Fes sites is shown in bold，and MB9 is italicized

M SRALRLEC ELIGLGGCLA APGQGLEWMG WINFNSGGTN

AEVQLVQSGA EVNKPGASVK YA，QKFQGWVT MTRDTSISTA $R A L Y N R N D R S$ PNWFDPWGQG TLVTVSSGSA SAPTLKLEEG EFSEARVQAV LTQPPSVSVA FGQTARITCG GNNIGSKSVQ WYQQKPGQAF VLVVYDDSDR PSGIFERFSG SNSGNMATLT ISRVEAGDEA DYYCQVWDSS SDHVVFGGGG KLTVLGQFKA APSVTLFPPS AAARTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE THKDDQLICV NENGGCEQYC SDKTGTKRSC RCHEGY3LLA DGVSCTPTVE YPCGKIPILE KRNA3KPQGR RKRRKRIVGG KVCPKGECPW QVLLIVNGAQ LCGGTLINTI WVVSAAHCFD KIKNMRNLIA VLGEKDLSEH DGDEQSRRVA QVIIPSTYVP GTTNYDIALL RLKQPVVLTD HVVPLCLPER TFSERTLAFV RFSLVSGWGO＿LLDRGATALE LMVLNVPRLN TQDCLQQ3RK VGDSPNITEY MFCAGYSDG3 KDSCKGDSGG PHATHYRGTW YLTGIVSvVGQ GCATVGHFGV YTRV3QYIEK LQKLMR3E PR FGVLLRAPFP GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEK＇ISKAK GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYP3DIAVE WESNGQPENN YKTTPPVLD3 DGSFFLYSKL TVDKSRt＇JQQG NVFSCSV䐓HE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSDKT HTCPPCPAPE LLGGP3VFLF PPEPKDTLMI SRTPEVTCVV VDVSHEDPEV KENNYVOGVE VHNAKTKPRE EQYNSEYRVV SVLTVLHQDW LNGKEYKCKV 3NKALPAPIE KITSKAKGQP REPQVYTLPP

5 SRDELTENQV SLTCLVKGEY PSDIAVENES NGQPENNYKT TPPVLD3DGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALE NHYTQKSLSL SPGK

FVn-053 DNA sequence gt.ct.cctgca aggcttctgg gcccetggac aagggcttga tatgcacaga agtttcaggg tacatggagc tgagcaggct
cgtgctttgt ataaccggaa accetggtca ccgtctcctc gaattitcag aagcocgcgt ccaggaraga cggccaggat tggtaccagc agaagccagg ccctcaggga tccct.gagcg atcagcaggg t.egaage egg agtgatcatg tggtattegg gcccectcgg tcactctgtt tcttacagtg atggggacca gaccagctcc agtcctatat acgcacaagg atgaccagct agtgaccaca cgggcaccaa gacggggtgt cctgcacacc aaaagaaa tg ccagcaaacc aaggtgtgcc cca aagggga ttgtgtgggg aaaatcaaga gacggggatg ggcaccacca catgtggtgc cgcttcccat ctcatggtcc gtgggagact aaggactcct tacctgacgg tacaccaggg ccaggagtcc ggtggcggcg gacaaaactc acacatgccc tgcgtggtgg tggacgtgag ggcgtggagg tgcataatgc cgtgtggtca gcgtcctcac tgcaagg tct ccaacaaagc gggcagcccc gagaaccaca aaccaggtca gcctgacctg tgggagagca atgggcagcc gacggctcct tcttcctcta aacgtcttct catgctccgt ctctccctgt etcegggtaa ggttccggtg gegggggate ctcctgggag gaccaccagt tcccggaccc ctgaggtcac aagttcaact ggtacgtgga gagcagtaca acagcacgt.a ctgaatggca aggagtacaa $\begin{array}{ll}\text { aaaaccatct } & \text { ccaaagccaa } \\ \text { tcccgcgatg } & \text { agctgaccaa }\end{array}$ tcccgcgatg agctgaccaa cccagcgaca tcgccgtgga acgcctcccg tgttggactc
gcicctctgc cttctgcttg ggcttcaggg gtctggagct gaggtgaata agectgggge atacaccttc accggctact atatgeactg gtggatggga tggatcaacc ctaacagtgg ctgggtcacc atgaccaggg acacgtccat gagatctgac cgaccggtcc agggagtgea acaggc tgtg ctgactcagc tacctgtggg ggaaacaaca ccaggcccc t qtgctiggtcg t attctctggc tccaactetg g ggat.gaggcc g eggagggace cccgccgtcc gtgtgcctca ctgcttctgc gatctgtgcg gcgctcctgt caragttgaa t ccatgtg gaaaaatacc gtgt.eeatgg ggaagaggat. caacaccat.c tgggtggtct ccgcggccca cctgatcgcg gtgctgggcg agcacgacct gcgggtggcg caggtcatca tccccagcac cgcgctgctc cgcctgcacc agcccgtggt gcccgaacgg ctggggccag ccggctgatg caeggagtae cagtggaggc ctggggccag catcgagtgg cccatttccc gggtggatca accgtgccca caaggacacc ccacgaagac caagacaaag cgtcctgcac cctcccagcc ggt.gt.acacc cctggtcaaa ggagaacaac cagcaagctc gatgeatgag aggtggcggc cgacaaaact cttcct.ct+c cccccaaaac atgcgtggtg gtggacg tga cggcgtggag ccgtgtggt.c gtgcaaggtc agggcagccc gaaccaggtc gtgggagagc cgacggctcc
ctgcctggct ctcagtga ag ggtgcgacag tggcacaaac cagcaccgcc tgegagagge gggccaggga tgaagaaggt gtcagtggcc aagtgtgcag tagegacegg caccctgacc ggatagtagt gcccaaggct gttctggatt ctcctgcaag gaactgtgag gcagtactgc tctgctggca tattctagaa tg tggggggc tggagctcag ctgtttcgac cagcgagcac gtacgtcceg cctcactgac ggccttcgtg ggccctggag gtcacggaag ggatggcagc gggcacgtgg ctttggggtg agagccacgc aggtgggtcc egggggatec accgtcagtc tgaggtcaca gtacgtggac cagcacgtac ggagtacaag caaagecaaa gctgacca ag cgccgtggag gttggactcc gcagcagggg gcagaagagc aggcggtgga agcacctgaa cct.eatgatc ccctgaggtc gecgegggag ccaggactgg ccccatcgag cctgccecca aggcttctat ctacaagacc caccgtggac

| aagagcaggt. ggcagcaggg gaacgtcttc tcatgctccg | t.gatgcatga ggctctgcac |
| :--- | :--- | :--- | :--- |
| aaccactaca cgcagaagag cctctccctg tctccgggta aatga |  |

FVII-044 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or PS4 to Fc region is underlined, linker connecting both Fes sites is shown in bold, and PS4 peptide is italicized

MSFARLCC MLGGLGCLZ
 MKKDDQLICV NENGGCEQYC SDHTGTKRSC R.CHEGYSLLA DGVSCTPTVE KRNASKPQGR IVGGKVCPKG NLIAVLGEHD LSEHDGDEQS LPERTFSERT LAFVRFSLVS

FVII-044 D id A sequence
atggtctccc aggocctcag getcctc tgc ettctgettg ggcttcaggg ctgcetggc t gcgttcctgg aggagctgeg tccttcgagg tcttacagtg atggggacca agtcctatat
gacggggtgt aaaagaaatg cctgcacacc ccagcaaacc gaçtoticcat ggcaggtcc atcaacacca t.ctgqqtggt cggcgggtgg cgcaggtcat atcgcgctgc ctgcccgaac ggctggggcc ggacgttctc agctgctgga tgacccagga acatqt gcccacatgc agggc tgege tacatcgaqt ggctgcaaaa qccccatttc gggggtggat ccaccgtgcc cccaaggaca agecacgaag gecaagacaa accgtcctqc gccetcccag caggtgtaca tgcctggtca
caggcggtgg cagctccgga ccctcatgat accctgaggt agecgeggga accaggactg cccccatcga gaatac ccctgccccc atecegggat aaggc ttcta tcccagcgac
ggegtec tgc ctggagaggg gaegeggaga agtccatgcc ctccctgcct aacgagaacg cggtgccacg tatccatqtq attgtggggg aatggagハtc cactgtttcg ctcagcgagc acgtacgt.ee gt.eetea ctg ctggccttcg acggccetgg cagtcaegga tcqgatggca eggggcacqt cactttgggg tcagagccac ggaggtgggt ggeggggqat ggaccgtcag cctgaggtca tggtacgtgg aacagcacgt aaggagtaca tccaaagcca gagctgacca atcgccgtgg
accggcgccg gcgcgccaac agtgcaagga ggagcagtgc ggacgaagct. gttctggatt. aga atggggg ctcctgca ag tegagggecg gaactgtgag gcggctgtga geagtactge aggggtactc tetgetggea gaaaatacc tattctagaa gcaaqqtqtg ccccaaaggg agttgtgtgg ggqgaccctg acaaaatcaa gaactggagg aegaegggga tgagcagagc cgggcaccac caaccacgac accat.gt.ggt. gcccctctge. tgcgcttctc attggtcagc agctcatggt cctcaacgtg aggtgggaga ctccccaaat gcaaqqactc ctgcaagggg ggtacctgac gggcatcgtc tgtacaccag ggtctcccag geccaggagt cctectgega ceggtggegg eggatcagg t ccgacaaaac tcacacatge. tcttcctctt ccccccaa aa catgcgtggt ggtggacgtg acggcgtgga ggtgcataat accgtgtggt. cagcgtcctc agtgcaaggt ctccaacaaa aagggcagee ccgagaacca agaaccaggt caqcctgacc agtggqagag caatgggcag
propeptide is double underlined, linker region connecting FVII or OS! to Fc region is underlined, linker connecting both Fes sites is shown in bold, and OS1 peptide is italicized

| IS SOALEXTJC | . LLLGL2GCLA | AVEVTQEEAH | CVIHERFRAN | AFLEELRPGS | LERECKEEQC |
| :---: | :---: | :---: | :---: | :---: | :---: |
| SEGEAREIFK | DAERTKLFMI. | SYSDGDQCAS | SPCQHGGSCK | DQLQ3YICFC | LPAFEGRNCE |
| THKDDQLICV | NENGGCEQYC | 3 DHTGTKRSC | RCHEGY3LLA | DGVSCTPTVE | YPCGKIPILE |
| KRNASKPQGR | IVGGKVCPKG | ECPWQVLLLV | NGAQLCGGTL | INTIWWSAA | HCFDKIKNinR |
| NLIAVLGEHD | LSEHDGDEQS | RRVAOVIIPS | TYVPGTTNHD | IALLRLHQPV | VLTDHVVPLC |
| LPERTFSERT | LAFVRFSLVS | GSGQLLDRGA | TALE LMVLNV | PRLMTQDCLQ | QSRKVGDSPN |
| ITEYMFCAGY | SDGSKDSCKG | DSGGPHATI-IY | RGTWYLTGIV | SWGQGCATVG | HFGVYTRVSQ |
| YIEWLQKLMR | SEPRPGVLLR | AFFPGGGGSG | GGGSGGGGSG | GGGSGGGGSG | GGGS DKTHTC |
| PPCPAPELLG | GPSVFLFPPK | PKDTLMI SRT | PEVTCWVDV | SHEDPEVKFIM | WYVDGVEVHN |
| AKTKPREEQY | IMSTYRVVSVL | TVLIQ DSLIG | KEYKCKV3NK | ALPAPIEKTI | SKAKGQPREP |
| QVYTLPP5RD | ELTKNQVSLT | CLVKGFYPSD | JAVEne SNGQ | PENNYKTTPP | VLDSDGSFFL |
| YSKIMVDKSR | WQQGNVFSCS | VMHEALHAHY | TQKSL3L3PG | KGGGG3GGGG | SGGGGSGGGG |
| SDKTHTCPPC | PAPELLGGPS | VFLFPPKPKD | TLMISRTPEV | TCVWDVSHE | DPEVKFNWYV |
| DGVEVHNAKT | KPREEQYN3T | YRVVSVLTVL | HQ DWLNGKEY | KCKV3NKALP | APIEKTISKA |
| KGOPREPQVY | TLPP3RDELT | KNQVSLTCLV | KGFYPSDIAV | EWESNGQPEK | KYKTTPPVLD |
| SDG3FFLY3K | LTVDKSRWQQ | GNVFSCSVMH | EALHNHYTQK | SLSLSPGKGG | GG3GGGGSGG |
| GGSGGGGSAC | TEPMALHNLC | $G G$ |  |  |  |


| ccggagaaca | actacaagac |
| :--- | :--- |
| tacagcaagc | tcaccgtgga |
| gtgatgcatg | aggctctgca |
| aaaggtggcg | gcggatcagg |
| tcagacaaaa | ctcacacatg |
| gtcttcctct | tccccccaaa |
| acatgcgtgg | tggtggacgt |
| gacggcgtgg | aggcgcataa |
| taccgtgtgg | tcagcgtcct |
| aagtgcaagg | tctccaacaa |
| aaagggcagc | cccgagaacc |
| aagaaccagg | tcagcctgac |
| gagtgggaga | gcaatgggca |
| tccgacggct | ccttcttcct |
| gggaacgtct | tctcatgctc |
| agcctccccc | tgtctccggg |
| ggaggttccg | gtggcggggg |
| ggcgggtga |  |

cacgcctccc
caagagcagg
caaccactac
tgggggtgga
cccaccgtgc
acccaaggac
gagccacgaa
tgccaagaca
caccgtcctg
agccctccca
acaggtgtac
ctgcctggtc
gccggagaac
ctacagcaag
cgtgatgcat
caaaggtggc
atcagcccgc

| gtgttggact | ccgacggctc | cttcttcctc |
| :--- | :--- | :--- |
| tggcagcagg | ggaacgtctt | ctcatgctcc |
| acgcagaaga | gcctctccct | gtctccgggt |
| tcaggcggtg | gaggttccgg | tggcggggga |
| ccagcacctg | aactcctggg | aggaccgtca |
| accctcatga | tcccccggac | ccctgaggcc |
| gaccccgagg | tcaagttcaa | ctggtacgtg |
| aagccgcggg | aggagcagta | caacagcacg |
| caccaggact | ggctgaatgg | caaggagtac |
| gcccccatcg | agaaaaccat | ctccaaagcc |
| accctgcccc | catcccgcga | tgagctgacc |
| aaaggcttct | at.cccagcga | catcgccgtg |
| aactacaaga | ccacgcctcc | cgtgttggac |
| ctcaccgtcg | acaagagcag | gtggcagcag |
| gaggctctgc | acaaccacta | cacgcagaag |
| ggcggaccag | gtgggggtgg | atcaggcggt |
| accgagcggt | gggccctgca | caacctgcgc |

FVII-045 amino acid sequence. Signal sequence is shown in dotted underline,
 THKDDQLICV NENGGCEQYC KRNASKPQGR IVGGKVCPKG NLAVLGEAD

FVII-045 DNA sequence atggtctccc aggecctcag gcagtcttcg taacccagga gcgttcctgg aggagctgeg tccttcgagg aggeceggga tcttacagtg atggggacca gaccagctcc ag:cctatat aegcacaagg atgaccagct agtgaccaca cgggcaccaa gacggggtgt cctgcacacc aaaagaaatg ccagcaaacc gagtgtccat ggcaggtcct atcaacacca tctgggtggt aacctgatcg cggtgctggg cggcgggtgg cgcaggtcat atcgcgctgc tccgcctgca ctgcccgaac ggacgttctc ggctggggcc agetgctgga ccccggctga tgacccagga
gctcctctg ggaagcccac gccgggctcc gatcttcaag gtgtgcctca ctgcttctgc gatctgtgtg gcgctcctgt cacagttgaa ccaaggccga gttgttggtg ctccgcggcc cgagcacgac catccccagc ccagcccgcg tgagaggacg ccgtggcgcc ctgcctgcag
ettctgettg ggcgtcctgc ctggagaggg gaegeggaga agtccacgcc ctccctgcct aacgagaacg cggtgccacg tatccatgtg attgtggggg aatggagctc cactgtttcg ctcagcgagc acgtacgtcc gtcctcactg ctggccttcg acggccctgg cagtcaegga
ggcttcagg accggcgccg agtgcaagga ggagcagtgc ggacgaagct gttctggatt agaatggggg ctcctgcaag tegagggecg gaactortyac gcggctgtga geagtactge aggggtactc tct.gct.ggca gaaaaatacc tattctagaa gcaaggtgtg ccccaaaggg agttgtgtgg gggqaccctg acaaatcaa gaactggagg aegaegggga tgagcagagc cgggcaccac caaccacgac accatgtggt gcccctccgc tgcgcttctc attggtcagc agetcatggt cctcaacg tq aggtgggaga ct:ccccaaat.
AKTKPREEQY NSTYRWSVL

QVYTLPP3RD ELTKNQVSLT Y3KLTVDK3R WQQGNVFSCS SDKThTCPPC PAPELLGGP3
3DGSFFLY3k LTVDKSRwQ ${ }^{\text {GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GG3GGGGSGG }}$ GG3GGGG3AC TERDALHNLC GG

|  |  |  |  |
| :---: | :---: | :---: | :---: |
|  |  | ggtactgac |  |
|  |  | tgtacaccag |  |
| catgcg | tcagagc |  | ga |
| gctecggc | ggaggtgggt | ccg | gt |
|  | gge | cc |  |
| cctggg | ggacegtcag | tct |  |
| cocggace | cctgaggtca | catgcgtggt | g9 |
| caagttcaa |  | acg | ggtgcat |
| agcagta | aacagcacgt | accg tg | 硡t |
| tgaatgg | aaggagtaca | agtgcaag | ccaac |
| gaaaaccat | tccaaa | gg | ccgagaa |
| ccggg | gagc | agaac | gctgac |
| cagcga | atcgce | agtggga | caatgggcas |
| gcat | gtgt | ccgacgg | cttcttc |
| ag |  | egt | ctcat |
| accact | aegcagaag | gcctctcc | ccgg |
| ggggtgg | teaggeggt | gaggttce | cgeggga |
|  | ccagc | aactect | ag |
| aagga | acce eatga | tceccog | ccetgag |
| gccacga | gaccot | tea agtt | ctggtac |
| tgccaagaca | aagcegc | aggagca |  |
|  | caccagg | ggctgaa |  |
| agcectccea | gccecc | aga | ctccaagage |
| ag | accctg | catcco | gag |
|  |  |  |  |
| cgagaac | tacaaga | ccacgectc |  |
| ctacagcaag | ctcaccgtc |  |  |
| gatgea | gag |  |  |
| taaggtgge |  |  |  |
|  | accgageg |  |  |

FVII-G46 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or OS2 to Fc region is underlined, linker connecting both Fes sites is shown in bold, and OS2 peptide is italicized
MYSORARLLC. LLEGEQGCLAR AVFVTOEEAH GVLHRRRRAM AFLEELRPGS LERECKEEQC SFEEAREIFK DAERTKLFWI SYBDGDQCAS SPCQNGG3CK DQLQSYICFC LPAFEGRKCE TKKDDoLICV NENGGCEQYC SDhtGTkRSC RCKEGYSLLA DGVSCTPTVE YFCGKIPILE ECPWQVLLLV NGAQLCGGTL INTIWW SAA HCFDKIKNWR RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC GwGelldrga tale levisv primtedcle esrkvgdspn D3GGPHathy rgtwyltgiv sïjgegcatvg hfgvytrvse APFPGGGGSG GGGSGGGSG GGGSGGGGSG GGGSDThTC PKDTLMISRT PEVTCVVVDV SREDPEVKFN WYVDGVEVHN TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP CLVkgyysd mavewesigg pennykttpr vidsdgsfal
 vflfppkpkd tlmisrtpev tcwvdvshe dpevkfnwyv YRWSVLTVL HQDWLNGKEY KCKVSNKALP AFIEKTISKA kNQVSLTCLV KGFYPSDIAV EWESNGQPEIM NYKTTPPVLD givvesc svain ealhnhytek SlsLSPGK gG GG3GGGGSGG

60 FV11-G46 DivA sequence atggtctccc aggecctcag gcagtctteg taacccagga gcgtecctgg aggagctgeg tccttcgagg aggeceggga
tcttacagtg gaccagctcc acgcacaagg agtgaccaca gacggggtgt aaaagaaatg gagtgtccat atcaacacca aacc $\quad$ gatcg cggcgggtgg atcgegctgc ctgcccgaac ggctggggcc ccccggctga atcacggagt gacagcggag agctggggcc tacatcgagt gccccattc gggggtgqat ccaccgtgcc cccaaggaca agccacgaag gccaagacaa accgtcctgc gccctcccag caggtgcaca tgcctggtca ccggagaaca tacagcaagc gtgatgca :.g aaaggtggcg tcagacaaaa gtcttcctct acatgcgtgg gacggcgtgg taccgtgtgg aagtgcaagg aaagggcagc aagaaccagg qagtgggaga tccgacggct gggaaegtet agcctctccc ggaggttccg

FViI-047 amino acid sequence. Signal sequence is shown in doited underline, propeptide is double underlined, linker region connecting FVII or PS4 to Fc regions is underlined, and PS4 peptide is italicized

|  | Cutat | AVFVTOLEAH | AN |  | ERECKEEQC |
| :---: | :---: | :---: | :---: | :---: | :---: |
| SFEEAREIFK | DAERTKLFWI | SY3DGDQCAS | SPCQNGGSCK | DQLQSYICFC | CE |
| TKKDDQLICV | NENGGCEQYC | SDHTGTKRSC | RCKEGYSLLA | DGVSCTPTVE | YPCGKIPILE |
| KRNASKPQGR | IVGGKVCPKG | ECPWQVLLLV | NGAQLCGGTL | INTI開W SAA | HCFDKIKNinR |
| NLIAVLGEHD | LSEHDGDEQS | RRVAQVIIPS | TYVPGTTNHD | IALLRLKQPV | VLTDHWPLC |
| LPERTFSERT | LAFVRFSLVS | GWGQLLDRGA | TALELVVIV | PRLMTQDCLQ | QSRKVGDSPN |
| ITEYMFCAGY | SDGSKDSCKG | D 3GGPHATHY | RGTWYLTGIV | S ${ }^{\text {V }}$ JGQGCATVG | HFGVYTRVSQ |
| YJ EWLQKLMR | SEPRPGVLLR | APFPGGGGSG | GGGSGGGGSG | GGGSGGGGSG | GGGS DKTHTC |
| PPCPAPELLG | GPSVFLFPPK | PKDTLMISRT | PEVTCVVVDV | SREDPEVKFN | WYVDGVEVHN |
| A.KTKPREEQY | NSTYRWSVL | TVLHQDWLNG | KEYKCEVSNK | ALPAPIEKTI | SKAKGQPREP |


| QVYTLPPSRD | EITKNOVSLT | CLVKGFYP3D | 1AVEWESNGQ | PENNYKï ï PP | VLDSDGSFFL |
| :---: | :---: | :---: | :---: | :---: | :---: |
| YSKITVDKSR | WOQGNVFSCS | VMHEALHNHY | TQKSLSL3PG | KGGGGSGGGg | SGGGGSGGGG |
| SGGGGSGGGG | SACWERWALH | $N L$ cGGg SGG | GGSGGGGSGG | GGSGGGGSGG | G G S DRTTHTC P |
| PCPAPELLGG | PSVFLFPPKP | KDTLMI SRTP | EVTCVVVDVS | REDPEVKFNW | YV DGVEVHNA |
| KTKPREEQYN | 3TYRVVSVLT | VLHQDWLNGK | EYKCKVSNKA | LPAPIEKTIS | KAKGQPREPQ |
| VYtLPPSRDE | LTKNQVSLTC | LVKGFYPSDI | AVEWESNGQP | eNNYkttppl | LDSDGSFFLY |
| SKLTVDKSRW | QQGNVF8CSV |  | QKSLSLSFGK |  |  |

FVII-047 DNA sequence
atggtctccc aggccetcag gcagtcttcg taacccagga gcgttcctgg aggagctgcg tccttcgagg aggcccggga tcttacagcg atggggacca qaccagctcc agtcotatat acgcacaagg agtgaccaca gacggggtgt aaaagaaatg gagtgtccat tcaacacca aacctgatcg cggcgggtgg atcgcgctgc ctgcccgaac ccccggctga tgacccagga atcacggagt. acatgttctg gacagtggag gcccacatgc agctggggcc agggctgcgc tacatcgagt ggctgcaaaa gccccatttc ccggtggcgg gggggtggat caggcggtgg ccaccgtgcc cagctccgga cccaaggaca ccctcatgat agccacgaag accctgaggt gccaagacaa agccgcggga accgtcctgc gccctcccag caggtgtaca tgcctggtca aaggcttcta ccggagaaca actacaagac tacagcaagc tcaccgtcga gtgatgcatg aggctctgca aaaggcgg tig gcggttcagg gagggtcagg aacctgtgcg gtggcggtgg ggtggatcag gcggtggagg ccgtgcccag caccggaact aaggacaccc tcatgatctc cacgaagacc ctgaggtcaa aagacaaagc cgcgggagga gtcctgcacc aggactggct ctcccagccc ccatcgagaa gtgtacaccc tgcccccatc ctggtcaaag gcttctatcc gagaacaact acaagaccac agcaagctca ccgtggacaa atgeatgagg ctctgcacaa tga
gctcctctgc ggaagcccac gccgggctcc gatcttcaag gegtgcctca ctgcttctgc gacctgtgtg gcgctcctgt cacagttgaa ccaaggccga gttgttggtg ctccgcggcc cgagcacgac catccccagc ccagcccgtg tqagaggacg ccgtggcgcc ctgcctgcag tgccggctac cacccactac aaccgtgggc gctcatgcgc tggctccggc aggttccggc actcctggga ctcccggacc caagttcaac ggagcagtac gctgaatggc gaaaaccatc atcccgggat Lcccagcgac cacgcctccc caagagcagg caaccactac tqqaggaggg cggtggcgga ctccggcgga ttccggtggc cctgggcgga ccggacccct gttcaactgg gcagtacaac gaatggcaag aaccatctcc cegggatgag cagcgacatc gcctcccgtg gagcaggtgg ccactacacg
cttctgcttg ggcgtcctgc ctggagaggg gacgcggaga agtccatgcc ctccctgcct aacgagaacg cggtgccac tatccatgtg attgtggggg aatggagctc cactgtttcg ctcagcgagc acgtacgtcc gtcctcactg ctggcct:cg acggccctgg cagtcacgga tcggatggca cggggcacgt cactttgggg tcagagccac ggaggtgggt ggcgggggat ggaccgtcag cctgaggtca tggtacgtqg aacagcacgt aaggagtaca tccaaagcca gagctgacca atcgccgtgg gtgttggact tggcagcagg acgcagaaga teaggegq t:g tcagcctgca ggt.gggtccg gggggatccg ccgtcagtct gaggtcacat tacgtggacg agcacgeacc gagtacaagt aaagccaaag ctgaccaaga gccgtggaqt ttggactccg cagcagggga cagaagagee tctccctgtc
ctgcctggct gcgcgccaac ggagcagtgc gttctggact ctcctgcaag gaactgtgag gcagtactgc tctgctggca tattctagaa ccccaaaggg ggggaccctg gaactggagg tgagcagagc caaccacgac gccoctctgc attggtcagc cctcaacgtq ctccccaaat ctgcaagggg gggcatcgtc ggtctcccag cctcctgcga cggatcaggt tcacacatgc ccccccaaaa ggtggacgtq gqtgcataat cagcgtcctc ctccaacaaa ccgagaacca cagcctgacc caatgggcag cttcttcctc ctcatgctcc gtctccgggt egggggegga ggccctgcac atcaggtggg cacatgccca cccaaaaccc ggacgtgagc geataatgee cgtcctcacc caacaaagcc agaaccacag cctgacctgc tqqgcagccg cttcctctac atgctccgtg teegggtaaa

5 FVll-048 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or OS1 to Fc regions is underlined, and OS1 peptide is italicized
 SFEEAREIFK DAERTKLFWI THKDDQLTCV IMENGGCEQYC KRNASKPQGR IVGGKVCPKG IJLIAVL GEHD LSEHDGDEQS LPERTFSERT LAFVRFSLVS TיIEYMFCA.G Y SDGSKDSCKG YIENLQKLNR 3EPRPGVLLR PPCPAPELLG GPSVFLFPPK AKTKPREEQY NSTYRWSVL QVYTLPPSRD ELTKNQVSLT YSKLTVDKSR WQQGNVF3CS 5GGGGSGGGG SACTER*1A LH PCPAPELLGG PSVFLFPPKP KTKPREEQYN 3 TYRVVS VLT vytuppsRDE L'TRNQVSLTC SKLTVDKSRW QQGNVFSCSV

## DNA sequetice of FVII-048

atggtctccc aggccctcag gcagtc ttcg taacccagga gcgttcctgg aggagctgeg tccttcgagg aggeceggga tcttacagtg atggggacca gaccagctcc agtcctatat aegcacaagg atgaccagct agtgaccaca cgggcaccaa gacggggtgt cctgcacacc aaaagaaatg ccagcaaacc gagtoteccat ggcaggtcc atcaacacca t.ctgggtggt aacctgatcg cggtgctggg cggcgggtgg cgcaggtcat atcgcgctgc tccgcctgca ctgcccgaac ggacgttctc ggctggggcc agctgctgga ccccggctga tgacccagga atcacggagt acatgttctg gacagtggag gcccacatgc agetggggee agggctgege tacatergagt ggctgcaaaa gccccatttc ccggtggcgg gggggtggat caggcggtgg ccaccgtgcc cagctccgga cccaaggaca ccctcatgat agecacgaag accctgaggt gecaagacaa agecgeggga accgtcctgc accaggactg gccetcccag cccccatcga caggty:aca ccctgccccc tgcctggtca aaggc ttcta ceggagaaca actacaagac tacagcaagc tcaccgtcga gtgatgcatg aggctctgea aaaggcggtg gcggttcagg tccggtggcg gagggtcagg aacctgtgcg gtggcgatgg

AVFVTQEEAH GVLHRRRRAN | SY3DGDQCAS | SPCQNGGSCK |
| :--- | :--- |
| SDHTGTKRSC | RCHEGYSLLA | ECPWQVLLLV NGAQLCGGTL RRVAQVITES TYVPGTTNHD GWGQLLDRGA MA LE LMVILNV DSGGPHATHY RG?WYTYGIV APFPGGGGSG GGGSGGGSG SKGQGCATVG PKDTLMISRT PEVTCVVVDV TVLHODWLNG KWUNG CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL VMIEALHNHY TQKSLSLSFG KGGGGSGGGG SGGGGSGGGG WXCGGGGSGG GG3GGGG3GG GGSGGGG3GG GG3DKTHTCP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ LVKGFYPSDI AVSWE SNGQP ENNYRTTPPV LD3DGSFFLY MHEALHNHY: QK3LSLSPGK

gctcctctigc ettctgettg ggaagcccac gccgggctcc gatcttcaag gtgtgcctca ctgcttctgc gatctgtgtg gcgctcctgt cacagttgaa ccaaggccga gttgttggtg ctccgcggcc cgagcacgac catccccagc ccagcccgtg tgagaggacg ccgtggcgcc ctgcctgcag tgccggctac cacccactac aaccgtgggc gcteatgege tggctccggc aggttccggt actcctggga ctcccggacc caagttcaac ggagcagtac gctgaatggc aaggagtaca gaaaacca :o tccaaagcca atecegggat gagetrgacca tcccagcgac atcgccgtgg cacgcctccc gt.gtt.ggact caagagcagg tggcagcagg caaccactiac acgcagaaga tggaggaggg teaggeggtg cggtggcgga tcagcctgca ctccggcgga ggtgggtccg
ggovtcaggg ctgcctggc t accggcgccg gcgcgccaac agtgcaagga ggagcagtgc ggacgaagct. gttctggatt. agaatggggg ctcctgcaag tcgagggccg gaactgtgag gcggctgtga gcagtactgc aggggtactc tetgetggea gaaaaatacc tattctagaa gcaaggtgtg ccccaaaggg agt..gt.gtgg ggggaccctg acaaaatcaa gaactggagg aegaegggga tgagcagage. cgggcaccac caaccacgac accatgtggt. gcccctctgc tgcgcttctc attggtcagc agctcatggt. cctcaacgtg aggtgggaga ctccccaaat gcaaggactc ctgcaagggg ggtacctgac gggcatcgtc tgtacaccag ggtctcccag geccaggagt cctectgega ccggtggcgg cggatcaggt ccgacaaaac tcacacatgc tcttcctctt ccccccaaaa catgcgtggt. ggtggacgtg acggcgtgga ggtgcataat accgtgtggt cagcgtcctc agtgcaaggt ctccaacaaa aagggcagee ccgagaacca agaaccaggt cagcctgacc agtgggagag caat.gggcag ccgacggctc cttcttcctc gga aegtett ctcatgctcc gcctctccct. gtctccgggt gtggatccgg egggggegga ccgageggat. ggccctgcac gtggcggcgg atcaggtggg

FVII-049 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FV1l or OS2 to Fc regions is underlined, and OS2 peptide is italicized

SFEEARE IFK DAERTKJFWI THKDDQLICV NENGGCEQYC
KRNASKPQGR IVGGKVCPKG
NLIAVLGEHD LSEHDGDEQS
LPERTFSERT LAFVRFSLVS
ITEYMFCAGY SDGSKDSCKG
YIEWLQKLMR SEPRFGVLLR
PPCPAPELLG GPSVFLFPPK
AKTKPREEQY NSTYRVV3VL
QVYTLPP3RD ELTKNQVSLT
YSKLTVDKSR WQQGNVFSCS SGGGG 3GGGG SACTERDALH $\begin{array}{ll}\text { PCPAPELLGG } & \text { P3VFLFPPKP } \\ \text { KTKPREEOYN } & \text { STYRVVSVLT }\end{array}$ VYTLPPSRDE LTKNQVSLTC SKLTVDKSRW QQGNVFSCSV

| AVFVTQEDAH | GVLAPPRRRAN | AFLEELRPGS | LERECKEEQC |
| :---: | :---: | :---: | :---: |
| SY3DGDQCAS | SPCQNGG3CK | DOLOSYICFC | LPAFEGRNCE |
| SDHTGTKRSC | RCHEGYSLLA | DGVSCTPTVE | YPCGKIPILE |
| ECPWOVLLLV | NGAQLCGGTL | IKTISVVSAA | HCFDKIKNWR |
| RRVAQVIIPS | TYVEGTMNHD | IALLRLKOPV | VLTDHVVPLC |
| GWGOLLDRGA | TALELMVLNV | PRLMTQDCLQ | QSRKVGDSPN |
| DSGGPHATHY | RGTWYLTGIV | SWGQGCATVG | HFGVYTRVSQ |
| APFPGGGGSG | GGGSGGGGSG | GGGSGGGGSG | GGGS DKTHTC |
| PKDTLMI SRT | PEVTCVVVDV | SHEDPEVKFN | WYVDGVE VH? |
| TVLUQDWLNG | KEYKCKVSNK | ALDAPIEKTI | SKAKGQPREP |
| CLVKGFYPSD | IAVEWESNGQ | PENNYKTTPP | VLD3DGSFFL |
| VMAEALHNHY | YQKSLSLSPG | KGGGG SGGGG | SGGGGSGGGG |
| MXCGGGGSGG | GGSGGGG3GG | GGSGGGGSGG | GG SOKJHTICP |
| KD'TLMISRTP | EVTCVWDVS | HEDPEVKFNA | YVDGVEVHNA |
| VLHODWLNGK | EYKCKVSNKA | LPAPIEKTI3 | KAKGOPREPQ |
| LVKGFYPSDI | AYENESNGQP | ENNYKTTPPV | LDSDG3FFLY |
| MHEALHNI-IYT | QKSLSLSPGK |  |  |


#### Abstract

gggggatccg acaaaactca cacatgccca ccgtcagtct tcctcttccc cccaaaaccc gaggtcacat gcgtggtggt ggacgtgagc tacgtggacg gcgtggaggt gcataatgcc agcacgtacc gtgtggtcag cgtcctcacc gagtacaagt gcaaggtctc caacaaagcc aaagccaaag ggcagccecg agaaccacag ctgaccaaga accaggtcag cctgacccgc gccgtggagt gggagagcaa tgggcagccg ctggactccg acggcccctt cttcctctac cagcagggga acgtcttctc atgctccgtg cagaagagcc tctccetgtc tccgggtaaa


| ccgtgcccag | caccggaact |
| :--- | :--- |
| aaggacaccc | tcatgatctc |
| cacgaagacc | ctgaggtcaa |
| aagacaaagc | cgcgggagga |
| gtcctgcacc | aggactggct |
| ctcccagccc | ccatcgagaa |
| gtgtacaccc | tgcccccatc |
| ctggtcaaag | gcttctatcc |
| gagaacaact | acaagaccac |
| agcaagctca | ccgtggacaa |
| atgcatgagg | ctctgcacaa |
| tga |  |

40 DNA sequence of FVII-049 atggtctccc aggccctcag gcagtcttcg taacccagga gcgttcctgg aggagctgcg tccttcgagg aggcccggga tcttacagtg atggggacca gaccagctcc agccctatat acgcacaagg atgaccagct agtgaccaca cgggcaccaa gacggggcgt cctgcacacc gaatg ccagcaaace gagtgtccat ggcaggtcct atcaacacca tctgggtggt aacctgatcg cggtgctggg cggcgggtgg cgcaggtcat atcgcgetgc tccgcctgca ctgcccgaac ggacgttctc ggctggggcc agccgctgga ccccggctga tgacccagga ateaeggagt acatgetctg gacagtggag gcccacatgc agetggggee agggctgege tacatcgagt ggctgcaaaa gccccatttc ccggtggegg gggggtggat caggcggtgg

getgc gccagcccac gatcttcaag gtgtgectca ctgcttccgc gatctgtgtg gcgctcctgt cacagttgaa ccaaggccga gttgttggtg ctccgcggcc cgagcacgac catccocagc ccagcccgeg cgagaggacg ccgtggcgec ctgcctgcag tgccggctac cacccactac aaccgtgggc gctcatgcgc tggctccggc aggttccggt
cttctgcttg ggcttcaggg ctgcctggct ggcgtcctgc accggcgecg gcgcgccaac ctggagaggg agtgcaagga ggagcagtgc gacgcggaga ggacgaagct gttctggatt agtccacgcc agaatggggg ctcctgcaag ctccctgcct tcgagggceg gaactgtgag aacgagaacg gcggctgtga gcagtactgc cggtgccacg aggggcactc tctgctggca tatccat:g tg gaaaatacc tactctagaa attgtggggg gcaaggtgtg ccccaaaggg aatggagctc agttgtgtgg ggggaccctg cactgtttcg acaaaatcaa gaactggagg ctcagcgagc aegaegggga tgagcagagc acgtacgtcc cgggcaccac caaccacgac gtcctcactg accatgtggt gcccctccgc ctggccctcg tgcgcttctc attggtcagc acggccotgg agcccatggt cctcaacgcg cagtcaegga aggtgggaga ctccccaaat ccggatggca gcaaggactc ctgcaagggg eggggcaegt ggtacctgac gggcatcgtc cactttgggg tgtacaccag ggtctcccag tcagagccac geccaggagt cctcctgcga ggaggtgggt ccggtggcgg eggatcaggt ggegggggat cogacaaaac tcacacatgc

| actcctggga | ggaccgtcag | tcttcctctt | ccccccaa ad |
| :---: | :---: | :---: | :---: |
| caagttca ac | tggtacgtgg | acggcgtgga | ggtgcata at |
| ggagcagtac | aacagcacgt | accgtgtggt | cagcgtcctc |
| gctgaatggc | aaggagtaca | agtgcaaggt | ctccaacaa |
| gaaaaccatc | tccaaagcca | aagggcagee | ccgagaacca |
| atecegggat | gagctgacca | agaaccaggt | cagcctgacc |
| tcccagcgac | atcgccgtgg | agtgggagag | caatgggcag |
| cacgectccc | gtgttggact | ccgacggctc | ottcttccto |
| caagagcagg | tggcagcagg | ggaacgtett | etcatge サ- |
| caaccactac | aeccagadya | gcctctccet | gtctccgggt |
| tggaggaggg | teaggeggtg | gtggatccgg | egggggegga |
| cggtggcgga | tcagcctgca | ccgagcggga | cgccetgcac |
| ctccggcgga | ggtgggtccg | gtggcggcgg | atcaggtggg |
| ttccggtggc | gggggatccg | acaaaactca | cacatgccea |
| cctgggcgga | ccgtcagtct | tcctcttccc | cccaaaaccc |
| ccggacccet | gaggtcacat | gcgtggtggt | ggacgtgagc |
| gttcaactgg | tacgtggacg | gcgtggaggt | geataatgee |
| gcagtacaac | agcacgtacc | g toytactcag | cgtcctcacc |
| gaatggcaag | gagtacaagt | gcaaggtctc | caacaaagcc |
| aaccatctcc | aaagccaaag | ggcagccceg | agaaccacag |
| cegggatgag | ctgaccaaga | accaggtcag | cctgacctgc |
| cagcgacatc | gccgtggagt | gggagagcaa | tgggcagccg |
| gcctccogtg | ggactccg | acggctcctt | cttcctci;ac |
| gagcaggtgg | cagcagggga | acgtcttctc | atgctccgtg |
| ccactacacg | cagaagagee | tctccctgtc | teegggtaaa |

FVn-01 1 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlmed, Gla domam is italicized, linker region connecting FVll to Fc region is underlined, and linker connecting both Fes sites is shown in bol

|  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| EAREI | DAERTKLFS | SYSDGDQCAS | 3PCQNGG3CK | Delesyicfo | lpafsgrnce |
| GkDDLIICV | neng cisquc | SDhtgTkRSC | RCMEGYSLLA | dgvsctptve | ypCgkipile |
| kRNASKPQGR | IVGGKvcpig | ECFWQVLLLV | ngadicgerl | IntIwwSAA | hCFDKIKNWR |
| NLIAVLGE ${ }^{\text {d }}$ | LSEHDGDEQS | RRVAQVIIPS | tyvpgitnhd | IALLRLHQPV | vlidhhvvplc |
| LPERTFSERT | LAFVRFSLVS | GwGQLIDRGA | talelmvinv | PRLMTQDCLQ | Q3RKvgdspn |
| ITEYMFCAGY | sdgskdsckg | DS | RGTelyditiv | SwGqGCATVG | HF |
| YeEwLekimf | SEPRPGVLLR | APFPGGGGSG | GGGSGGGG3G | GGGSGGGGSG | gGG dimpht |
| ppCPAPELLG | GPsvflfppk | PRDTLMISR | Pevtcuwdv | SHEDPEVKEN | wYVDGVEVHN |
| aKtkpreeqy | kstyrvvsvi | TVLhQDiling | ksykckvsmk | ALPAPIEkTI | SKAKGQPREP |
| QVYTLPPSRD | eltinevslt | Clviggrypsd | IA veresmge | Pennykttpp | VLDSDGSFFL |
| YSKI.TVDKS | WQQGnvfscs | vmhe mbhnay | tgkslslibg | KGGGGSGGGG | SGGGGSGGGG |
| sdKthtcpp | PAPELLGGPS | vFlfppkpkd | timisrtpev | TCVWDVSHE | DPEvkFNvvYV |
| DGvevinakt | KPREEQYNST | YRVVSVLTVL | HQ DWLINGKEY | KCKVSsmal | ApIEKTIS |
| QPREP |  |  |  |  | nykt ${ }^{\text {depvid }}$ |

FV11-G1 I DNA sequence atggtctcce aggecctcag gcagtcttcg taacccagga gcgttcctgg aggagctgeg tccttcgagg aggeceggga tcttacagtg atggggacca gaccagctcc agtcctatat
aegcacaa agtgaccac gacgggtgt aaaagaaatg gagtgtccat ggcaggtcct

| gctcctctgc | ettctgettg | ggcttcaggg | ctgcctggct |
| :--- | :--- | :--- | :--- |
| ggaagcccac | ggcgtcctgc | accggcgccg | gcgcgccaac |
| geeggge tec | ctggagaggg | agtgcaagga | ggagcagtgc |
| gatcttcaag | gaegeggaga | ggacgaagc t gttctggatt |  |
| gtgtgcc tca | agtccatgcc | agaatggggg | ctcctgcaag |
| ctgcttctgc | ctccctgcct | tegagggecg | gaactgtgag |
| gatctgtgtg | aegagaacg | gcggctgtga | geagtactge |
| gcgctcctgt | cggtgccacg | aggggtactc | tetgetggea |
| cacagttgaa | tatccatgtg | gaaaaatacc | tattctagaa |
| ccaaggccga | attgtggggg | gcaaggtgtg | ccccaaaggg |
| gttgttggtg | aatggagctc | agtegtgtgg | ggggaccctg |

atcaacacca aacctgatcg cggcgggtgg atcgcgctgc ctgcccgaac ccccgqctqa atcacggagt gacagtggag agctggggcc
gccecatttc gggggtggat ccaccgtgcc cccaaggaca agccacgaag gccaagacaa accgtcctgc gccct:cccag caggt:gt:aca tgcctggt.ca ccggagaaca tacagcaagc gtgatgcatg aaaggtggcg tcagacaaaa gtcttcctct acatgcgtgg gacggeg t.gg taccgtgtgg aagtgcaagg aaagggcagc aagaaccagg gagtgggaga tccgacggct. gggaacgtct agcctctccc

cactgtttcg
ctcagcgagc acgtacgtcc gtcctcactg ctggccttcg acggccctgg cagtcacgqa tcggatggca cggggoacot cactttgggg tcagagccac ggaggtgggt. ggcgggggat ggaccgtcag cctgaggtca tggtacgtgy aacagcacgt aaggagcaca

## tccaaagcca

gacetgacca atcgccgtgg gt.gttggact tggcagcagg aegcagaaga teaggeggtg ccagcacctg accetcatqa gaccctgagg aagccgcgqq caccaggact gcceccatcg auctigcucc aaaggectet aactacaaga ctcaccgtgg gaggctctgc
aca aatcaa gaactggagg aegaegggga tgagcagage. cgggcaccac caaccacgac accatgtggt gcccctctgc tqcqcttctc attggtcagc agcccatggt cctcaacgtg aggtgggaga ctccccaaat gcaaggactc ctgcaagggg ggtacctgac gggcatcgtc tgtacaccag qgtctcccag geccaggagt cctcctgcga ccggtggcgg cggatcaggt ccgacaaaac tcacacat.gc. tcttcctctt ccccccaaa catgcgtggt ggtggacgtg acggcgtgga gqtqcataat accgtgtugt cagcgtcctc agtgcaaggt ctccaacaaa aagggcagee ccgagaacca agaaccaggt cagcctgacc agtgggagag caatgggcag ccgacggctc cttcttcctc gga aegtett ctcatgctcc gcctctccct gtctccgggt gaggttcegg tggcggggga aactcctggg aggacegtea tctccoggac ccetgaggtc tcaaqttcaa ctggtacgcg aggagcagta caacagcacg ggctgaatgg caaggagtac.
agaaaaccat ctccaaagcc catcccgcga Cgagctgacc atcccagcga categcegtg ccacgcotcc egtgttggac acaagagcag gtggcagcag acaaccacta caegcagaag

B domain deleted FVTTI amino acid sequence: Signal peptide underlined; 14 amino a.cid linker (containing the remaining $B$ domain) between the HC and IIC sequence is double underlined, with the S743/Q1638 fusion site indicated in bold.

$\begin{array}{ll}\text { CLLRECESA } & \text { TRRYYLGAVE } \\ \text { SW YKKET_F } & \text { VSFTDHLFNI } \\ \text { SHPVSLHAV } & \text { GVSYWKASEG }\end{array}$ GVSYWKASEG $\begin{array}{ll}\text { KENGPMASDP } & \text { LCLTYSYLSH } \\ \text { HKFILLFAV } & \text { FDEGKSWHSE }\end{array}$ PPGLIGCHRK SVYWHV IGMG FIrrAQ ITJMM EAEDYDDDLT 351 EPQLR1MKNNE 401 WVHYIAAEEE DWDYAPLVLA DSEMDVVRFD PDDRSYKSQY LLYGEVGDTL PILPGEIFKY KNTVTVEDGP J. QLEDPEFQAS NEMHSJNGYV MTALLTVSSC DKNTGDYYED R-HIREITRTTL QSDQEEIDYD LWDYGMSSSP LGLLGP YIRA FVKPNE'TKI'Y LVCHTNTLNP
QMEDPTFKEN QMEDPTFKEN

GELPVDARFP LGPTIQAEVY EKEDDKVFPG LIGALLVCRE AA SA RAWPKM EGKTFLVRNH AYVKVDSCPE RSVAKKHPKT KYKKVRFMAY PYNIYPHGIT TKSDPRCLTR NV ILFSVFDE FDSLOLSVCL PFSGETVFMS SYEDISAYLL DTIISVEMKKE HVLRNRAQSG EVEDNI MVTF FWKVQHHTVAP A. RGRQVTVQE YRFHAI NGYI

| 1101 | YNLYPGVFET | VEMT, PSKAG I | WRVECLIGEH |
| :---: | :---: | :---: | :---: |
| $1 \pm 51$ | GVASGMIRD F | QITASGQYGQ | WAPKLARLKY |
| 1201 | LAPMTIKGIK | TQGARQKFSS | LYISQFIIMY |
| 1251 | FFGNVDSSGI | KINIFNPPII | ARYIRLHPT |
| 1301 | MPLGMESKA I | SDAQITASSY | FTNMFATWSP |
| 1351 | PKEWLQVDFQ | KTMKVTGVTT | QGVKSLLTSM |
| 140 l | QNGKVKVFQG | NQDSFTPVVN | SLDPPLLTRY |

IHFSGHVFTV LHAGMSTLFL SGSINAWSTK SLDGKKWQTY YSIRSTLRME SKARLKLQGR YVKEFLISSS IFIHPQSWVH

RKKE تykmad VYS $\mathrm{N} K \mathrm{KCQTPL}$ EPFSWIKVDI RGNS TGTLMV LMGCDLNSCS SNAWRPQVNN QDGHQWTLFF QtalRMEVLG

Full length FVITI amino a.cid sequence: Sigral peptide underlined

| ; | MQIELSTCFF | LCLLRFCFSA |
| :---: | :---: | :---: |
| 51 | PRVPKSFPFN | TSWYKKTLF |
| 101 | DTWITLKKM | ASHPVSLHAV |
| 151 | GSHTYVWQVL | KENGPMASDP |
| 201 | GSLAKEKTQT | LHKFILLFAV |
| 251 | HTVNGYVNR S | LPGLIGCHRK |
| 301 | RQASLEISPI | TFLTAQTLLM |
| 351 | EPQLRMKNNE | EAEDYDDDLT |
| 401 | WVHYIAAE ${ }^{\text {V }}$ 河 | DWDYAPLVLA |
| 451 | TDETFKTREA | IQHSSGILGP |
| $50 \%$ | DVRPLYS RRL | PKGVKHLKDF |
| 551 | YYSSFVNMER | DLASGLIGPL |
| 601 | NRSWYLTENI | QRFLPNPAGV |
| $65 \%$ | HEVAYWYILS | IGAQTDFLSV |
| 701 | MENPGLWILG | CHNSDFRNRG |
| 751 | SKNNAIEPRS | FSQNSRHPST |
| 001 | IQNVSSSDLL | MLLROSPTPH |
| 851 | SEMTHFRPQL | HHSGDMVFTP |
| 301 | SNNLISTIPS | DNLAAGTDNT |
| 951 | SGGPLSLSEE | NNDSKLLESG |
| $100 \%$ | ALLTKDNALF | KVS J SLLKTN |
| 1051 | ILESDTEFKK | VTPLIFDRMI. |
| 1101 | EGPIPPDAQN | PDMSFFKMLF |
| 115 T | LGPEKSVSGQ | NFJ:SBKNKW |
| 1201 | LHENNTKNQE | KKIQHEIEKK |
| 125. | TRQNVEG SYD | GAYAPVLQDF |
| 1301 | NQTKQIVEKY | ACTTRISPNT |
| 1351 | IVDDTSTQWS | KNRMKHETPST |
| 1401 | QANRS JLPIA | KVSSFPSIRP |
| 1451 | SHFLQGAKKN | NLSLAILTLE |
| $150 \%$ | KPDLPKTSGK | VELLPKVHIY |
| 1551 | A IKWNEANRP | GKVPFLRVAT |
| 1601 | SQEKSPEKTA | FKKKDT ILSL |
| 1651 | TERLCSQNPP | VLKRKQRE T |
| 1701 | EDENQSPRSF | QKKTRHYF IA |
| 175:. | KVVFQEFTDG | SFTOPLYRGE |
| 1001 | PYSFYSSLIS | YEEDOROGAE |
| 1051 | CKAWAYFSDV | DLEKDVHSGL |
| 1901 | IFDETKSWYF | TENMSRNCRA |
| 1951 | LVMAQDQRIR | WYLLSMGSNE |
| 2001 | VFETVEMLPS | KAGIWRVECL |
| 2051 | IRDFQITASG | QYGQWAPKLA |
| 2101 | HGIKTQGARQ | KFSSLYISQF |
| 2151 | SSGIKHNIFK | PPIIARYIRL |
| 2201 | SKAISDAQIT | ASSYFTNMFA |
| 2251 | VDFQKTMKVT | GVTTQGVKSL |
| 2301 | VFQGNQDSFT | PVVNSLDPPL |
| 2351 | Y |  |

TRRYYLGAVE LSWDYMOSDI VEFTDHLFNI AKPRPPWMGL GVS YWKAS EG AEYDDQTSQR LCLTYSYLSH VDLVKDLNSG FDEGKS WHSE TKNSLMQDRD SVYWHVIGMG TTPEVHSIFL DLGQFLLFCE ISSHQHDGME D SEMDWRFD DDNSPSFIQI PDDRSYKSQY LNNGPQRIGR LLYGEVGDTL LIIFKNQASR PILPGEIFKY KWTVTVEDGP LICYKESVDQ RGNQIMSDKR QLEDPEFQAS NIMHSINGYV FFSGYTFKKK MVYEDTLTLF MTALLKVSSC DKNTGDYYED RQKQFNATTI PEND IEKTDP GLSLSDLQEA KYETFSDDPS ESGLQLRLNE KLGTTAATEL SSLGPPSMPV HYDS QLDTTL LMNSQESSWG KTSNNSATNR MDKNATALRL LPESARWIQR VGKGE FTKDV STLIQENVVL RSLNDSTNRT SQQNFVTQRS LTQIDYNEKE rYLTRVLFQD MTGDQREVGS OKDLFPTETS ESSAKTPSKL NACESNHAIA RTTLQSDQEE AVERLWDYGM LNEHLGLLGP PRKNFVKPNE IGPLLVCHTN PCNIQMEDPT NIHSIHFSGH IGEHLHAGMS RLHYSGS INA WSTKEPFSWI KVDLLAPMI I IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD HPr!HYSIRST LRMELMGCDL NSCSMPLGME TWS PSKARLK LQGRSNAMRP QVNNPKEWLQ LTSMYVKEFL ISSSQDGHQW TLFFONGKVK. LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL

FIX amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined
 ERECMEEKCS FEEAREVFEN TERTTEFWKQ YVDGDQCESN PCLNGGS CKD DINSYECWCP FGFEGKNCEL DVTCNIKNGR CEQFCKNSAD NKWCSCTEG YRLAENOKSC EPAVPFPCGR VSVSQTSKLT RAETVFPDVD YVNSTEAETI LDNITQSTQS FNDFTRWGG EDAKPGQFPW

QVVLnigkvda fcgas IVnek wivtanhcve tgvkitvvag EHNIEETEHT EQKRNVIRI I PHHNYNAAIN KYNHDIALLE LDEPLVLNSY VTPICIADKE YTNIFLKFGS GYVSGWGRVF HKgRSARVLQ YLRVPLVDRA TCL.RSTKETY YNNMFC.AGFK EGGRDSCQGD SGGPKVTEVE GTSFITGIIS WGE EC7-VMKGK YGIYTKVSRY VNWIKEKTKL T

FIX dna sequence

| atgt.acagtt | t | atgaaaaege | acaaaatt |
| :---: | :---: | :---: | :---: |
| aattcaggt | aaat.t.ggaag | agtttgttc | agggaatct |
| gt.gt.agt | tttgaagaag | cacgagaagt | tt.t.t.gaaa ac |
| gaagcag | tatgttgatg | gatcagtg | agtccaat |
| gcaaggat | cattaatt | cctatgaatg | tggtgtccc |
| ctgtgaatta | gatgtaacat | gtaacattaa | gaatggcaga |
| tagtgetgat | aacaaggtgg | tttgctcctg | tactgaggga |
| gaagtcctgt | aaccagcag | tgccatttcc | atgtggaaga |
| aagctcacc | gtgc tgaga | ctgtttttcc | gatgtggac |
| gaaacca tt | ggataaca | tcac tcaag | caat |
| ggtgga | gaagatgeca | aaccaggtca | attccettgg |
| agt.t.gat.gca | ttctgtggag | gctctatcgt | aat.gaaa |
| tgtgttgaa | actggtgtt | aattacagt | gtegcaggt |
| gaacataca | gagcaaaagc | gaaatgtgat | cgaattatt |
| tattaat | aagtacaacc | atgacattgc | cttctggaa |
| agctac | gttacaccta | tttgcattgc | gacaaggaa |
| tttggatct | ggctatgta | gtggctgggg | aagagtcttc |
| tcttcag | taccttagag | ttccacttgt | tgaccgagcc |
| gtt caeca tc | aca | tctgtgc | tt.eea |
| caaggaga | gggggac | cccatgttac | gaagtggaa |
| attattagc | ggggtgaag | agtgtgcaat | aaaggcaaa |
| ateceggtat | gtcaactgga | ttaaggaaaa | acaaagctc |

FX amino acid sequence. Signal sequence is shown in dote d underline, propeptide is double underlined
MGRFFHVYL GASLAGLIII GESLFIREEO ANNLLARYIE TCS YEEAREV FEDSDKTNEF WMKYKDGDQC ETSPCQKQGK Celftrklc8 LDngdcdefc Heeqinsvvcs cargytladn KR8V7VQATSS SGEAPDSITW KFYDAADLD? TENPFDLLDF CKDGEC PWQA LLINEENEGF CGGTILSEFY ILTAAHCLYQ AVHEVEVVIK HNRFTKETYD FDIAVLRLKT PITFRMNVAP VSGFGRTHEK GRQSTRLKML EVPYVDRMSC KLSSSFIITQ G? HV TRFK.DT YFVTGIVSWG VJ.J.SSPLK

50 FX DNA sequence
atggggegce cactgcacet ggggaaagtc tgttcatccg gccaattcct ttcttgaaga acctgctcat
tggaataaat acaaagatgg tgtgaattat cacgaggaac agaactctgt ggcaaggect gcattcccac aagaggtcag tggcccaggc aagccatatg aaccagacgc tgcaaggacg tgtggtggaa agectgagag gggagtgt.ee
cgtcctgctc cagggagcag gatgaagaaa ccgegaggtc cgaccagtgt atacacctgc gctctgcagc ggtgtgctcc agggecctac caccagcagc cctggaccoc gggegacaac ctggcaggec cgagttctac

| agtgcctccc | tggctggcct | cctgctgctc |
| :--- | :--- | :--- |
| gccaacaaca | tectggegag | ggtcacgagg |
| ggacacctc.g | aaagagagtg | catggaagag |
| tttgaggaca | gegacaagae | gaatgaattc |
| gagaccagtc | cttgccagaa | ccagggcaaa |
| acctgtttag | aaggattcga | aggcaaaaac |
| ctggacaacg | gggactgtga | ccagttctgc |
| tgcgcccgcg | ggtacaccct | ggctgacaac |
| ccctgtggga | aacagaccct | ggaaegcagg |
| ageggggagg | cccctgacag | catcacatgg |
| accgagaacc | ccttcgacct. | gcttgacttc |
| aacctcacca | ggatcgtggg | aggecaggaa |
| ctgctcatca | atgaggaaaa | cgagggttte. |
| atectaaegg | cagcccactg | tctctaccaa |


| eggaacaegg | agcaggagga |
| :--- | :--- |
| cacaaccggt | tcacaaagga |
| cccatcacct. | tccgcatgaa |
| tccacgctga | tgacgcagaa |
| ggccggcagt | ccaccaggct |
| aagctgtcca | gcagcttcat |
| caggaggatg | cctgccaggg |
| tacttcgtga | caggcatcgt |
| atetacaeca | aggtcaccgc |
| ttgeccaagg | ccaagagcca |

gggcggtgag
gacctatgac
cgt.ggcgcct
gaeggggatt
caagatgetg
catcacccag
ggacagcggg cagctgggga cttcctcaag tgccccggag

DNA sequence of GVI1--066
ATGGTC TCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG
CTGCCTGGCT
B1 GCAGTCTTCG TAACCCAGGA ! 21 GCGTrCCTGG AGGAGCTGCG 131 TCCTTCGAGG AGGCCCGGGA 241 TCTTACAGTG ATGGGGACCA 301 GACCAGCTCC AGTCCTATAT 361 ACGCACAAGG ATGACCAGCT 421 AGTGACCACA CGGGCACCAA 431 GACGGGGTGT CCTGCACACC 541 AAAAGAAATG CCAGCAAACC 601 GAGTGTCCAT GGCAGGTCCT 661 ATCAACACCA TCTGGGTGGT 721 AACCTGATCG CGGTGCTGGG 731 CGGCGGGTGG CGCAGGTCAT 841 ATCGCGCTGC TCCGCCTGCA 901 CTGCCCGAAC GGACGTTCTC 961 GGCTGGGGCC AGCTGCTGGA 1.021 CCCCGGCTGA TGACCCAGGA - 0.01 ATCACGGAGT ACATGTTCTG -1. 11 GACAGTGGAG 1201 AGCTGGGGCC -. 261 TACATCGAGT J. 321 GCCCCATTTC 1. 351 GGGGGTGGAT -. 441 CCACCGTGCC 1. 501 CCCAAGGACA ]. 561 AGCCACGAAG - .621 GCCAAGACAA ]. 631 ACCGTCCTGC -. 741 GCCCTCCCAG CAGGTGTACA CCCTGCCCCC ]. 861 TGCCTGGTCA AAGGCTTCTA - 921 CCGGAGAACA ACTACAAGAC - 931 TACAGCAAGC TCACCGTCGA 2041 GTGATGCATG AGGCTCTGCA 2 01 AAACGGCGCC GCCGGAGCGG 2161 TCCGGTGGCG GGGGATCCGG 2221 AAGAGGGCGC AGGTGGAGGT 2231 CTGAGACTCT CCTGTGCAGC 234]. CGCCAGGCTC CAGGGAAGGG 2401 ACATACTACG CAGACTCCGT 2461 ACGCTGTATC TGCAAATGAA 2521 CGGGGCGCCA CCTACACCAG 2531 GGCCAGGGAA CCCTGGTCAC 2641 GGTGAATTTT CAGAAG CACG 2701 rTGGGACAGA CAGTCAGGAT 2761 TGGTACCAGC AGAAGCCAGG 2821 CCCTCAGGGA TCCCAGACCG 283]. ATCACTGGGG CTCAGG CGG 2941 GGCCAGCAGG GCGTGTTCGG 3001. GCCCCCTCGG TCACTCTGTT ? 061 GGTGGGTCCG GTGGCGGCGG

GGAAGCCCAC GGCGTCCTGC GCCGGGCTCC CTGGAGAGGG GAT CTTCAAG GACGCGGAGA GTGTGCCTCA AGTCCATGCC CTGCTTCTGC CTCCCTGCCT GATCTGTGTG AACGAGAACG GCGCTCCTGT CGGTGCCACG CACAGTTGAA TATCCATGTG CCAAGGCCGA ATTGTGGGGG GTTGTTGGTG AATGGAGCTC CTCCGCGGCC CACTGTTTCG CGAGCACGAC CTCAGCGAGC CATCCCCAGC CCAGCCCGTG TGAGAGGACG CCGTGGCGCC CTGCCTGCAG TGCCGGCTAC CACCCACTAC AACCGTGGGC GCTCATGCGC TGGCTCCGGC AGGTTCCGGT ACTCCTGGGA CTCCCGGACC CCTGAGGTCA CAAGTTCAAC TGGTACGTGG GGAGCAGTAC AACAGCACGT GCTGAATGGC AAGGAGTACA G A, AAACC ATC TCCAAAGCCA ATCCCGGGAT GAGCTGACCA TCCCAGCGAC ATCGCCGTGG CACGCCTCCC GTGTTGGACT CAAGAGCAGG TGGCAGCAGG CAACCACTAC ACGCAGAAGA TGGCGGCGGA TCAGGTGGGG CGGTGGAGGT TCCGGTGGGG GCAGGAGTCT GGGGGAGGCT CTCTGGATTC ATGTTTAGCA GCCAGAGTGG GTCTCAGGTA GAAGGGCCGG TTC ACCGTCT CAGCCTGAGA GCCGAGGACA CCGGAGCGAC GTGCCCGACC CGTCTCCTCA GGGAGTGCAT CGTATCTGAA CTGACTCAGG CACATGCCAA GGAGACAGCC ACAGGCCCCT ACTCTTGTCA ATTCTCTGCC TCCAGCTCAG AGATGAGGCT CGGCGGCACC CCCGCCCTCT ATCAGGTGGG

ACCGGCGCCG GCGCGCCAAC AGTGCAAGGA GGAGCAGTGC GGAC GAAGCT GTTCTGGATT AGAATGGGGG CTCCTGCAAG TCGAGGGCCG GAACTGTGAG GCGGCTGTGA GCAGTACTGC AGGGGTACTC TCTGCTGGCA GAAAAATACC TATTCTAGAA GCAAGGTGTG CCCCAAAGGG AGTTGTGTGG GGGGACCCTG ACAAAATCAA GAACTGGAGG ACGACGGGGA TGAGCAGAGC CGGGCACCAC CAACCACGAC ACCATGTGGT GCCCCTCTGC TGCGCTTCTC ATTGGTCAGC AGCTCATGGT CCTCAACGTG AGGTGGGAGA CTCCCCAAAT GCAAGGACTC CTGCAAGGGG GGTACCTGAC GGGCATCGTC TGTACACCAG GGTCTCCCAG GCCCAGGAGT CCTCCTGCGA CCGGTGGCGG CGGATC AGGT CCGACAAAAC TCACAC ATGC TCTTCCTCTT CCCCCCAAAA CATGCGTGGT GGTGGACGTG ACGGCGTGGA GGTGCATAAT ACCGTGTGGT CAGCGTCCTC AGTGCAAGGT CTCCAACAAA AAGGGCAGCC CCGAGAACC A AGAACCAGGT CAGCCTGACC AGTGGGAGAG CAATGGGCAG CCGACGGCTC CTTCTTCCTC GGAACGTCTT CTCATGCTCC GCCTCTCCCT GTCTCCGGGT GTGGATCAGG CGGTGGAGGT GTGGATCAAG GAAGAGGAGG TGGTACAGCC TGGGGGGTCC GGTATGCCAT GAGCTGGGTC TTAGTGGTAG TGGTGGTAGT CCAGAGACAA TTCCAAGAAC CGGCTGTATA TTACTGCGCC AGACCAGCTT CGACTACTGG CCGCCCCAAA GCTTGAAGAA ACCCTGCTGT GTCTGTGGCC TCAGAAACTT TTATGCAAGC TCTATGGTTT AAGTAAAAGG GAAACACAGC TTCCTTGACC GCCTGCTGTA CTACGGCGGC TCCTACGTCA GCCCAAGGCT GTGGCGGTGG CTCCGGCGGA GCGGTGGAGG TTCCGGTGGC

| 3.21 | GGGGGATCAG | ACAAAACTCA | CACATGCC.CA | CCGTGCCCAG | CACCGGAACT | CCTGGGCGGA |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 3161 | CCGTCAGTCT | TC.CTCTTCCC | CCCAAAACCC | AA_GGA.CAC.CC | TCATGATCTC | CCGGACCCCT |  |
| 3241 | GAGGTCACAT | GC.GTGGTGGT | GGACGTGAGC | CACGAAGACC | CTGAGGTCAA | GTTCAACTGG |  |
| 3301 | TACGTGGACG | GCGTGGAGGT | GCATAATGCC | AAGACAAAGC | CGCGGGAGGA | GCAGTACAAC |  |
| 3361 | AGCACGTACC | GTGTGGTCAG | CGTCCTCACC | GTCCTGCACC | AGGACTGGCT | GAATGGCAAG |  |
| 3421 | GAGTACAAGT | GC.AAGGTCTC | CAACAAAGCC | CTCCCAGCCC | CCATCGAGAA | AACCATCTCC |  |
| 3481 | AAAGCCAAAG | GGCAGCCCCG | AGAACCACAG | GTGTACAC.CC | TGCCCCCATC | CCGGGATGAG |  |
| 3541 | CTGACCAAGA | ACCAGGTCAG | CCTGACCTGC | CTGGTCAAAG | GCTTCTATCC | CAGCGACATC |  |
| 3601 | GCCGTGGAGT | GGGAGAGCAA | TGGGCAGCCG | GAGAACAACT | ACAAGACCAC | GCCTCCCGTG |  |
| 3661 | TTGGACTCCG | ACGGCTCCTT | CTTCC.TCTAC | AGCAAGCTCA | CCGTGGACAA | GAGCAGGTGG |  |
| 3721 | CAGCAGGGGA | AC.GTCTTCTC | ATGCTCCGTG | ATGCATGAGG | CTCTGCACAA | CCACTACACG |  |
| 3751 | CAGAAGAGCC | TC.TCCCTGTC | TCCGGGTAAA | TGA |  |  |  |

FVH-066 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVI or SCB5 to Fc region is underlined, and linker with proprotein converlase processing sites is shown in bold

1 MVSQALRLLC LLLGLQGCLA AVVV REEAEGVGFRRRRAN AFLEELRPGS LERECKEEQC

61 SFEEAREIFK DAERTKLTW SYSDGDQCAS SPCQNGGSCK DQLQSY CFC 121 THKDDQLICV NENGGCEQYC SDiZGTKRSC RCHEGYSLLA 131 KRUASFPQGR IVGGKVCPKG ECPiJQVLLLV NGAQLCGGTL 241 NLl AVLGEHD LSEHDGDEQS RRVAQVI IPS TYVPGTTNHD 30.1 LPERTPSERT LAF'VRF'SLVS GWGQLLDRGA TALELMVLNV 361 ITEYMPCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV 42.1 Y1EWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG 43.1 PPC 601 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL 661 YSKLTVDKSR SQQGNVFSCS VMHEALfiNHY TQKSL3LSPG KRRRRSGGGG SGGGGSGGGG 721 SGGCGSGGGG SGGGGSRKRR KRAQVQLQES GGGLVQPGGS LRLSCAASGF MFSRYAMSWV 731 RQAPGKGPEW VSGISGSGGS TYYADSVKGR FTVSRDNSKN TLYLOMNSLR AEDTAVYYCA 841 RGATYTSRSD VFDOTSFDYW GQGTLVTVSS GSASAPKLEE GEFSEARV3E LTQDPAVSVA 901 LGQTVRITCQ GDSLRNFYAS SYQQKPGQAP TLVIYGLSKR PSGIPDRF3A 3SSGNTASLT 961 ITGAQAEDEA DYYCLLYYGG GQQGVFGGGT KLTVLRQFKA APSVTLFPPS SftAGGGGSGG 1021 GGSGGGG3GG GGSGGGGSGG GG3DKTHTCP PCPAPELLGG PSVFLFPPKP KDTLM1SRTP 1031 EVTCVVVDVS EEDPEVKFNW YVDGVEVHMA KTKPREEQYN STYRVVSVLT VLHQDWLNGK 1141 EYKCKVSNKA LFAPIEKTIS KAKGQPREPO VYTLPPSRDE LTKNOVSLTC LVKGFYPSDI 1201 AVEWESNGQF ENMYKTTPPV LD3DGSFFLY SKLTVDKSRS QOGNVFSC3V MfiEALHMfiYT 1261 OKSLSLSPGK *

SEVTCVVVDV SEEDPEVKF'N WYVDGVEVHN DQLQSY1CFC DGVSCTPTVE YPCGK1 PILE RLLRLHQPV VLTDHWPLC PRLMTQDCLQ QSRKVGDSPN SWGQGCATVG HFGVYIPVSQ GGGSGGGGSG GGGSDKTHTC

DNA sequence for FV1 I-0 57
ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CT「CTGC TG GGCTTCAGGG CTGCCTGGCT

GCAGTCTTCG 121 GCGTTCCTGG 131 TCCTTCGAGG 241 TCTTACAGTG 301 GACCAGCTCC 361 ACGCACAAGG 421 AGTGACCACA 431 GACGGGGTGT 601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA 661 GACAAAACTC ACACATGCCC ACCGTGCCCA 721 TTCCTCTTCC CCCCAAAACC CAAGGACACC 731 TGCGTGGTGG 841 GGCGTGGAGG 901 CGTGTGGTCA
961 TGCAAGGTCT 1021 GGGCAGCCCC 1031 AACCAGGTCA G 1141 TGGGAGAGCA A 1201 GACGGCTCCT 1261 AACGTCTTCT 1.321 CTCTCCCTGT 331 GGTTCCGGTG GCGGGGGATC CGGCGGTGGA 1441 GGTTCAAGCG T 1501 AAAGGGGAGT GTCCATGGCA GGTCCTGTTG TTGGTGAATG GAGCTCAGTT GTGTGGGGGG 1561 ACCCTGATCA ACACCATCTG GGTGGTCTCC GCGGCCCACT GTTTCGACAA AATCAAGAAC

DNA sequence for FVIi－058
1 ATGGTCTCCC
61 GCAGTCTTCG 121 GCGTTCCTGG 181 TCCTTCGAGG 241 TCTTACAGTG 301 GACCAGCTCC 361 ACGCACAAGG 421 AGTGACCACA 481 GACGGGGTGT 541 AAAAGAAATG 601 GGTGGCGGCG 661 GACAAAACTC 721 TTCCTCTTCC 781 TGCGTGGTGG 841 GGCGTGGAGG 91 CGTGTGGTCA 961 TGCAAGGTCT 1021 GGGCAGCCCC 1081 AACCAGGTCA

1681 CAGAGCCGGC 1741 CACGACATCG 1801 CTCTGCCTGC 1 © 61 GTCAGCGGCT 1921 AACGTGCCCC 1981 CCAAATATCA 2041 AAGGGGGACA 2． 01 ATCGTCAGCT 2 TCCCAGTACA 2221 CTGCGAGCCC 2281 TCAGGTGGGG 2341 ACATGCCCAC 2401 CCAAAACCCA 2461 GACGTGAGCC 2521 CATAATGCCA 2581 GTCCTCACCG 2641 AACAAAGCCC 2701 GAACCACAGG 2761 CTGACCTGCC 2821 GGGCAGCCGG 2881 「［ワTC＂ 2881 CClCTACA 3001 CCGGGTAAAT

621 TGGAGGAACC TGATCGCGGT GCTGGGCGAG CACGACCTCA
TGATCGCGGT GCTGGGCGAG CACGACCTCA GGGTGGCGCA GGTCATCATC CCCAGCACGT CGCTGCTCCG CCTGCACCAG CCCGTGGTCC CCGAACGGAC GGGGCCAGCT GGCTGATGAC CGGAGTACAT GTGGAGGCCC GGGGCCAGGG TCGAGTGGCT CATTTCCCGG GTGGATCAGG CGTGCCCAGC AGGACACCCT ACGAAGACCC AGACAAAGCC TCCTGCACCA TCCCAGCCCC TGTACACCCT TGGTCAAAGG AGAACAACTA GCAAGCTCAC TGCATGAGGC GA

GCGAGCACGA CGGGGATGAG ACGTCCCGGG CACCACCAAC TCACTGACCA TGTGGTGCCC CCTTCGTGCG CTTCTCATTG CCCTGGAGCT CATGGTCCTC CACGGAAGGT GGGAGACTCC ATGGCAGCAA GGACTCCTGC GCACGTGGTA CCTGACGGGC TTGGGGTGTA CACCAGGGTC AGCCACGCCC AGGAGTCCTC GTGGGTCCGG TGGCGGCGGA GGGGATCAGA CAAAACTCAC CGTCAGTCTT CC7CTTCCCC AGGTCACATG CGTGGTGGTG ACGTGGACGG CGTGGAGGTG GCACGTACCG TGTGGTCAGC AGTACAAGTG CAAGGTCTCC AAGCCAAAGG GCAGCCCCGA TGACCAAGAA CCAGGTCAGC CCGTGGAGTG GGAGAGCAAT TGGACTCCGA CGGCTCCTTC AGCAGGGGAA CGTCTTCTCA AGAAGAGCCT CTCCCTGTCT

FVH－057 amino acid sequence．Signal sequence is shown in dotted underline，propeptide is double underlined， linker region connecting FVIf light chain or heavy chain to Fc region is underlined，linker region connecting the Fc and the Factor X〕a cleavage site is shown in bold，and the Factor Xja cleavage site is shown in dashed underline

|  | MVSQALRLLC | LLLGLQGCLA | － | RA | AFLEELRPGS | LERECKEEQC |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 61 | SFEEAREIFK | DAER＇IKLEWL | SYSDGDQCAS | SPCQNGGSCK | DQLQSY1CFC | LPAFEGRNCE |
| 121 | THKDDQLICV | NENGGCEQYC | S DiH？GTKRSC | RCHEGYSLLA | DGVSCTPTVE | YPCGKI PlLE |
| 131 | KRNASKPQGR | GGGGSGGGGGS | GGGGSGGGGS | GGGGSGGGGS | DKTHTCPPCP | APELLGGPS $V$ |
| 24 | FLFPPKPKDT | T | C | D | GVE VENAKTK | $\underline{\square}$ |
| 301 | RVVSVLTVLH | QDBLNGKEYK | CKVSNKALPA | P1ER ${ }^{\text {Pr }}$ ISKAK | GQPRE PQVY | LPPSRDELTK |
| 36 | NQVSLTCLVK | GFYPSDIAVE | 㽚 S NGQPENN | YKT＇TPPVLDS | DGSFFLYSRL | T＇VDKSRBQQG |
| 421 | W | A HHNHPTQKS | ISTSEGKGGG | GSGGGGSEGG | GSGGCGSGEG | GSGGGGSGGG |
| 431 | GSSVSQT3KL | TRIVGGKVCP | KGECFKQVLL | LVNGAQLCGG | TLINTIWVVS | AAHCFDKIKN |
| 541 | WR．NLI AVLGE | HDLSEHDGDE | QSRRVAQVI I | PSTYVPGTTN | HDIALLRLHQ | PVVLTDHVVP |
| S0 1 | LCLPERTFSE | RTLAFVRFSL | VSGHGQLLDR | GATALE LMVi： | NVPRLMTQDC | LQOSRK／GDS |
| 661 | PNITEYMFCA | GYSDGSKDSC | KGDSGGPHAT | HYRGTWYLTG | IVSWGQGCAT | VGHFGVYTRV |
| 721 | SQYI ENLQRL | MRSEPRPGVL | LRAPFP GGGG | SGGGGSGGGG | SGGGGSGGGG | SGGGGS DKTH |
| 731 | TCPPCPAPEL | LGGPSVFLFP | P KP KDTLMIS | RTPEVTCVW | DVSHEDPEVK | F＠miYVDGVEV |
| 841 | HNAKTKPREE | QYNSTYRWS | VLTVLHODSvL | NGKEYKCKVS | NKALPAPIEK | TISKAKGOPR |
| 901 | EPQVYTLPPS | RDELTKNQVS | LTCLVKGFYP | SDIAVESESN | GOPPENNYKTT | PPVLDSDGSF |
| 961 | FLYSKLTVDK | SRWQQGNVFS | CSVMHEALHN | HYTQK3LSLS | PGK＊ |  |

TAACCCAGGA GGAAGCCCAC AGGAGC＇${ }^{2}$＇GCG GCCGGGCTCC AGGCCCGGGA GATCTTCAAG ATGGGGACCA GTGTGCCTCA AGrCCPATAT CTGCTTCTGC ATGACCAGCT GATCTGTGTG CGGGCACCAA GCGCTCCTGT CCTGCACACC CACAGTTGAA CCAGCAAACC CCAAGGCCGA GATCAGGTGG GGGTGGATCA ACACATGCCC ACCGTGCCCA CCCCAAAACC CAAGGACACC TGGACGTGAG CCACGAAGAC TGCATAATGC CAAGACAAAG GCGTCCTCAC CGTCCTGCAC CCAACAAAGC CCTCCCAGCC GAGAACCACA GGTGTACACC GCCTGACCTG CCTGGTCAAA

CTTCTGCTTG GGCGTCCTGC CTGGAGAGGG GACGCGGAGA AGTCCATGCC CTCCCTGCCT AACGAGAACG CGGTGCCACG TATCCATGTG GGTGGCGGTG GGCGGTGGAG GCTCCGGAAC CTCATGATC ${ }^{n}$ CCTGAGGTCA CCGCGGGAGG CAGGACTGGC CCCATCGÂGA CTGCCCCCAT CCCGGGATGA GCTGACCAAG GGCTTCTATC CCAGCGACAT CGCCGTGGAG

| ]. 141 | TGGGAGAGCA | ATGGGCAGCC |
| :---: | :---: | :---: |
| 1201 | GACGGCTCCT | TCTTCCTCTA |
| 1261 | AACGTCTTCT | CATGCTCCGT |
| 1.21 | CTCTCCCTG? | CTCCGGGTAA |
| 1361 | GGTTCCGGTG | GCGGGGGATC |
| 1441 | GGTTCAGACT | TCCTGGCCGA |
| 1501 | AAAGGGGAGT | GTCCATGGCA |
| 1561 | ACCCTGATCA | ACACCATCTG |
| 1621 | TGGAGGAACC | TGATCGCGGT |
| 1681 | CAGAGCCGGC | GGGTGGCGCA |
| 1741 | CACGACATCG | CGCTGCTCCG |
| 1801 | CTCTGCCTGC | CCGAACGGAC |
| 1861 | GTCAGCGGCT | GGGGCCAGCT |
| 1921 | AACGTGCCCC | GGCTGATGAC |
| 1981 | CCAAATATCA | CGGAGTACAT |
| 2041 | AAGGGGGACA | GTGGAGGCCC |
| 201 | ATCGTCAGCT | GGGGCCAGGG |
| 21.61 | TCCCAGTACA | TCGAGTGGCT |
| 2221 | CTGCGAGCCC | CATTTCCCGG |
| 2281 | TCAGGTGGGG | GTGGATCAGG |
| 2341 | ACATGCCCAC | CGTGCCCAGC |
| 2401 | CCAAAACCCA | AGGACACCCT |
| 2461 | GACGTGAGCC | ACGAAGACCC |
| 2521 | CATAATGCCA | AGACAAAGCC |
| 2581 | G ${ }^{1}$ 'CC ${ }^{1} \mathrm{~T}$ CACCG | TCCTGCACCA |
| 2641 | AACAAAGCCC | TCCCAGCCCC |
| 270 1. | GAACCACAGG | TGTACACCCT |
| 2761 | CTGACCTGCC | TGGTCAAAGG |
| 2821 | GGGCAGCCGG | AGAACAACTA |
| 2881 |  | GCAAGCTCAC |
| 2941 | TGCTCCGTGA | TGCATGAGGC |
| 3001 | CCGGGTAAAT | GA |

$\begin{array}{ll}\text { GGAGAACAAC } & \text { TACAAGACCA } \\ \text { CAGCAAGCTC } & \text { ACCGTCGACA }\end{array}$ GATGCATGAG GCTCTGCACA AGGTGGCGGC GGATCAGGTG CGGCGGTGGA GGTTCCGGTG GGGCGGCGGC GTGCGGATTG GGTCCTGTTG TTGGTGAATG GGTGGTCTCC GCGGCCCACT GCTGGGCGAG CACGACCTCA GGTCATCATC CCCAGCACGT CCTGCACCAG CCCGTGGTCC GTTCTCTGAG AGGACGCTGG GCTGGACCGT GGCGCCACGG CCAGGACTGC CTGCAGCAGT GTTCTGTGCC GGCTACTCGG ACATGCCACC CACTACCGGG CTGCGCAACC GTGGGCCACT GCAAAAGCTC ATGCGCTCAG TGGCGGTGGC TCCGGCGGAG CGGTGGAGGT TCCGGTGGCG ACCTGAACTC CTGGGAGGAC CATGATCTCC CGGACCCCTG TGAGGTCAAG TTCAACTGGT GCGGGAGGAG CAGTACAACA GGACTGGCTG AAT GGCA $\lambda_{\mathrm{AG} \mathrm{G}}$ CATCGAGAAA ACCATCTCCA GCCCCCATCC CGGGATGAGC CTTCPATCCC AGCGACATCG CAAGACCACG CCTCCCGTGT CGTGGACAAG AGC AGGTGG C TCTGCACAAC CACTACACGC

CGCCTCCCGT GTTGGACTCC AGAGCAGGTG GCAGCAGGGG ACCACTACAC GCAGAAGAGC GGGGTGGATC AGGCGGTGGA GGGGTGGATC AGGAGGAGGT TGGGGGGCAA GGTGTGCCCC GAGCTCAGTT GTGTGGGGGG GTTTCGACAA AATCAAGAAC GCGAGCACGA CGGGGATGAG ACGTCCCGGG CACCACCAAC TCACTGACCA TGTGGTGCCC CCTTCGTGCG CTTCTC ATTG CCCTGGAGCT CATGGTCCTC CACGGAAGGT GGGAGACTCC ATGGCAGCAA GGACTCCTGC GCACGTGGTA CCTGACGGGC TTGGGGTGTA CACCAGGGTC AGCCACGCCC AGGAGTCCTC GTGGGTCCGG TGGCGGCGGA GGGGATCAGA CAAAACTCAC CGTCAGTCTT CCTCTTCCCC AGGTCACATG CGTGGTGGTG ACGTGGACGG CGTGGAGGTG GCACGTACCG TGTGGTCAGC AGTACAAGTG CAAGGTCTCC AAGCCAAAGG GCAGCCCCGA TGACCAAGAA CCAGGTCAGC CCGTGGAGTG GGAGAGCAAT TGGACTCCGA CGGCTCCTTC AGCAGGGGAA CGTCTTCTCA AGAAGAGCCT CTCCCTGTCT

FVH-058 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, and the thrombin cleavage site is shown in dashed underline

| 1 | MVSQALRLLC | LLLGLQGCLA | AVF TOEEAI | GVLHRRRKAN | AFLEELRPGS | LERECKEEQC |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 61 | SFEEAREIFK | DAERTKLFWI | SY3DGDQCAS | SPCQNGGSCK | DQLQSYICFC | LPAFEGRNCE |
| 121 | THKDDQLICV | NENGGCEQYC | SDHTGTKRSC | RCHEGYSLLA | DGV5CTPTVE | YPCGKI PILE |
| 131 | KRNASKPQGR | GGGGSGGGGS | GGGGSGGGGS | GGGGSGGGGS | DKTHTCPPCP | APELLGGPSV |
| 241 | FLFPPKPKDT | LMISRTPEVT | CVVVDVSHED | PEVKFNTYVD | GVEVHNAKTK | PREEQYNSTY |
| 301 | RVVSVLTVLH | QDKLNGKEYK | CKVSNKALPA | PIEKTISKAK | GOPREPQVYT | LPFSRDELTK |
| 361 | NQVSLTCLVK | GFYPSDIAVE | WESNGQPENT | YKTTPPVLDS | DGSFFLYSKL | TVDKSRSvOQG |
| 421 | NVFSCSVMEE | A A HRYTOKS | TAEISEGKGGG | GSGGCGSEGG | GSGGGGSCGG | GSGGCGSGEG |
| 481 | GSDFLAEGGG_ | VRTVGGKVCP | YGECP鳥QVLL | LVNGAQLCGG | TLINTIWVVS | A $A$ HCEDK TKJ |
| 541 | NRLLI AVLGE | HDLSEHDGDE | QSRRVAOVI I | PSTYVPGTTN | HDIALLRLHQ | PVVLTDHVVP |
| 601 | LCLPERTFSE | RTLAFVRFSL | VSGSGQLLDR | GATALELMVL | NVPRLMTQDC | LQQSRKVGDS |
| 661 | PNITEYMFCA | GYSDGSKDSC | KGDSGGPHAT | HYRGTKYLTG | IVSSGQGCAT | VGHFGVYTRV |
| 721 | SQYIEWLQKL | MRSEPRPGVL | LRAPFPGGGG | SGGGG3GGGG | SGGGGSGGGG | 3GGGGSDKTH |
| 731 | TCPPCPAPEL | LGGPS VFLFP | PKPKDTLMIS | RTPEVTCVVV | DVSHEDPEVK | FNWYVDGVEV |
| 841 | HNAKTKPREE | QYNSTYRVVS | VLTVLHODSSvL | NGKEYKCKVS | MKALPAPIEK | TISKAKGOPR |
| 901 | EPQVYTLPPS | RDELTKNQVS | LTCLVKGFYP | SDI AVESESN | GQPENNYKTT | PPVLDSDGSF |
| 961 | FLYSKLTVDK | SRKQQGNVFS | CSVMHEALHR | HYTQKSLSLS | PGK* |  |

DNA sequence for FVIi-059
1
61
121
ATGGTCTCCC
131
241
GCGTTCCTTCGAGG
301
361
TCTTACAGTG
421 ACGCACAGAGGG GCTCCTCTGC CTTCTGCTTG GGCTTCA TAACCCAGGA GGAAGCCCAC .AGGAGCTGCG GCCGGGCTCC AGGCCCGGGA GATCTTCAAG ATGGGGACCA GTGTGCCTCA AGTCCTATAT CTGCTTCTGC ATGACCAGCT GATCTGTGTG CGGGCACCAA GCGCTCCTGT CCTGCACACC CACAGTTGAA CCAGCAAACC CCAAGGCCGA GATCAGGTGG GGGTGGATCA GGCTTCAGGG GGCGTCCTGC ACCGGCGCCG CTGGAGAGGG AGTGCAAGGA GACGCGGAGA GGACGAAGCT AGTCCATGCC AGAATGGGGG CTCCCTGCCT TCGAGGGCCG AACGAGAACG GCGGCTGTGA CGGTGCCACG AGGGGTACTC TATCCATGTG GAAAAATACC GAAAAATACC GGCGG GTTCCGGTGG TCCTGGGAGG CTGCCTGGCT GCGCGCCAAC GGAGCAGTGC GTTCTGGATT CTCCTGCAAG GAACTGTGAG GCAGTACTGC TCTGCTGGCA TATTCTAGAA AGGTGGGTCC CGGGGGATCC ACCGTCAGTC TGAGGTCACA

| 81 | TGCGTGGTGG | TGGACGTGAG | CCACGAAGAC | CCTGAGGTCA | AGTTCAACTG | GTACGTGGAC |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 841 | GGCGTGGAGG | TGCATAATGC | CAAGACAAAG | CCGCGGGAGG | AGCAGTACAA | C |
| 901 | CGTGTGGTCA | GCGTCCTCAC | CGTCCTGCAC | CAGGACTGGC | TGAATGGCAA | GGAGTACAAG |
| 96 | TGCAAGGTCT | CCAACAAAGC | CCTCCCAGCC | CCC ATCGAGA | AA ACCAT CTC | CAAAGCCAAA |
| 102 | GGGCAGCCCC | GAGAACCACA | GG | CTGCCCCCAT | CCCGGGATGA | G |
| 1 | AACCAGGTCA | GCCTGACCTG | CCTGGTCAAA | GGCTTCTATC | CCAGCGACAT | CGCCGTGGAG |
|  | TGGGAGAGCA | ATGGGCAGCC | GGAGAA | TAC | CGCCTCCCGT | GTTGGACTCC |
| 1201 | G | TCTTCCTCTA | CAGCAAGCTC | A | AGAGCAGGTG | GCAGCAGGGG |
| 12 | AACGTCTTCT | CATGCTCCGT | G | GCTCTGCACA | C | C |
| 1 | CTCTCCCTG? | CTCCGGGTAA | AGG | GGATCAG | GGGGTGGATC | AGGCGGTGGA |
| 1 | GGTTCCGGTG | GCGGGGGATC | CGGCGGTGGA | GGTTCCGGTG | GGGGTGGATC | T |
| 1 | GGTTCAACCA | C | GC | GTGGGGGGCA | AGGTGTGCCC | G |
|  | TGTCCATGGC | AGGTCCTGTT | GTTGGTGAAT | GGAGCTCAGT | TGTGTGGGGG | GACCCTGATC |
|  | AACACCATCT | GGGTGGTCTC | CGCGGCCCAC | TGTTTCGACA | AAATCAAGAA | CTGGAGGAAC |
| 1 | C | T | G | AGCGAGCACG | ACGGGGATGA | G |
| 1 | CGGGTGGCGC | AGGTCATCAT | CCCCAGCACG | TACGTCCCGG | GCACCACCAA | CCACGACATC |
|  | GCGCTGCTCC | GCCTGCACCA | CCC | CTCACTGACC | C | CCTCTGCCTG |
| 1 | CC | C | G | GCCTTCGTGC | GCTTCTCATT | G |
| 1 | TGGGGCCAGC | TGCTGGACCG | TGGCGCCACG | GCCCTGGAGC | TCATGGTCCT | CAACGTGCCC |
| 19 | CGGCTGATGA | CCCAGGACTG | CCTGCAGCAG | TCACGGAAGG | TGGGAGACTC | CCCAAATATC |
| - 9 | AC | TGTTCTGTGC | C | G | AGGACTCCTG | CAAGGGGGAC |
| 20 | AGTGGAGGCC | CACATGCCAC | CCACTACCGG | GGCACGTGGT | ACCTGACGGG | CATCGTCAGC |
| 2. | TGGGGCCAGG | GCTGCGCAAC | CGTGGGCCAC | TTTGGGGTGT | CACCAGGGT | CTCCCAGTAC |
| 21 | ATCGAGTGGC | TGCAAAAGCT | CATGCGCTCA | GAGCCACGCC | CAGGAGTCCT | CCTGCGAGCC |
| 222 | CCATTTCCCG | GTGGCGG | CTCCGGCGGA | GGTGGGTCCG | GTGGCGGCGG | ATCAGGTGGG |
| 22 | GGTGGATCAG | GCGGTGGAGG | TTCCGGTGGC | GGGGGATCAG | AC-AAAACTCA | CACATGCCCA |
| 23 | CCGTGCCCAG | CACCTGAACT | CCTGGGAGGA | CCGTCAGTCT | TССТСТTССС | CCCAAAAC-CC |
| 24 | AAGGACACCC | TCATGATCTC | CCGGACCCCT | GAGGTCACAT | GCGTGGTGGT | GGACGTGAGC |
| 2461 | CACGAAGACC | CTGAGGTCAA | GTTCAACTGG | TACGTGGACG | GCGTGGAGGT | GCATAATGCC |
| 2521 | AAGACAAAGC | CGCGGGAGG | GCAGTACAAC | AGCACGTACC | GTGTGGTCAG | CGTCCTCACC |
| 2581 | G ${ }^{1} \mathrm{CC}{ }^{1} \mathrm{~T}$ GCACC | AGGACTGGCT | GAATGGCAAG | GAGTACAAGT | GCAAGGTCTC | CAACAAAGCC |
| 2641 | CTCCCAGCCC | CCATCGAGAA | AACCATCTCC | AAAGCCAAAG | GGCAGCCCCG | AGAACCAC-AG |
| 27 | GTGTACACCC | TGCCCCCATC | CCGGGATGAG | CTGACCAAGA | ACCAGGTCAG | CCTGACCTGC |
| 2761 | C ${ }^{1}$ 'GG ${ }^{1} \mathrm{CAAAG}$ | GCriTCAATCC | CAGCGACATC | GCCGTGGAGT | GGGAGAGCAA | TGGGCAGCCG |
| 2821 | GAGAACAACT | ACAAGAGCAC | GCCTCCCGTG | TTGGACTCCG | ACGGCTCCTT | CTTCCTCTAC |
| 2881 | AGCAAGCTCA | CCGTGGACAA | GAGCAGGTGG | CAGCAGGGGA | ACGTCTTCTC | ATGCTCCGTG |
| 2941 | ATGCATGAGG | CTCTGCACAA | CCACTACACG | CAGAAGAGCC | TCTCCCTGTC | TCCGGGTAAA |
| 00 |  |  |  |  |  |  |

FVIf-059 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVYI light chain or heavy chain to Fc region is underlined, linker region connecting the Fe and the thrombin cleavage site is shown in bold, and the thrombin cleavage site is shown in dashed underline

1 MVSQALRLLC LLLGLQGCLA AVFVTQEEf H GVLHRRPRf $\mathbb{N}$ AFLEELRPGS LERECKEEQC
S 1 SFEEAREIFK DAERTK"LFWI SY3DGDQCAS SPCQNGGS CK DQLQSYICFC LPAFEGRNCE 121 THKDDQLICV NEMGGCEQYC SDHTGTKRSC RCHEGY SLLA DGVSCTPTVE YPCGKI PILE 131 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV 241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNNYVD GVEVfiNAKTK PREEOYMSTY 301 RVVSVLTVLH QDSvLNGKEYK CKVSNKALPA PIEKTISKAK GOPREPQVYT LPFSRDELTK 361 NQVSLTCLVK GFYPSDIAVE NESNGQPENV YKTTPPVLDS DGSFFLYSKL TVDKSRSvOQG 421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GAGGOGSGGG GSGGGGSGGG GSGGEGSGGG 481 GSTTK1KPE. VGGKVCPKGE CPSQVLLLVN GAQLCGGTLI NTI WVVSAAH CFDKIKNKRN 541 LIAVLGEHDL SEHDGDEQSR RVAQVI I FST YVPGTTNHDI ALLRLHQPVV LTDHVVPLCL 601 PERTFSERTL AFVRFSLVSG KGQLLDRGAT ALE IMVLNVP RLMTODCLQQ SRKVGDS PNI 661 TEYMFCAGYS DGSKDSCKGD SGGPHATHYR GTKYLTGIVS KGQGCATVGH FGVYTRVSQY 721 IEWLQKLMRS EFRPGVLLRA PFPGGGGSGG GG3GGGGSGG GGSGGGGSGG GGSDKTHTCP 731 PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNTV YVDGVEVfiNA 841 KTKPREEQ IN STYRVVSVLT VLHQDIALNGK EYKCKVSNKA LPAPIEKTIS KAKGOPREPQ 901 VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY 961 SKLTVDK3RW QGGVVi'SCSV MEIEALHNHYT QK3LSLSFGK *

DNA sequence for FV1 I --0 60
1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT 1 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC 131 TCCTTCGAGG AGGCCCGGGA GATC ${ }^{2} T C A A G$ GACGCGGAGA GGACGAAGCT GTTCTGGATT 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG

5

FVII-060 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting $\mathrm{FV}_{\mathrm{ii}}$ light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, and the thrombin cleavage site is shown in dashed underline

|  | MV | Lliglegcla | AVFVTQEE AK: | Lhrreran | LRPGS | eec |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 61 | SFEEAREIFK | DAERTKLFWI | SYSDGDQCAS | SPCQNGGSCK | DQLQSYICFC | LPAFEGRNCE |
| 121 | THKDDQLICV | CEQYC | SDHTGTKR3C | RCHEGYSLLA | DGVSCTPTVE | Le |
| 181 | RRNASK.PQGR | GGGG5GGGGS | GGGGSGGGGS | GGGGSGGGGS | P | APELLGGPSV |
| 241 | FL | MISRTPEVT | CVVVDVSHED | PEVEFNWYVD | тK | PEEEQY |
| 301 | RWS | QDKLNGKEYK | SNKA | PIEKTISKAK | GQ | K |
| 361 | nQvSltclvk | GFYPSDIAV | SESNGQPENN | YKтtppvids | DGSFFlyskl | TVD |
| 421 | NVFSC3VMKE | ALHNHYTQKS | IJSLSPGugg | GSGGGGSGGG | GSGGGGSGGG | GSG |
| 481 | GSALRPRVVG | GAVVGGKı | KGECPWQVLL | LVNGAQLCGG | ws | AAhCFDKikn |
| 541 | WRNLTAV̈LGE | HDLSEHDGDE | QSRRVAQVTI | PSTYVPGTTN | HD TALLRLHe | PWLTDHw? |
| 601 | LC | RTLAFVRFSL | vSGWGQLLDR | gatalelmvi | nVPRLMTQDC | LQQ3RKvGDS |
| 661 | PNITEXM | GY3DGSkdSc | KGDSGGPhat | HYRGTKYLTG | IVSWGQGCAT | vghfgvytrv |
| 721 | SQYIEWLQKL | MR3EPRPGVL | LRAPFPGGGG | SGGGgSgGg | SGGGG5GGGG | 5GGGG5DKTH |
| 781 | TCPPCPAPEL | LGGPSVFLFP | PKPKDTLMIS | RTPEVTCVVV | DVSHEDPEVK | FNWYVDGVEV |
| 841 | hnaktkpree | QYMSTYRVVS | vLTVLHQ ${ }^{\text {dew }}$ | ngKelkekv | NKALPAP1EK | ISKAKGQPR |
| 901 | EPQVYTLPPS | RDELtKNQVS | LTCLVKGFYP | Sotavewes | GQPENN | PPVLDSDGSF |
| 961 | FLYSKLTVDK | SRWQQGNVF | CSVMHEALHE | HYTQKSLSL | PGK* |  |

61 SFEEAREIFK DAERTKLFWİ $\frac{\text { SYSDGDQCAS SPCONGGSCK }}{}$ 121 THKDDQLICV NENGGCEQYC SDhtGTKR3C RCHEGYSLLA DGVSCTPTVE 181 RRNASEPQGR GGGG5GGGGS GGGGSGGGGS GGGGSGGGGS
 301 RWSVLTVLH QDKLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT 421 VFFSC3VMKE ALHNHYTOKS 481 GSALRPRVVG GAVVGGKVCP 541 WRNLTAV̈LGE HDLSEHDGDE 601 LCLPERTfSE RTLAFVRFSL 661 PNI7EXMFCA GY3DGSKDSC 721 SQYIEWLQKL MR3EPRPGVL 81 TCPPCPAPEL OMS RDELTKNQVS SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK*

821 GGGCAGCCGG 2881 TTCCTCTACA 2941 TGCTCCGTGA 3001 CCGGGTAAAT

ATGACCAGCT CGGGCACCAA CCTGCACACC CCAGCAAACC GATCAGGTGG ACACATGCCC CCCCAAAACC TGGACGTGAG TGCATAATGC GCGTCCTCAC CCAACAAAGC GAGAACCACA GCCTGACCTG ATGGGCAGCC TCTTCCTCTA CATGCTCCGT CTCCGGGTAA GCGGGGGATC TGCGGCCCCG GTCCATGGCA ACACCATCTG TGATCGCGGT GGGTGGCGCA CGCTGCTCCG CCGAACGGAC GGGGCCAGCT GGCTGATGAC CGGAGTACAT GTGGAGGCCC GGGGCCAGGG TCGAGTGGCT CATTTCCCGG GTGGATCAGG CGTGCCCAGC AGGACACCCT ACGAAGACCC AGACAAAGCC TCCTGCACCA TCCCAGCCCC TGTACACCCT TGGTCAAAGG AGAACAACTA GCAAGCTCAC TGCATGAGGC GA

GATCTGTGTG gСgctcctgt CACAGTTGAA CCAAGGCCGA GGGTGGATCA ACCGTGCCCA CAAGGACACC CCACGAAGAC CAAGACAAAG CgTcctgcac CCTCCCAGCC GGTGTACACC CCTGGTCAAA GGAGAACAAC CAGCAAGCTC GATGCATGAG AGGTGGCGGC CGGCGGTGGA GGTGGTGGGC GGTCCTGTTG GGTGGTCTCC GCTGGGCGAG gGtcatcatc CCTGCACCAG GTtctctag GCTGGACCGT CCAGGACTGC GTTCTGTGCC ACATGCCACC CTGCGCAACC GCAAAAGCTC tGGCGGTGGC CGGTGGAGGT АССтGAACTC CATGATCTCC TGAGMTC』AG GCGGG AGGAG CAGTACAACA GGACTGGCTG AATGGCAAGG CATCGAGAZA ACCATCTCCA GCCCCCATCC CGGGATGAGC CTTCTATCCC AGCGACATCG CДAGACCACG CCTCCCGTGT CGTGGACAAG AGCAGGTGGC TCTGCACAAC CACTACACGC

| AACGAGAACG | GCGGCTGTGA | GCAGTACTGC |
| :---: | :---: | :---: |
| CGGTGCCACG | AGGGGTACTC | TCTGCTGGCA |
| TATCCATGTG | GAAAAATACC | TATTCT AGAA |
| GGTGGCGGTG | GCTCCGGCGG | AGGTGGGTCC |
| GGCGGTGGAG | GTTCCGGTGG | CGGGGGATCC |
| GCTCCGGAAC | TCCTGGGAGG | ACCGTC AGTC |
| CTCATGATCT | CCCGGACCCC | TGAGGTCACA |
| CCTGAGGTCA | AGTTCAACTG | GTACGTGGAC |
| CCGCGGGAGG | AGCAGTACAA | CAGCACGTAC |
| CAGGACTGGC | TGAATGGCAA | GGAGTACAAG |
| CCC ATCGAGA | AAACCATCTC | CAAAGCCAAA |
| CTGCCCCCAT | CCCGGGATGA | GCTGACCAAG |
| GGCTTCTATC | CCAGCGACAT | CGCCGTGGAG |
| TACAAGACCA | CGCCTCCCGT | GTTGGACTCC |
| ACCGTCGACA | AGAGCAGGTG | GCAGCAGGGG |
| GCTCTGCACA | ACCACTACAC | GCAGAAGAGC |
| GGATCAGGTG | GGGGTGGATC | AGGCGGTGGA |
| GGTTCCGGTG | GGGGTGGATC | AGGAGGAGGT |
| GGCGCCGTGG | TGGGGGGCAA | GGTGTGCCCC |
| TTGGTGAATG | GAGCTCAGTT | GTGTGGGGGG |
| GCGGCCCACT | GTTTCGACAA | AATCAAGAAC |
| CACGACCTCA | GCGAGCACGA | CGGGGATGAG |
| CCCAGCACGT | ACGTCCCGGG | CACCACCAAC |
| CCCGTGGTCC | TCACTGACCA | TGTGGTGCCC |
| AGGACGCTGG | CCTTCGTGCG | CTTCTCATTG |
| GGCGCCACGG | CCCTGGAGCT | CATGGTCCTC |
| CTGCAGCAGT | CACGGAAGGT | GGGAGACTCC |
| GGCTACTCGG | ATGGCAGCAA | GGACTCCTGC |
| CACTACCGGG | GCACGTGGTA | CCTGACGGGC |
| GTGGGCCACT | TTGGGGTGTA | CACCAGGGTC |
| ATGCGCTCAG | AGCCACGCCC | AGGAGTCCTC |
| TCCGGCGGAG | GTGGGTCCGG | TGGCGGCGGA |
| TCCGGTGGCG | GGGGATCAGA | CAAAACTCAC |
| CTGGGAGGAC | CGTCAGTCTT | CCTCTTCCCC |
| CGGACCCCTG | AGGTCACATG | CGTGGTGGTG |
| TTCAACTGGT | ACGTGGACGG | CGTGGAGGTG |
| CAGTACAACA | GCACGTACCG | TGTGGTCAGC |
| AATGGCAAGG | AGTACAAGTG | CAAGGTCTCC |
| ACCATCTCCA | AAGCCAAAGG | GCAGCCCCGA |
| CGGGATGAGC | TGACCAAGAA | CCAGGTCAGC |
| AGCGACATCG | CCGTGGAGTG | GGAGAGCAAT |
| CCTCCCGTGT | TGGACTCCGA | CGGCTCCTTC |
| AGCAGGTGG C | AGCAGGGGAA | CGTCTTCTCA |
| CACTACACGC | AGAAGAGCCT | CTCCCTGTCT |

GCAGTACTGC TCTGCTGGCA tattct agan AGGTGGGTCC CGGGGGATCC ACCGTC AGTC TGAGGTCACA GTACGTGGAC CAGCACGTAC GGAGTACAAG CAAAGCCAAA GCTGACCAAG cGccgtgeag GTTGGACTCC GCAGCAGGGG GCAGAAGAGC AGGCGGTGGA AGGAGGAGGT GGTGTGCCCC GTGTGGGGGG AATCAAGAAC CGGGGATGAG CACCACCAAC tGTGGTGCCC CtTCTCATtG CATGGTCCTC GGGAGACTCC GGACTCCTGC CCTGACGGGC CACCAGGGTC AGCagtcctc TGGCGGCGGA СААААСТСАС сСтстtсссС CGTGGTGGTG CGTGGAGGTG TGTGGTCAGC CAAGGTCTCC GCAGCCCCGA CCAGGTCAGC GGAGAGCAAT CGGCTCCTTC cgтстtстса CTCCCTGTCT

5
DNA sequ ence for FVII-061
1 дTGGTCTCOC AGGCOCTGAG GCTCCTCTGC CTTCTGCTTG GGOTROAGGG OTGCCTGGOT 6]. GOAGTCTTCG TAACOCAGGA GGAAGCCOAC GGOGTCOTGC ACOGGOGCOG GCGCGOCAAC 121 GCGTTCCTGG AGGAGCTGCG GCOGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC 131 TGCTTCGAGG AGGCOCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGOT GTTCTGGATT 241 TCTRACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG 301 GACCAGCTCQ AGTCOTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCOG GAACTGUGAG 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AAOGAGAACG GCGGCTGTGA GCAGTACTGC 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACRC TCTGCTGGCA 461 GACGGGTGT COGGACACC CACAGTTGAA FATCCATGTG GAAAAATACC TATTCTAGAA 541 AAAAGAAATG COAGOAAACC CCAAGGCOGA GGTGGCGGTG GCTCOGGCGG AGGTGGGTCC 601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGRGGAG GTTCOGGTGG CGGGGGATCC 661 GACAAAACTC ACACATGCCC ACCGTGCCOA GCTCCGGAAC TCOTGGGAGG ACCGTCAGTC 721 TTCCTCTTCC COCCAAAACC CAAGGACACC CTOATGATCT CCOGGACCOC TGAGGTCAOA 781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC 041 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGOAGTACAA CAGCACGTAC 901 CGTGTGGTCA GCGTOCTGAC CGTCGTGGAC CAGGACTGGC TGAATGGCAA GGAGTACAAG 961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCRC CAAAGCCAAA 021 GGGCAGCCOC GAGAACCACA GGTGTACACC CTGCCCCOAT CCOGGGATGA GCTGACCAAG 0 O1 AACCAGGTCA GCCTGACOTG CCTGGTCAAA GGOTTCTATC CCAGCGACAT CGCCGTGGAG 141 TGGGAGAGCA A?GGGCAGCC GGAGAACAAC TACAAGACCA CGOCTCCOGI GTPGGACTGC 20J GACOGOTCOT JCrroCrom CAGCAAGCDC ACOGTCGACA AGAGCAGGTG GCAGCAGGOG 261 AACOMCTUC? CADGOTCGO GATGCATGAG GCICTGCACA ACOACPACAC GCAGAAGAOC



 561 ACOCTGATCA ACACOATOPGGOGGTCOCO GOGGCOCACD GTMPCGACAA AATCAAGAAC 621 rGGAGGAACC JGATGGOGGT GCTGOGCGAG CACGACOMCA GOGAGCACGA CGGGGAGGAG 681 CAGAGCOGGC GGGTGGCGCA GOTCATCADC COCACOACGT ACGTCCOGGG OACCACOAAO

 861 GTOAGCGGOT GGGGOOAGCT GCTGGACOGT GOCGCOACGG COOTGAGOT OATGGTCODO 921 AAOCTGCOCC GGCTGATGAC CCAGGACPGC OTGOAGCAGI OACGGAAGGJ GGGAGACTOC 95] CCAAADATCA CGGAGRACAT GruTDGJGCC GOCWACJCGG ATGGCAGCAA GGACJOCJGC
 101 ATCOTCAGC? GGGGOCAGGG CMGCOCAACO GTGGCOCACJ mrGGGGTGTA OACOAGGGTC 161 rCCCAGTACA JCGAGTGGCP GCAAAAGODC ATGCGCJCAG AGOCACGCCO AGGAGYCOMC 221 CTGCGAGCOC CATrmCOOGG TGGCGGTGGO TOCGGOGGAG GTGGGTOCGG TGOCGGOGGA 2281 rCAGGDGGGG GRGGATCAGG CGGJGAGGT TCOGGDGGCG GGGGATCAGA CAARAORCAC
 2401 COAAAACOCA AGGACACOCR CATGATCPCC CGGACOCCHG AGGRCAOATG GGOGGTGGTG 461 GACGTGAGCC ACGAAGACCC JGAGGRCAAG prcaACJGGJ ACGTGGCGG CGTGGAGGM 521 CAmAADCCOA AGACAARGCC GCGGGAGGAG CAGTACAACA GCACOTACOG TGTGGTCACC 2581 GTCOPCACOG JCOTGCACCA GGACMGGCTG AATGGCAAGG AGTACAAGG CAAGGROTOC 641 ARCAMAGCCC ICCCAGCCCC CADCGAGAAA ACCACDCCA ARGCCRAAGG GCAGCCCOGA

 821 GGGCAGCOGG AGAACAACHA CAAGACCACG CCICCCGOGI MGGACPCCGA GGGCICCIMC 881 TMCCTOTACA GCAZGCTCAC CGMGACAAG AGCAGGTGGC AGCAGGGGAA OGOCTMCTCA
 3001 CCGCGMAAA GA

FVII-061 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVU light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, and the thrombin cleavage site is shown in dashed underline

1 MVSQATRITAC LTLGIQGCLA AVFVTQEE A.H GVLHRRRRAN A.FLEELRPGS LERECKEEQC
61 BFEFARETFK DAERTKLFWI 3 YSDGDQCA.S SPCQNGGSCK DQLQSYICFC LPAFEGRNCE 121 THRDDQLICV NENGGCEQYC SDHTGTKR3C RCHEGYSLLA DGVSCTPTVE YPCGKI PILE 151 RRNASREQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS 241 ETFEPKEKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD DRTHTCPECP APELLGGPSV 301 QVVSVT TYYTH QDKLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT PREEQRDELTK 361 WOVODTOTH GFYPSD1AVE 421 NVFSCSVMKE ALHNHYTQKS TSLSPGKGGG GSGGGGSGGG GSGGGGSEGG GSGGEGSGGG 481 GSALRPRVVG GAIVGGKVCP KGECPWQVLL LVNGAQLCGG TLINTIWWS AAHCEJJJKN 541 WRNLIf VIGE EDTSEHDGDE QSRRVAQVT1 PSTYVPGTTN HDTALIRLHQ PVVLTDHVVP 601 ICIPERTFSE RTLAFVRFSL VSGWGQLLDR GAILALMTMVL NVPRLMTQDC LQQSRKVGDS

661 PNI7EXMFCA GY3DGSKDSC 721 SYIE雨LQKL MR3EPRPGVL 781 TCPPCPAPEL LGGPSVFLFP 841 HNAKTKPREE QYNSTYRWS 901 EPQVYTLPPS RDELTKNQVS 961 FLYSKLTVDK SRKQQGNVFS

Kgdsgaphat hyrgt yleg tvswgegcat vghfgvytrv LRAPFPGGGG SGGGGSGGGG SGGGGSGGGG SGGGGS DKTH PKPREDLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV vLtVLHQDVL NGKEYKCKVS NKALPAPTEK TISKAKGQPR ltclvkgryp Sdiavewe ${ }^{\text {N }}$ GQPENNYKTT PPVLDSDGSF CSVMheailhy hytqkslsls PGK*

DNA sequence for FVYI-062
1 ATGGTCTCCC AGGCCCTCAG
61 GCAGTCTTCG TAACCCAGGA 121 GCGTTCCTGG AGGAGCTGCG 131 TCCTTCGAGG AGGCCCGGGA 241 TCTTACAGTG ATGGGGACCA 301 GACCAGCTCC AGTCCTATAT 361 ACGCACAAGG ATGACCAGCT 421 AGTGACCACA CGGGCACCAA 431 GACGGGGTGT CCTGCACACC 541 AAAAGAAATG CCAGCAAACC 601 GGTGGCGGCG GATCAGGTGG 661 GACAAAACTC ACACATGCCC 721 TTCCTCTTCC CCCCAAAACC 761 TGCGTGGTGG TGGACGTGAG 841 GGCGTGGAGG TGCATAATGC 901 CGTGTGGTCA GCGTCCTCAC 961 TGCAAGGTCT CCAACAAAGC 1 -1 21 GGGCAGCCCC GAGAACCACA 1051 AACCAGGTCA GCCTGACCTG 141 TGGGAGAGCA 1201 GACGGCTCCT 1261 AACGTCTTCT 1.21 CTCTCCCTG? 1.351 GGTTCCGGTG 1441 GGTTCAGGTG 1501 TGGCAGGTCC 1561 ATCTGGGTGG 1621 GCGGTGCTGG 1681 GCGCAGGTCA 1741 CTCCGCCTGC 1.01 CGGACGTTCT 1 G1 CAGCTGCTGG 1921 ATGACCCAGG 1981 TACATGTTCT 2041 GGCCCACATG 2. 01 CAGGGCTGCG 2161 TGGCTGCAAA 2221 CCCGGTGGCG 2281 TCAGGCGGTG 2341 CCAGCACCTG 2401 ACCCTCATGA 2461 GACC CTGAGG 2521 AAGCCGCGGG 2581 CACCAGGACT 2641 GCCCCCATCG 270 J. ACCCTGCCCC 2761 AAAGGC?TC? 2821 AACTACAAGA 2881 CTCACCGTGG 2941 GAGGCTCTGC

ATGGGCAGCC TCTTCCTCTA CATGCTCCGT CTCCGGGTAA GCGGGGGATC GTGGAGGATC TGTTGTTGGT TCTCCGCGGC GCGAGCACGA TCATCCCCAG ACCAGCCCGT CTGAGAGGAC ACCGTGGCGC ACTGCCTGCA GTGCCGGCTA CCACCCACTA CAACCGTGGG AGCTCATGCG GTGGCTCCGG GAGGTTCCGG AACTCCTGGG TCTCCCGGAC TCAAGTTCAA AGGAGCAGTA GGCTGAATGG AGAAAACCAT CATCCCGGGA ATCCCAGCGA CCACGCCTCC ACAAGAGCAG ACAACCACTA

GCTCCTCTGC
GGAAGCCCAC GCCGGGCTCC GATCTTCAAG GTGTGCCTCA CTGCTTCTGC GATCTGTGTG GCGCTCCTGT CACAGTTGAA CCAAGGCCGA GGGTGGATCA ACCGTGCCCA CAAGG ACACC CCACGAAGAC CAAGACAAAG CGTCCTGCAC CCTCCCAGCC GGTGTACACC CCTGGTCAAA GGAGAACAAC CAGCAAGCTC GATGCATGAG AGGTGGCGGC CGGCGGTGGA CATTGTGGGG GAATGGAGCT CCACTGTTTC CCTCAGCGAG CACGTACGTC GGTCCTCACT GCTGGCCTTC CACGGCCCTG GCAGTCACGG CTCGGATGGC CCGGGGCACG CCACTTTGGG CTCAGAGCCA CGGAGGTGGG TGGCGGGGGA AGGACCGTCA CCCTGAGGTC CTGGTACGTG CAACAGCACG TACCGTGTGG CAAGGAGTAC AAGTGCAAGG CTCCAAAGCC AAAGGGCAGC T GAGCTGACC AAGAACCAGG CATCGCCGTG GAGTGGGAGA CGTGTTGGAC TCCGACGGCT GTGGCAGCAG GGGAACGTCT CACGCAGAAG AGCCTCTCCC

CTTCTGCTTG GGCGTCCTGC CTGGAGAGGG GACGCGGAGA AGTCCATGCC CTCCCTGCCT AACGAGAACG CGGTGCCACG TATCCATGTG GGTGGCGGTG GGCGGTGGAG GCTCCGGAAC CTCATGATCT CCTGAGGTCA CCGCGGGAGG CAGGACTGGC CCC ATCGAGA CTGCCCCCAT GGCTTCTATC TACAAGACCA ACCGTCGACA GCTCTGCACA GGATCAGGTG GGTTCCGGTG GGCAAGGTGT CAGTTGTGTG GACAAAATCA CACGACGGGG CCGGGCACCA GACCATGTGG GTGCGCTTCT GAGCTCATGG AAGGTGGGAG AGCAAGGACT TGGTACCTGA GTGTACACCA CGCCCAGGAG TCCGGTGGCG TCAGACAAAA GTCTTCCTCT ACATGCGTGG GACGGCGTGG AGGTGCATAA TCAGCGTCCT TCTCCAACAA CCCGAGAACC TCAG CCTGAC GCAATGGGCA CCTTCTTCCT TCTCATGCTC TGTCTCCGGG

| GGCTTCAGGG | CTGCCTGGCT |
| :---: | :---: |
| ACCGGCGCCG | GCGCGCCAAC |
| AGTGCAAGGA | GGAGCAGTGC |
| GGAC GAAGCT | GTTCTGGATT |
| AGAATGGGGG | CTCCTGCAAG |
| TCGAGGGCCG | GAACTGTGAG |
| GCGGCTGTGA | GCAGTACTGC |
| AGGGGTACTC | TCTGCTGGCA |
| GAAAAATACC | TA |
| GCTCCGGCGG | AGGTGGGTCC |
| GTTCCGGTGG | CGGGGGATCA |
| TCCTGGGCGG | ACCGTCAGTC |
| CCCGGACCCC | TGAGGTCACA |
| AGTTCAACTG | GTACGTGGAC |
| AGCAGTACAA | CAGCACGTAC |
| TGAATGGCAA | GGAGTACAAG |
| AA ACCAT CTC | CAAAGCCAAA |
| CCCGGGATGA | GCTGACCAAG |
| CCAGCGACAT | CGCCGTGGAG |
| CGCCTCCCGT | GTTGGACTCC |
| AGAGCAGGTG | GCAGCAGGGG |
| ACCACTACAC | GCAGAAGAGC |
| GGGGTGGATC | AGGCGGTGGA |
| GGGGTGGATC | AGGAGGAGGT |
| GCCCCAAAGG | GGAGTGTCCA |
| GGGGGACCCT | GATCAACACC |
| AGAACTGGAG | GAACCTGATC |
| ATGAGCAGAG | CCGGCGGGTG |
| CCAACCACGA | CATCGCGCTG |
| TGCCCCTCTG | CCTGCCCGAA |
| CATTGGTCAG | CGGCTGGGGC |
| TCCTCAACGT | GCCCCGGCTG |
| ACTCCCCAAA | TATCACGGAG |
| CCTGCAAGGG | GGACAGTGGA |
| CGGGCATCGT | CAGCTGGGGC |
| GGGTCTCCCA | GTACATCGAG |
| TCCTCCTGCG | AGCCCC ATTT |
| GCGGATCAGG | TGGGGGTGGA |
| CTCACACATG | CCCACCGTGC |
| TCCCCCCAAA | ACC CAA GGAC |
| TGGTGGACGT | GAGCCACGAA |
| AGGTGCATAA | TGCCAAGACA |
| TCAGCGTCCT | CACCGTCCTG |
| TCTCCAACAA | AGCCCTCCCA |
| CCCGAGAACC | AC7-vGGI'GTAC |
| TCAG CCTGAC | CTGCCTGGTC |
| GCAATGGGCA | GCCGGAGAAC |
| CCTTCTTCCT | CTACAGCAAG |
| TCTCATGCTC | CGTGATGCAT |
| TGTCTCCGGG | TAAA ${ }^{\text {J }}$. GA |

GGCTTCAGGG CTGCCTGGCT GCGCGCCAAC GGAGCAGTGC GTTCTGGATT CTCCTGCAAG GCAGTACTGC TCTGCTGGCA TATTCTAGAA AGGTGGGTCC ACCGTCAGTC TGAGGTCACA GTACGTGGAC CAGCACGTAC GGAGTACAAG GCTGACCAAG CGCCGTGGAG CTCC GCAGAAGAGC AGGCGGTGGA AGGAGGAGGT GATCAACACC GAACCTGATC CCGGCGGGTG CCTGCCCGAA CGGCTGGGGC GCCCCGGCTG ATCACGGAG GGACAGTGGA GTACATCGAG AGCCCC ATTT TGGGGGTGGA ACC CAAGGAC GAGCCACGAA TGCCAAGACA AGCCCTCCCA AC7-vGG17'GTAC GCCGGAGAAC CGTGATGCAT TAAA'J.GA

FVH-062 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVlia light chain or heavy chain to Fc region is underlined, and linker region connecting the Fc and the FVIia heavy chain is shown in bold

1 MVSQALRLLC LLLGLQGCLA. AVFvTQEEAE GVLFRRRRRA过 AFLEELRPGS LERECKEEQC
61 SEEEAPEIFK DAETTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE 121 THKDDQLICV NEMGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKI PILE 131 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV 241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVEFNWYVD GVEVHNAKTK PREEOYMSTY 301 RVVSVLTVLH QD6VLNGKEYK CKVSNKALPA PIEKTISKAK GOPREPQVYT LPFSRDELTK

FVH-090 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVYI light chain or heavy chain to Fc region is underlined, linker region connecting the Fc and the thrombin cleavage site is shown in bold, and the thrombin cleavage site is shown in dashed underline

1 MVSQALRLLC LLLGLQGCLA AVFTQEERH GVLHRRREf AFLEELRPGS LERECKEEQC

DNA sequence for FVH-100
1 ATGGTCTCCC 61 GCAGTCTTCG 121 GCGT TCCTGG 181 TCCTTCGAGG 241 TCTTACAGTG 301 GACCAGCTCC 361 ACGCACAAGG 21 AGIGACCACA 481 GACGGGGTGT 541 AAAAGAAATG 601 GGTGGCGGCG 661 GACAAAACTC 721 TTCCTCTTCC 781 TGCGTGGTGG 841 GGCGTGGAGG 901 CGTGTGGTCA 961 TGCAAGGTCT

2161 ATGCGCTCAG 1081 AACCAGGTCA 1141 TGGGAGAGCA 1201 GACGGCTCCT 1261 AACGTCTTCT 1321 CTCTCCCTGT 1381 GGTTCCGGTG 1441 GGTTCAGCCC 1501 TGGCAGGTCC 1561 ATCTGGGTGG 1621 GCGGTGCTGG 1681 GCGCAGGTCA 1741 CTCCGCCTGC 1801 CGGACGTTCT 1861 CAGCTGCTGG 1921 ATGACCCAGG 1981 GGCTACTCGG 2041 CACTACCGGG 2101 GTGGGCCACT 2221 TCCGGCGGAG 2281 TCCGGTGGCG 2341 CTGGGAGGAC 2401 CGGACCCCTG 2461 TTCAACTGGT 2521 CAGTACAACA 2581 AATGGCAAGG 2641 ACCATCTCCA 2701 CGGGATGAGC 2761 AGCGACATCG 2821 CCTCCCGTGT 2881 AGCAGGTGGC 2941 CACTACACGC

AGGCCCTCAG GCTCCTCTGC TAACCCAGGA AGGAGCTGCG AGGCCCGGGA ATGGGGACCA AGTCCTATAT ATGACCAGCT CGGGCACCAA CCTGCACACC CCAGCAAACC GATCAGGTGG ACACATGCCC CCCCAAAACC TGGACGTGAG TGCATAATGC GCGTCCTCAC CCAACAAAGC GAGAACCACA GCCTGACCTG ATGGGCAGCC TCTTCCTCTA CATGCTCCGT CTCCGGGTAA GCGGGGGATC TGCGGCCCCG TGTTGTTGGT TCTCCGCGGC GCGAGCACGA TCATCCCCAG ACCAGCCCGT CTGAGAGGAC ACCGTGGCGC ACTGCGAGGC ATGGCAGCAA GCACGTGGTA TTGGGGTGTA AGCCACGCCC GTGGGTCCGG GGGGATCAGA CGTCAGTCTT AGGTCACATG ACGTGGACGG GCACGTACCG AGTACAAGTG AAGCCAAAGG TGACCAAGAA CCGTGGAGTG TGGACTCCGA AGCAGGGGAA AGAAG $A$ GCCT

GGAAGCCCAC GCCGGGCTCC GATCTTCAAG GTGTGCCTCA CTGCTTCTGC GATCTGTGTG GCGCTCCTGT CACAGTTGAA CCAAGGCCGA GGGTGGATCA ACCGTGCCCA CAAGGZCACC CCACGAAGAC CAAGACAAAG CGTCCTGCAC CCTCCCAGCC GGTGTACACC CCTGGTCAAA GGAGAACAAC CAGCAAGCTC GATGCATGAG AGGTGGCGGC CGGCGGTGGA GATTGTGGGG GAATGGAGCT CCACTGTTTC CCTCAGCGAG CACGTACGTC GGTCCTCACT GCTGGCCTTC CACGGCCCTG CAGCTACCCC GGACTCCTGC CCTGACGGGC CACCAGGGTC AGGAGTCCTC TGGCGGCGGA CAAAACTCAC CCTCTTCCCC CGTGGTGGTG CGTGGAGGTG TGTGGTCAGC CAAGGTCTCC GCAGCCCCGA CCAGGTCAGC GGAGAGCAAT CGGCTCCTTC CGTCTTCTCA CTCCCTGTCT

CTTCTGCTTG GGCGTCCTGC CTGGAGAGGG GACGCGGAGA AGTCCATGCC CTGCCTGGCT AACGAGAACG cgatgccacg tatclatgtg GgtGgctgta GGCGGTGGAG GCTCCGGAAC CTCATGATCT CCTGAGGTCA CCGCGGGAGG CAGGACtGGC CCCATCGAGA ctgcccccat GGCTTCTATC TACAAGACCA ACCGTCGACA GCtctgcaca GGATCAGGTG Ggttccgatg GGC AAGGTGT CAGTTGTGTG GACAAAATCA CACGACGGGG ccGGGCACCA GACCATGTGG GTGCGCtTCT gagctcatg GGCAAGATCA AAGGGGGACA ATCGTCAGCT TCCCAGTACA CTGCGAGCCC TCAGGTGGGG ACATGCCCAC CCAAAACCCA GACGTGAGCC CATAATGCCA GTCCTCACCG A GAACCACAGG CTGACCTGCC gGgcagccge tтсСТСтаса tgCtccatga CCGGGTAAAT

GgCttcag ctacctgect ACcGGCGCCG GCGCGCCAAC AgTGCAAGGA GGAGCAGTGC GGACGAAGCT GTTCTGGATT AgAAtgGgg ctcctgcang tCGAGGGCCG GAACTGTGAG GCGGCTGTGA GCAGTACTGC AGGGGTACTC TCTGCTGGCA gAAAAATACC TATTCTAGAA GCTCCGGCGG AGGTGGGTCC GTTCCGGTGG CGGGGGATCC TCCTGGGAGG ACCGTCAGTC CCCGGACCCC TGAGGTCACA Agttcanctg gtacgtganc AGCAGTACAA CAGCACGTAC tgantgacan ggagtachag AAACCATCTC CAAAGCCAAA CCCGGGATGA GCTGACCAAG CCAGCGACAT CGCCGTGGAG CGCCTCCCGT GTTGGACTCC AgAGCAGGTG GCAGCAGGGG accactacac gcagangagc GGGGTGGATC AGGCGGTGGA GGGGTGGATC AGGAGGAGGT GCCCCAAAGG GGAGTGTCCA GGGGGACCCT GATCAACACC aganctggag gancctgatc ATGAGCAGAG CCGGCGGGTG CCAACCACGA CATCGCGCTG tGCCCCTCTG CCTGCCCGAA CATTGGTCAG CGGCTGGGGC tCCTCAACGT GCCCCGGCTG CGGAGTACAT GTTCTGTGCC GTGGAGGCCC ACATGCCACC GGGGCCAGGG CTGCGCAACC TCGAGTGGCT GCAAAAGCTC CATtTCCCGG tGGCGGTGGC GTGGATCAGG CGGTGGAGGT CGTGCCCAGC ACCTGAACTC AGG ${ }^{2} \mathrm{CACCCT}$ CATGATCTCC ACGAAGACCC TGAGGTCAAG AGACAAAGCC GCGGGAGGAG tCCtGCACCA GGACTGGCTG TCCCAGCCCC CATCGAGAAA tGTACACCCT GCCCCCATCC TGGTCAAAGG CTTCTATCCC agad chacta cangaccacg GCAAGCTCAC CGTGGACAAG tGCATGAGGC TCTGCACAAC GA

FVIT－： 00 amino acid sequence．Signal sequence is shown in dotted underline，propeptide is double underlined， linker region connecting FV H light chain or heavy chain to Fc region is underlined，linker region connecting the Fc and the thrombin cleavage site is shown in bold，the thrombin cleavage site is shown in dashed underline，and the trypsin 170 loop region is wave underlined

| 1 | MySQATRLIC | IJLGTOGCJA | AVFVTQEEAH | GVLHRRRRAN | AFLEELRPGS | LERECKEEQC |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 61 | SFEEAREIFK | DAERTKLFWI | SYSDGDQCAS | SPCQNGGSCK | DQLQSYICFC | LPAFEGRNCE |
| 121 | THKDDQLICV | NENGGCEQYC | SDHTGTKRSC | RCHEGYSLLA | DGVSCTPTVE | YPCGKT PILE |
| 181 | KRNASKPQGR | GGGGSGGGGS | GGGGSGGGGS | GGGGSGGGG5 | DKTHTCPPCP | APELLGGPSV |
| 241 | FLFPPKPKDT | LMI3RTPEVT | CVVVDVSHED | PEVKFNSYVD | GVEVHNAKTK | PREEQYNSTY |
| 301 | RW5VLTVLH | QDKLNGKEYK | CKVSNKALPA | PTEKTISKAK | GQPREPQVYT | LPPSRDELTK |
| 361 | NQVS ${ }^{\text {NutcluvK }}$ | GFYPSDIAVE | 䀦ESNGQPEN | YK T．TPPVLDS | DGSFFLYSEI | 「＇VDKSENQQ |
| 421 | NvFSCSVMHE | ALHNHYTQKS | LSLS GKGGG | GSGGGGSGGG | GSGGGGSGGG | GSGGGGSGGG |
| 481 | GSALRPRIVG | GKVCPKGECP | SQVLILVNGA | QLCGGTLINT | Invvsfallcy | DKIKNWPELI |
| 541 | AVLGEHDLSE | HDGDEQSP．RV | AQVITPSTYV | PGTPNHDIAI | LRLHQPVVLT | DHVVPLCLPE |
| 601 | RTFSERTLAF | VRFSLVSGWG | QLLDRGATAL | EJMVJINVERL | PITQ DCEASYP | GK1TEYMFCA |
| 661 | GYSDGSKDSC | KGDSGGP KAT | HYRGTKYLTG | TVSWGQGCAT | VGKFGVYTRV | 8QYTENLQKL |
| 721 | MP．SE PRPGVL | LRAPFP GGGG | SGGGGSGGGG | SGGGGSGGGG | SGGGGSDKTH | 「＇С PPCPAPEL |
| 781 | LGGPSVFLFP | PKPKDTLMTS | RTPEVTCVW | DVSHEDPEVK | FNWYVDGVEV | HNAKTKPREE |
| 841 | QYNSTYRVVS |  | NGKEYKCKVS | NKALPAPTEK | TISKAKGQPR | EPOVYTLPPS |
| 91 | RDELTKMQVS | LTCLVKGFYP | SDI．AVEWESN | GQPENNYKTT | PPVLDSDGSF | FLYSKLTVDK |
| 961 | SPNQQGNVFS | CsVmbeathr | HYTQKSL3LS | PGE＊＊ |  |  |

DNA．sequence for FVIT－1．5
30 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCTTCG 121 GCGTTCCTGG 181 TCCTTCGAGG 241 TCTTACAGTG 3.1 GACCAGCTCC 361 ACGCACAAGG
421 AGTGACCACA
481 GACGGGGTGT
S41 AAAAGAAATG

601 GGTGGCGGCG
661 GACAA动ACTC
721 TTCCTCTTCC
781 TGCGTGGTGG
841 GGCGTGGAGG
901 CGTGTGGTCA
961 TGCAAGGTCT
021 GGGCAGCCCC
1081 AACCAGGTCA
1141 TGGGAGAGCA
1201 GACGGCTCCT
261 AACGTCTTCT
321 CTCTCCCTGT
331 GGTTCCGGTG
441 GGTTCAGCCC
501 TGGCAGGTCC
561 ATCTGGGTGG S21 GCGGTGCTGG S31 GCGCAGGTCA

TAACCCAGGA GGAAGCCCAC AGGAG CTGCG AGGCCCGGGA GATCTTCAAG ATGGGGACCA GTGTGCCTCA AGrCCPATPAT CTGCTTCTGC AtGACCAGCT GATCTGTGTG CGGGCACCAA GCGCTCCTGT CCTGCACACC CACAGTTGAA CCAGCAAACC CCAAGGCCGA GATCAGGTGG GGGTGGATCA GGGTGGATCA CAAGGACACC CCACGAAGAC CAAGACAAAG CGTCCTGCAC CCTCCCAGCC GGTGTACACC CCTGGTCAAA GGAGAACAZC CAGCAAGCTC GATGCATGAG AGGTGGCGGC CGGCGGTGGA GATTGTGGGG GAATGGAGCT CCACTGTTTC TCTCCGCGGC GCGAG CACGA CCICAGCGAG GGTCCTCACT GCTGGCCTTC ACCGTGGCGC ACTGCCTGCA GTGCCGGCTA CCACCCACTA CAACCGTGGG AGCTCATGCG GTGGCTCCGG GAGGTTCCGG AACTCCTGGG TCTCCCGGAC TCAAGTTCAA

CTGGAGAGGG GACGCGGAGA AGTCCATGCC CTGCCTGGCT $A A C$ GAGAACG CGGTGCCACG TATCCATGTG GGTGGCGGTG GGCGGTGGAG GCTCCGGAAC TCATGAT CT CCTGAGGTCA CCGCGGGAGG CAGGACTGGC CCCATCGAGA CTGCCCCCAT GGCTTCTATC TACAAGACCA ACCGTCGACA GCTCTGCACA GGATCAGGTG GGTTCCGGTG GGCAAGGACT CAGTTGTGTG GACAAAATCA CACGACGGGG CCGGGCACCA GACCATGTGG GTGCGCTTCT GTACTCCAAG AAGGTGGGAG AGCAAGGACT TGGTACCTGA GTGTACACCA CGCCCAGGAG TCCTCCTGCG TCCGGTGGCG GCGGATCAGG TCAGACAAAA GACGGCGTGG TGGTGGACGT

GCGCGCCAAC GGAGCAGTGC GTTCTGGATI CTCCTGCAAG GAACTGTGAG GCAGTACTGC TCTGCTGGCA tATtCTAGAA AGGTGGGTCC CGGGGGATCA ACCGTCAGTC TGAGGTCACA GTACGTGGAC CAGCACGTAC GGAGTACAAG CAAAGCCAAA GCTGACCAAG CGCCGTGGAG GTTGGACTCC GCAGCAGGGG GCAGAAGAGC AGGCGGTGGA AGGAGGAGGT GGAGTGTCCA GAT C $A \mathrm{ACACC}$ GIACCTGAT C CCGGCGGGTG CATCGCGCTG CCTGCCCGAA CGGCTGGGGC GCCCCGGCTG TAT CACGGAG GGACAGTGGA CAGCTGGGGC GTACATCGAG AGCCCCATTT TGGGGGTGGA CCCACCGTGC ACCCAAGGAC GAGCCACGAA TGCCAAGACA

FVII-1 15 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVIf light chain or heavy chain to Fc region is underlined, linker region connecting the Fc: and the thrombin cleavage site is shown in bold, the thrombin cleavage site is shown in dashed underline, and the three point mutations in FVIia (V158D, E2.96V and M298Q) are in bold and underlined

|  | MVSQALRLLC | LLGUGGCLA | AVETOEEAS | gVi.frrrran | AFLEELRPGS | LERECKEEQC |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 61 | SFEEAREIFK | DAERTKLFW1 | SYSDGDQCAS | SPCQNGGSCK | DQLQSY!CFC | LPAFEGRNCE |
| 121 | TKKDDQLICV | NENGGCEQYC | S DitGTKRSC | RCHEGYSLTA | DGVSCTPTVE | YPCGKI PILE |
| 181 | KRNASKPQGR | GGGGSGGGGS | GGGGSGGGGS | gGGGSGGGGS | DKT | APELLGGPSV |
| 1 | FLFPP | LM | CVWD | PEVKFNYYVD | GVE | PREEQYNS |
| 1 | RVVSVLTVLH | QDBLNGKEYK | CKVSNKALPA | P1 | GQPRE PQ | LPPSRDELTK |
| 361 | NovSLTCLVK | GFYPSDI | WESNGQPENN | YKTTPPVLDS | DG | TVD |
| 421 | NVESCSVMife | ALHNHYTQRS | LSLSEGKgge | gscgegscge | gsggegseg | G |
| 431 | GSALRPRIVG | GKDCPKGECP | BQVLLLVNGA | QLCGGTLINT | IKVV | DK |
| 541 | avLGEHDLSE | HDGDEQSRRV | AQV1 1PSTYV | PGTTNHDIAL | LRLHQPVVL? | DfivvPLCLPE |
| 601 | RTFSERTLAF | vRFSLVsGBG | QlLDrgatal | VLQVLNVFRL | MTQDCLQQ3R | Kighs enite |
| 661 | YMFCAGYSDG | SKDSCKGDSG | GPaAtHyRGI' | WYL? GIVSBG | QGCATVGHFG | VYTRVSQY1E |
| 721 | WLQKLMRSEP | RPGVLLRAPF | PGGGGSSGGGG | SGGGGSGGGG | SGGGGSGGGG | 8DKTHTCPPC |
| 731 | PAPELLGGPS | VFLFPPKPKD | TLMISRIPEV | TCVVVDVSHE | DPE VKFNWYV | DgVEVHNAK? |
| 1 | KPREEQYMST | YRVVSVLTVL | HQDilngkey | KCKVS \#KALP | AP1EKTISKA | KGQPRE PQVY |
| 1 | TLPPSRDELT | KNQVSLTCLV | KGFYPSDIAV | EWESNGQPEN | HYKTTPPVLD | BDGSFFL.YSK |
| 96 | LTVDKSRbQO | GNVESCSVMy | EALHNHYTQK | SLSLSPGK* |  |  |

1 ATGGTCTCCC AGGCCCTCAG GCT'CCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT

61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC 121 GCGTTCCTGG AGGAGC'MCG GCCGGGCTCC 131 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG 241 ?C?ACAGIG ATGGGGACCA GTGTGCCTCA 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC 361 ACGCACAAGG ATGACCAGCN GATCTGTGTG 421 AG? GACCACA CGGGCACCAA GCGCTCCTGT 431 GACGGGGTGT CCTGCACACC CACAGTTGAA 541 AAAAGAAATG CCAGCAAACC CCAAGGCGCC 601 TGCCCCAAAG GGGAGTGTCC ATGGCAGGTC 661 GGGGGGACCC TGATCAACAC CATCTGGGTG 721 AAGAACTGGA GGAACCTGAT CGCGGTGCTG 731 GATGAGCAGA GCCGGCGGGT GGCGCAGGTC 841 ACCAACCACG ACATCGCGCT GCTCCGCCTG 901 GTGCCCCTCT GCCTGCCCGA ACGGACGTTC 961 TCATTGGTCA GCGGCTGGGG CCAGCTGCTG 1021 GTCCTCAACG TGCCCCGGCT GATGACCCAG 1031 GACTCCCCAA 1141 TCCTGCAAGG 1201 ACGGGCATCG 1261 AGGGTCTCCC 1321 GTCCTCCTGC 1331 GGCGGATCAG 1441 ACTCACACAT转 621 GAGGTGCATA 631 GTCAG 1741 GTCTCCAACA AAGCCCTCCC AGCCCCCATC $1 \approx 01$ CCCCGAGAAC CACAGGTGTA CACCCTGCCC 1861 GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC 1921 AGCAATGGGC AGCCGGAGAA CAACTACAAG A 1931 TCCTTCTTCC TCTACAGCAA GCTCACCGTG 2041 TTCTCATGCT CCGTGATGCA TGAGGCTCTG

GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC C GGAGAGG AGTGCAAGGA GGAGCAGTGC GACGCGGAGA GGACGAAGCT GTMCGAI? AGTCCATGCC AGAATGGGGG CTCCTGCAAG CTCCCTGCCT TCGAGGGCCG GAACTGTGAG AACGAGAACG GCGGCTGTGA GCAGTACTGC CGGTGCCACG AGGGGTACTC TCTGCTGGCA ? ATCCATGTG GAAAAATACC TATTCTAGAA CTGCGGCCCC GGATTGTGGG GGGCAAGGTG CTGTTGTTGG TGAATGGAGC TCAGTTGTGT GTCTCCGCGG CCCACTGTTT CGACAAAATC GGCGAGCACG ACCTCAGCGA GCACGACGGG ATCATCCCCA GCACGTACGT CCCGGGCACC CACCAGCCCG TGGTCCTCAC TGACCATGTG TCTGAGAGGA CGCTGGCCTT CGTGCGCTTC GACCGTGGCG CCACGGCCCT GGAGCTCATG GACTGCCTGC AGCAGTCACG GAAGGTGGGA TGTGCCGGCT ACTC GGATGG CAGCAAGGAC GCCACCCACT ACCGGGGCAC GTGGTACCTG GCAACCGTGG GCCACTTTGG GGTGTACACC AAGCTCATGC GCTCAGAGCC ACGCCCAGGA GGTGGCTCCG GCGGAGGTGG GTCCGGTGGC GGAGGTTCCG GTGGCGGGGG ATCCGACAAA GAACTCCTGG GCGGACCGTC AGTCTTCCTC ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTCAAGTTCA ACTGGTACGT GGACGGCGTG GAGGAGCAGT ACAACAGCAC GTACCGTGTG TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GAGAAAACCA TCTC CAAAGC CAAAGGGCAG CCATCCCGGG ATGAGCTGAC CAAGAACCAG TATCCCAGCG ACATCGCCGT GGAGTGGGAG ACCACGCCTC CCGTGTTGGA CTCCGACGGC GACAAGAGCA GGTGGCAGCA GGGGAACGTC CACAACCACT ACACGCAGAA GAGCCTCTCC

| 2201 | CTGTCTCCGG | GTAAAGGTGG | CGGCGGATCA | GGTGGGGGTG | GATCAGGCGG | TGGAGGTTCC |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 2161 | GGTGGCGGGG | GATCAGACAA | AACTCACACA | TGCCCACCGT | GCCCAGCACC | TGAACTCCTG |  |
| 221 | GGAGGACCGT | CAGTCTTCCT | CTTCCCCCCA | AAACCCAAGG | ACACCCTCAT | GATCTCCCGG |  |
| 2281 | ACCCCTGAGG | TCACATGCGT | GGTGGTGGAC | GTGAGCCACG | AAGACCCTGA | GGTCAAGTTC |  |
| 2341 | AACTGGTACG | TGGACGGCGT | GGAGGTGCAT | AATGCCAAGA | CAAAGCCGCG | GGAGGAGCAG |  |
| 2401 | TACAACAGCA | CGTACCGTGT | GGTCAGCGTC | CTCACCGTCC | TGCACCAGGA | CTGGCTGAAT |  |
| 2461 | GGCAAGGAGT | ACAAGTGCAA | GGTCTCCAAC | AAAGCCCTCC | CAGCCCCCAT | CGAGAAAACC |  |
| 2521 | ATCTCCAAAG | CCAAAGGGCA | GCCCCGAGAA | CCACAGGTGT | ACACCCTGCC | CCCATCCCGC |  |
| 2581 | GATGAGCTGA | CCAAGAACCA | GGTCAGCCTG | ACCTGCCTGG | TCAAAGGCTT | CTATCCCAGC |  |
| 2641 | GACATCGCCG | TGGAGTGGGA | GAGCAATGGG | CAGCCGGAGA | ACAACTACAA | GACCACGCCT |  |
| 2701 | CCCGTGTTGG | ACTCCGACGG | CTCCTTCTTC | CTCTACAGCA | AGCTCACCGT | GGACAAGAGC |  |
| 2761 | AGGTGGCAGC | AGGGGAACGT | CTTCTCATGC | TCCGTGATGC | ATGAGGCTCT | GCACAACCAC |  |
| 2821 | TACACGCAGA | AGAGCCTCTC | CCTGTCTCCG | GGTAAATGA |  |  |  |

FVII-18 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, the thrombin cleavage site is shown in dashed underline, the linker region connecting Fivtheavy chain to Fc region is underlined, and the linker region connecting the Fc regions is shown in bold

1 MVSQALRLLC LLLGLQGCLA AVFVIOEEM GVLHRPRRAN AFLEELRPGS LERECKEEQC
61 SFEEAREIFK DAERTRIFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC
121 THKDDQLICV NENGGCEQYC SDHYGTKRSC RCHEGYSLLA 131 KRNASKPQGA LRPRI VGGKV CPKGECPWQV LLLVNGAOLC 241 KNMRNLIAVL GEHDLSEHDG DEQSRRVAQV IIPSTYVPGT 301 ypLCLPERTF SERTLAFVRF SLVSGBGQLL DRGATALELM DGVSCTPTVE LPAFEGRNCE SERTLAFVRF SLVSGBGQLL DRGATALELM TNEDI ALLRL HQPVVLTDHV 421 RVSQYTEWLQ KLMRSEPRPG 431 '1HTCPPCPAP ELLGGPSVFL 541 EVHNAKTKPR Sol PREPQVYTLP 661 SFFLYSKLTV 721 GGGGSDKTHT 731 NWYVDGVEVH 841 ISKAKGQPRE EEQ YNSTYRV VL LRAPFPGG GGSGGGGSGG FPPKPKDTLM ISRTPEVTCV GGSGGGGSGG ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PSRDELTKNQ VSLTCLVKGF YPSDILIKCK VSNKALPAPI YPS DIAVEWE SNGQPENNYK HNEYTQKSLS LSPGKGGGGS KPKDTLMISR TPEVTCVVVD LTVLHQDWLN GKE YKCKV3N 901 PVLDSDG3FF PQVYTLPPSR DELTKNOVSL TCL VKGFYPS DIAVEWESNG TTPPVLDSDG GGGGSGGGGS VSHEDFEVKF KALPAPIEKT QPENNYKTTP GK*

1 ATGGTCTCCC
61 GCAGTCTTCG 121 GCGTTCCTGG 131 TCCTTCGAGG 241 TCTTACAGTG 301 GACCAGCTCC 361 ACGCACAAGG 421 AGTGACCACA 431 GACGGGGTGT 541 AAAAGAAATG S01 GTGGGGGGCA 661 GGAGCTCAGT 721 TGTTTCGACA 731 AGCGAGCACG 841 TACGTCCCGG 901 CTCACTGACC 961 GCCTTCGTGC 1021 GCCCTGGAGC 1031 TCACGGAAGG 1.41 GATGGCAGCA 1201 GGCACGTGGT 1261 TTTGGGGTGT 1321 GAGCCACGCC 1331 GGTGGGTCCG 1441 GGGGGATCCG 1501 CCGTCAGTCT 1561 GAGGTCACAT 1621 TACGTGGACG 1631 AGCACGTACC 1741 GAGTACAAGT 1861 CTGACCAAGA ACCAGGTCAG CCTGACCTGC C
$1 \% 01$ AAAGCCAAAG GGCAGCCCCG AGAACCACAG

AGGCCCTCAG TAACCCAGGA AGGAGCTGCG GCCGGGCTCC AGGCCCGGGA GATCTTCAAG ATGGGGACCA GTGTGCCTCA AGTCCTATAT CTGCTTCTGC ATGACCAGCT GATCTGTGTG CGGGCACCAA GCGCTCCTGT CCTGCACACC CACAGTTGAA CCAGCAAACC CCAAGGCGGA AGGTGTGCCC TGTGTGGGGG AAATCAAGAA ACGGGGATGA GCACCACCAA ATGTGGTGCC GCTTCTCATT TCATGGTCCT TGGGAGACTC CAACGTGCCC AGGACTCCTG ACCTGACGGG ACACCAGGGT CAGGAGTCCT GTGGCGGCGG ACAAAACTCA TCCTCTTCCC CCCAAAACCC GCGTGGTGGT GGACGTGAGC GCGTGGAGGT GCATAATGCC GTGTGGTCAG CGTCCTCACC GCAAGGTCTC CAACAAA.GCC

GGCGTCCTGC TGGAGAGGG ACCGGCGCCG AGTGCAAGGA AGTCCATGCC AGACGAAGCT CTCCCTGCCT TCGAGGGCCG AACGAGAACG GCGGCTGTGA CGGTGCCACG AGGGGTACTC TATCCATGTG GGAGGTGGTT TGTCCATGGC AGGTCCTGTT AACACCATC" GGGTGGTCTC CTGATCGCGG CGGGTGGCGC AGGTCATCAT GCGCTGCTCC GCCTGCACCA CCCGAACGGA CGTTCTCTGA TGGGGCCAGC TGCTGGACCG CGGCTGATGA CCCAGGACTG ACGGAGTACA TGTTCTGTGC AGI'GGAGGCC CACATGCCAC TGGGGCCAGG GCTGCGCAAC ATCGAGTGGC TGCAAAAGCT CCATTTCCCG GTGGCGGTGG GGTGGATCAG CCGTGCCCAG CTCCGGAACT

CTGCCTGGCT GCGCGCCAAC GGAGCAGTGC GTTCTGGATT CTCCTGCAAG GAACTGTGAG GCAGTACTGC TCTGCTGGCA TATTCTAGAA GCCCCGGATT gTtGGTGAAT CGCGGCCCAC GCACGACCTC CCCAGCACG GCCCGTGGTC GAGGACGCTG GGGGGCCAGG CCTGCAGCAG CGGCTACTCG CCACTACCGG CGTGGGCCAC CATGCGCTCA CTCCGGCGGA TTCCGGTGGC CTGGGCGGA CCGGACCCCT GTTCAACTGG GCAGTACAAC GAATGGCAAG AACCATCTCC CCGGGATGAG CAGCGACATC

5

DNA sequence for FVYi-1 27

1 ATGGTCTCCC
61 GCAGTCTTCG 121 GCGTTCCTGG 131 TCCTTCGAGG 241 TCTTACAGTG 301 GACCAGCTCC 361 ACGCACAAGG 421 AGTGACCACA 431 GACGGGGTGT 541 AAAAGAAATG 601 TGCCCCAAAG 661 GGGGGGACCC 721 AAGAACTGGA 731 GATGAGCAGA 841 ACCAACCACG 901 GTGCCCCTCT 961 TCAMJGGTCA 1021 GICCICAACG TGCCCCGGCT 1031 ACGGAGTACA TGTTCTGTGC 1. 41 AgTGGAGGCC 1201 TGGGGCCAGG 1261 ATCGAGTGGC 1321 CCATTTCCCG 1331 GGTGGATCAG 1441 CCGTGCCCAG 1501 AAGGACACCC 1561 CACGAAGACC 1621 AAGACAAAGC

AGGCCCTCAG GCTCCTCTGC TAACCCAGGA GGAAGCCCAC -AGGAGCTGCG GCCGGGCTCC AgGCCCGGGA GATCTTCAAG ATGGGGACCA GTGTGCCTCA -AGTCCTATAT CTGCTTCTGC -ATGACCAGCT GATCTGTGTG CGGGCACCAA GCGCTCCTGT CCTGCACACC CACAGTTGAA CCAGCAAACC CCAAGGCGCC GGGAGTGTCC ATGGCAGGTC TGA-TCAACAC CATCTGGGTG GGa-ACCTGAT CGCGGTGCTG GCCGGCGGGT GGCGCAGGTC ACATCGCGCT GCTCCGCCTG GCCTGCCCGA ACGGACGTTC GCGGCTGGGG CCAGCTGCTG 1631 GTCCTGCACC 1741 CTCCCAGCCC

CACATGCCAC GCTGCGCAAC TGCAAAAGCT GTGGCGGTGG GCGGTGGAGG CTCCGGAACT TCATGATCTC CTGAGGTCAA CGCGGGAGGA AGGACTGGCT CCATCGAGAA

GGCGTCCTGC CTGGAG-AGGG AGTGCAAGGA GACGCGGAGA GGACGAAGCT AGTCCATGCC AGAATGGGGG CTCCCTGCCT TCGAGGGCCG AACGAGAACG GCGGCTGTGA CGGTGCCACG AGGGGTACTC TATCCATGTG GAAAAATACC CTGCGGCCCC GGATTGTGGG CTGTTGTTGG TGAATGGAGC GTCTCCGCGG CCCACTGTTT GGCGAGCACG ACCTCAGCGA ATCATCCCCA GCACGTACGT CACCAGCCCG TGGTCCTCAC TCTGAGAGGA CGCTGGCCTT GACCGTGGCG CCACGGCCCT GACTGCGAGG CCAGCTACCC GATGGCAGCA AGGACTCCTG GGCACGTGGT ACCTGACGGG TTTGGGGTGT ACACCAGGGT GAGCCACGCC CAGGAGTCCT GGTGGGTCCG GTGGCGGCGG GGGGGATCAG ACAAAACTCA CCGTCAGTCT TCCTCTTCCC GAGGTCACAT GCGTGGTGGT TACGTGGACG GCGTGGAGGT AGCACGTACC GTGTGGTCAG GAGTACAAGT AAAGCCAAAG

CTGCCTGGCT GCGCGCCAAC GGAGCAGTGC GTTCTGGAT T CTCCTGCAAG GAACTGTGAG GCAGTACTGC TCTGCTGGCA TATTCTAGAA GGGCAAGGTG TCAGTTGTGT CGACAAAATC GCACGACGGG CCCGGGCACC TGACCATGTG CGTGCGCTTC GGAGCTCATG CGGCAAGATC CAAGGGGGAC C-AtCGTCAGC CTCCCAGTAC CCTGCGAGCC ATCAGGTGGG CACATGCCCA CCCAAAACCC GGACGTGAGC GCATAATGCC CGTCCTCACC CAACAAAGCC
A GAAC CACAG

45 DNA sequence for FVIi-125 shown in bold

| 1801 | GTGTACACCC | TGCCCCCATC | CCGGGATGAG | CTGACCAAGA | ACCAGGTCAG | CCTGACCTGC |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 1861 | CTGGTCAAAG | GCTTCTATCC | CAGCGACATC | GCCGTGGAGT | GGGAGAGCAA | TGGGCAGCCG |
| 1921 | GAGAACAACT | ACAAGACCAC | GCCTCCCGTG | TTGGACTCCG | ACGGCTCCTT | CTTCCTCTAC |
| 1981 | AGCAAGCTCA | CCGTGGACAA | GAGCAGGTGG | CAGCAGGGGA | ACGTCTTCTC | ATGCTCCGTG |
| 2041 | ATGCATGAGG | C7CTGCACAA | CCACTACACG | CAGAAGAGCC | TCTCCCTGTC | TCCGGGTAAA |
| 2101 | GGTGGCGGCG | GATCAGGTGG | GGGTGGATCA | GGCGGTGGAG | GTTCCGGTGG | CGGGGGATCA |
| 2161 | GACAAAACTC | ACACATGCCC | ACCGTGCCCA | GCACCTGAAC | TCCTGGGAGG | ACCGTCAGTC |
| 2221 | TTCCTCTTCC | CCCCAAAACC | CAAGGACACC | CTCATGATCT | CCCGGACCCC | TGAGGTCACA |
| 2281 | TGCGTGGTGG | TGGACGTGAG | CCACGAAGAC | CCTGAGGTCA | AGTTCAACTG | GTACGTGGAC |
| 2341 | GGCGTGGAGG | TGCATAATGC | CAAGACAAAG | CCGCGGGAGG | AGCAGTACAA | CAGCACGTAC |
| 2401 | CGTGTGGTCA | GCGTCCTCAC | CGTCCTGCAC | CAGGACTGGC | TGAATGGCAA | GGAGTACAAG |
| 2461 | TGCAAGGTCT | CCAACAAAGC | CCTCCCAGCC | CCCATCGAGA | AAACCATCTC | CAAAGCCAAA |
| 2521 | GGGCAGCCCC | GAGAACCACA | GGTGTACACC | CTGCCCCCAT | CCCGCGATGA | GCTGACCAAG |
| 2581 | AACCAGGTCA | GCCTGACCTG | CCTGGTCAAA | GGCTTCTATC | CCAGCGACAT | CGCCGTGGAG |
| 2641 | TGGGAGAGCA | ATGGGCAGCC | GGAGAACAAC | TACAAGACCA | CGCCTCCCGT | GTTGGACTCC |
| 2701 | GACGGCTCCT | TCTTCCTCTA | CAGCAAGCTC | ACCGTGGACA | AGAGCAGGTG | GCAGCAGGGG |
| 2761 | AACGTCTTCT | CATGCTCCGT | GATGCATGAG | GCTCTGCACA | ACCACTACAC | GCAGAAGAGC |
| 2821 | CTCTCCCTGT | CTCCGGGTAA | ATGA |  |  |  |

FVIi-127 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, the thrombin cleavage site is shown in dashed underline, the trypsin 170 loop region is wave underlined, the linker region connecting FVII heavy chain to Fc region is underlined, and the linker region connecting the Fc regions is

|  | MVSQALRLLC |  | AVFVT34EEAH |  | AFLEELRPGS | LERECKEEQC |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 61 | SFEEAREIFK | DAERTRLEW | SYSDGDQCAS | SPCQNGGS | DQLQSY1CFC | LP |
| 121 | THKDDQLICV | NEN | SD | RC |  | YPCGK1PILE |
|  | KRNASKPQGA | LRPRIVGGKV | CPKGECPWQV | LLLVNGAOLC | GGTLINTTBV | VSAAHCFDKI |
|  | KNo | GEHDL5EHDG | DEQSRRVAQV | GT | TNED IALLRL |  |
|  | VP | SE |  | DR | VL | DCEASYPGKI |
|  | TEYMFCAGYS | DG |  |  |  | FGVYTRVSQY |
| 21 | IEWLQKLMRS | E | PFP | GGSGGGGSGG | GGS | GGS |
|  | PCPAPELLGG | PSVFLFPPKP | KDTMM | EVTCVVVDVS | HEDUEVKFM | YVDGVE VITNA |
|  | KT | ST | V | EYKCKVSNKA | LPAPIEKTIS | PQ |
|  | VYTLPP | LTKNQVSLTC | LVK | AV | ENN | LDSDGSFFLY |
| 661 | SKLTVDK3RW | QGGVIISCSV | MEEALHN | QK3LSLSFGK | GGGGSGGGGS | GGGGSGGGGS |
|  | DKTHTCPPCP | APELLGGPSV | FLFPPKPKDT | LMI SRTPEVT | CVVVDVSHE | EVKFNBYVD |
|  | GVEVHNAKTK | PREEQYNS | RVVSVLTV | QDBLNGKEYK | CKVSNKALP | PIERTISKAK |
|  | GQPREPQVYT | LPPSRDE | NQVS | GFY PSDIAVE | WESNGQUENN | S |
|  |  |  |  |  |  |  |

1 ATGGTCTCCC
61 GCAGTCTTCG 121 GCGTTCCTGG 181 TCCTTCGAGG 241 TCTTACAGTG 301 GACCAGCTCC 361 ACGCACAAGG 421 AGTGACCACA 431 GACGGGGTGT 541 AAAAGAAATG S01 GAGTGTCCAT $6_{6} 1$ ATCAACACCA 721 AACCTGATCG 751. CGGCGGGTGG 841 ATCGCGCTGC 901 CTGCCCGAAC 961 GGCTGGGGCC 1021 CCCCGGCTGA 10:3J. ATCACGGAGT 1. 41 GACAGTGGAG 1201 AGCTGGGGCC 1261 TACATCGAGT 1321 GCCCCATTTC 1331. GGGGGTGGAT 1441 GCCGCCCCCA 150J. CGGAGCCTGC 1561 CAGAGCCCCC 1621 TTCAGCGGCA S31 GACGTGGGCG

AGGCCCTCAG TAACCCAGGA AgGAGCTGCG AGGCCCGGGA ATGGGGACCA AGTCCTATAT ATGACCAGCT CGGGCACCAA CCTGCACACC CCAGCAAACC GGCAGGTCCT TCTGGGTGGT CGGTGCTGGG CGCAGGTCAT TCCGCCTGCA GGACGTTCTC AGCTGCTGGA TGACCCAGGA ACATGTTCTG GCCCACATGC AGGGCTGCGC GGCTGCAAAA CCGGTGGCGG CAGGCGGTGG GCGTGCCCGT TGCACAGCAA AGCTGCTGAT GCGGCAGCGG TGTACTACTG

GCTCCTCTGC GGAAGCCCAC GCCGGGCTCC GATCTTCAAG GTGTGCCTCA CTGCTTCTGC GATCTGTGTG GCGCTCCTGT CACAGTTGAA CCAAGGCCGA GTTGTTGGTG CTCCGCGGCC CGAGCACGAC CATCCCCAGC CCAGCCCGTG TGAGAGGACG CCGTGGCGCC CTGCCTGCAG TGCCGGCTAC CACCCACTAC AACCGTGGGC GCTCATGCGC TGGCTCCGGC AGGTTCCGGT GACCCCCGGC CGGCAACACC CTACCGGATG CACCGCCTTC CATGCAGCAC

| CTTCTGCTTG | GGCTTCAGGG | CTGCCTGGCT |
| :--- | :--- | :--- |
| GGCGTCCTGC | ACCGGCGCCG | GCGCGCCAAC |
| CTGGAGAGGG | AGTGCAAGGA | GGAGCAGTGC |
| GACGCGGAGA | GGACGAAGCT | GTTCTGGATT |
| AGTCCATGCC | AGAATGGGGG | CTCCTGCAAG |
| CTCCCTGCCT | TCGAGGGCCG | GAACTGTGAG |
| AACGAGAACG | GCGGCTGTGA | GCAGTACTGC |
| CGGTGCCACG | AGGGGTACTC | TCTGCTGGCA |
| TATCCATGTG | GAAAAATACC | TATTCTAGAA |
| ATTGTGGGGG | GCAAGGTGTG | CCCCAAAGGG |
| AATGGAGCTC | AGTTGTGTGG | GGGGACCCTG |
| CACTGTTTCG | ACAAAATCAA | GAACTGGAGG |
| CTCAGCGAGC | ACGACGGGGA | TGAGCAGAGC |
| ACGTACGTCC | CGGGCACCAC | CAACCACGAC |
| GTCCTCACTG | ACCATGTGGT | GCCCCTCTGC |
| CTGGCCTTCG | TGCGCTTCTC | ATTGGTCAGC |
| ACGGCCCTGG | AGCTCATGGT | CCTCAACGTG |
| CAGTCACGGA | AGGTGGGAGA | CTCCCCAAAT |
| TCGGATGGCA | GCAAGGACTC | CTGCAAGGGG |
| CGGGGCACGT | GGTACCTGAC | GGGCATCGTC |
| CACTTTGGGG | TGTACACCAG | GGTCTCCCAG |
| TCAGAGCCAC | GCCCAGGAGT | CCTCCTGCGA |
| GGAGGTGGGT | CCGGTGGCGG | CGGATCAGGT |
| GGCGGGGGAT | CCGACATCGT | GATGACCCAG |
| GAGAGCGTGA | GCATCAGCTG | CCGGAGCAGC |
| TACCTGTGCT | GGTTCCTGCA | GCGGCCCGGC |

5 ＂ 21 SS＊

201 そうに AAGATCAGCT 521 CAGCGGCCCG 1981 AAGTACAACG AGCTGAGCAG 101 GAGTACGGCA 2161 AGCAGCTGA

FVII－－125 amino acid sequence．Signal seguesice is shown in dotted underline， propeptide is double und．erli．ned，and linker region connecting FVIT to APS is bold，and AP3 scFv is italicized

1 MVSORYRLLC．LILGIQGCYA AYEYMOERM GYHWRERAN AFLEELRPGS LERECKEEQC 61 SFEEAREIFK DAERTKLFHI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE 1－1 THKDDQL JCV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE 13〕 KRNA3KPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL IRT IWW SAA HCFDKIKBUNR 2ヶ1 NLTAVLGEFD LSEHDGDEQS RRVAQVTIPS TYVFGTTNHD IALLRLHQPV VLTDHVVPLC 301 LPERTFSEET L．AFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ Q8RKVGDSPN 361 ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRV8Q 421 YIEWLQKLLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGS DI VMTQ 4！31．AAPSVPVTPG ESVSISCRSS RSLLHSNGNT YLCWFLQRPG QSPQLLIYRM SNLASGVPDR 541 FSGSGSGTAF TLRISRVEAE DVGVYYCMQH LEYPFTFGSG TKLEIKRGGG GSGGGGSGGG 601 GSQVQLQOSG AELVRPGTSV KISCKASGYT FTNYWLGWVK QRPGHGLENI GDIYPGGGYN 661 KYNENFKGKA TLTADTSSST A YMQLSSLTS EDSA．VYFCAR EYGNYD YAMD SWGQGTSVTV 5i AGCCACGAAG 1621 GCCAAGACAA 1681 ACCGTCCTGC $1 \% 41$ GCCCTCCCAG $1 \S 01$ CAGGTGTACA 185 J ．TGCCTGGTCA 1921 CCGGAGAACA 1951 TACAGCAAGC 2041 GTGATGCATG 2101 AAAGGTGGCG 2151．TCAGACAAAA 2221 GTCTTCCTCT 223J．ACATGCGTGG 2341 GACGGCGTGG 2401 TACCGTGTGG 2461 AAGTGCAAGG 2521 AAAGGGCAGC 2531 AAGAACCAGG 2641 GAGTGGGAGA 2701 TCCGACGGCT 2761 GGGAACGTCT 821 AGCCTCTCCC 28！1．GGCGGATCAG
DNA secuence for FVI 1－067
1 ATGGTCTCCC
61 GCAGTCTTCG TAACCCAGGA 121 GCGTTCCTGG 181 TCCTTCGAGG 241．TCTTACAGTG 301 GACCAGCTCC 361 ACGCACAAGG 421 AGTGACCACA
 G41 AAAAGAAATG 601 GAGTGTCCAT 651．ATCAACACCA 721 AACCTGATCG 7\＆ 1 CGGCGGGTGG 84J．ATCGCGCTGC 901 CTGCCCGAAC 961．GGCTGGGGCC 1021 CCCCGGCTGA 10s 1 ATCACGGAGT 1141 GACAGTGGAG 1201 AGCTGGGGCC 1261 TA 1321 GCCCCATTTC 1381 GGGGGTGGAT 1381 GGGGGIGGAT 441 CCACCGTGCC GGA CCCTCATGAT ACCCTGAGGT AGCCGCGGGA ACCAGGACTG CCCCCATCGA CCCTGCCCCC AAGGCTTCTA ACTACAAGAC TCACCGTGGA AGGCTCTGCA GCGGATCAGG CTCACACATG TCCCCCCAAA TGGTGGACGT AGGTGCATAA TCAGCGTCCT TCTCCAACAA CCCGAGAACC TCAGCCTGAC GCAATGGGCA CCTTCTTCCT TCTCATGCTC TGTCTCCGGG GTGGGGGTGG

GCTCCTCTGC GGAAGCCCAC GCCGGGCTCC GATCTTCAAG GTGTGCCTCA CTGCTTCTGC GATCTGTGTG GCGCTCCTGT CACAGTTGAA CCAAGGCCGA GTrGTTGGTG CTCCGCGGCC CGAGCACGAC CATCCCCAGC CCAGCCCGTG TGAGAGGACG CCGTGGCGCC CTGCCTGCAG TGCCGGCTAC CACCCACTAC AACCGTGGGC GCTCATGCGC TGGCTCCGGC AGGTTCCGGT ACTCCTGGGC CTCCCGGACC CAAGTTCAAC GGAGCAGTAC GctGAATGGC GAAAACCATC ATCCCGGGAT TCCCAGCGAC CACGCCTCCC CAAGAGCAGG CAJiCCACTAC TGGGGGTGGA CCCACCGTGC ACCCAAGGAC GAGCCACGAA
TGCCAAGACA CACCGTCCTG AGCCCTCCCA ACAGGTGTAC CTGCCTGGTC GCCGGAGAAC CTACAGCAAG CGTGATGCAT GAGGCTCTGC TAAAGGTGGC GGTGGCTCCG ATCAGGCGGT GGAGGTTCCG

GGCTTCAGGG ACCGGCGCCG AGTGCAAGGA GGACGAAGCT AGAATGGGGG TCGAGGGCCG GCGGCTGTGA AGGGGTACTC GAAAAATACC GCAAGGTGTG AGTTGTGTGG ACAAAATCAA ACGACGGGGA CGGGCACCAC ACCATGTGGT TGCGCTTCTC AGCTCATGGT AGGTGGGAGA GCAAGGACTC GGTACCTGAC TGTACACCAG GCCCAGGAGT CCGGTGGCGG CCGACAAAAC TCTTCCTCTT CATGCGTGGT ACGGCGTGGA ACCGTGTGGT AGTG CAAGGT AAGGGCAGCC AGAACCAGGT AGTGGGAGAG CCGACGGCTC GGAACGTCTT GCCTCTCCCT GAGGTTCCGG AACTCCTGGG TCTCCCGGAC TCAAGTTCAA AGGAGCAGTA GGCTGAATGG AGAAAACCAT CATCCCGCGA ATCCCAGCGA CCACGCCTCC ACAAGAG CAG ACAACCACTA GCGGAGGTGG GTGGCGGGGG

CTGCCTGGCT GCGCGCCAAC GGAGCAGTGC GTTCTGGATI CTCCTGCAAG GAACTG TGAG GCAGTACTGC TCTGCTGGCA TATTCTAGAA CCCCAAAGGG GGGGACCCTG GAACTGGAGG TGAGCAGAGC CAACCACGAC GCCCCTCTGC ATTGGTCAGC CCTCAACGTG CTCCCCAJVIT CTGCAAGGGG GGGCATCGTC GGTCTCCCAG CCTCCTGCGA CGGATCAGGT TCACACATGC CCCCCCAAAA GGTGGA CGTG GGTGCATAAT CAGCGTCCTC CTCCAACAAA CCGAGAACCA CAGCCTGACC CAATGGGCAG CTTCTTCCTC CTCATGCTCC GTCTCCGGGT TGGCGGGGGA AGGACCGTCA CCCTGAGGTC CTGGTACGTG CAACAGCACG CAAGGAGTAC CTCCAAAGCC TGAGCTGACC CATCGCCGTG CGTGTTGGAC GTGGCAGCAG CACGCAGAAG GTCCGGTGGC ATCAGCGCAG

5

20 FVII-067 arnino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVIIa to Fc region is underlined, linker connecting both f'c regions is dashed underlined and linker connecting the E'c region to $\Xi C E S$ is in bold

1 MVGQALRLLC LLLGLQGCLA AVEMELEAK, $\mathbf{F}$ LHPQPRAN AFLEELRPGS LERECKEEQC 61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS 121 TKKDDQL ICV WERGGCEQYC 131 KRNA SKPOGR IVGGKVCPKG 241 NLIAVLGEKD LSEHDGDEQS 301 LPERTFSERT LAFVRFSLVS 361 ITEYMFCAGY SDGSKDSCKG 421 YIEWIQKLMR 3EPRPGVLLR 43]. PPCPAPELLG 541 AKTKPREEQY 601 QVYTLPPSED 661 YSKLTVDKSR 721 SDKTHTCPPC 731 D̈GVEVHKAKT 641 KGQPREPQVY 901 SDGSFFLY8K 961 GGSGGGGSGG 1021 GKGPEWVSGI 1031 YTSRSDVPDQ 1:41 VRITCQGDSL 1201 QAEDEADYYC

AGGAGTCTGG CTGGATTC.AT CAGAGTGGGT AGGGCCGGTT GCCTGAGAGC GGAGCGACGT TCTCCTCAGG TATCTGAACT CATGCCAAGG AGGCCCCTAC TCTCTGCCTC ATGAGGCTGA GCGGCACCAA CGCCCTCTTC

GGGAGGCTTG GTTTAGCAGG CTCAGGTATT CACCGTCTCC CGAGGACACG GCCCGACCAG GAGTGCATCC GACTCAGGAC AGACAGCCTC TCTTGTCATC CAGCTCAGGA CTATTACTGC CTGCTGTACT GCTG.ACCGTC CTACGTCAGC TGCGGCCTGA TATGCCATGA AGTGGTAGTG AGAGACAATT GCTGTATATT ACCAGCTTCG GCCCCAAAGC CCTGCTGTGI AGAAACTTTT TATGGTTTAA

GGGGGTCCCT ACGGCGGCGG CCAAGGCTGC GGGGGTCCCT
GCTGGGTCCG GTGGTAGTAC CCAAGAACAC ACTGCGCCCG ACTACTGGGG TTG.AAG.AAGG CTGTGGCCTT ATGCAAGCTG GTAAAAGGCC CCTTGACCAT

GAGACTCTCC CCAGGCTCC.A ATACTACGCA GCTGTATCTG GGGCGCCACC CCAGGGAACC TGAATTTTCA GGGACAGACA GTACCAGCAG CTCAGGGATC CACTGGGGCT CCAGCAGGGC CCCCTCGGTC

SDHTGTKRSC ECPWQVLLLV RRVAQVIIPS GKGQLLDRGA DSGGPHATHY APFPGGGGSG GGGSGGGGSG GGGSDKIHIC PKDTLMISRT PEVTCVVVV STEDPEVKFN WYVDGVEVFN GFSVFLFPPK NSTYEVV 3VL ELTKNQVSLT WQQGNVPSCS PAPELLGGPS VF TLPPSRDELT KNQVSLTCLV LTVDKS RWQQ GNVFSCSVMH GGSGGGGSAQ VQLQESGGGL SGSGGSTYYA DSVKGRFTVS TSFDYWGQGT LVTVSSGSAS RNFYASWYQQ LLYYGGGQQG VFGGGTKLTV

NA seeriience for FVI1-094
1 ATGGTCTCCC AGGCCCTCAG
61 GCAGTCTTCG 721 GCGTTCCTGG 131 TCCTTCGAGG AGGCCCGGGA 241 TCTTACAGTG 301 GACCAGCTCC 361 ACGCACAAGG 421 AGTGACCACA 481 GACGGGGTGT 541 AAA $A G A R A T G$ 601 GAGTGTCCAT 661 ATCAACACCA 721 AACCTGATCG 781 CGGCGGGTGG 841 ATCGCGCTGC 901 CTGCCCGAAC 9Gi 1 GGCTGGGGCC J. 021 CCCCGGCTGA 1081 ATCACGGAGT J... 41 GACAGTGGAG 1201 AGCTGGGGCC 1261 TACATCGAGT 1321 GCCCCATTTC 1381 TCAGGTGGGG 1441 CTGCAGGAGT 1501 GCCTCTGGAT 1561 GGGCCAGAGT 1621 GTGAAGGGCC 1681 AACAGCCTGA 1741 AGCCGGAGCG 601 ACCGTCTCCT 1861 CGCGTATCTG 1921 ATCACATGCC 1981 GGACAGGCCC 2041 CGATTCTCTG

GCTCCTCTGC GGAAGCCCAC GCCGGGCTCC GATCTTCAAG GTGTGCCTCA CTGCTTCTGC GATCTGTGTG GCGCTCCTGT CACAGTTGAA CCAAGGCCGA GTTGTTGGTG CTCCGCGGCC CGAGCACGAC CATCCCCAGC CCAGCCCGTG TGAGAGGACG CCGTGGCGCC CTGCCTGCAG TGCCGGCTAC CACCCACTAC AACCGTGGGC GCTCATGCGC TGGCGGTGGC CGGTGGAGGT CTTGGTACAG CAGGTATGCC TATTAGTGGT CTCCAGAGAC CACGGCTGTA CCAGACCAGC ATCCGCCCCA GGACCCTGCT GGACCCTGCT
CCTCAGAAAC CATCTATGGT AGGAAACACA GCTTCCTTGA

GGCTTCAGGG CTGCCTGGCT ACCGGCGCCG GCGCGCCAAC AGTG CAAGGA GGAGCAGTGC GGACGAAGCT GTTCTGGATT AGAATGGGGG CTCCTGCAAG TCGAGGGCCG GAACTGTGAG GCGGCTGTGA GCAGTACTGC AGGGGTACTC TCTGCTGGCA GAAAAATACC TATTCTAGAA GCAAGGTGTG CCCCAAAGGG AGTTGTGTGG GGGGACCCTG ACAAAATCAA GAACTGGAGG ACGACGGGGA TGAGCAGAGC CGGGCACCAC CAACCACGAC ACCATGTGGT GCCCCTCTGC TGCGCTTCTC ATTGGTCAGC AGCTCATGGT CCTCAACGTG AGGTGGGAGA CTCCCCAAAT GCAAGGACTC CTGCAAGGGG GGTACCTGAC GGGCATCGTC TGT.ACACCAG GGTCTCCCAG GCCCAGGAGT CCTCCTGCGA GTGGGTCCGG TGGCGGCGGA GGGGATCAGC GCAGGTGCAG CCCTGAGACT CTCCTGTGCA TCCGCCAGGC T'CCAGGGAAG GTACATACTA CGCAGACTCC ACACGCTGTA TCTGCAAATG CCCGGGGCGC CACCTACACC GGGGCCAGGG AACCCTGGTC AAGGTGAATT TTCAGAAGCA CCTTGGGACA GACAGTCAGG GCTGGTACCA GCAGAAGCCA GGCCCTCAGG GATCCCAGAC CCATCACTGG GGCTCAGGCG

## DNiA sequence for FVr I-02.

I. ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CrTTCTGCTTG GGCTTCAGGG CTCCCTGGCT

61 GCOGAAGTGC AGCTGGTGCA GTCTGGAGCT 321 GTCTCCTGCA AGGCTTCTGG ATACACCTTC 131. GCCCCTGGAC A 241 TATGCACAGA 301 TACATGGAGC 361 CGrGCTITIGI 421 raccracirch 431 GAATTTTCAG 541 CCAGGACAGA 601. TGGTACCAGC 661 CCOTCAGGGA 721 ATOAGCAGGO 731 AGTGATCATG 841 GCOCOCTCGG 901 TCTTACAGTG 361 GACCACCICC 1021 aCGCACARGG 1031 AGTGACCACA 1341 GACGGGGTM 120J дAAAGAAATG 1261 GAGTGTCCAT 1321. АТСАДСАССА 1331 AACCTGATCG 1441 CGGCGGOTGG 1E01 ATCGCGCTGQ 1561 CTCCCCGAAC 1.621. GGCTGGGGCC 1631 CCCCGOCTGA 17.1 ATCACGOAGT 1801 GACAGTGGAG 1861 AGCTGGGGCC 1521. TACATCGAGT 1981 GCCCCATTTC 2041 GGGGGroGAT 2101 CACOGTGCC $26 G 1$ CCCAACGACA 2221 मGCCACCAAG 2281 GCCAAGACAA 2341 ACCGrcclrgc 2401 GCCCTCCCAG 2461 CAGTGTACA 2E21. TGCCTGGTCA 250.1 CCGGAGAACA 264J TACAGCAAGC 2701 GTGATGCATG 2761 AAAGGTGGGG 2G21 TCCGACAAAA 2881 GTCTTCOTCT 254. ACATGCGTGG 3001 GAccGccrec 3061 TMCCG 3121 AAGTGCAAGG

210נ GAAGATGAGG CTGZCTATTA CTGCOTGCTG TACTACGGCG GOGGCCAGCA GGOCGTGTVC 2161 GGCGGCGGCA COAAGCIGAC CGTCCTACGI CAGCCCAAOG CIGCCCOCTC GGMCACTCHE 2221 TrCOCOCT THOGGGO GTA

EVIT-094 amino acid sequence. Signal sequence is shown in dot ted underine,
propeptide 1 s double und.erii.ned,. and 1 inker region connecting i s underlined

I MYSQAYRLEC LILGLOGCYA
 GYSDGDQOAS GPCQNGGGCK K I $A \mathrm{~A}$
DO
D AI THKDDOIJCV NENGGCEOYO 1.31. KRNASKPQGR IVGGKVCEKG 241 NTIAVIGEHD JGSHDGDEQS 301 LDERTESERS LREVRESLVS 361 ITEYMFCAGY SLGSKDGGKS 4 AT YJEWIOKIMR SEPRPGVLIR 431 LQESGGGJVQ PGGSJRLSCA ARGFWFSRYA MSWVRQARGK GPFWVSGTSG SGGSTYYZDS 541 VKGRFTVSRD NSENTLYLQU NSLRAPDTAY YYCARGATYT SRSDVPDQTS EDYWGQGTLV EO1 FWSSGSRSAD KLEEGEFSEA RVSEDTODPA VSVALGQTVE ITCQGDSLEN EYASWYQKP GG1 GOAPTLVIYG LSERPSGIDD RESASSSGNY ASLTITGAQA EDERDYYCLI YYGGGQQGVE 721 GGGTKTVLR QUK\&APSVIL FPPSSAA* QEQSICFC IPAFEGRNGE DHTGTKRGC RCERGYSLLA DGVOCTPTVF YPCGKTPTHE ECPWQVILILV NOAQLCGGTL LNTITVVSAA HCPDKIKKWR RRVAQVIIPS TVVEGTMNHD JALIRTJQPV VITTDFVVELC GWGOLLDRGZ WALELMVLNV PRLMTQDCLQ QSRKVGDSPN DSGGPHATHY RGTWYLTGIV SWGOGOATVG KFGVYTRVSQ FWPDTGGGG BGGGGOGOGG SGGGGGGGGG BGOGGGAQVO PPSSAA*CGAGGTGAATA AGCCTGGGGC CPGZGTGAAG ACCGGCTACT ATATGCACTG GGTGOGACAG $\begin{array}{ll}\text { ACCGGCTACT } & \text { ATATGCACTG GGTGGACAG } \\ \text { TGGATCAACC } & \text { CTAACAGTGG TGGACAдAC }\end{array}$ ATGACCAGGG ACACGTCCAT CAGCACCGCG GACACGGCCG TGTATTACTG TGOGAGAGCO ACACGGCCG TGTATTACTG TGCGAGAGGC GGGCCAOSGA GGGCCAOGGA
TGAAGAAGGT GTCZGTGGOC AAGTGTGCAG AAGTGNGCAG
TAGCGACCGG CACOCTCACC GGATAGTAGT GCCCAAGGOT GTTCTGGATT TCOTGCAAG GAACMGTGAG GCAGTACTGC TCTGCTGGCA 1ATTOTAGAA CCGAAAGGG GGGCACCCTG GAACTGGZGG TGAGCAGAGC CAACOACGAC GCCCOTCTGC TTGGTCAGG CCMCARCGTG PCCCCAAAT CTOCAAGGGG GGGCATCGTC GGTCTCOCAG COPCCTGCGA CGGATCAGGI MCACACAIGC CCACACAIGC GGTGGACGTG GGTGCATAAT CAGCGTCCTC CTCCAACAAA CCGACARCCA CAGCOTGACC AATGGGCAG TTCTTCCTC СTCATGCTCC G?CTCCOBGI TGGCGGGGGA AGGACCGTG CCCTGAGGTC CTGGTACGTG CAACAGCACO CARGGAGTRC CTCCAAAGCC Iq AAAGGGcมG CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGCGA TGAGTTGCC 324 J A AGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG

5
$\begin{array}{ll}3301 & \text { GAGTGGGAGA } \\ 3361 & \text { TCCGACGGCT } \\ 3421 & \text { GGGAACGTCT }\end{array}$ 3481 AGCCTCTCCC

GCAATGGGCA CCTTCTTCCT TCTCATGCTC TGTCTCCGGG

GCCGGAGAAC AACTACAAGA CCACGCCTCC CGTGATGCAT TAAATGA

GGTGTTGGAC
GTGGCAGCAG
CACGCAGAAG
FVIT-028 amino acid sequence. Signal sequence is shown in dotted underline,
linker region connecting EVII to $E^{\prime} c$ region is underlined, linker connecting
both Fes sites is shown in bold., and M39 is italicized
1 MVSGALRLG LTLGLQGGA AEVQLVQSGA EVNKPGASVK VSCKASGYTF TGYYVHWVRQ
6.1 APGQGLEWMG WINPNSGGTN YAQKFQG WVT MTRDTSISTA YMELSRLRSD DTA.VYYCARG
121 RAL YNR.NDRS PNNFDP WGQG TT, TTVSSGSA SAPTLKLEEG EFSEARVQAV LTQPPSVSVA
I\&. $\operatorname{PGQTARITCG~GNNIGSKSVQ~WYQQKPGQAP~VLVVYDDSDR~PSGIPERFSG~SNSGNNATLT~}$
241 ISRVEAGDEA. DYYCQVWDSS SDHVVFGGGT KLTVLGQPKA APSVTLFPPS AAARTKLFN1
301 SYSDGDQCSS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE THKDDQLICV NENGGCEQYC
361 SDHTGTKR3C RCHEGYSLLA DGV3CTPTVE YPCGKIPILE KRNASKPQGR IVGGKVCPKG
42 J ECPWQVLLLV NGAQLCGGTL INTIWWSAA HCFDKIKNWR NLIAVJGEHD LSEHDGDEQS
481 RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VI』DHVVDLA LPERTFSERT LAFVRFSLVS
541 GKGQLLDRG.A TALE LMVLNV PRLMTODCLQ QSRKVGDSPN 1TEYMFCAGY SDGSKDSCKS
601 DSGGPHATHY RGTKYLTG IV SWGQGCATVG HFGVYTRVSQ YIEWLQKLMR SEPRPGVLLR
6€ 1 APFPGGGG5G. . $G G G S G G G G S$ G GGSGGGGSG GGGDKTETG PPCPAPELLG GPSVFLFPPK
721. PKDTJUMISRT PEVTCVYVDV 5HEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRW SVT,
781 TVLHQDSLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPFSRD ELTKNQVSLT
841 CLVKGFYPSD IAVE玥SNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS
901 VMHEALHNKY TQWSLSLSPG KGGGGSGGGG SGGGGSGGGG SDKTHTCPPC PAPELLGGPS
961 VFLFPPKPKD TLMISRTPEV TCWVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
301021 YRWSVLTVL EQDWLNGKEY KCKV SNKALP APIEKTI SKA KGQPREPQVY TLPPSRDELT
IO8l KNGVST.TCIV KGFYPSDIAV ENESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
1141 GNVESCSVMH EALHNHYTQK SLSLSPGK*
DNA sequence F'VII~039

1 ATGGTCTCCC AGGCCCTCAG
GI GCAGTCTTCG 121．GCGTTCCTGG 181 TCCTTCGAGG 241 TCTTACAGTG 301 GACCAGCTCC 361 ACGCACAAGG 421 AGTGACCACA 481 GACGGGGTGT 541 AAAAGAAATG 601 GGCAAGGTGT 66i1 CAGTTGTGTG 721．GACAAAATCA 78！CACGACGGGG 841 CCGGGCACCA 901 GACCATGTGG $9 \mathrm{G}_{1} 1$ GTGCGCTTCT 1021 GAGCTCATGG I0日l AAGGTGGGAG 1．．．4J．AGCAAGGACT 1201 TGGTACCTGA 1261 GTGTACACCA 1321 CGCCCAGGAG I 3g．t TCCGGTGGCG 144］．TCCGACAAAA 1501 GTCTTCCTCT 1561 ACATGCGTGG 1621 GACGGCGTGG 1681 TACCGTGTGG 1．741．AAGTGCAAGG 1801 AAAGGGCAGC 1861 AAGAACCAGG 1921 GAGTGGGAGA 1981 TCCGACGGCT 204 J GGGAACGTCT 2！！AGCCTCTCCC 235 J GGAGGTTCCG 2221 GAACTCCTGG 22 \＆ 1 ATCTCCCGGA 2341 GTCAAGTTCA 2401 GAGGAGCAGT 2461 TGGCTGAATG 252． 2581 CCATCCCGCG 2641 TATCCCAGCG 2701 ACCACGCCTC 275 J GACAAGAGCA

TAACCCAGGA AGGAGCTGCG AGGCCCGGGA ATGGGGACCA AgTCCTATAT ATGACCAGCT CGGGCACCAA CCTGCACACC CCAGCAAACC GCCCCAAAGG GGGGGACCCT AGAACTGGAG ATGAGCAGAG CCAACCACGA TGCCCCTCTG CATTGGTCAG TCCTCAACGT ACTCCCCAAA CCTGCAAGGG CGGGCATCGT GGGTCTCCCA TCCTCCTGCG GCGGATCAGG CTCACACATG TCCCCCCAAA TGGTGGACGT AGGTGCATAA TCAGCGTCCT TCTCCAACAA CCCGAGAACC TCAGCCTGAC GCAATGGGCA CCTTCTTCCT TCTCATGCTC TGTCTCCGGG GTGGCGGGGG GAGGACCGTC CCCCTGAGGT ACTGGTACGT ACAACAGCAC GCAAGGAGTA TCTCCAAAGC ATGAGCTGAC ACATCGCCGT CCGTGTTGGA GGTGGCAGCA

GCtCctctac GGAAGCCCAC GCCGGGCTCC GATCTTCAAG GTGTGCCTCA CTGCTTCTGC GATCTGTGTG GCGCTCCTGT CACAGTTGAA CCAAGGCCGA GGAGTGTCCA GATCAACACC GAACCTGATC CCGGCGGGTG CATCGCGCTG CCTGCCCGAA CGGCTGGGGC GCCCCGGCTG TATCACGGAG GGACAGTGGA CAGCTGGGGC GTACATCGAG AGCCCCATTT TGGGGGTGGA CCCACCGTGC ACCCAAGGAC GAGCCACGA．A TGCCAAGACA CACCGTCCTG AGCCCTCCCA ACAGGTGTAC CTGCCTGGTC GCCGGAGAAC CTACAGCAAG CGTGATGCAT TAAAGGTGGC A TCAG ACAAA AGTCTTCCTC CACATGCGTG GGACGGCGTG GTACCGTGTG CAAGTGCAAG CAAAGGGCAG CAAGAACCAG GGAGTGGGAG CTCCGACGGC GGGGAACGTC

GGCGTCCTGC TGGGAGAGGG GACGCGGAGA AGTCCATGCC CTCCCTGCCT AACGAGAACG CGGTGCCACG TATCCATGTG GGCGGAGGAG TGGCAGGTCC ATCTGGGTGG GCGGTGCTGG GCGCAGGTCA CTCCGCCTGC CGGACGTTCT CAGCTGCTGG ATGACCCAGG TACATGTTCT GGCCCACATG CAGGGCTGCG TGGCTGCAAA CCCGGTGGCG TCAGGCGGTG CCAGCTCCGG ACCCTCATGA GACCCTGAGG AAGCCGCGGG CACCAGGACT GCCCCCATCG ACCCTGCCCC AAAGGCTTCT AACTACAAGA CTCACCGTGG GAGGCTCTGC GGCGGATCAG ACTCACACAT TTCCCCCCAA GTGGTGGACG GAGGTGCATA GTCAGCGTCC GTCTCCAACA CCCCGAGAAC GTCAGCCTGA AGCAATGGGC TCCTTCTTCC TTCTCATGCT

GGCTTCAGGG ACCGGCGCCG GCGCGCCAAC AGTGCAAGGA GGAGCAGTGC GGACGAAGCT AGAATGGGGG CTCCTGCAAG TCGAGGGCCG GAACTGTGAG GCGGCTGTGA GCAGTACTGC AGGGGTACTC TCTGCTGGCA GAAAAATACC TATTCTAGAA ACTTCACTCG GGTTGTGGGG TGTTGTTGGT GAATGGAGCT TCTCCGCGGC CCACTGTTTC GCGAGCACGA CCTCAGCGAG TCATCCCCAG CACGTACGTC ACCAGCCCGT GGTCCTCACT CTGAGAGGAC GCTGGCCTTC ACCGTGGCGC CACGGCCCTG ACTGCCTGCA GCAGTCACGG GTGCCGGCTA CTCGGATGGC CCACCCACTA CCGGGGCACG CAACCGTGGG CCACTTTGGG AGCTCATGCG CTCAGAGCCA GTGGCTCCGG CGGAGG TGGG GAGGTTCCGG TGGCGGGGGA AACTCCTGGG CGGACCGTCA TCTCCCGGAC CCCTGAGGTC TCAAGTTCAA CTGGTACGTG AGGAGCAGTA CAACAG CACG GGCTGAATGG CAAGGAGTAC AGAAAACCAT CTCCAAAGCC CATCCCGGGA TGAGCTGACC ATCCCAGCGA CATCGCCGTG CCACGCCTCC CGTGTTGGAC ACAAGAGCAG GTGGCAGCAG ACAACCACTA CACGCAGAAG GTGGGGGTGG ATCAGGCGGT GCCCACCGTG CCCAGCACCT AACCCAAGGA CACCCTCATG TGAGCCACGA AGACCCTGAG ATGCCAAGAC AAAGCCGCGG TCACCGTCCT GCACCAGGAC AAGCCCTCCC AGCCCCCATC CACAGGTGTA CACCCTGCCC CCTGCCTGGT CAAAGGCTTC AGCCGGAGAA CAACTACAAG TCTACAGCAA GCTCACCGTG CCGTGATGCA TGAGGCTCTG

282 J CACAACCACT ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAATGA


DNA sequence for FVII-040
1 ATGGTCTCCC AGGCCCTCAG
61 GCAGTCTTCG TAACCCAGGA AGGAGCTGCG AGGCCCGGGA ATGGGGACCA AGTCCTATAT ATGACCAGCT CGGGCACCAA CCTGCACACC CCAGCAAACC CCAAAGGGGA GGACCCTGAT ACTGGAGGAA AGCAGAGCCG ACCACGACAT CCCTCTGCCT TGGTCAGCGG TCAACGTGCC CCCCAAATAT GCAAGGGGGA GCATCGTCAG TCTCCCAGTA TCCTGCGAGC GATCAGGTGG ACACATGCCC CCCCAAAACC TGGACGTGAG TGCATAATGC GCGTCCTCAC CCAACAAAGC GAGAACCACA GCCTGACCTG ATGGGCAGCC TCTTCCTCTA CATGCTCCGT CTCCGGGTAA GCGGGGGATC GACCGTCAGT CTGAGGTCAC GGTACGTGGA ACAGCACGTA AGGAGTACAA CCAAAGCCAA AGCTGACCAA TCGCCGTGGA TGTTGGACTC GGCAGCAGGG CGCAGAAGAG

GGAAGCCCAC GCCGGGCTCC GATCTTCAAG GTGTGCCTCA CTGCTTCTGC GATCTGTGTG GCGCTCCTGT CACAGTTGAA CCAAGGCGGC GTGTCCATGG CAACACCATC CCTGATCGCG GCGGGTGGCG CGCGCTGCTC GCCCGAACGG CTGGGGCCAG CCGGCTGATG CACGGAGTAC CAGTGGAGGC CTGGGGCCAG CAT'CGAGTGG CCCATTTCCC GGGTGGATCA ACCGTGCCCA CAAGGACACC CCACGAAGAC CAAGACAAAG CGTCCTGCAC CCTCCCAGCC GGTGTACACC CCTGGTCAAA GGAGAACAAC CAGCA.AGCTC GATGCATGAG AGGTGGCGGC AGACAAAACT CTTCCTCTTC ATGCGTGGTG CGGCGTGGAG CCGTGTGGTC GTGCAAGGTC AGGGCAGCCC GAACCAGGIC GTGGGAGAGC CGACGGCTCC GAACGTCTTC TCTCCGGGTA

TTTCTGCTTG GGCTTCAGGG CTGCCTGGCT GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC GGACGAAGCT GTTCTGGATT AGAATGGGGG CTCCTGCAAG TCGAGGGCCG GAACTGTGAG GCGGCTGTGA GCAGTACTGC AGGGGTACTC TCTGCTGGCA GAAAAATACC TATTCTAGAA TCACTCGGGT TGTGGGGGGC TGTTGGTGAA TGGAGCTCAG CCGCGGCCCA CTGTTTCGAC AGCACGACCT CAGCGAGCAC TCCCCAGCAC GTACGTCCCG AGCCCGTGGT CCTCACTGAC AGAGGACGCT GGCCTTCGTG GTGGCGCCAC GGCCCTGGAG GCCTGCAGCA GTCACGGAAG CCGGCTACTC GGATGGCAGC CCCACTACCG GGGCACGTGG CCGTGGGCCA CTTTGGGGTG TCATGCGCTC AGAGCCACGC GCTCCGGCGG AGGTGGGTCC GTTCCGGTGG CGGGGGATCC TCCTGGGCGG ACCGTCAGTC CCCGGACCCC TGAGGTCACA AGTTCAACTG GTACGTGGAC AGCAGTACAA CAGCACGTAC TGAATGGCAA GGAGTACAAG AAACCATCTC CAAAGCCAAA CCCGGGATGA GCTGACCAAG CCAGCGACAT CGCCGTGGAG CGCCTCCCG! GTTGGACT'CC AGAGCAGGTG GCAGCAGGGG ACCACTACAC GCAGAAGAGC GGGGTGGATC AGGCGGTGGA CACCGTGCCC AGCACCTGAA CCAAGGACAC CCTCATGATC GCCACGAAGA CCCTGAGGTC CCAAGACAAA GCCGCGGGAG CCGTCCTGCA CCAGGACTGG CCCTCCCAGC CCCCATCGAG AGGTGTACAC CCTGCCCCCA GCCTGGTCAA AGGCTTCTAT CGGAGAACAA CTACAAGACC ACAGCAAGCT CACCGTGGAC TGATGCATGA GGCTCTGCAC AATGA

FVII-040 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, the FXIa cleavage site is shown in dashed underlined, and the linker region connecting the Fcegions is shown in bold

5

FIX--042 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, and linker region connecting the Fc region $s$ is underlined



5

1. ATGCAGCGCG 1 GGATATCTAC 121 GCTTGCCTTT 131 GATTTGACAG 241 AACATCACAG 301 AAAAACAAAG 361 CTAAAGAATT 421 TGAATCGGCC 431 AGAGAGAATG 541 CTGAAAGAAC 601. CATGTTTAAA 661 TTGGATTTGA 721 GCGAGCAGTT 731 ATCGACTTGC 8:1 TTTCTGTTTC 901 ATGTAAATTC 96. TTAATGACTT 1021 AGGTTGTTTT 1081 GGATTGTAAC 1上 1 AACATAATAT 1201 CTCACCACAA 1261 TGGACGAACC 132 ] ACACGAACAT 1381 ACAAAGGGAG 1441 CATGTCTTCG 1501 AAGGAGGTAG $15 \mathrm{E}_{1} 1$ GGACCAGTTT 1621 ATGGAATATA 1681 CTGACAAAAC 17』 J TCTTCCTCTT 1201 CATGCGTGGT 1 1\} 1 ACGGCGTGGA 1921 ACCGTGTGGT 1981 AGTGCAAGGT 20! J. AAGGGCAGCC 2101 AGAACCAGGT 2161 AGTGGGAGAG 2221 CCGACGGCTC 22 घl GGAACGTCTT 23.]. GCCTCTCCCT 2401 GTGGATCAGG 2461 GTGGATCAAG 2521 TGGTACAGCC 2581 GGTATGCCAT 2641. TTAGTGGTAG 2701 CCAGAGACAA 2761 CGGC TGTATA 2821 AGACCAGCTT 28 \& 1 CCGCCCCAAA 294 J ACCCTGCTGT 3001 TCAGAAACTT 305 J. TCTATGGTTT 3121 GAAACACAGC 3!81 GCCTGCTGTA 3241. TCCTACGTCA 3301 GTGGCGGTGG 3351. GCGGTGGAGG 3421 CACCGGAACT 34 S1 TCATGATCTC 3541 CTGAGGTCAA 3601 CGCGGGAGGA 3661 AGGACTGGCT

181 VSVSQTSKIT RAFTVFPDVD YVNSTEAETI LDNTTOSTQ FNDFTRWGG 44 RAETVFPDVD YVNSTEAETI LDNITOSTQS FCGGSYVNEK WIYTAAHCVE LDEPLVLNSY YLRVPLVDRA TCLRSTKFTI $\begin{array}{ll}\text { YLRVPLVDRA } & \text { TCLRSTKFTI } \\ \text { WGEECAMKGK. YGIYTKVSRY }\end{array}$ TLMISRTPEV TCVWDVSHE HQDWLNGKEY HDWLNGKEY KGFYPSDIAV EALENNHYTQK FPPKPKDTLN VSVLTVLHQD SLTCLVKGF FSCSVMHEAI - 06

068
-06AACATGAT TCAGTGCTGA TAGATATAGA CAGATATTGAA CAATATTGAA ATTTTGGCTC ACTTTCTTAA ATTCTTTTAC AAAGAGGTAT TATGGAAGAA AACTGAATTT TGGCGGCAGT AGGAAAGAAC TTGTAAAAAT AGAAAACCAG ACAAACTTCT TACTGAAGCT CACTCGGGTT GAATGG TAAA TGCTGCCCAC TGAGGAGACA CTACAATGCA CTTAGTGCTA CTTCCTCAAA ATCAGCTTTA ATCTACAAAG AGATTCATGT CTTAACTGGA TACCAAGGTG TCACACATGC CCCCCCAAAA GGTGGACGTG GGTGCATAAT CAGCGTCCTC CTCCAACAAA CCGAGAACCA CAGCCTGACC CAATGGGCAG CTTCTTCCTC CTCATGCTCC GTCTCCGGGT CGGTGGAGGT GAAGAGGAGG TGGGGGGTCC GAGCTGGGTC TGGTGGTAGT TTCCAAGAAC TTACTG CGCC CGACTACTGG GCTTGAAGAA GTCTGTGGCC TTATGCAAGC AAGTAAAAGG TTCCTTGACC CTACGGCGGC GCCCAAGGCT CTCCGGCGGA TTCCGGTGGC CCTGGGCGGA CCGGACCCCT  G GAATGG CAAG

## CATGGCAGAA

 ATGTACAGGT AATATCTGAT GAGTCTAACA CATGCCCTAA GAGATGTAAA ATTTCAGTTT AATTCAGGTA AAGTGTAGTT TGGAAGCAGT TGCAAGGATG TGTGAATTAG AGTGCTGATA AAGTCCTGTG AAGCTCACCC GAAACCATTT GTTGGTGGAG GTTGATGCAT TGTGTTGAAA GAACATACAG GCTATTAATA AACAGCTACG TTTGGATCTG GTTCTTCAGT TTCACCATCT CAAGGAGATA ATTATTAGCT TCCCGGTATG CCACCGTGCC CCCAAGGACA AGCCACGAAG GCCAAGACAA ACCGTCCTGC GCCCTCCCAG CAGGTGTACA TGCCTGGTCA CCGGAGAACA TACAGCAAGC GTGATGCATG AAACGGCGCC TCCGGTGGCG AAGAGGGCGC CTGAGACTCT CGCCAGGCTC ACATACTACG ACGCTGTATC CGGGGCGCCA GGCCAGGGAA GGTGAATTTT TTGGGACAGA TGGTACCAGC CCCTCAGGGA ATCACTGGGG GGCCAGCAGG GCCCCCTCGG GGTGGGTCCG GGGGGATCAG CCGTCAGTCT GAGGTCACAT ${ }^{\prime}$ TACGTGGACG AGCACGTACC GAGTACAAGTTCACCAGGC TTGTTTCCTT GCTGTCTTCT GCCAGCACGC AGAGAAATTG ATTTTCATGA TTCTTGATCA AAT TGGAAG A TTGAAGAAGC ATGTTGATGG ACATTAATTC ATGTAACATG ACAAGGTGGT AACCAGCAGT GTGCTGAGAC TGGATAACAT AAGATGCCAA TCTGTGGAGG CTGGTGTTAA AGCAAAAGCG AGTACAACCA TTACACCTAT GCTATGTAAG ACCTTAGAGT ATAACAACAT GTGGGGGACC GGGGTGAAGA TCAACTGGAT CAGCTCCGGA CCCTCATGAT ACCCTGAGGT AGCCGCGGGA ACCAGGACTG CCCCCATCGA CCCTGCCCCC AAGGCTTCTA ACTACAAGAC TCACCGTCGA AGGCTCTGCA GCCGGAGCGG GGGGATCCGG AGGTGCAGCT CCTGTGCAGC CAGGGAAGGG CAGACTCCGT TGCAAATGAA CCTACACCAG CCCTGGTCAC CAGAAGCACG CAGTCAGGAT AGAAGCCAGG TCCCAGACCG CTCAGGCGGA GCGTGTTCGG TCACTCTGTT GTGGCGGCGG ACAAAACTCA TCCTCTTCCC GCGTGGTGGT GCGTGGAGGT GTGTGGTCAG GCAAGGTCTC

TCATCACCAT TTTTAAAATA GilA GTACTG TGGG IATITGGAI TGAAAACGCC AACAAAATTC GTTTGTTCAA ACGAGAAGTT AGATCAGTGT GAGTCCAATC CTATGAATGT TGGTGTCCCT TAACATTAAG AATGGCAGAT TTGCTCCTGT ACTGAGGGAT GCCATTTCCA TGTGGAAGAG TGTTTTTCCT GATGTGGACT CACTCAAAGC ACCCAATCAT ACCAGGTCAA TTCCCTTGGC CTCTATCGTT AATGAAAAAT AATTACAGTT GTCGCAGGTG AAATGTGATT CGAATTATTC TGACATTGCC CTTCTGGAAC TTGCATTGCT GACAAGGAAT TGGCTGGGGA AGAGTCTTCC TCCACTTGTT GACCGAGCCA GTTCTGTGCT GGCTI'CCATG CCATGTTACT GAAGTGGAAG GTGTGCAATG AAAGGCAAAT TAAGGAAAAA ACAAAGCTCA ACTCCTGGGC GGACCGTCAG CTCCCGGACC CCTGAGGTCA CAAGTTCARC TGGTACGTGG GGAGCAGTAC AACAGCACGT GCTGAATGGC AAGGAGTACA GAAAACCATC TCCAAAGCCA ATCCCGGGAT GAGCTGACCA TCCCAGCGAC ATCGCCGTGG CACGCCTCCC GTGTTGGACT CAAGAGCAGG TGGCAGCAGG CAACCACTAC ACGCAGAAGA TGGCGGCGGA TCAGGTGGGG CGGTGGAGGT TCCGGTGGGG GCAGGAGTCT GGGGGAGGCT CTCTGGATTC ATGTTTAGCA GCCAGAGTGG GTCTCAGGTA GAAGGGCCGG TTCACCGTCT CAGCCTGAGA GCCGAGGACA CCGGAGCGAC GTGCCCGACC CGTCTCCTCA GGGAGTGCAT CGTATCTGAA CTGACTCAGG CACATGCCAA GGAGACAGCC ACAGGCCCCT ACTCTTGTCA ATTCTCTGCC TCCAGCTCAG AGATGAGGCT GACTATTACT CGGCGGCACC AAGCTGACCG CCCGCCCTCT TCTGCGGCCG ATCAGGTGGG GGTGGA TCAG CACATGCCCA CCGTGCCCAG CCCAAAACCC AAGGACACCC GGACGTGAGC CACGAAGACC GCATAATGCC AAGACAAAGC CGTCCTCACC GTCCTGCACC CAACAAAGCC CTCCCAGCCC

3721 CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC 3731 TGCCCCCATC CCGCGATGAG CTGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG 3841 GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT 3901 ACAAGACCAC GCCTCCCGTG TTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA 396.1 CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG 402 J . CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA TGA


DNA sequence for FIX-088
1 ATGCAGCGCG TGAJiCAT'GAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCTTT'TA
61 GGATATCTAC TCAGTGCTGA ATGTACAGGT TTGTTTCCTT TTTTAAAATA CATTGAGTAT 121 GCTTGCCTTT TAGATATAGA AATATCTGAT GCTGTCTTCT TCACTAAATT TTGATTACAT 1.31 GATTTGACAG CAATATTGAA GAGTCTAACA GCCAGCACGC AGGTTGGTAA GTACTGTGGG 241 AACATCACAG ATTTTGGCTC CATGCCCTAA AGAGAAATTG GCTTTCAGAT TATTTGGATT 301 AAAAACAAAG ACTTTCTTAA GAGATGTAAA ATTTTCATGA TGTTTTCTTT TTTGCTAAAA 361 CTAAAGAATT ATTCTTTTAC ATTTCAGTTT TTCTTGATCA TGAAAACGCC AACAAAATTC 421 TGAATCGGCC AAAGAGGTAT AATTCAGGTA AA TYGGAAGA GTTTGTTCAA GGGAATCTAG $43 \mathrm{~J} . A G A G A G A A T G$ TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA 54. 1 CTGAAAGAAC AACTGAATTT TGGAAGCAGT ATGTTGATGG AGATCAGTGT GAGTCCAATC 601 CATGTTTAAA TGGCGGCAGT TGCAAGGATG ACATTAATTC CTATGAATGT TGGTGTCCCT 661 TTGGATTTGA AGGAAAGAAC TGTGAATTAG ATGTAACATG TAACATTAAG AATGGCAGAT 721 GCGAGCAGTT TTGTAAAAAT AGTGCTGATA ACAAGGTGGT TTGCTCCTGT ACTGAGGGAT 731 ATCGACTTGC AGAAAACCAG AAGTCCTGTG AACCAGCAGT GCCATTTCCA TGTGGAAGAG §41 TTTCTGTTTC ACAAACTTCT AAGCTCACCC GTGCTGAGAC TGTTTTTCCT GATGTGGACT 50 J. ATGTAAATTC TACTGAAGCT GAAACCATTT TGGATAACAT CACTCAAAGC ACCCAATCAT 961 TTAATGACTT CACTCGGGTT GTTGGTGGAG AAGATGCCAA ACCAGGTCAA TTCCCTTGGC 1021 AGGTTGTTTT GAATGGTAAA GTTG.ATGCAT TCTGTGGAGG CTCTATCGTT AATGAAAAAT 1031 GGATTGTAAC TGCTGCCCAC TGTGTTGAAA CTGGTGTTAA AATTACAGTT GTCGCAGGTG 141 AACATAATAT TGAGGAGACA GAACATACAG AGCAAAAGCG AAATGTGATT CGAATTATTC $1201 . C T C A C C A C A A$ CTACAATGCA GCTATTAATA AGTACAACCA TGACATTGCC CTTCTGGAAC 1261 TGGACGAACC CTTAGTGCTA AACAGCTACG TTACACCTAT TTGCATTGCT GACAAGGAAT 1321 ACACGAACAT CTTCCTCAAA TTTGGATCTG GCTATGTAAG TGGCTGGGGA AGAGTCTTCC 1331 ACAAAGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTT GACCGAGCCA 1441 CATGTCTTCG ATCTACAAAG TTCACCATCT ATAACAACAT GTTCTGTGCT GGCTTCCATG 150 J AAGGAGGTAG AGATTCATGT CAAGGAGATA GTGGGGGACC CCATGTTACT GAAGTGGAAG 1561 GGACCAGTTT CTTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCAAAT 162 ]. ATGGAATATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAAGAAAAA ACAAAGCTCA 1631 CTGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG 1741 TCTTССТСТT CCCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA J. 801 CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG 1861 ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT J. 92 J . ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA 1931 AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA 2041 AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT GAGCTGACCA 2101 AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG 2 : 11 AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGTTGGACT 2221 CCGACGGCTC CTTCTTCCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG 2281 GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA 2341 GCCTCTCCCT GTCTCCGGGT AAAGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGCGGTG 2401 GAGGTTCCGG TGGCGGGGGA TCAGACAAAA CTCACACATG CCCACCGTGC CCAGCACCTG 461 AACTCCTGGG AGGACCGTCA GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA 2521 TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG

5 3961 CCAAGGCTGC
2531 TCAAGTTCA 2641 AGGAGCAGTA 2701 GGCTGAATGG 2751 . AGAAAACCAT 2821 CATCCCGCGA 283]. ATCCCAGCGA 2941 CC.ACGCCTCC 3001 ACAAGAGCAG 3061 ACAACCACTA 3221 GCGGAGGTGG 3..31. GTGGCGGGGG 324.1 GGGGGTCCCT 3301 GCTGGGTCCG 3361 GTGGTAGTAC 3421 CCAAGAACAC 3431 ACTGCGCCCG 3541 ACTACTGGGG 3601 TTGAAGAAGG 3661 CTGTGGCCTT $3 \% 1$ ATGCAAGCTG 3731 GTAAAAGGCC 3841 CCTTGACCAT 3901 ACGGCGGCGG

CTGGTACGTG GACGGCGTGG CAACAG CACG CAAGGAGTAC AAGTGCAAGG CTCCAAAGCC AAAGGGCAGC TGAGCTGACC AAGAACCAGG CATCGCCGTG GAGTGGGAGA CGTGTTGG.AC TCCGACGGCT GTGGCAGCAG GGGAACGTCT CACGCAGAAG AGCCTCTCCC GTCCGGTGGC GGCGGATCAG ATCAGCGCAG GTGCAGCTGC GAGACTCTCC TGTGCAGCCT CCAGGCTCCA GGGAAGGGGC ATACTACGCA GACTCCGTGA GCTGTATCTG CAAATGAACA GGGCGCCACC TACACCAGCC CCAGGGAACC CTGGTCACCG TGAATTTTCA GAAGCACGCG GGGAC.AGACA GTCAGGATCA GTACCAGCAG AAGCCAGGAC CTCAGGGATC CCAGACCGAT CACTGGGGCT CAGGCGGAAG CCAGCAGGGC GTGTTCGGCG CCCCTCGGTC ACTCTGTTCC

AGGTGA TCAGCGTCCT TCTCCAACAA CCCGAGAACC TCAGCCTGAC GCAATGGGCA CCTTCTTCCT TCTCATGCTC TGTCTCCGGG GTGGGGGTGG AGGAGTCTGG CTGGATTCAT CAGAGTGGGT AGGGCCGGTT GCCTGAGAGC GGAGCGACGT TCTCCTCAGG TATCTGAACT CATGCCAAGG AGGCCCCTAC tCTCTGCCTC ATGAGGCTGA GCGGCACCAA CGCCCTCTTC

TGCCAAGACA CACCGTCCTG AGCCCTCCCA ACAGGTGTAC CTGCCTGGTC GCCGGAGAAC CTACAGCAAG CGTGATGCAT TAAAGGTGGC ATCAGGCGGT GGGAGGCTTG GTTTAGCAGG CTCAGGTATT CACCGTCTCC CGAGGACACG GCCCGACCAG GAGTGCATCC GACTCAGGAC AGACAGCCTC TCTTGTCATC CAGCTCAGGA CTATCACTGC cegaccgic tGcGGCCTGA

AAGCCGCGGG
CACCAGGACT
GCCCCCATCG ACCCTGCCCC AAAGGCTTCT AACTACAAGA CTCACCGTCG GAGGCTCl' GC GGTGGCTCCG GGAGGTTCCG GTACAGCCTG TATGCCATGA AGTGGTAGTG AGAGACAATT GCTGTATATT ACCAGCTTCG GCCCCAAAGC CCTGCTGTGT AGAAACTTTT TATGGTTTAA AACACAGCTT CTGCTGTACT

30 FIX--083 awino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined., linker connecting both Fc regions is under Iined and Jnker connecting the Fc region to SCE5 is in bold

1 MQRVNVIMAE SPGLITICLL GYLLSAECTV FLDHENANKI LNR EKEYNSG KLEEFVQGNL 61 ERECMEEKCS FEEAREVFEN TERTTE THKQ YVDGDQCE SN PCLNGGSCKD DINSYECWCP 121 FGFEGKNCEL DVTCNIKNGR CEQFCKNSAD NKWCSCTBG YRLAENQKSC EPAVPFPCGR 181 VSVSQTSKLT RAETVFPDVD Y ${ }^{3 / 3}$ STEAETI LDKITQSTQS FNDFTRVVGG EDAKPGQFPW 241. QVVLNGKVDA FCGGSEVNEK NIVTAAKCVE TGVKI TVVAG EHNIEETEHT EQKRNVIRII 301 PKHNYNAAIN KYNEDTALLE LDEPLVLNSY VTPICIADKE YTNIFLKFG3 GYVSGWGRVF 361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMEAGH EGGRDSCQGD SGGPHVTEVE 421 GTSFLTGIIS WGEECAMKGK YGIYTKVSRY VNVIKEKTKL TDKTHTCPPC PAPELLGGPS 481 VFLFPPKPKD TLMIGRTPEV TCWVDV SHE DFEVKFNWYV DGVEVHNAKT KPREEQYNST 541 YRW3VLTVL KQDWLNGKEY KCKVSNKALP APIEKTJSKA K.GQPREPQVY TLPPSRDELT 601 KNQVSLTCLV KGFYPSDIAV ESESNGOPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ 661 GNVFSCSVMH EALHKHYTQK SLSLSPGKGG GCSGOGGSGG GGSGGGGSDK THTCPPCPAP 721 ELLGGPSVFL FPPKPKDTLM İSRTPEVTCV VVDVSHEDPE VKFNKYVDGV EVHKAKTKPR 781 EEOYNSTYRV VSVLTVLHQD 841 PSRDEI.TKNQ VSLTCLVKGF 901 DKSRNQQGNV FSCSVMHEAL 95]. GGGGSAQVQL QESGGGLVQP 1021 GGSTY ${ }^{*} A D S V$ KGRFTVSRBN 1081 DYWGQGTLVT VSSGSASAPK 1141 YASWYQQKPG QAPTLVIYGL 1201 YGGGQQGVFG GGTKLTVLRQ

WLNGKEYKCK HNDIAVEWE SNGQPENNTYK. TTPPVLDSDG GGSIRDSLS LSPGKGGGGS GGGGSGGGGS GSLRLSCAA SKNTLYLQME LEEGEFSEAR SKRPSGIPDR PKAAPSVTLF PPSSAA*

UNA sequence for FIX-089

1 GGATATCTAC 12 I GCTTGCCTTT 131. GATTTGACAG 241 AACATCACAG 301 AAAftACftAAG 361 CTAAAGAATT 421 TGAATCGGCC 431 AGAGAGAATG 541 CTGAAAGAAC 601 CATGTTTAAA 661 TTGGATTTGA 721 GCGAGCAGTT 731. ATCGACTTGC 841 TTTCTGTTTC 901. ATGTAAATTC 961 TTAATGACTT 1021 AGGTTGTTTT 1031 GGATTGTAAC lifl AACATAATAT 1201. CTCACCACAA 1261 TGGACGAACC 1321 ACACGAACAT 1331 ACAAAGGGAG 144! CATGTCTTCG 1501 AAGGAGGTAG

TGAACATGAT TCAGTGCTGA TAGATATAGA CAATATTGAA ATTTTGGCTC ACTTTCTTAA ATTCTTTTAC AAAGAGGTAT TATGGAAGAA AACTGAATTT TGGCGGCAGT AGGAAAGAAC TTGTAAAAAT AGAAAACCAG ACAAACTTCT TACTGAAGCT CACTCGGGTT GAATGG TAAA TGCTGCCCAC TGAGGAGACA CTACAATGCA CTTAGTGCTA CTTCCTCAAA ATCAGCTTTA ATCTACAAAG AGATTCATGT

ATGTACAGGT AATATCTGAT GAGTCTAACA CATGCCCTAA ACCAGCACGC GAGATGT. AATGGAGA TGGAAGCAGT ATGTTGATGG TGCAAGGATG ACATTAATTC TGTGAATTAG .ATGTAJiCATG AgTGCTGATA ACAAGGTGGT AAGTCCTGTG AACCAGCAGT AAGCTCACCC GTGCTGAGAC GAAACCATTT TGGATAACAT GTTGGTGGAG AAGATGCCAA GTTGATG CAT TCTGTGGAGG TGTGTTGAAA CTGGTGTTAA GAACATACAG AGCAAAAGCG GCTATTAATA AGTACAACCA AACAGCTACG TTACACCTAT TTTGGATCTG GCTATGTAAG GTTCTTCAGT ACCTTAGAGT TTCACCATCT ATAACAACAT CAAGGAGATA GTGGGGGACC

TTTTAAAATA TCACTA AGGTTGGTAA GCTTTCAGAT TGTTITCIT TGAAAACGCC GTTTGTTCAA ACGAGAAGTT AGATCAGTGT CTATGAATGT TAACATTAAG TTGCTCCTGT GCCATTTCCA TGTTTTTCCT CACTCAAAGC ACCAGGTCAA CTCTATCGTT AATTACAGTT AAATGTGATT TGACATTGCC TTGCATTGCT TGGCTGGGGA TCAGA GTTCTGT CCATGTTAC

5

| 155 I | GGACCAQTTT | CTTAACTGGA |
| :--- | :--- | :--- |
| 1621 | A!GGAATATA | TACCAAGGTG |
| 1681 | CTGACAAAAC | TCACACATGC |
| 1.74 J | TCTTCCTCTT | CCCCCCAAAA |
| 1801 | CATGCGTGGT | GGTGGACGTG |
| 1861 | ACGGCGTGGA | GGTGCATAAT |
| 1921 | ACCGTGTGGT | CAGCGTCCTC |
| 1931 | AGTG CAAGG T | CTCCAACAAA |
| 2041 | AAGGGCAGCC | CCGAGAACCA |
| 2101 | AGAACCAGGT | CAGCCTGACC |
| 21.6 I | AGTGGGAGAG | CAATGGGCAG |
| 2221 | CCGACGGCTC | CTTCTTCCTC |
| 2231 | GGAACGTCTT | CTCATGCTCC |
| 2341 | GCCTCTCCCT | GTCTCCGGGT |
| 2401 | GTGGATCCGG | CGGGGGCGGA |
| 2451 | CCGAGCGGAT | GGCCCTGCAC |
| 2521 | GTGGCGGCGG | ATCAGGTGGG |
| 2531 | ACAAAACTCA | CACATGCCCA |
| 2641 | TCCTCTTCCC | CCCAAAACCC |
| 2 "01 | GCGTGGTGGT | GGACGTGAGC |
| 2761 | GCGTGGAGGT | GCATAATGCC |
| 2821 | GTGTGGTCAG | CGTCCTCACC |
| 283 J | GCAAGGTCTC | CAACAAAGCC |
| 2941 | GGCAGCCCCG | AGAACCACAG |
| 3001 | ACCAGGTCAG | CCTGACCTGC |
| 3061 | GGGAGAGCAA | TGGGCAGCCG |
| $3: 21$ | ACGGCTCCTT | CTTCCTCTAC |
| 32.3 J | ACGTCTTCTC | ATGCTCCGTG |
| 3241 | TCTCCCTGTC | TCCGGGTAAA |


| GGGGTGAAGA | GTGTGCAATG | AAAGGCAAAT |
| :--- | :--- | :--- |
| TCA.ACTGGAT | TAAGGAAAAA | ACAAAGCTC.A |
| CAGCTCCGGA | ACTCCTGGGA | GGACCGTCAG |
| CCCTCATGAT | CTCCCGGACC | CCTGAGGTCA |
| ACCCTGAGGT | CAAGTTCAAC | TGGTACGTGG |
| AGCCGCGGGA | GGAGCAGTAC | AACAGCACGT |
| ACCAGGACTG | GCTGAATGGC | AAGGAG TAC.A |
| CCCCCATCGA | GAAAACCATC | TCCAAAGCCA |
| CCCTGCCCCC | ATCCCGGGAT | GAGCTGACCA |
| AAGGCTTCTA | TCCCAGCGAC | ATCGCCGTGG |
| ACTACAAGAC | CACGCCTCCC | GTGTTGGACT |
| TCACCGTCGA | CAAGAGCAGG | TGGCAGCAGG |
| AGGCTCTGCA | CAACCACTAC | ACGCAGAAGA |
| GCGGTTCAGG | TGGAGGAGGG | TCAGGCGGTG |
| GAGGGTCAGG | CGGTGGCGGA | TCAGCCTGCA |
| GTGGCGGTGG | CTCCGGCGGA | GGTGGGTCCG |
| GCGGTGGAGG | TTCCGGTGGC | GGGGGATCCG |
| CAC CGGAAC | CCTGGGCGGA | CCGTCAGTCT |
| TCATGATCTC | CCGGACCCCT | GAGGTCACAT |
| CTGAGGTCAA | GTTCAACTGG | TACGTGGACG |
| CGCGGGAGGA | GCAGTACAAC | AGCACGTACC |
| AGGACTGGCT | GAATGGCAAG | GAGTACAAGT |
| CCATCGAGAA | AACCATCTCC | AAAGCCAAAG |
| TGCCCCCATC | CCGGGATGAG | CTGACCAAG.A |
| GCTTCTATCC | CAGCGACATC | GCCGTGGAGT |
| ACAAGACCAC | GCCTCCCGTG | TTGGACTCCG |
| CCGTGGACAA | GAGCAGGTGG | CAGCAGGGGA |
| CTCTGCACAA | CCACTACACG | CAGAAGAGCC |
|  |  |  |


| ATTATTAGCT | GGGGTGAAGA | GTGTGCAATG |
| :--- | :--- | :--- |
| TCCCGGTATG | TCA.ACTGGAT | TAAGGAAAAA |
| CCACCGTGCC | CAGCTCCGGA | ACTCCTGGGA |
| CCCAAGGACA | CCCTCATGAT | CTCCCGGACC |
| AGCCACGAAG | ACCCTGAGGT | CAAGTTCAAC |
| GCCAAGACAA | AGCCGCGGGA | GGAGCAGTAC |
| ACCGTCCTGC | ACCAGGACTG | GCTGAATGGC |
| GCCCTCCCAG | CCCCCATCGA | GAAAACCATC |
| CAGGTGTACA | CCCTGCCCCC | ATCCCGGGAT |
| TGCCTGGTCA | AAGGCTTCTA | TCCCAGCGAC |
| CCGGAGAACA | ACTACAAGAC | CACGCCTCCC |
| TACAGCAAGC | TCACCGTCGA | CAAGAGCAGG |
| GTGATGC.ATG | AGGCTCTGCA | CAACCACTAC |
| AAAGGCGGTG | GCGGTTCAGG | TGGAGGAGGG |
| TCCGGTGGCG | GAGGGTCAGG | CGGTGGCGGA |
| AACCTGTGCG | GTGGCGGTGG | CTCCGGCGGA |
| GGTGGATCAG | GCGGTGGAGG | TTCCGGTGGC |
| CCGTGCCCAG | CACCGGAAC | CCTGGGCGGA |
| AAGGACACCC | TCATGATCTC | CCGGACCCCT |
| CACGAAGACC | CTGAGGTCAA | GTTCAACTGG |
| AAGACAAAGC | CGCGGGAGGA | GCAGTACAAC |
| GTCCTGCACC | AGGACTGGCT | GAATGGCAAG |
| CTCCCAGCCC | CCATCGAGAA | AACCATCTCC |
| GTGTACACCC | TGCCCCCATC | CCGGGATGAG |
| CTGGTCAAAG | GCTTCTATCC | CAGCGACATC |
| GAGAACAACT | ACAAGACCAC | GCCTCCCGTG |
| AGCAAGCTCA | CCGTGGACAA | GAGCAGGTGG |
| ATGCATGAGG | CTCTGCACAA | CCACTACACG |
| TGA |  |  | TGA

GGACCGICAG CCTGAGGTCA IGACACGIGG AGGAG TAC.A TCCAAAGCCA解 TGGCAGCAGG AcGCAGAAGA ICAGGCGGIG CAGCCTGCA GGGGGATCCG CCGTCAGTCT GAGGTCACAT AGCACGTACC GAGTACAAGT AAAGCCAAAG CIGACCAAG.A TTGGACTCCG CAGAAGAGCC

FIX-089 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is doufo.l. $\approx$ underlined., linker regions connecting 031 to Fc regions are underlined, and OS1 peptide is italicized

1 MQRVNVIMAE EPGITPICII GYTLSAECTV FLDHENANKI LNRPKRYNS G KLEEFVQGNL 61 ERECMEEKCS E'EEAREVFEK JERTTEFGKQ YVDGDOCESN PCLNGGSCKD DINSYECWCP 121 FGFEGKNCEL DVTCNIKJSIGR CEQFCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPFPCGR 181 VSVSQTSKLT RAETVFPDVD Y ${ }^{3 / 3} ; 3 T E A E T I$ LDNTTQSTQS FNDFTRWGG EDAKPGQFPW 24 J QVVLNGKVDA FCGGGTVNEK wTVTAAHCVE TGVKITWAG BHNIEETEHT EQKRNVIRII 301 PKHNYNAATN KYNWDIALLE LDEPLVLNSY VTPICIADKE YTNYFLKFG3 GYVSGWGRVF 361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YMMMFCAGFH EGGRDSCQGD SGGPHVTEVE 421 GTSFLTGIIS KGEEC.AMKGK YGIYTEVSRY VNAIKEKTEL TDKTHTCPPC PAPELLGGPS 481 VFLFPPKPKD TIMISRTPEV TCVVVDVSHE DPEVKPNWYV DGVEVHNAKT KPREEQYNST 541 YRW3VLTVL KODTLNGKEY KCKVSNKALP APIEKTJGKA KGQPREPQVY TLPPSRDELT 601 KNQVSLTCLV KGFYPSDIAV E $\bar{A} E S N G O P E N$ NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ 661 GNVF3CSVMH EALHNHYTQK SLSLS]?GKGG GGSGGGGSGG GGSGGGGSGG GGSGGGGSZC '721 TERMALHNLC GGGGSGGGGS GGGGSGGGOS GGGGSGGGGS DKTHTCPPCP APELLGGPSV "81 FLFPPKPKDT LVISRTPEVT CWVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY 84 I RW SVITVIH QDWLNG KEYK CKV SNEALPA PTEKTIGKAK GQPREPQVYT LPPSRDELTK 901 NQVSLTCLVK GFYPSDIAVE WESN $\mathrm{S}_{Q}$ PNN YKTTPPVLD3 DGSFFLYSKL TVDKSRWQQG 96J. KVPSCSVMHE ALHNHYTQKS LSLSFGK*

| 61 | ERECMEEKCS | E'EEAREVFEK | TERTTEFMKQ | YVDGDOCESN | PCLNGGSCKD | DINSYECWCP |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 121 | FGFEGKNCEL | DVTCNIKJSIGR | CEQFCKNSAD | NKVVCSCTEG | YRLAENQKSC | EPAVPFPCGR |
| 181 | VSVSQTSKLT | RAETVFPDVD | Y ${ }^{3 / 4}$; 3 TEAETI | LDNITQSTQS | FNDFTRWGG | EDAKPGQFPW |
| 241 | QVVLNGKVDA | FCGGSTVNEK | WTVTAAMCVE | TGVKITWAG | BHNIEETEHT | EQKRNV IRII |
| 301 | PKHNYNAA IN | KYnadeatue | LDEPLVLNSY | VTPICIADKE | Y TN IFLKFG3 | GYVSGWGRVF |
| 361 | HKGRSALVLQ | YLRVPLVDRA | TCLRSTKFTI | YMNMACAGFE | EGGRDSCQGD | SGGP HVTEVE |
| 421 | GTSFLTGIIS | KGEEC. AMKGK | YG IYTEVSRY | VNWIKEKTEL | TDKTHTCPPC | PAPELLGGPS |
| 481 | VFLFPPKPKD | TMMISRTPEV | TCVVVDVSHE | DPEVKPNWYV | DGV EVHNAKT | KPREEQYNST |
| 541 | YRW3VLTVL | KDWLNGKEY | KCKV SMKA LP | APIEKTIEKA | KGQ PRE PQVY | TLPPSRDELT |
| 601 | KNQVSLTCLV | KGFYPSDIAV | EmESNGOPEN | NYKTTPPVLD | SDGSFFLYSK | LTVDKSRWQQ |
| 661 | GNVF3CSVMH | EALHNHYTQK | SLSLS]?CKGG | GOSGOGGSGG | GGEGGGGSGG | GGSGGGGS 4 |
| '721 | TERMALHNLC | GGGOSGGGOS | GGGGSGGGGS | gGGGSGGGGS | DKTHTCPPCP | APELLGGPSV |
| " 81 | FLFPPKPKDT | LMISRTPEVT | CWVDVSHED | PEVKFNWYVD | GVEVHNAKTK | PREEQYNSTY |
| 841. | RW SVITVI.H | QD WING KEyK | CKV SNKA LPA | PIEKTISKAK | GQPREPQVYT | LPPSRDELTK |
| 901 | NQVSLTCLVK | GFYP SDIAVE | WESNGQPENN | YKTTPPVLD3 | DGSFFLYSKL | TVDKSRWQQG |
| 961. | KVPSCSVMHE | ALHNHYTQKS | LSLSFGK* |  |  |  |

NA sequence for FIX- 090

| 1 | ATGCAGCGCG | TGAACATGAT |
| ---: | :--- | :--- |
| 61 | GGATATCTAC | TCAGTGCTGA |
| 121 | GCTTGCCTTT | TAGATATAGA |
| 181 | GATTTGACAG | CAATATTGAA |
| 241 | AACATCACAG | ATTTTGGCTC |
| 301 | AAAAACAAAG | ACTTTCTTAA |
| 361 | CTAAAGAATT | ATTCTTTTAC |
| 42 J | TGAATCGGCC | AAAGAGGTAT |
| 481 | AGAGAGAATG | TATGGAAGAA |
| 541 | CTGAAAGAAC | AACTGAATTT |
| 601 | CATGTTTAAA | TGGCGGCAGT |
| 661 | TTGGATTTGA | AGGAAAGAAC |
| 72 I | GCGAGCAGTT | TTGTAAAAAT |
| 781 | ATCGACTTGC | AGAAAACCAG |
| 841 | TTTCTGTTTC | ACAAACTTCT |
| 901 | ATGTAAATTC | TACTG.AAGCT |
| 961 | TTAATGACTT | CACTCGGGTT |
| 1021 | AGGTTGTTTT | GAATGGTAAA |
| 1081 | GGATTGTAAC | TGCTGCCCAC |
| 114 J | AACATAATAT | TGAGGAGACA |
| 1201 | CTCACCACAA | CTACAATGCA |
| 1261 | TGGACGAJVCC | CTTAGTGCTA |
| 1321 | ACACGAACAT | CTTCCTCAAA |
| 1381 | ACAAAGGGAG | ATCAGCTTTA |
| 1441. | CATGTCTTCG | ATCTACAAAG |


| CATGGCAGAA | TCACCAGGCC | TCATCACCAT | CTGCCTrTTA |
| :--- | :--- | :--- | :--- |
| ATGTACAGGT | TTGTTTCCTT | TTTTAAAATA | CATTGAGTAT |
| AATATCTGAT | GCTGTCTTCT | TCACTAAATT | TTGATTACAT |
| GAGTCTAACA | GCCAGCACGC | AGGTTGGTAA | GTACTGTGGG |
| CATGCCCTA.A | AGAGAAATTG | GCTTTCAGAT | TATTTGGATT |
| GAGATGTAAA | ATTTTCATGA | TGTTTTCTTT | TTTGCTAAAA |
| ATTTCAGTTT | TTCTTGATCA | TGAAAACGCC | AACAAAATTC |
| AATTCAGGTA | AATTGGAAGA | GTTTGTTCAA | GGGAATCTAG |
| AAGTGTAGTT | TTGAAGAAGC | ACGAGAAGTT | TTTGAAAACA |
| TGGAAGCAGT | ATGTTGATGG | AGATCAGTGT | GAGTCCA.ATC |
| TGCAAGGATG | -ACATTAATTC | CTATGAATGT | TGGTGTCCCT |
| TGTGAATTAG | ATGTAACATG | TAACATTAAG | AATGGCAGAT |
| AGTGCTGATA | ACAAGGTGGT | TTGCTCCTGT | ACTGAGGGAT |
| AAGTCCTGTG | AACCAGCAGT | GCCATTTCCA | TGTGGAAGAG |
| AAGCTCACCC | GTGCTGAGAC | TGTTTTTCCT | GATGTGGACT |
| GAAACCATTT | TGGATAACAT | CACTCAAAGC | ACCCAATCAT |
| GTTGGTGGAG | AAGATGCCAA | ACCAGGTCAA | TTCCCTTGGC |
| GTTGATGCAT | TCTGTGGAGG | CTCTATCGTT | AATGAAAAAT |
| TGTGTTGAAA | CTGGTGTTAA | AATTACAGTT | GTCGCAGGTG |
| GAACATACAG | AGCAAAAGCG | AAATGTGATT | CGAATTATTC |
| GCTATTAATA | AGTACAACCA | TGACATTGCC | CTTCTGGAAC |
| AACAGCTACG | TTACACCTAT | TTGCATTGCT | GACAAGGAAT |
| TTTGGATCTG | GCTATGTAAG | TGGCTGGGGA | AGAGTCTTCC |
| GTTCTTCAGT | ACCTTAGAGT | TCCACTTGTT | GACCGAGCCA |

5

FIX-- 90 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, and linker regions c:onnecting FIX to SCE5 is Uxiderlined.

1 MORVMVIMA SPGLTYGLY GYLISREOCN FINAENANET LNEPKEYNSQ KLEEFVQGNI
61 ERECMEEKCS FEEAREVFEK TERTTEFMKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP 121 FGFEGKNCEL DVTCNIKNGR CEQFCKNSAD WKVVCSCTEG 181 VSVSQTSKLT RAETVFPDVD YVR3TEAETI LDKTTQSTQS 24J. QWLNG KVDA FCGGSTVNEX WIVTAA出CVE TGVKITWAG 301 PHHN Y ~ 361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YMNMFCAGFKr 421 GTSFLTGIIS WGEECAMKGK 48 SGGGGSGGGG S3AQVQLQESG 541 SGISGSGGST YYADSVKGRF 601 PDQTSFDYWG QGTLVTVSSG 661 DSYRNFYA 8 Y Y 721 YYCLLYYGGG QQGVFGGGTK

YGIYTKVSRY VNWIKEKTKL GGLVQPGGSI, RLSCAA3GFM TVSEDNSKNT LYLQWNSLRA SASAPKLEEG EFSEARVSEL LVIYGLSKRP SGIVDRFSA 3 SSG

CCATGTTACT GAAGTGGAAG GTGTGCAATG AAAGGCAAAT TAAGGAAAAA ACAAAGCTCA CGGATCAGGT GGGGGTGGAT GCAGCTGCAG GAGTCTGGGG TGCAGCCTCT GGATTCATGT GAAGGGGCCA GAGTGGGTCT OICCGMGAAG GGCCGGTTCA AATGAACAGC CTGAGAGCCG CACCAGCCGG AGCGACGTGC GGTCACCGTC TCCTCAGGGA AGCACGCGTA TCTGAACTGA CAGGATCACA TGCCAAGGAG GCCAGGACAG GCCCCTACTC AGACCGATTC TCTGCCTCCA GGCGGAAGAT GAGGCTGACT GTTCGGCGGC GGCACCAAGC TCTGTTCCCG CCCTCTTCTG

YRI:AENQKSC EPAVPFPCGR FNDFTRVVGG EDAKPGQFPW EHNIEETEHT EQKRNVIRII YTNIFLKFGS GYVSGn'GRVF EGGRDSCQGD SGGPHVTEVE TGGGG3GGGG $\quad$ GGGGGgGGGG FSRYAMSWV R OAPGKGPEWV EDTAVYYCAR GATYT3RSDV TQDFAVSVAL GQTVRJTCQG SGNTASLTI TGAQAEDEAD

DNA sequence for $F V r I-088$
$\pm$ ATGGTCTCCC AGGCCCICAG
61 GCAGTCTTCG 121 GCGTTCCTGG 131. TCCTTCGAGG 241 TCTTACAGTG 301 GACCAGCTCC 361 ACGCACAAGG 421 AGTGACCACA 431. GACGGGGTGT 541 AAAAGAAATG 601 GAGTGTCCAT 661 ATCAACACCA 721 AACCTGATCG 731 CGGCGGGTGG §41 ATCGCGCTGC 501. CTGCCCGAAC 961 GGCTGGGGCC 1021 CCCCGGCTG.A 1031 ATCACGGAGT 141 GACAGTGGAG 1201 AGCTGGGGCC 1261 TACATCGAGT 1321 GCCCCATTTC 1331 GGGGGTGGAT 1441 CCACCGTGCC 1501. CCCAAGGACA 1561 AGCCACGAAG 1621 GCCAAGACAA 1631 ACCGTCCTGC 1741 GCCCTCCCAG 1801. CAGGTGTACA $1 \S 61$ TGCCTGGTCA 1.921. CCGGAGAACA 1931 TACAGCAAGC 2041 GTGATGCATG 2101 AAACGGCGCC 2!61 TCCGGTGGCG $222 \mathrm{~J} . A A G A G G G A C A$

TAACCCAGGA AGGAGCTGCG AGGCCCGGGA ATGGGGACCA AGTCCTATAT ATGACCAGCT CGGGCACCAA CCTGCACACC CCAGCAAACC GGCAGGTCCT TCTGGGTGGT CGGTGCTGGG CGCAGGTCAT TCCGCCTGCA GGACGTTCTC AGCTGCTGGA TGACCCAGGA ACATGTTCTG GCCCACATGC AGGGCTGCGC GGCTGCAAAA CCGGTGGCGG CAGGCGGTGG CAGCTCCGGA CCCTCATGAT ACCCTGAGGT AGCCGCGGGA ACCAGGACTG CCCCCATCGA CCCTGCCCCC AAGGCTTCTA ACTACAAGAC TCACCGTCGA AGGCTCTGCA GCCGGAGCGG GGGGATCCGG TCGTGATGAC

GCTCCTCTGC GGAAGCCCAC GCCGGGCTCC GATCTTCAAG GTGTGCCTCA CTGCTTCTGC GATCTGTGTG GCGCTCCTGT CACAGTTGAA CCAAGGCCGA GTTGTTGGTG CTCCGCGGCC CGAGCACGAC CATCCCCAGC CCAGCCCGTG TGAGAGGACG CCGTGGCGCC CTGCCTGCAG TGCCGGCTAC CACCCACTAC AACCGTGGGC GCTCATGCGC TGGCTCCGGC AGGTTCCGGT ACTCCTGGGA CTCCCGGACC CAAGTTCAAC GGAGCAGTAC GCTGAATGGC GAAAACCATC ATCCCGGGAT TCCCAGCGAC CACGCCTCCC CA. AGAGCAGG CAACCACTAC TGGCGGCGGA CGGTGGAGGT CCAGGCCGCC

CTTCTGCTTG GGCGTCCTGC CTGGAGAGGG GACGCGGAGA AGTCCATGCC CTCCCTGCCT AACGAGAACG CGGTGCCACG TATCCATGTG ATTGTGGGGG AATGGAGCTC CACMGr"יMCG CTCAGCGAGC ACGTACGTCC GTCCTCACTG CTGGCCTTCG ACGGCCCTGG CAGTCACGGA TCGGATGGCA CGGGGCACGT CACTTTGGGG TCAGAGCCAC GGAGGTGGGT GGCGGGGGAT GGACCGTCAG CCTGAGGTCA TGGTACGTGG AACAGCACGT AAGGAGTACA TCCAAAGCCA GAGCTGACCA ATCGCCGTGG GTGTTGGACT TGGCAGCAGG ACG CAGAAG A rCAGGTGGGG TCCGGTGGGG CCCAGCGTGC

GGCTT'CAGGG CTGCCTGGCT ACCGGCGCCG GCGCGCCAAC AGTGCAAGGA GGAGCAGTGC GGACGAAGCT GTTCTGGATT AGAATGGGGG CTCCTGCAAG TCGAGGGCCG GAACTGTGAG GCGGCTGTGA GCAGTACTGC AGGGGTACTC TCTGCTGGCA GAAAAATACC TATTCTAGAA GCAAGGTGTG CCCCAAAGGG AGTTGTGTGG GGGGACCCTG ACAAAATCiiA GAACTGGAGG ACGACGGGGA TGAGCAGAGC CGGGCACCAC CAACCACGAC ACCATGTGGT GCCCCTCTGC TGCGCTTCTC ATTGGTCAGC AGCTCATGGT CCTCAACGTG AGGTGGGAGA CTCCCCAAAT GCAAGGACTC CTGCAAGGGG GGTACCTGAC GGGCATCGTC TGTACACCAG GGTCTCCCAG GCCCAGGAGT CCTCCTGCGA CCGGTGGCGG CGGATCAGGT CCGACAAAAC TCACACATGC TCTTCCTCTT CCCCCCAAAA CATGCGTGGT GGTGGACGTG ACGGCGTGGA GGTGCATAAT ACCGTGTGGT CAGCGTCCTC AGTGCAAGGT CTCCAACAAA AAGGGCAGCC CCGAGAACCA AGAACCAGGT CAGCCTGACC AGTGGGAGAG CAATGGGCAG CCGACGGCTC CTTCTTCCTC GGAACGTCTT CTCATGCTCC GCCTCTCCCT GTCTCCGGGT GTGGATCAGG CGGTGGAGGT CCGTGACCCC CGGCGAGAGC

5

DNA sequence for FVITT－041 361 ITEYMFCAGY SDGSKDSCKG 421 YIESLQKLMR SEPRPGVLLR 481 PPCPAPELLG GPSVFLFPPK 541 AKTKPREEQY NSTYEYV3YL 601 OVYTLPPSRD 661 Y3K． TS I CWFLQRPGQS PQLLIYRMSN 841 YPFTFGSGTK LEIKRGGGGS 1021 DKTHTCPPCP APELLGGPSV 1031 GVEVHNAKTK PREEQYKSTY 1さ41 GQFREFQVYT LPPSRDELTK 1201 DGSFFLYSKL TVDKSRWQQG

1．ATGCAAATAG AGCTCTCCAC
61 ACCAGAAGAT ACTACCTGGG 121 GGTGAGCTGC 131 ACCTCAGTCG 241 GCTAAGCCAA 301．GATACAGTGG 361 GGTGTATCCT 421 GAGAAAGAAG 431 AAAGAGAATG 541 GTGGACCTGG 601．GGGAGTCTGG 661 TTTGATGAAG 721 GCTGCATCTG 731 CTGCCAGGTC引き1 ACCACTCCTG 901 CGCCAGGCGT 961 GACCTTGGAC 1021 GCTTATGTCA 1081 GAAG CGGAAG 1： 1 GATGACAACT 1201 TGGGTACATT 1261 CCCGATGACA 1321．AAGTACAAAA

CTGTGGACGC TGTACAAAAA GGCCACCCTG TCATTACACT ACTGGAAAGC ATGATAAAGT GTCCAATGGC TAAAAGACTT CCAAGGAAAA GGAAAAGTTG CTCGGGCCTG TGATTGGATG AAGTGCACTC CCTTGGAAAT AGTTTCTACT AAGTAGACAG ACTATGATGA CTCCTTCCTT ACATTGCTGC GAAGTTATAA AAGTCCGATT

| 2231 | GTGAGCATCA | GCTGCCGGAG | CAGCCGGAGC |
| :--- | :--- | :--- | :--- |
| 2341 | TGCTGGTTCC | TGCAGCGGCC | CGGCCAGAGC |
| 2401 | CTGGCCAGCG | GCGTGCCCGA | CCGGTTCAGC |
| 2451. | CGGATCAGCC | GGGTGGAGGC | CGAGGACGTG |
| 2521 | TACCCCTTCA | CCTTCGGCAG | CGGCACCAAG |
| 2531 | GGCGGCGGCG | GCAGCGGCGG | CGGCGGCAGC |
| 2641 | CTGGTGCGGC | CCGGCACCAG | CGTGAAGATC |
| 2701 | AACTACTGGC | TGGGCTGGGT | GAAGCAGCGG |
| 2761 | ATCTACCCCG | GCGGCGGCTA | CAACAAGTAC |
| 2821 | ACCGCCGACA | CCAGCAGCAG | CACCGCCTAC |
| 2831. | AGCGCCGTGT | ACTTCTGCGC | CCGGGAGTAC |
| 2941 | GGCCAGGGCA | CCAGCGTGAC | CGTGAGCAGC |
| 3001 | GGTGGCGGCG | GATCAGGTGG | GGGTGGATCA |
| 3061 | GACAAAACTC | ACACATGCCC | ACCGTGCCCA |
| 3121 | TTCCTCTTCC | CCCCAAAACC | CAAGGACACC |
| 3131. | TGCGTGGTGG | TGGACGTGAG | CCACGAAGAC |
| 3241 | GGCGTGGAGG | TGCATAATGC | CAAGAC AAAG |
| 3301 | CGTGTGGTCA | GCGTCCTCAC | CGTCCTGCAC |
| 3361 | TGCAAGGTCT | CCftACAAAGC | CCTCCCAGCC |
| 3421 | GGGCAGCCCC | GAGAACCACA | GGTGTACACC |
| 3431. | AACCAGGTCA | GCCTGACCTG | CCTGGTCAAA |
| 3541 | TGGGAGAGCA | ATGGGCAGCC | GGAGAACAAC |
| 3601 | GACGGCTCCT | TCTTCCTCTA | CAGCAAGCTC |
| 3661 | AACGTCTTCT | CATGCTCCGT | GATGCATGAG |
| 3721 | CTCTCCCTGT | CTCCGGGTAA | ATGA |


| CTGCTGCACA | GCAACGGCAA | CACCTACCTG |
| :--- | :--- | :--- |
| CCCCAGCTGC | TGATCT．ACCG | GATGAGCAAC |
| GGCAGCGGCA | GCGGCACCGC | CTTCACCCTG |
| GGCGTGTACT | ACTGCATGCA | GCACCTGGAG |
| CTGGAGATCA | AGCGGGGCGG | CGGCGGCAGC |
| CAGGTGCAGC | TGCAGCAGAG | CGGCGCCGAG |
| AGCTGCAAGG | CCAGCGGCTA | CACCTTCACC |
| CCCGGCCACG | GCCTGGAGTG | GATCGGCGAC |
| AACGAGAACT | TCAAGGGCAA | GGCCACCCTG |
| ATGCAGCTGA | GCAGCCTGAC | CAGCGAGGAC |
| GGCAACTACG | ACTACGCCAT | GGACAGCTGG |
| GGTGGCGGTG | GCTCCGGCGG | AGGTGGGTCC |
| GGCGGTGGAG | GTTCCGGTGG | CGGGGGATCA |
| GCACCGGAAC | TCCTGGGCGG | ACCGTCAGTC |
| CTCATGATCT | CCCGGACCCC | TGAGGTCACA |
| CCTGAGGTCA | AGTTCAACTG | GTACGTGGAC |
| CCGCGGGAGG | AGCAGTACAA | CAGCACGTAC |
| CAGGACTGGC | TGAATGGCAA | GGAGTACAAG |
| CCC．ATCGAGA | AAACCATCTC | CAAAGCCAAA |
| CTGCCCCCAT | CCCGGGATGA | GCTGACCAAG |
| GGCTTCTATC | CCAGCGACAT | CGCCGTGGAG |
| TACAAGACCA | CGCCTCCCGT | GTTGGACTCC |
| ACCGTGGACA | AGAG CAGGTG | GCAGCAGGGG |
| GCTCTGCACA | ACC．ACT．ACAC | GCAGAAGAGC |

FVII－088 amino acid sequence．Signal sequence is shown in dotted underline， propeptide is double underlined，linker region connecting FVII or AP3 to Fc region is underlined，the AP3 scE＇v italicized，and linker with proprotein convertase processing sites is shown in bold
i MVSQALRLIC LILGLQGCLE AVYVTQEEAK GVHJRRRREN AFLEELRPGS LERECKEEQC
61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQKGGSCK DQLQSYICFC LPAFEGRKCE 121 TKKDDQL JCV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVS CTFTVE YPCGKIPILE 181 KRNASKPOGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL IRTIWWSAA HCPDKIKRSR 241 NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC 301 LPER．TFSERT LAFVRFSLVS GWGQI．TIDRGA TALELMVLNV PRLMTQDCLQ Q $3 R K V G D S P N$ WQQGNVPSCS GGGGSGGGGS QVQLQQSGAE LVRPGTSVKI SCKASGYTFT 901 NYWLGWVKQR PGHGLEWIGD I YPGGGYNKY NENFKGKATL TADTSSSTAY MQLSSLTSED 961 SAVYFCAREY GVYD YAMDSW GQGTSVTVSS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS FLFPPKPKDT LMISRTPEVT CVWDVSHED PEVKFNWYVT RWSVLTVLH QDWLKGKEYK CKVSKKALPA PIEKTISKAK NQVSLTCLVK GFYFSDIAVE WESNGQEENN YKTTPPVLDS NVESCSV让E ALHNHYTQK8 LSLSPGK＊

TGCAGTGGA AAGATTTCCT GACTCTGTTT GATGGGTCTG TAAGAACATG TTCTGAGGGA CTTCCCTGGT CTCTGACCCA GAATCAGC GACACAGACC GCACTCAGAA GCCTAAAATG CCACAGG AAA AATATTCCTC CTCGCCAATA GTTTTGTCAT CTGTCCAGAG TGATCTTACT TATCCAiiATT TGAAGAGGAG AAGTCAATAT TTGAACAATG TATGGCATAC ACAGATGAAA

CTGTCATGGG CCTAGAGTGC GTAGAATTCA CTAGGTCCTA GCTTCCCATC GCTGAATATG GGAAGCCATA CTGTGCCTTA CTCATTGGAG TTGCACAAAT ACAAAGAACT CACACAGTCA TCAGTCTATT GAAGGTCACA ACTTTCCTTA ATCTCTTCCC GAACCCCAAC GATTCTGAAA TGAAA CGCTCAGTTG

TGCGATTCTG CTTTAGTGCC ACTATATGCA AAGTGATCTC CAAAATCTTT TCCATTCiiAC CGGATCACCT TTTCAACATC CCATCCAGGC TGAGGTTTAT CTGTCAGTCT TCATGCTGTT ATGATCAGAC CAGTCAAAGG CATATGTCTG GCAGGTCCTG CCTACTCATA TCTTTCTCAT CCCTACTAGT ATGTAGAGAA TTATACTACT TTTTGCTGTA CCTTGATGCA GGATAGGGAT ATGGTTATGT AAACAGGTCT GGCATGTGAT TGGAATGGGC CATTTCTTGT GAGGAACCAT CTGCTCAAAC ACTCTTGATG ACCAACATGA TGGCATGGAA TACGAATGAA AAATAATGAA TGGATGTGGT CAGGTTTGAT CCAAGAAGCA TCCTAAAACT ATGCTCCCTT AGTCCTCGCC GCCCTCAGCG GATTGGTAGG CCTTTAAGAC TCGTGAAGCT
381. ATTCAGCATG AATCAGGAAT CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG

GATGTCCGTC 1E5]. CCAATTCTGC 1521 ACTAAATCAG 1631 GATCTAGCTT 1741 AGAGGAAACC 1801 AACCGAAGCT 1861 CAGCTTGAGG 19\% TTTGATAGTT 1931 ATTGGAGCAC 2041 ATGGTCTATG 2101 ATGGAAAACC 2161 ATGACCGCCT 2221 AGTTATGAAG 2231 TTCTCTCAAA 234.1 CAGTCAGATC 2401 GATTTTGACA 2461 CG.ACACTATT 2521 CATGTTCTAA 2531 . CAGGAATTTA 2641 TTGGGACTCC 2701 AGAAATCAGG 2761 CAGAGGCAAG $2 \S 21$ TTTTGGAAAG 2881 GCTTATTTCT 2941 CTGGTCTGCC 3001. TTTGCTCTGT 3061 GAAAGAAACT 3121 TATCGCTTCC 31 31 CAGGATCAAA 324.1 ATTCATTTCA 330 J . TACAATCTCT 3361 TGGCGGGTGG 3421 GTGTACAGCA 3431 CAGATTACAG 354 I TCCGGATCAA 3601 TTGGCACCAA 3661 CTCTACATCT 3721 CGAGGAAATT 3731 AAACACAATA 3841 TATAGCATTC 3501 ATGCCATTGG 396.1 TTTACCAATA 402]. AGTAATGCCT 4031 AAGACAATGA 4141 TATGTGAAGG 4201 CAGAATGGCA 4261 TCTCTAGACC 432 I. CAGATTGCCC 4331 CACACATGCC 4441 CCCCCAAAAC 4501 GTGGACGTGA 4561 GTGCATAATG 4621 AGCGTCCTCA 4681 TCCAACAAAG 4741 CGAGAACCAC 4801 AGCCTGACCT $4 \S 61$ AATGGGCAGC 4921 TTCTTCCTCT 4981 TCATGCTCCG TGATGCATGA GGCTCTGCAC 5041 TCTCCGGGTA AAGGTGGCGG CGGATCAGGT 5101 GGCGGGGGAT CAGACAAAAC TCACACATGC Bi 61 GGACCGTCAG TCTTCCTCTT CCCCCCAAAA 522 1. CCTGAGGTCA CATGCGTGGT GGTGGACGTG 5281 TGGTACGTGG ACGGCGTGGA GGTGCATAAT 534 J . AACAGCACGT ACCGTGTGGT CAGCGTCCTC A 5401 AAGGAGTACA AGTGCAAGGT CTCCAACAAA 546 TCCAAAGCCA AAGGGCAGCC CCGAGAACCA 5521 GAGCTGACCA AGAACCAGGT CAGCCTGACC 564 I . GTGTTGGACT CCGACGGCTC CTTCTTCCTC §701 TGGCAGCAGG GGAACGTCTT CTCATGCTCC 5761 ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAATG

5 FVII1－041 amino acid sequence．Signal seqilence ís shcwn in dotted underline： and linker region connecting the e＇c regioins is under．］．ined 51．T3WYK．KTLF VEFTDHLFWI AKPRPPWMGL LGPTTQAEVY 121 GVSYWKASEG 13J．VDLVKDLNSG 241 AASARAWPKM 301 RQASLEISPI 361 EAEDYDDDLT 421 PDDRSYKSQY 431 LIIFKNQASR 541 TKSDPRCLTR 601 KRSWYLTENI 661 IGAQTDFLSV 721 MTALLKVS3C 731 03DQEEIDYD 841 HVLRNRAQSG 901 RNQASRPY8F 961 AYFSDVDLEK 1021 ERNCRAPCNI 1031．IKFSGHVFTV İ4 VYSNKCQTPL 1201 LAPMTIHGIK TQGARQKFSS 1261 KHNIFNPPTI 1321 FTNMFATKSP 1331 YVKEFLISSS 1441 QIALRMEVLG 1501 VDVSHEDPEV 1561 SNKALPAPTE K 1621 NGQPENNYKT 1681 SPGKGGGGSG 1741 PEVTCWVDV 1801 KEYKCKVSNK 1661 IAVEWESNGQ 1921 TQKSLSLSPG

AEYDDQTSQR． LIGALLVCRE KTVNGYVNRS TFLTAQTLLM DIG DLGEIFCH DSEMDVVRFD DDNSPSFIQI LNNGPQRT．GR．KYKKVRFMAY PYNIYPHGIT DVRPLYSRRL YYSSFVNMER DLASGLIGPL QRELPNPAGV QLEDPEFQAS FFSGYTFKHK MVYEDTLTLF DKNTGDYYED DTISVEMKKE 3VPOFKKVVF YSSLISYEED DVHSGLIGPL QMEDPTFKEN RKKEEYKMAL TQGARQKFSS ARYIRLHPTH SKARLHLQGR QDGHQWTLFF CEAQDLYDKT KFNWYVDGVE KTISKAKGQP TPPVLDSDGS GGGS（3／4GGGSG． 3HEDPEVKFN ALPAPIEKTI WYVDGVEVHN P PE
K ＊

DNA sequence for FVITI－108
1 ATGCAAATAG AGCTCTCCAC 61 ACCAGAAGAT ACTACCTGGG 121 GGTGAGCTGC CTGTGGACGC 131 ACCTCAGTCG TGTACAAAAA 241 GCTAAGCCAA 301 GATACAGTGG 361 GGTGTATCCT 42 J GAGAAAGAAG 431 AAAGAGAATG 541 GTGGACCTGG 60 1．GGGAGTCTGG 661 TTTGATGAAG 72 J．GCTGCATCTG 731 CTGCCAGGTC §41 ACCACTCCTG 901 CGCCAGGCGT 961 GACCTTGGAC 1．021．GCTTATGTCA 1081 GAAG CGGAAG 1！ 1 GATGACAACT 1201 TGGGTACATT 126 l CCCGATGACA 1321．AAGTACAAAA 1381 ATTCAGCATG 1441 T＇TGATTATAT 1501 GATGTCCGTC 15 हil CCAATTCTGC 162 J．ACTAAATCAG 1681 GATCTAGCTT J． 74 J ．AGAGGAAACC 1801 AACCGAAGCT 1361 CAGCTTGAGG 1．921．TTTGATAGTT I 9母，ATTGGAGCAC 204〕．ATGGTCTATG 2101 ATGGAAAACC $2: 61$ ATGACCGCCT 2221 AGTTATGAAG 22日！TTCTCTCAAA 234］．CAGTCAGATC

TGCAGTGGAA AAGATTTCCT GACTCTGTTT GATGGGTCTG TAAGAACATG TTCTGAGGGA CTTCCCTGGT CTCTGACCCA GAATTCAGGC GACACAGACC GCACTCAGAA GCCTAAAATG CCACAGG A AATATTCCTC CTCGCCAATA GTTTTGTCAT CTGTCCAGAG TGATCTTACT TATCCAiiATT TGAAGAGGAG AAGTCAATAT TATGGCATAC CTTGGGACCT AGCAAGCAGA AAGGAGATTA ATTCAAATAT CCTGACCCGC TGGCCCTCTC AGACAAGAGG AGAGAATATA CCAAGCCTCC AGTTTGTTTG CCTITCTGTC CACCCTATTC GAT＇TCTGGG TTCTAGTTGT ATACTTGCTG CTTGAAACGC TGACTATGAT

CTGTCATGGG CCTAGAGTGC GTAGAATTCA CTAGGTCCTA GCTTCCCATC GCTGAATATG GGAAGCCATA CTGTGCCTTA CTCATTGGAG TTGCACAAAT ACAAAGAACT CACACAGTCA TCAGTCTATT GAAGGTCACA ACTTTCCTTA ATCTCTICCC GAACCCCAAC GATTCTGAAA GGCTCAGTTG GACTGGGACT TTGAACAATG ACAGATGAAA TTACTTTATG CCATATAACA CCARAGGGTG AAATGGACAG TATTACTCTA CTCATCTGCT AATGTCATCC CAACGCTTTC AACATCATGC CATGAGGTGG TTCTTCTCTG CCATTCTCAG TGCCACAACT GACAAGAACA AGTAAAAACA CATCAACGGG GATACCATAT

TGCGATTCTG CTTTAGTGCC ACTATATGCA AAGTGATCTC CiiAAfitctit tccattciiAC CGGATCACCT TTTCAACATC CCATCCAGGC TGAGGTTTAT CTGTCAGTCT TCATGCTGTT ATGATCAGAC CAGTCAAAGG CATATGTCTG GCAGGTCCTG CCTACTCATA TCTTTCTCAT CCCTACTAGT ATGTAGAGAA TTATACTACT TTTTGCTGTA CCTTGATGCA GGATAGGGAT ATGGTTATGT AAACAGGTCT GGCATGTGAT TGGAATGGGC CATTT＇CTTGT GAGGAACCAT CTGCTCAAAC ACTCTTGATG ACCAACATGA TGGCATGGAA TACGAATGAA AAATAATGAA TGGATGTGGT CAGGTTTGAT CCAAGAAGCA TCCTAAAACT ATGCTCCCTT AGTCCTCGCC GCCCTCAGCG GATTGGTAGG CCTTTAAGAC TCGTGAAGCT GGGAAGTTGG AGACACACTG TCTACCCTCA CGGA．ATCACT TAAAACATTT GAAGGATTTT tGACTGTAGA AGATGGGCCA GTtTCGTTAA TATGGAGAGA ACAAAGAATC TGTAGATCAA TGTTTTCTGT ATTTGATGAG TCCCCAATCC AGCTGGAGTG ACAGCATCAA TGGCTATGTI CATACTGGTA CATTCTAAGC GATATACCTT CAAA．CACAAA GAGAAACTGT CTTCATGTCG CAG．ACTTTCG GAACAGAGGC CTGGTGATTA TTACGAGGAC ATGCCATTGA ACCAAGAAGC AAATAACTCG TACTACTCTT CAGTTGAAAT GAAGAAGGAA

2401 GATTTTGACA TTTATGATGA GGATGAAAAT CAGAGCCCCC GCAGCTTTCA AAAGAAAACA 2461 CGACACTATT TTATTGCTGC AGTGGAGAGG CTCTGGGATT ATGGGATGAG TAGCTCCCCA 2521 CATGTTCTAA GAAACAGGGC TCAGAGTGGC AGTGTCCCTC AGTTCAAGAA AGTTGITTTC 25 31. CAGGAATTTA CTGATGGCTC CTTTACTCAG C 2641 TTGGGACTCC TGGGGCCATA TATAAGAGCA 2701 AGAAATCAGG CCTCTCGTCC CTATTCCTTC 2751 CAGAGGCAAG GAGCAGAACC TAGAAAAAAC 2821 TTTTGGAAAG 2831 GCTTATTHCT 2941 CTGGTCTGCC 300 J . TTTGCTCTGT 3061 GAAAGAAACT 3121 TATCGCTTCC 3131 CAGGATCAAA 3241 ATTCATTTCA 3301 TACAATCTCT 3361 TGGCGGGTGG 3421 GTGTACAGC.A 3431 CAGATTACAG 3541 TCCGGATCAA 3601 TTGGCACCAA 3661 CTCTACATCT 372 I CGAGGAAATT 3731 AAACACAATA 3341 TATAGCATTC 3901 ATGCCATTGG 3961 TTTACCAATA 4021 AGTAATGCCT 4031 AAGACAATGA 42!1 TATGTGAAGG 4201 CAGAATGGCA 4261 TCTCTAGACC 432 I. CAGATTGCCC 4381 CACACATGCC 4441 CCCCCAAAAC CCAAG 4501 GTGGACGTGA GCCACGAAGA CCCTGAGGTC 4561 GTGCATAATG CCAAGACAAA GCCGCGGGAG 4621 AGCGTCCTCA CCGTCCTGCA CCAGGACTGG 4681 TCCAACAAAG CCCTCCCAGC CCCCATCGAG 4741 CGAGAACCAC AGGTGTACAC CCTGCCCCCA 4801 AGCCTGACCT GCCTGGTCAA AGGCTTCTAT $4 \xi \xi_{i} 1$ AATGGGCAGC CGGAGAACAA CTACAAGACC 492 J . TTCTTCCTCT ACAGCAAGCT CACCGTCGAC A 4981 TCATGCTCCG TGATGCATGA GGCTCTGCAC A 504 J TCTCCGGGTA 5101 GGTGGAGGTT 52. 1 A 522 ]. GGGGGGTCCC TGAGACTCTC CTGTGCAGCC 5281 AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG 534 J . GGTGGTAGTA CATACTACGC AGACTCCGTG 5401 TCCAAGAACA CGCTGTATCT GCAAATGAAC 5461 TACTGCGCCC GGGGCGCCAC CTACACCAGC 5521 GACTACTGGG GCCAGGGAAC CCTGGTCACC 55:1 CTTGAAGAAG GTGAATTTTC AGAAGCACGC 5641 . TCTGTGGCCT TGGGACAGAC AGTCAGGATC 5701 TATGCAAGCT GGTACCAGCA GAAGCCAGGA 5761 AGTAAAAGGC CCTCAGGGAT CCCAGACCGA 5821 TCCTTGACCA TCACTGGGGC TCAGGCGGAA 5381 TACGGCGGCG GCCAGCAGGG CGTGTTCGGC 594 J. CCCAAGGCTG CCCCCTCGGT CACTCTGTTC 6001 TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA 6061 TCCGGTGGCG GGGGATCAGA CAAAACTCAC 6121 CTGGGCGGAC CGTCAGTCTT CCTCTTCCCC 6181 CGGACCCCTG AGGTCACATG CGTGGTGGTG 624 J TTCAACTGGT ACGTGGACGG CGTGGAGGTG 6301 CAGTACAACA GCACGTACCG TGTGGTCAGC 635 J . AATGGCAAGG AGTACAAGTG CAAGGTCTCC 6421 ACCATCTCCA AAGCCAAAGG GCAGCCCCGA 6481 CGGGATGAGC TGACCAAGAA CCAGGTCAGC 654 . AGCGACATCG CCGTGGAGTG GGAGAGCAAT 6601 CCTCCCGTGT TGGACTCCGA CGGCTCCTTC 6661 AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA 6721 CACTACACGC AGAAGAGCCT CTCCCTGTCT

CCCTTATACC GAAGTTGAAG ATAATATCAT G TATTCTAGCC TTTGTCAAGC CTAATGAAAC AGTTTGACTG GATGTGCACT CA GCTCATGGGA GAGGCCTGAT TGGACCCCTT GACAAGTGAC AGTACAGGAA ACCAAAAGCT GGTACTTCAC TGAAAATATG CAGATGGAAG ATCCCACTTT TAAAGAGAAT ATGGATACAC TACCTGGCTT AGTAATGGCT AGCATGGGCA GCAATGAAAA CATCCATTCT CGAAAAAAAG AGGAGTATAA AATGGCACTG GTGGAAATGT TACCATCCAA AGCTGGAATT CTACATGCTG GGATGAGCAC ACTTTTTCTG GGAATGGCTT CTGGACACAT TAGAGATTTT TGGGCCCCAA AGCTGGCCAG ACTTCATTAT GAGCCCTTTT CTTGGATCAA GGTGGATCTG ACCCAGGGTG CCCGTCAGAfi GTTCTCCAGC AGTCTTGATG GGAAGAAGTG GCAGACTTAT TTCTTTGGCA ATGTGGATTC ATCTGGGATA GCTCGATACA TCCGTTTGCA CCCAACTCAT TTGATGGGCT GTGATTTAAA TAGTTGCAGC TTGATGGGCT GTGATTTAAA TAGTTGCAGC TCAAAAGCTC GACTTCACCT CCAAGGGAGG CCAAAAGAGT GGCTGCAAGT GGACTTCCAG CAGGGAGTAA AATCTCTGCT TACCAGCATG CAAGATGGCC ATCAGTGGAC TCTCTTTTTT AATCAAGACT CCTTCACACC TGTGGTGAAC CTTCGAATTC ACCCCCAGAG TTGGGTGCAC TGCGAGGCAC AGGACCTCTA CGACAAAACT CTCCTGGGAG GACCGTCAGT CTTCCTCTTC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG AAGTTCAACT GGTACGTGGA CGGCGTGGAG GAGCAGTACA ACAGCACGTA CCGTGTGGTC CTGAATGGCA AGGAGTACAA GTGCAAGGTC AAAACCATCT CCAAAGCCAA AGGGCAGCCC TCCCGCGATG AGCTGACCAA GAACCAGGTC CCCAGCGACA TCGCCGTGGA GTGGGAGAGC ACGCCTCCCG TGTTGGACTC CGACGGCTCC AAGAGCAGGT GGCAGCAGGG GAACGTCTTC AACCACTACA CGCAGAAGAG CCTCTCCCTG GGCGGCGGAT CAGGTGGGGG TGGATCAGGC GgTGGAGGTT CCGGTGGGGG TGGATCAAGG CAGGAGTCTG GGGGAGGCTT GGTACAGCCT TCTGGATTCA TGTTTAGCAG GTATGCCATG CCAGAGTGGG TCTCAGGTAT TAGTGGTAGT AAGGGCCGGT TCACCGTCTC CAGAGACAAT AGCCTGAGAG CCGAGGACAC GGCTGTATAT CGGAGCGACG TGCCCGACCA GACCAGCT'TC GTCTCCTCAG GGAGTGCATC CGCCCCAAAG GTATCTGAAC TGACTCAGGA CCCTGCTGTG ACATGCCAAG GAGACAGCCT CAGAAACTTT CAGGCCCCTA CTCTTGTCAT CTATGGTTTA TTCTCTGCCT CCAGCTCAGG AAACACAGCT GATGAGGCTG ACTATTACTG CCTGCTGTAC GGCGGCACCA AGCTGACCGT CCTACGTCAG CCGCCCTCTT CTGCGGCCGG TGGCGGTGGC TCAGGTGGGG GTGGATCAGG CGGTGGAGGT ACATGCCCAC CGTGCCCAGC ACCGGAACTC CCAAAACCCA AGGACACCCT CATGATCTCC GACGTGAGCC ACGAAGACCC TGAGGTCAAG CATAATGCCA AGACAAAGCC GCGGGAGGAG GTCCTCACCG TCCTGCACCA GGACTGGCTG AACAAAGCCC TCCCAGCCCC CATCGAGAAA GAACCACAGG TGTACACCCT GCCCCCATCC CTGACCTGCC TGGTCAAAGG CTTCTATCCC GGGCAGCCGG AGAACAACTA CAAGACCACG TTCCTCTACA GCAAGCTCAC CGTGGACAAG TGCTCCGTGA TGCATGAGGC TCTGCACAAC CCGGGTAAAT GA

55 DNA sequence for pSYN-FVIII-049

| i ATGCAAATAG | AGCTCTCCAC | CTGCTTCTTT | CTGTGCCTTT | TGCGATTCTG | CTTTAGTGCC |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 5]. ACCAGAAGAT | ACTACCTGGG | TGCAGTGGAA | CTGTCATGGG | ACTATATGCA | AAGTGATCTC |
| Al GGTGAGCTGC | CTGTGGACGC | AAGATTTCCT | CCTAGAGTGC | CAAAATCTTT | TCCATTCAAC |
| 15] ACCTCAGTCG | TGTACAAAAA | GACTCTGTTT | GTAGAATTCA | CGGATCACCT | TTTCAACATC |
| 241 GCTAAGCCAA | GGCCACCCTG | GATGGGTCTG | CTAGGTCCTA | CCATCCAGGC | TGAGGTTTAT |
| 301 GATACAGTGG | TCATTACACT | TAAGAACATG | GCTTCCCATC | CTGTCAGTCT | TCATGCTGTT |
| 361 GGTGTATCCT | ACTGGAAAGC | TTCTGAGGGA | GCTGAATATG | ATGATCAGAC | CAGTCAAAGG |
| 4 l GAGAAAGAAG | ATGATAAAGT | CTTCCCTGGT | GGAAGCCATA | CATATGTCTG | GCAGGTCCTG |
| 43J. AAAGAGAATG | GTCCAATGGC | CTCTGACCCA | CTGTGCCTTA | CCTACTCATA | TCTTTCTCAT |
| 54.1 GTGGACCTGG | TAAAAGACTT | GAATTCAGGC | CTCATTGGAG | CCCTACTAGT | ATGTAGAGAA |
| 601 GGGAGTCTGG | CCAAGGAAAA | GACACAG. ACC | TTGCACAAAT | TTATACTACT | TTTTGCTGTA |
| 661 TTTGATGAAG | GGAAAAGTTG | GCACTCAGAA | ACAAAGAACT | CCTTGATGCA | GGATAGGGAT |
| 721 GCTGCATCTG | CTCGGGCCTG | GCCTAAAATG | CACACAGTCA | ATGGTTATGT | AAACAGGTCT |
| 731. CTGCCAGGTC | TGATTGGATG | CCACAGGAAA | TCAGTCTATT | GGCATGTGAT | TGGAATGGGC |
| 641 ACCACTCCTG | AAGTGCACTC | AATATTCCTC | GAAGGTCACA | CATTTCTTGT | GAGGAACCAT |
| 901 CGCCAGGCGT | CCTTGGAAAT | CTCGCCAATA | ACTTTCCTTA | CTGCTCAAAC | ACTCT'TGATG |
| 261 GACCTTGGAC | AGTTTCTACT | GTT'TTGTCAT | 7-vrctcticcc | ACCAACATGA | TGGCATGGAA |
| 1021 GCTTATGTCA | AAGTAGACAG | CTGTCCAGAG | GAACCCCAAC | TACGAATGAA | AAATAATGAA |
| 1031. GAAGCGGAAG | ACTATGATGA | TGATCTTACT | GATTCTGAAA | TGGATGTGGT | CAGGTTTGAT |
| 1.4.1 GATGACAACT | CTCCTTCCTT | TATCCAAATT | CGCTCAGTTG | CCAAGAAGCA | TCCTAAAACT |

1201. TGGQTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT 1261 CCCGATGACA GAAGTTATAA AAGTCAATAT T 1321 AAGTACAAAA 1.331. ATTCAGCATG 1441 TTGATTATAT 150 J GATGTCCGTC 1561 CCAATTCTGC 1621 ACTAAATCAG 1631 GATCTAGCTT 1741 AGAGGAAACC 1.801. AACCGAAGCT 1861 CAGCTTGAGG 1921 TTTGATAGTT 1931 ATTGGAGCAC 2041 ATGGTCTATG 2. 0 J ATGGAAAACC 2161 ATGACCGCCT 2221 AGTTATGAAG 2231 TTCTCTCAAA 2341 CAGTCAGATC 2401 GATTTTGACA 2461 CGACACTATT 252 J CATGTTCTAA 2531 CAGGAATTTA 2641 TTGGGACTCC 2701 . AGAAATCAGG 2761 CAGAGGCAAG 282 J. TTTTGGAAAG 2831 GCTTATTTCT 29:1 CTGGTCTGCC 3001 TTTGCTCTGT 3061 GAAAGAAACT 3 3. 2 J TATCGCTTCC 3181 CAGGATCAAA 3241 ATTCATTTCA 3301 TACAATCTCT 33 G1 TGGCGGGTGG 342 ] GTGTACAGCA 3481 CAGATTACAG 3541 TCCGGATCAA 3601 TTGGCACCAA 3661 CTCTACATCT 372 J. CGAGGAAATT 3781 AAACACAATA 384]. TATAGCATTC 3901 ATGCCATTGG 3961 TTTACCAATA 402 I. AGTAATGCCT $40 \& 1$ AAGACAATGA 43.4 J TATGTGAAGG 4201 CAGAATGGCA 4261 TCTCTAGACC 4321 CAGATTGCCC 43 CACACATGCC 4441 CCCCCAAAAC 4501 GTGGACGTGA 4561 GTGCATAATG 4621 AGCGTCCTCA 4681 TCCAACAAAG 474 J. CGAGAACCAC 4801 AGCCTGACCT 4 961 AATGGGCAGC 4921 TTCTTCCTCT $49 \xi 1$ TCATGCTCCG 504 J. TCTCCGGGTA $=101$ GGTGGAGGTT 5? 5] AAGAGGAGGA S22 1 GGCGGACCGT CAGTCTTCCT CTTCCCCCCA 5281 ACCCCTGAGG TCACATGCGT GGTGGTGGAC 534 J . AACTGGTACG TGGACGGCGT GGAGGTGCAT 5401 TACAACAGCA CGTACCGTGT GGTCAGCGTC 546 J. GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC 5521 ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA 5581 GATGAGCTGA CCAAGAACCA GGTCAGCCTG 5641 GACATCGCCG TGGAGTGGGA GAGCAATGGG C 5701 CCCGTGTTGG ACTCCGACGG CTCCTTCTTC 576 J. AGGTGGCAGC AGGGGAACGT CTTCTCATGC


#### Abstract

GGAACAATG GCCCTCAGCG


 ACAGATGAAA CCTTTAAGAC TTACTTTATG GGGAAGTTGG CCATATAACA TCTACCCTCA CCAAAAGGTG TAAAACATTT AAATGGACAG TGACTGTAGA TATTACTCTA CTCATCTGCT AATGTCATCC CAACGCTTTC AACATCATGC CATGAGGTGG TTCTTCTCTG CCATTCTCAG TGCCACAACT CAGACTTTCG GACAAGAACA CTGGTGATTA AGTAAAAACA ATGCCATTGA CATCAACGGG AAATAACTCG GATACCATAT CAGTTGAAAT CAGAGCCCCC GCAGCTTTCA CTCTGGGATT ATGGGATGAG AGTGTCCCTC AGTTCAAGAA GTGGAGAACT GAAGTTGAAG ATAATATCAT TATTCTAGCC TTATTTCTTA TTTGTCAAGC CTAATGAAAC ACTAAAGATG AGTTTGACTG GATGTGCACT CAGGCCTGAT GCTCATGGGA GACAAGTGAC ACCAAAAGCT GGTACTTCAC CAGATGGAAG ATCCCACTTT ATGGATACAC TACCTGGCTT AGCATGGGCA GCAATGAAAA CGAAAAAAAG AGGAGTATAA GTGGAAATGT TACCATCCAA CTACATGCTG GGATGAGCAC GGAATGGCTT CTGGACACAT TGGGCCCCAA AGCTGGCCAG CTTGGATCAA CCCGTCAGAA GGAAGAAGTG ATGTGGATTC TCCGTTTGCA GTGATTTAAA agattactac GACTTCACCT GGCTGCAAGT AATCTCTGCT ATCAGTGGAC CTTCACACC ACCCCCAGAG GACCGTCAGT CTGAGGTCAC GGTACGTGGA ACAGCACGTA CCAAAGCCAA aGCTGACCAA TCGCCGTGGA TGTTGGACTC GGCAGCAGGG GCAGAAGGG CAGGTGGGGG CCGGTGGGGG GCCCAGCTCC AAGACCCTGA AAAGCCGCG TGCACCAGGA CAGCCCCCAT ACACCCTGCC CCATCCCGG TCAAAGGCTT CT.ATCCCAGC ACAACTACAA GACCACGCCT AGCTCACCGT GGACAAGAGC ATGAGGCTCT GCACAACCAC玉؛321 TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGA

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FVIII-049 amino acid sequence. Signal sequence is shown
in dotted underline, and linker with proprotein
convertase processing sites is shown in bold
```

|  |  |  | TRRYYLGA.VE | LSWDYMQSDI | GELFVD | PRVPKSFPFN |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 61. | TSW | VEFtDHLFNI | A KPr | LGPTIOAEV | DTW J.T. LKNM | ASHPVSLHAV |
| 121 | GVSYwKASEG | AEYDDQTSQR | EKE | GSHTYWQVL | KENGPM | LCLTYSYLSH |
| 131 | vDLVKDLNSG | LIGALLVCRE | GSLAKEKTG | LHKFILLfa | FDEGKSWHS | TKNS |
| 24 | AA | HT |  |  | T |  |
| 301 | RQASLETERI | TFLTAQTLLM | DLGQFLLF | xSShQHDGM | AYVKVDSCE | EP |
| 3). | EAEDYDDDLT | DSEMDWRFD | DDNSPSFIQ | RSVAKKKPK | wVHY JAAE | DWDY |
| 42 | PDDRSYKSQY | Lang | KYKKVRFMA | - | 12 |  |
| 43 | LIIMKNQASR | PYNIYPHGIT | DVRPLYSRR | PigGikhled | PILP | KWTVIVEDGP |
| 541 | TKSDPRCLTR | YYSGFVNMER | DLASGLIGE | LICYKESVDQ | RGNQIMSDKR | mvilfsvede |
|  | NRSWYLT | QRFLPNPA | QLEDPEFQAS | NIM | FDSLQLS | HEVAYWYils |
| S61 | IGAQTDFLSV | FFSGYtfkhk | Mvyedtitle | PfSGETVFM | MENPGLITI | CHN |
| 721 | MTALLKVSSC | DKNTGDYYE | SYEDISAYL | SKNNAIEP | FsQenp | HQR |
| 3. | QSDQEEIDY | Dtisvemkk | DFDIYDEDE | QSPRSFQKK | RHYFIAAV | LWDYGMSSSP |
| 241 | HVLRNRAQSG | SVPQFiKKVVF | QEFTDGSFTQ | Plyrgelne | LGLLGPYIR | EVED |
| 901 | RNQASRPYSF | YSSLISYEED | QRQGAE PRKN | FVKPNETK | FWKVQHHM | TKDEFDCKAW |
| 961 | AYFSD | VHSGLI | LVC | AHG | FALFFTIFD | TKSWYFTENM |
| 10.1 | ERNCRAPCN | QMEDPTFKEN | YRFHATNGY | mbtapgingm | QDQR | SMGSI |
| 31 | IHfSGHVFTV | RKKEEYEMAL | YNLYFGVFET | VEMLPSKAGI | WRVEC | LKAGM |
| 11 | VYSNKCOI | GMASGHIRDF | QITASGOYG | WA | SGBxM | EPFSWIEVDI |
| 1201 | LAPMITHGIR | TQGARQKFSS | LYISQFİM | SLDGKKWQTY | RGNStGTL | FFGN |
| 61 | Khnvensp | ARYIRLHP | YSIRSTLRME | LMGCDLNS | MPLGMESK | SDAQ ITASEY |
| 321 | FTNMFATMSP | 3KARLHLQGR | 3NAWRPQVI | PKEWLQVDF | KTMKVTGV | QGV |
| 1381. | YVKEFLISSS | QDGHQWTLFE | ongrukyp | NQDSFTPWN | SLDPPLLIT | LRI |
| 1441 | QIALRME | CEAQDLYDKT | HTCPPCPAP | LLGGPSVF | PPKFKDTL | SRTPEVTC |
| 1501 | vDVSKEDPEV | KFNWYVDGVE | VHNAKTKPR | EQYNSTYRV | SVLTVLHQD | LNGKEYKC |
| 1561 | SNKALPAPIE | KTIEKAKGQ | REPQVYTLP | SRDELTKNQV | SLTCLVKGFY | PSDIAVEK |
| 1621 | NGQ FENAYKT | TPPVLDSDGS | YSKLTV | KSRWQQGNV | SCSVMHEA | \%HYTQKSI.SL: |
| 1.633. | SPGKRRRRSG | GGGSGGGG | GGGSGGG | GGGSGGGGSR | KRRRRDKTHT | CPPCPAPE |
| 1741 | GGFSVFLFP | KPKDTLMISR | TPEVTCVWD | VSHEDPEVKE | WWYVDGVEV | NAKTK |
| 1.801 | YnSTYRWSV | LTVLHQDinLN | GKEYKCRV SN | KALPAPIEKT | ISKAKGQP | PQV |
| 1861 | DELTKNGVSL | TCLVKGFYPS | DIAVEKESKG | QPE | PVLBSDGSFF | LYSKLTVDKS |
| 1921 | RWQQGNVFSC | VMEEA | YTQKSLSLS |  |  |  |

What is claimed is:

1. A chimeric clotting factor which comprises i) a clotting factor selected from the group consisting of FVII, FIX and FX and ii) a targeting moiety which binds to platelets and, optionally, iii) a spacer moiety between the clotting factor and the targeting moiety.

The chimeric clotting factor of claim 1 , which comprises a structure represented by the formula A B C, wherein A is the clotting factor; wherein B is a spacer moiety; and wherein C is at least one targeting moiety which binds to platelets.

The chimeric clotting factor of claim 2, which comprises a structure from amino terminus to carboxy terminus represented by a formula selected from the group consisting of: A B C; C B A.

The chimeric clotting factor of claim 1 , wherein said chimeric clotting factor exhibits increased generation of thrombin in the presence of platelets as compared to an appropriate control lacking the at least one targeting moiety.

The chimeric clotting factor of claim 1 , further comprising a scaffold moiety and, optionally, a second spacer moiety.

The chimeric clotting factor of claim 5, further comprising D and E , wherein D is a spacer moiety; and E is a scaffold moiety and wherein the chimeric clotting factor comprises a structure from ammo terminus to carboxy terminus represented by a formula selected from the group consisting of: A B CDE;ADEBC;EDABC;CBADE;EDCB A; and C B E D A.
7. The chimeric clotting factor of claim 6, wherein E is a dimeric Fc region comprising a first Fc moiety, F 1 and a second Fc moiet ${ }^{\prime}$, F 2 .

The chimeric clotting factor of claim 7, wherein the clotting factor is expressed as a polypeptide comprising a cleavable scFc ( cscFc ) linker interposed between two Fc moieties, wherein the cscFc linker is adjacent to at least one enzymatic cleavage site which results in cleavage of the cscFc polypeptide linker.
9. The chimeric clotting factor of claim 8 , wherein the $\operatorname{cscFc}$ linker is adjacent to at least one enzymatic cleavage site which results in cleavage of the cscFc linker.

The chimeric clotting factor of claim 9, wherein the at least one enzymatic cleavage site is an intracellular processing site.

The chimeric clotting factor of claim 9, wherein the polypeptide linker is flanked by two enzymatic cleavage sites which are recognized by the same or by different enzymes.

The chimeric clotting factor of claim 9, wherein the polypeptide linker has a length of about 10 to about 50 amino acids.

The chimeric clotting factor of claim 9 , wherein the polypeptide linker has a length of about 20 to about 30 amino acids.

The chimeric clotting factor of claim 9, wherein the polypeptide linker comprises agly/ser peptide.

The chimeric clotting factor of claim 14 , wherein the gly/ser peptide is of the formula $\left(\mathrm{Gly}_{4} \mathrm{Ser}\right) \mathrm{n}$, or $\operatorname{Ser}\left({\mathrm{G} 3 y_{4}} \mathrm{Ser}\right) \mathrm{n}$ wherein n is a positive integer selected from the group consisting of $1,2,3,4,5,6,7,8,9$ and 10.

The chimeric clotting factor of claim 15 , wherein the $\left(\mathrm{Giy}_{4} \mathrm{Ser}\right) \mathrm{n}$ linker is selected from the group consisting of $\left(\mathrm{Gly}_{4} \operatorname{Ser}\right) 6, \operatorname{Ser}\left(\mathrm{G1y}_{4}\right.$ Ser)6, $\left(\right.$ Gly $\left._{4} \operatorname{Ser}\right) 4$ and $\operatorname{Ser}\left(\right.$ Gly $\left._{4} \operatorname{Ser}\right) 4$.

The chimeric clotting factor of claim 8 , wherein the clotting factor comprises two polypeptide chains.

The chimeric clotting factor of claim 7, wherein the chimeric clotting factor has a struture selected from the group consisting of: A linked to Fl via a spacer moiety and C linked to F2; A linked to F1 via a spacer moiety and C linked to F 2 via a spacer moiety; A linked to F and C is linked to F 2 via a spacer moiety; A linked to F 1 via a spacer moiety and C is linked to F 2 via a spacer moiety.
19. The chimeric clotting factor of claim 18, which comprises two polypeptides wherein the first polypeptide comprises the moieties A B F ; A B Fl; A B Fl, A B Fl D C and the second polypeptide comprises the moieties C F2: C D F2;:F2 D C, F2 D C. wherein the two polypeptide chains form an Fc region.
20. moiety is fused to at least one of the polypeptide chains of the Fc region
23. The chimeric clotting factor of claim 7, wherein the targeting moiety is fused to at least one of F1 and F2 directly.

The chimeric clotting factor of claim 7, wherein the targeting moiety is fused to at least one of F 1 and F 2 via a spacer moiety.

The chimeric clotting factor of claim 7, wherein the targeting moiety is fused to at least one of F1 and F2 via a cleavable linker.

The chimeric clotting factor of claim 1 , wherein the targeting moiety is selected from the group consisting of: an antibody molecule, an antigen binding fragment of an antibody molecule, an scFv molecule, a receptor binding portion of a receptor, a peptide.

The chimeric clotting factor of claim $1,2,5$ or 7 wherein the targeting moiety binds to resting platelets.

The chimeric clotting factor of $1,2,5$ or 7 wherein the targeting moiety selectively binds to activated platelets.

The chimeric clotting factor of claim 25 , wherein the targeting moiety selectively binds to a target selected from the group consisting of: GPTba, GPVI, and the nonactive form of GP!b/[I].

The chimeric clotting factor of claim 26 , wherein the targeting moiety selectively binds to a target selected from the group consisting of: the active form of GPIIb/IIIa, P selectin, GMP-33, LAMP-1, LAMP-2, CD40L, and LOX-1.

The chimeric clotting factor of claim 25, wherein the targeting moiety binds to the GPIb complex

The chimeric clotting factor of claim 25 wherein the targeting moiety is a peptide selected from the group consisting of: PS4, OS1, and OS2.
31. The chimeric clotting factor of claim 25, wherein the targeting moiety comprises an antibody variable regions from an antibody selected from the group consisting of: SCE5, MB9, and AP3.

The chimeric clotting factor of claim $1,2,5$, or 7 wherein the clotting factor is Factor VII.
33. clotting factor is a high specific activity variant of Factor VII.

The chimeric clotting factor of claim $1,2,5$, or 7 wherein the clotting factor is Factor IX.

The chimeric clotting factor of claim $1,2,5$, or 7 wherein the clotting factor is a high specific activity variant of Factor IX.

The chimeric clotting factor of claim $1,2,5$, or 7 wherein the clotting factor is Factor X.

The chimeric clotting factor of claim $1,3,4$, or 5 wherein the clotting factor is a high specific activity variant of Factor X .

The chimeric clotting factor of claim 8 , wherein the clotting factor is secreted by a cell in active form.

The chimeric clotting factor of claim 1 , wherein the clotting factor is activated in vivo.

The chimeric clotting factor of claim 39 wherein the chimeric clotting factor comprises a heterologous enzymatic cleavage site not naturally present in the clotting factor.

The chimeric clotting factor of claim 40, wherein enzymatic cleavage site is genetically fused to the amino terminus of the heavy chain moiety of the clotting factor.

The chimeric clotting factor of claim 5 , wherein the scaffold moiety is a protein molecule which increases the hydrodynamic radius of the chimeric clotting factor.

The chimeric clotting factor of claim 42, wherein the scaffold moiety, if present, is selected from the group consisting of albumin and XTEN ${ }^{\circledR}$

A polypeptide comprising FVII, which FVII comprises a heterologous enzymatic cleavage site activatable by a component of the clotting cascade.

The polypeptide of claim 44 , wherein the polypeptide comprises a scaffold moiety and, optionally, a spacer moiety.

The chimeric clotting factor of claim 45 , wherein the scaffold moiety is a dimeric Fc region comprising a first Fc moiety, Fl and a second Fc moieiy, F2.
47. The chimeric clotting factor of claim 44, wherein the clotting factor comprises two polypeptide chains.

The chimeric clotting factor of claim 50 , wherein the at feast one enzymatic cleavage site is an intracellular processing site.
53. The chimeric clotting factor of claim 50 , wherein the cscFc linker is flanked by two enzymatic cleavage sites which are recognized by the same or by different enzymes.

The chimeric clotting factor of claim 49 , wherein the cscFc linker has a length of about 10 to about 50 amino acids.
55. The chimeric clotting factor of claim 49, wherein the cscFc linker has a length of about 20 to about 30 amino acids.
56. The chimeric clotting factor of claim 49 , wherein the cscFc linker comprises agly/ser peptide.

The chimeric clotting factor of claim 56 , wherein the gly/ser peptide is of the formula $\left(\mathrm{Gly}_{4} \mathrm{Ser}\right) \mathrm{n}$, or $\operatorname{Ser}\left(\mathrm{Gly}_{4} \mathrm{Ser}\right) \mathrm{n}$ wherein n is a positive integer selected from the group consisting of $1,2,3,4,5,6,7,8,9$ and 10.
58. The chimeric clotting factor of claim 56 , wherein the $\left(\mathrm{Gly}_{4} \mathrm{Ser}\right)$ n linker is selected from the group consisting of $\left({\mathrm{G} 3 y_{4}}^{4} \operatorname{Ser}\right) 6, \operatorname{Ser}\left(\mathrm{Gly}_{4}\right.$ Ser)6, (Gly $\left.{ }_{4} \operatorname{Ser}\right) 4$ and $\operatorname{Ser}\left(\mathrm{Gly}_{4} \mathrm{Ser}\right) 4$.
59. is a high specific activity variant of Factor VII.
60. The chimeric clotting factor of claim 44 wherein the heterologous enzymatic cleavage site not naturally present in the clotting factor is cleaved at the site of clot formation.

The chimeric clotting factor of claim 44, wherein the cleavage site is selected from the group consisting of: a factor XIa cfeaveage site, a factor Xa cleavage site, and a thrombin cleavage site.

The chimeric clotting factor of claim 59 , wherein enzymatic cleavage site is genetically fused to the amino terminus of the heavychain moiety of the clotting factor.

The chimeric clotting factor of claim 48 wherein the targeting moiety binds to resting platelets.

The chimeric clotting factor of 48 , wherein the targeting moiety selectively binds to activated platelets.

The chimeric clotting factor of claim 63, wherein the targeting moiety selectively binds to a target selected from the group consisting of: GPIba, GPV1, and the nonactive form of GPIMb/[1]a.

The chimeric clotting factor of claim 64, wherein the targeting moiety selectively binds to a target selected from the group consisting of: the active form of GPTb/IIa, P selectin, GMP-33, LAMP-!, LAMP-2, CD4GL, and LOX-1.

The chimeric clotting factor of claim 64, wherein the scaffold moiety is a protein molecule which increases the hydrodynamic radius of the chimeric clotting factor.
68. The chimeric clotting factor of claim 67 , wherein the scaffold moiety, if present, is selected from the group consisting of albumin and XTEN ${ }^{\circledR}$

The chimeric clotting factor of claim 44 , which comprises a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: A B C; C B A; A B CDE; A DEBC, E D A B C, CBADE, EDCBA, CBEDA, wherein A an activatabie clotting factor, B is absent or is a linker, C is a targeting moiety, D is absent or is a linker, and E is a scaffold moiety.
70. comprises a light and heavy chain of a clotting factor and each of the light and heavy chains are expressed as separate polypeptide chains.

A nucleic acid molecule encoding a chimeric ciotting factor of any one of claims $1,2,5,7,44,45,45$ and 58.

The nucleic acid molecule of claim 71, wherein the nucleic acid molecule is present in a vector.

The vector of claim 72 wherein the vector further comprises a nucleotide sequence encoding an enzyme which cleaves at least one of the enzymatic cleavage sites.

A host cell comprising the expression vector of claim 72.
A host cell comprising the expression vector of claim 72, wherein the host cell expresses an enzyme capable of intracellular processing.

The host cell of claim 75, wherein the enzyme is endogenous to the cell.

The host cell of claim 75, wherein the enzyme is heterologous to the cell.

A method for producing a chimeric ciotting factor comprising cuituring the host cell of claim 76 or 77 in culture and recovering the chimeric clotting factor from the medium.

A processed, heterodimeric polypeptide comprising two polypeptide chains, wherein said processed, heterodimeric polypeptide is made by expressing the vector of claim 78 in a cell cultured in cell culture medium and isolating the mature, heterodimeric polypeptide from the culture medium.
80. A composition comprising a chimeric clotting factor of any one of claims $1,2,5,7,44,45,48$ and 58 and a pharmaceutically acceptable carrier.

A composition comprising the nucleic acid molecule of claim 71 and a pharmaceutically acceptable carrier.
82. A method for improving hemostasia in a subject, comprising administering the composition of claim 80 or 81 ,
Generic examples of Enhanced Cloting Factor Fc fusions:
Platelet targeting for enhanced efficacy

Fig. 1
Generic examples of Enhanced Cloting Factor Fc fusions

Generic examples: Platelet targeting with Gla replaceldeletion to decrease clearance
Gla-less FVII (or FVIla, FIX, etc)
Targeting moiety (platelet
receptor scFv, peptide, etc.)

| Optional spacer linker or cleavable |
| :--- |
| linker (e.g. Xla, Xa, thrombin |
| cleavage site, etc.), may include |
| additional spacer linkers on |
| either/both sides (e.g. Gly Ser |
| spacers) |

Fc moiety
Processing site A, B (could be same
or different)
Generic examples: Platelet targeting with Gla replaceldeletion to decrease clearance, with single chain cleavable linkers
Gla-less FVII (or FVIIa, FIX, etc)
\$ Targeting moiety (platelet receptor
scFv, peptide, etc.)
Optional spacer linker or cleavable
linker (e.g. Xla, Xa, thrombin cleavage
site, etc.), may include additional
spacel linkers on eitherlboth sides
(e.g. GlySer spacers)
Fc moiety
5/68
Generic examples: Activated FVII constructs, activatable FVII constructs
Cleavable linker (e.g. Xla, Xa,
thrombin cleavage site, etc., may
include additional spacer linkers on N -
terminus, but requires direct fusion at
C-terminus to HC
Fc moiety
Optional spacer linkers

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Generic examples of Enhanced Clotting Factor (nonFc fusions): Platelet targeting for enhanced efficacy
E)
F) G)

D-G) can apply to FVIII, other non-gammacarboxylated cloting factors which
require N -terminal propeptide cleavage

Generic examples: Platelet targeting with Gla replaceldeletion to decrease clearance, to $\mathrm{FVI}, \mathrm{FX}, \mathrm{FX}$
(non-Fc fusions

D)

Q Glanless FVII (or FVIla, FIX, etc)
Targeting moiety (platelet receptor
scFv, peptide, etc.)
Optional spacer linker or cleavable
linker (e.g. Xla, Xa, thrombin cleavage
site, etc.), may include additional
spacer linkers on either/both sides
(e.g. GlySer spacers)
Scaffold, e.g., XTEN, albumin,
or other polypeptide sequence
A, D-F) can apply to FVII, FIX, FX as N -terminal fusion is now possible in Gla domain deleted versions
Fig. 8

## Activated FVII constructs, activatable FVI constructs with non.Fc fusions



1068
SDS PAGE for purification and activation of FVIL-011

| 1. Seeblue2 |
| :--- |
| 2. emply |
| 3. FVIFc-011 Q Load |
| 4. FVIFc-011 QF.T |
| 5. FVIFc-011 Q wash |
| 6. FVIFc-011 Q <br> elution pool |
| 7. FVIFc-011 Q strip <br> pool |
| 9. Seeblue2 |
| 10. emply |
| 11. FcRn Load |
| 12. FcRn FT |
| 13. FCRn wash |
| 14. FcRn elution |
| 15. FcRn Strip pool |


Fig. 10

## 11/68

HIC capture step, non reducing gel

$$
123456789101112131415
$$

FCRn column purification, non-reducing gel


| 1. Seeblue2 |
| :--- |
| 2. Hanest |
| 3. FVIFc-053 HIC Load |
| 4. FVIFc-053 HIC FT |
| 5. FVIFc-053 HIC wash |
| Fraction \#A2 |
| 6. FVIFC-053 HIC wash |
| Fraction \#A3 |
| 7. FVIFC-053 HIC wash |
| Fraction \#A4 |
| 8. FVIFc-053 HIC wash |
| Fraction \#A5 |
| 9. FVIFC-053 HIC wash |
| Fraction \#A6 |
| 9. FVIFC-053 HIC wash |
| Fraction \#A6 |
| 10. FVIFc-053 HIC wash |
| Fraction \#B7 |
| 11. Emply |
| 12. FVIFC-053 HIC Elution |
| Fraction \#B6 |
| 13. FVIFC-053 HIC Elution |
| Fraction \#B5 |
| 14. FVIIFC-053 HIC Elution |
| Fraction \#B4 |
| 15. FVIIFc-053 HIC Elution |
| Fraction \#B3 |

4-20\%Trisglycine SDSPAGE (reducing)

Fig. 11

## Binding of FVIL.011 and FVII-027 to activated platelets determined by FACs



Binding of FVIl-011 and FVIl-027 to activated platelets determined by FACs


Fig. 12B

Thrombin generation assay to measure activity of FVII-027, FVIl-011 and Novoseven in the presence of activated platelets


Thrombin generation assay to measure activity of FVII-027, FVIL-011 and Novoseven in the presence of activated platelets


Fig. 13B

Thrombin generation assay to measure activity of FVIL-027, FVIL.011 and Novoseven in the presence of activated platelets


Fig. 13C

Thrombin generation assay to measure activity of FVIl-027, FVll-011 and Novoseven in the presence of activated platelets


PAC- 1 eliminates increased binding to platelets and increased rates of thrombin generation associated with FVII-027


PAC-1 eliminates increased binding to platelets and increased rates of thrombin generation associated with FVII-027


Fig. 14B

Thrombin generation assay to measure activity of FVIl-037 and Novoseven in the presence of activated platelets


Thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets


Fig. 16A


Thrombin generation assay to measure activity of FVI-037 and Novoseven in the presence of activated platelets


Fig. 16C

Thrombin generation assay to measure activity of FVII.044, FVII-045, FVII-046, FVII-011 and Novoseven in the presence of activated platelets
FVII-044
FVII-045
FVII-046


FC
6x(GGGGS)spacer linker
$\bigcirc$ Targeting Peptide against GPb *

| Peptide* | Affinty <br> $K_{\text {p }}$ nM | FVIFC |
| :---: | :---: | :---: |
| PS4 | 64 | -044 |
| OS1 | 0.74 | -045 |
| OS2 | 31 | -046 |

*Benard et al. Biochemistry 2008, 47: 4674-4682
Fig. 17

Thrombin generation assay to measure activity of FVII-044, FVIL-045, FVIL-046, FVIL-011 and Novoseven in the presence


Fig. 18A
Thrombin generation assay to measure activity of FVII-044, FVII-045, FVII-046, FVIL-011 and Novoseven in the presence


Fig. 18B

## 2068

Thrombin generation assay to measure activity of FVIl-044, FVIL-045, FVII-046, FVI.011 and Novoseven in the presence of activated platelets


Thrombin generation assay to measure activity of FVII-045, FVII-011 and Novoseven in the presence


Thrombin generation assay to measure activity of FVIl-04\%, FVII-048, FVII-049, FVII-011 and Novoseven in the presence of activated platelets
FVII-047
FVII-048
FVIL-049
T $6 x(G G G G S)$ linker

OTargeting Peptide against GPib *

| Peptide* | Affinty <br> $K_{b}$, nM | FVIFc |
| :---: | :---: | :---: |
| PS4 | 64 | -047 |
| OS1 | 0.74 | -048 |
| OS2 | 31 | -049 |

*Benard et al. Biochemistry 2008, 47: 4674-4682
Fig. 19

Thrombin generation assay to measure activity of FVII-047, FVII-048, FVII-049, and FVII-011 in the


Fig. 20B

Thrombin generation assay to measure activity of FVIL.047, FVIL.048, FVIL-049, and FVIL.011 in the presence of activated platelets


## Fig. 20C

Thrombin generation assay to measure activity of FVII-047, FVIL-048, FVII-049, and FVII-011


Fig. 20D

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## Thrombin generation assay to measure activity of FVII.053 and FVII-011 in the presence of activated platelets

FVlla truncation lacking residues 1.35 insertion following residue R152 (WT FVII numbering) for intracellular activation


FVIIO53

FC moiety

Nargeting moiety MB9: scFv against activated GPlblla


4x(GGGGS)CscFo linker
\} $6 \times(G G G G S)$ spacer linker

Fig. 21

Thrombin generation assay to measure activity of FVIL-053 and FVII-011 in the presence of activated platelets


Fig. 22A
Thrombin generation assay to measure activity of FVIL.053 and FVII-011 in the presence of activated platelets


Thrombin generation assay to measure activity of $\mathrm{FVI}-053$ and FVIL-011 in the presence of activated platelets


Fig. 22C

Thrombin generation assay to measure activity of FVII-053 and FVII. 011 in the presence of activated platelets



PAC-1 elminates increased rate of thrombin generation associated with FVII-053


## Westem blot analysis of FVIF transfection of HEK 293 cells and protein A pulldown



FVII-011
Heavy chain and light chain expressed as single chain


FVII. 018
Heavy chain-Fc and light chain-Fc
expressed separately


FVII-010
Heavy chain-scFc and light chain expressed separately


FVIL-003
Heavy chain and light chain expressed as single chain


FVII-013
Heavy chain and light chain-scFce expressed separately
© FVllight chain

FVIl heavy chain

FC

$16 x(G G G G S)$ spacer linker

## Fig. 24

Western blot analysis of FVIFc species following transient transfection of HEK 293 cells and protein A pulldown


FVII light chain western
123456

98
64

non reducing

Interpretation of bands
A. FV/l-scFC
8. Light chain-scfo
C. Light chain Fc
D. FVll Fc
E. Fo
F. FVIIFCFC
G. Fo dimer
H. Light chain Fe dimer

1. Light chain-Fc:HC-FC
J. Light chair-scfe dimer

## Proteins loaded in gel

1. FVII-010
2. FVII-011
3. FV/l-013
4. FVII-018
5. FVII-003
6. No DNA in transfection

Fig. 25

## 3068

Western blot of protein A immunoprecipitation following transient transfection of $\mathrm{PSYN}-\mathrm{FVIL}-024$ with or without $\mathrm{pSYN}-\mathrm{PC5}-003$. Lane 1, Seeblue Plus2 marker; lane 2, PSYNFVIl 024 , non reducing; lane 3, pSYN.FVII-024+pSYN.PC5-003, non reducing; lane 4, $\mathrm{pSYN} . \mathrm{FVII-024}$, reducing; lane $5, \mathrm{pSYN} F \mathrm{FVII-024+pSYN-PC5-003}$, reducing.


FVII. 024

© FVIl light chain
FVII heavy chain

FC moiety

\} 6x(GGGGS)spacer inker

- Cleavage site RRRR

现 Cleavage site RKRRKR
$\leftarrow$ Nonprocessed single chain (lane 4)
Processing intermediate
FVIl heavy chain-Fc
FVIl light chain Fc

## Fig. 26

Western blot analysis (Fc western) of FVIFc species following transient transfection of HEK 293 cells and protein A pulldown


## 32168

FVIL.039 and FVII-040 Treatment by FXla © FVIl light chain FVII lightchain with R152 deletion


FV1-039


FVIl-040

FVII heavy chain with 1153 V mutation

- GGGDFTR (FXla cleavage sile) FC moiely


6x(GGGGS)spacer linker


Fig. 28

## Human platelets, purified components





Mouse platelet rich plasma





SCE 5
-scrvagainstache GPliblla

- Crossreacts with mouse and human receptors



## 3468



Fig. 30


## Activatable Constructs

FXla cleavage stes
LC7x(GGGGS)-SVSOTSKLTRIVGG: FVII-057
Thrombin cleavage sites
LC.7x(GGGGS)-DFLAEGGGVR-IVGG FVII-058
LC-7x(GGGGS)-TTKKPR-NGG: FVII. 659
LC-7x(GGGGS)ALRPRVVGGA-WGG: FVIH060
LC-7x(GGGGS)-ALRPRWGGA-VGG:FVIIO6!
Negative control
LC-8x(GGGGS)-IVGG: FVI-062


## Fig. 31


Fig. 32

픈

|  | FVII-089 |  |  |
| :--- | :--- | :--- | :--- |
|  |  |  |  |
| Thrombin (nM) | 5 | 25 | $125 \quad 625$ |

250
143
$\longrightarrow$
$\xrightarrow{\text { LCFFVIFC }}$
Fig. 33


## Fig. 34

## 39168



## Fig. 35



Fig. 36


Fig. 37

## Amidolytic activity of activatable FVIIF activated with thrombin



Fig. 38

## Activation of FX by FVIla



10 uM FX and FVlla titration

- 400 nM
- 200 nM
$\Delta 100 \mathrm{nM}$
v 50 nM
- 25 nM
$\diamond$ No FVlla
- No FX, 400nM FVlla

Fig. 39
$44 / 68$
FXa generation activity by "activatable" FVIFc

FXa chromogenic substrate
Fig. 40
45168
Thrombin cleavage sile
(ALRPR)


4668
Compare efficiency of thrombin cleavage of monomer (FVI-118,-119) vs
heterodimer (FVIL090) activatable


thrombin
Fig. 42

## $47 / 68$

Thrombin generation assay to compare wild type activatable FVIIFc (FVII. 118) to high specific activity variant (FVII-127)


- FVII-118200nM
- FVII 118200 nM + Thrombin
$\Delta$ FVIl-127 200nM
- FVIl-127 $200 \mathrm{nM}+5 \mathrm{MM}$ Thrombin
- No FVII
$\diamond$ No FVII $+5 n \mathrm{M}$ Thrombin

Fig. 43

## Target FVlla to active conformation of Gllblla via scFv (SCE5)


$\square$-Targeting moiely
SCE5: scFv against active conformation of GPIlblla
Fig. 44A

49168
Thrombin generation assays in plateletrich FVIII-deficient plasma


12.5 nM


Fig. 44B

Binding or rFVllaFc variants to platelets by FACs


Fig. 44C

## Target FVlla to all conformations of Gllblla via scFv (AP3)



[^0]
## Fig. 45A

52168

Thrombin generation assays in plateletrich FVIII-deficient plasma

12.5 nM Thrombin


Fig. 45B

Binding or rFVIlaFc variants to platelets by FACs


Fig. 45C

## Targeting FVlaFc to GPIb with peptides



- Targeting moielies PS4, OS1 and OS2: peptides against GPlb-alpha

| N-terminus | Crterminus |
| :---: | :---: |
| FVII-047 (PS4) | FVII-044 (PS4) |
| -FVIL-048 (051) | FVII.045 (OS1) |
| FFVIL-049 (OS2) | FYV1-046 (OS2) |

## Fig. 46A

Thrombin generation assays in platelet-rich FVill-deficient plasma


50 nM FVIla

12.5 nM FVIla


Fig. 46B
$56 / 68$
Thrombin generation assays in platelet-rich FVIII-deficient plasma

12.5 nM FVIla


Fig. 47A

Thrombin generation assays in plateletrich FVIIl-deficient plasma Comparison of FVII.045 and FVII-048


Fig. 47B

Binding of FVII-045, FVIl-048 and wild type
FVIlaFc to platelets determined by FACS


Affinity for the targeting peptides reported in Benard et al. Biochemistry 2008, 47: 4674-4682

| Peptide | Affinity <br> $K_{D}, \mathrm{nM}$ |
| :---: | :---: |
| PS4 | 64 |
| OS1 | 0.74 |
| OS2 | 31 |

Fig. 48

## 59168

FVII-108

$\square$-SCE5: scFv against active conformation of GPIblla

## FVIH041



Fig. 49A

## Thrombin generation assays in FVIII deficient plateletrich plasma



Assay was activated by platelet activation


Fig. 49B

Halflife (hours)


Fig. 50

ety SCE5: scFv against active conformation of
=Ig. $51 A$
$63 / 68$
Thrombin generation assays in plateletrich FIX -deficient plasma


Fig. 51B
$64 / 68$


Fig. 51C

Thrombin generation assays in platelet-rich FIX-deficient plasma




Fig. 52A

6668


## Fig. 52B

Target GPlb
(all platelets)


Specific activities<br><br>-FIX-042: 6 U/nmol<br>FIX-089: 2.4 lUhmol

FIX-089

- Targetin moiely OS1;peptide
against GPlb-alpha
Fig. 53A
$67 / 68$
Thrombin generation assays in plateletrich FIX deficient plasma


Fig. 53B

68168


Fig. 53C


## $X$ Further documents are listed in the continuation of Box $C$.

$X \quad$ See patent family annex.
Special categories of cited documents :
" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
" O " document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
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Chavanne, Franz

| Date of the actual completion of the international search | Date of mailing of the international search report |
| :---: | :---: |
| 3 November 2011 | $11 / 11 / 2011$ |
| Name and mailing address of the ISA/ <br> European Patent Office, P.B. 5818 Patentlaan 2 <br> NL-2280 HV Rijswijk <br> Tel. (+331-7) 340-2040, <br> Fax: $(+31-70) 340-3016$ | Authorized officer |

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| Category ${ }^{*}$ | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| :---: | :---: | :---: |
| X | YANG X ET AL: "An ordered sequential mechani sm for Factor IX and Factor IXa binding to platel et receptors in the assembly of the Factor X -acti vati ng compl ex" , <br> BIOCHEMICAL JOURNAL, THE BIOCHEMICAL SOCIETY, LONDON, GB, <br> vol . 390, no. 1, <br> 15 August 2005 (2005-08-15) , pages 157-167, XP009118579, <br> ISSN : 0264-6021, DOI : 10. 1042/BJ20050029 [retri eved on 2005-03-09] <br> the whole document | 1-82 |
| X, P | us 2010/330059 AI (STAFFORD DARREL W [US] ET AL) 30 December 2010 (2010-12-30) <br> abstract paragraphs [0003], [0015] - [0022], [0058], [0072] , [0074], [0079] examples I,IV,V | 1-6, 24-29 32 ,33 , 35, 37-45 47,49, 59-82 |
| A, P | wo 2010/115866 AI (NOVO NORDISK AS [DK] ; OESTERGAARD HENRIK [DK] ; PUSATERI TONY [US] ; BAR) 14 October 2010 (2010-10-14) abstract <br> page 1, Iine 10 - I ine 14 <br> page 2, 1ine 13-1ine 24 <br> page 8, line 23 - I ine 29 <br> page 11, line 18 - page 19, line 19 <br> page 21, I ine 4 <br> exampl es $1-3,5,9,10,38,40,42-46$ | 1-82 |

INTERNATIONAL SEARCH REPORT
Information on patent family members
International application No
-
PCT/US2011/043597

| Patent document cited in search repor |  | Publication date date |  | Patent family member(s) |  | $\begin{aligned} & \text { Publication } \\ & \text { date } \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| wo 2009140598 | AI | 19-11-2009 | CA | 2724630 | AI | 19-11-2009 |
|  |  |  | CN | 102112144 | A | 29-06-2011 |
|  |  |  | EP | 2288365 | Al | 02-03-2011 |
|  |  |  | JP | 2011520913 | A | 21-07-2011 |
|  |  |  | KR | 20110022604 | A | 07-03-2011 |
|  |  |  | US | 2011077202 | Al | 31-03-2011 |
| US 2010330059 | AI | 30-12-2010 | W0 | 2010151736 | AI | 29-12-2010 |
| Wo 2010115866 | AI | 14-10-2010 | NON |  |  |  |


[^0]:    $\square$ Targeting moiety AP3: scFv against GPlblla (all confomations)

