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(54) **Title**: CHIMERIC CLOTTING FACTORS

(57) **Abstract**: Chimeric clotting factors which localize the therapeutic to sites of coagulation (e.g., by being targeted to platelets or being activatable at sites of coagulation), have reduced clearance rates, have improved manufacturability, have reduced thrombogenicity, 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, 2011; U.S. Provisional Patent Application No. 61/442,150 filed February 11, 2011; 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, 2011; and U.S. Provisional Patent Application No. 61/491,762 filed May 31, 2011. 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 VIIa. This complex then converts Factors IX and X to their active forms. Factor Xa converts limited amounts of prothrombin to thrombin on the tissue factor-bearing cell. This resulting thrombin is then able to diffuse away from the tissue-factor bearing cell and activate platelets, and Factors V and VIII, making Factors Va and VIIIa. During the propagation phase of coagulation, Factor Xa is generated by Factor IXa (in complex with factor VIIIa) on the surface of activated platelets. Factor Xa, in complex with the cofactor 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 VIIa 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 Ile residue at amino acid position 153. In the presence of tissue factor, phospholipids and calcium ions, the two-chain Factor VIIa 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 TF-bearing 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 FVIIa 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 factor-independent

[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 IXaβ (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 IXaβ (mol wt approximately equal to 46,000). Factor IXaβ 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 Willebrand'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 manufacturability, have reduced thrombogenicity, 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 manufacturability, 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, 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.

[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; E D A B C; C B A D E; E D C B A; and C B E D A.

[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 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.

[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 gly/ser peptide.

[0023] In one embodiment, the gly/ser peptide is of the formula $(Gly_4Ser)n$, or $Ser(Gly_4Ser)n$ 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 $(Gly_4 Ser)n$ linker is selected

from the group consisting of $(Gly_4\ Ser)6$, $Ser(Gly_4\ Ser)6$, $(Gly_4\ Ser)4$ and $Ser(Gly_4\ Ser)4$.

[0024] In one embodiment, the clotting factor comprises two polypeptide chains.

[0025] In one embodiment, the chimeric clotting factor has a struture selected from the group consisting of: A linked to F1 via a spacer moiety and C linked to F2; A linked to F1 via a spacer moiety and C linked to F2 via a spacer moiety; A linked to F1 and C is linked to F2 via a spacer moiety; A linked to F1 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 F1; 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. In 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, OS1, 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] In 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 clotting 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 hydrodynamic 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 F1; 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 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 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 $(Gly_4Ser)n$, or $Ser(Gly_4Ser)n$ 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 $(Gly_4 Ser)n$ linker is selected from the group consisting of $(Gly_4 Ser)6$, $Ser(Gly_4 Ser)6$, $(Gly_4 Ser)4$ and $Ser(Gly_4 Ser)4$.

[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, GMP-33, 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 XTEN®

[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 C D E; A D E B C, E D A B C, C B A D E, E D C B A, C B E D A, 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] In 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 pertains 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 pertains 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 pertains 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 light 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 moiety; 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 a chimeric clotting factor, which comprises a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: ABC; ABCDE; ADEBC, EDABC, CBADE, EDCBA, 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 moiety, D is absent or is a linker, and E is a scaffold moiety.

[0060] In yet another aspect, the invention pertains to a chimeric clotting factor, which comprises a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: ABF1:F2; ABF1:CDF2; ABF1:F2DC, ABF1DC:F2DC, 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 moiety, D is absent or is a linker, and F1 and F2 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 a 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.

[0061a] Definitions of specific embodiments of the invention as claimed herein follow.

[0061b] According to a first embodiment of the invention, there is provided a chimeric clotting factor comprising:

(i) a clotting factor selected from the group consisting of factor VII (FVII), factor IX (FIX) and factor X (FX);

(ii) a targeting moiety which binds to platelets; and

(iii) a first Fc moiety and a second Fc moiety,

wherein the clotting factor is fused to the first Fc moiety, and

wherein the targeting moiety is fused to the second Fc moiety.

[0061c] According to a second embodiment of the invention, there is provided a nucleic acid molecule encoding the chimeric clotting factor of the first embodiment.

[0061d] According to a third embodiment of the invention, there is provided an expression vector comprising the nucleic acid molecule of the second embodiment.

[0061e] According to a fourth embodiment of the invention, there is provided an isolated host

cell comprising the expression vector of the third embodiment.

[0061f] According to a fifth embodiment of the invention, there is provided a method of producing a chimeric clotting factor, said method comprising culturing the host cell of the fourth embodiment in culture medium and recovering the chimeric clotting factor from the medium.

[0061g] According to a sixth embodiment of the invention, there is provided a processed, heterodimeric polypeptide comprising two polypeptide chains, wherein said processed, heterodimeric polypeptide is made by expressing the vector of the third embodiment in a cell cultured in cell culture medium and isolating the heterodimeric polypeptide from the cell culture medium.

[0061h] According to a seventh embodiment of the invention, there is provided a composition comprising the chimeric clotting factor of the first embodiment and a pharmaceutically acceptable carrier.

[0061i] According to an eighth embodiment of the invention, there is provided a composition comprising the nucleic acid molecule of the second embodiment and a pharmaceutically acceptable carrier.

[0061j] According to a ninth embodiment of the invention, there is provided a method of improving hemostasis in a subject, said method comprising administering to the subject the composition of the seventh or eighth embodiments.

[0061k] The term "comprise" and variants of the term such as "comprises" or "comprising" are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

[0061l] Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge in Australia.

BRIEF DESCRIPTION OF THE DRAWINGS

[0062] Figure 1 illustrates exemplary chimeric clotting factor constructs 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 cleavable 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 constructs 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 Gla domain. These exemplary constructs comprise a cleavable 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.

[0066] Figure 5 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 (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 constructs comprise a cleavable 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.

[0068] Figure 7 illustrates exemplary chimeric clotting factor constructs comprising a targeting domain.

[0069] Figure 8 illustrates exemplary chimeric clotting factor constructs 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 10 shows SDS PAGE for purification and activation of FVII-011.

[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 FVII-027 to activated platelets determined by FACS.

[0074] Figure 13 shows thrombin generation assay to measure activity of FVII-027, FVII-011 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, FVII-045, FVII-046, FVII-011 and Novoseven in the presence of activated platelets shown in Figure 18.

[0079] Figure 18 shows a thrombin generation assay to measure activity of FVII-044, FVII-045, FVII-046, FVII-011 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-011 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-011 and Novoseven in the presence of activated platelets.

[0082] Figure 21 shows the construct used in the thrombin generation assay to measure activity of FVII-053 and FVII-011 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-011 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 FVIIFc 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 pSYN-FVII-024 with or without pSYN-PC5-003. Lane 1, pSYN-FVII-024, non reducing; lane 2, pSYN-FVII-024, non reducing; lane 3, pSYN-FVII-024, reducing; lane 4, pSYN-FVII-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 FVII-040 treatment by FXIa.

[0090] Figure 29 shows that an FVIIaFc variant targeted to active form of GPIIbIIIa shows an increased rate of thrombin generation.

[0091] Figure 30 shows a Rotation Thromboelastometry (ROTEM) assay to measure the activity of FVII-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 contstruct 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 FVIIa 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°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 FVIIaFc can be detected.

[00101] Figure 40 shows FXa generation activity by "activatable FVIIFc." 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 (10uM) 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-118, FVII-119, and FVII-127.

[00103] Figure 42 illustrates the efficiency of thrombin cleavage of activatable constructs, specifically monomeric (FVII-118 and -119) as compared to the heterodimeric (FVII-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 GPIIbIIIa was included at various sites in the contruct. Figure 44B illustrates the results of thrombin generation assays in platelet-rich FVIII-deficient plasma using these constructs. N7 is the Novoseven control. Figure 44C illustrates the binding of recombinant FVIIaFc variants to platelets by FACs.

[00106] Figure 45A illustrates several targeted FVIIa constructs which include AP3, an scFv against GPIIbIIIa present on resting and activated platelets. Figure 45B shows the results of thrombin generation assays in platelet-rich FVIII-deficient plasma. Figure 45C shows the results of binding of rFVIIaFc variants to platelets by FACS.

[00107] Figure 46A shows several targeted FVIIa constructs that target GPIb-alpha 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 platelet-rich 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.

[00109] Figure 48 shows the binding of FVII-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 FVIII construct. Figure 49B shows the results of a thrombin generation assay in FVIII 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 (FVII-028).

[00112] Figure 51A shows several FIX construct comprising targeting moieties, in this case SCE5 scFv. Figure 51B shows the results of thrombin generation assays in platelet-rich FIX-deficient plasma using the constructs of Figure 51A. 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 assay comparing FIX-090 and Benefix. Figure 52B shows that the activity of FIX-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 FIX-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 clotting factor can be enhanced. For example, in the case of factor VII, unlike endogenous FVII that is likely activated by tissue factor (TF) at endothelial cell surfaces to generate activated factor X (FXa), exogenous FVIIa likely generates FXa/FIXa in a TF independent manner, most effective at the surface of activated platelets where other clotting factors are localized. However, physiologically FVIIa acts at the surface of a TF-bearing cell, such as an endothelial cell, and has low affinity for platelets. It has been hypothesized that therapeutic recombinant FVIIa 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 FVIIa is dosed at supra-physiological levels. Therefore, in the case of FVIIa, 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, FVIIa has a relatively short half-life (~2.3 hours) in humans. This short half-life likely contributes to the need to dose recombinant FVIIa multiple times to control a bleed. Thus, targeting clotting factors, and in particular FVIIa, to platelets improves efficiency.

[00117] The targeting moiety can be positioned at a number of places in a chimeric cloting 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 clotting 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, TTKIKPR, 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.

[00119] 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 cleavage 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 cleavage 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 (ORF) 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 C-terminal 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 manufacturability. 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 trans-Golgi apparatus.

[00125] In one embodiment, a cell expressing a construct encoding a polypeptide of the invention endogenously 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 construct 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 construct 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 hemostasis 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 (Gln or Q); glutamic acid (Glu or E); glycine (Gly or G); histidine (His or H); isoleucine (Ile or I); 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 (Trp 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 Ellman *et al.* Meth. Enzym. 202:301-336 (1991). To generate such non-naturally occurring amino acid residues, the procedures of Noren *et al.* 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.

[00132] 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 chains 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 monomeric subunits or polypeptides (e.g., two identical Fc moieties or two identical biologically active moieties). In another embodiment, the dimers of the invention are heterodimers, comprising two non-identical monomeric 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 scFc linker being cleavable (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 $(Gly_4\ Ser)_n$ (SEQ ID NO:4) Another exemplary gly/ser polypeptide linker comprises the amino acid sequence $S(Gly_4\ Ser)_n$.

[00137] In one embodiment, n=1. In one embodiment, n=2. In another embodiment, n=3, i.e., $(Gly_4\ Ser)_3$. In another embodiment, n=4, i.e., $(Gly_4\ Ser)_4$ (SEQ ID NO:6). In another embodiment, n=5. In yet another embodiment, n=6. In another embodiment, n=7. In yet another embodiment, n=8. In another embodiment, n=9. In yet another embodiment, n=10. Another exemplary gly/ser polypeptide linker comprises the amino acid sequence $Ser(Gly_4Ser)_n$ (SEQ ID NO:26). In one embodiment, n=1. In one embodiment, n=2. In a preferred embodiment, n=3. In another embodiment, n=4. In another embodiment, n=5. In yet another embodiment, 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 and PCR-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 non-essential 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, covalent 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 so joined 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 IgG, taking the first residue of heavy chain constant region to be 114) and ending at the C-terminus of the antibody. Accordingly, a complete Fc domain comprises at least a hinge domain, a CH2 domain, and a CH3 domain.

[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 CH4 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 CH2 domain, and a CH3 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 CH2 domain (or portion thereof) fused to a CH3 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 CH3 domain (or portion thereof). In another embodiment, a Fc moiety consists of a CH2 domain (or portion thereof) and a CH3 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 CH2 domain (e.g., all or part of a CH2 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 α-phase and longer β-phase. The α-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 β-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 β-phase. The typical β 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. In 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.

[00151] 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 "glycosylation" refers to the covalent linking of one or more carbohydrates to a polypeptide. Typically, glycosylation is a posttranslational event which can occur within the intracellular milieu of a cell or extract therefrom. The term glycosylation includes, for example, N-linked glycosylation (where one or more sugars are linked to an asparagine residue) and/or O-linked glycosylation (where one or more sugars are linked to an amino acid residue having a hydroxyl 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 glycosylation 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 CH1 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 general, 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 DNA elements which are derived from animal viruses such as bovine papilloma virus, 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 bacterial 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 al.* (1988), *Nature*, 331:543; Better *et al.* (1988) *Science*, 240:1041; Mullinax *et al.*, (1990). *PNAS,* 87:8095).

[00158] The term "host cell" 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, recovery 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 of CVI with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), PerC6 cells), HAK (hamster kidney line), SP2/O (mouse myeloma), P3x63-Ag3.653 (mouse myeloma), BFA-1c1BPT (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 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.

[00159] In addition to prokaryotes, 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 including *Pichia pastoris*. For expression in Saccharomyces, the plasmid YRp7, for example, (Stinchcomb *et al.*, (1979), *Nature*, 282:39; Kingsman *et al.*, (1979), *Gene*, 7:141; Tschemper *et al.*, (1980), *Gene*, 10:157) 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, (1977), *Genetics*, 85:12). The presence of the *trp1* 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.

[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-Golgi compartment. Intracellular processing enzymes cleave polypeptides prior to secretion of the protein from the cell. Examples of such processing sites include, e.g., those targeted by the PACE/furin (where PACE is an acronym for Paired basic Amino acid Cleaving Enzyme) family of endopeptidases. These enzymes are localized to the Golgi membrane and cleave proteins on the carboxyterminal side of the sequence motif Arg-[any residue]-(Lys or Arg)-Arg. As used herein the "furin" family of enzymes includes, e.g., furin, PC2, PC1/Pc3, PC4, PACE4, PC5/PC6, 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 non-synthetically 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-covalently 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 recombinantly 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] Hemostasis, 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 Naegeli (Glanzmann thrombasthenia). In liver

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

[00171] The chimeric molecules of the invention can be used prophylactiely. 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.

[00173] 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.

II. Clotting Factors

[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 cleavage 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 FVII 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 recombinantly 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.

[00179] 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 $CaCl_2$ 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 colocalizes with active processing enzymes in the trans-Golgi 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.

[00187] 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 Gla domain, two EGF domains (EGF-1 and EGF-2), and a serine protease domain (or peptidase S1 domain) that is highly conserved among all members of the peptidase S1 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 uncleaved 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. J. Biol. Chem. 276:17229; Soejima et al. 2002. J. Biol. Chem. 247:49027. In one embodiment, a variant form of FVII

includes the mutations Exemplary mutations include V158D-E296V-M298Q. In another embodiment, a variant form of FVII includes a replacement of amino acids 608-619 (LQQSRKVGDSPN, corresponding to the 170- loop) from the FVII mature sequence with amino acids EASYPGK 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 A1-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 negatively 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 A1- and A3-domains. In plasma, FVIII is stabilized by binding to von Willebrand factor. More specifically, the FVIII light chain is bound by noncovalent interactions to a primary binding site in the amino terminus of von Willebrand factor. Upon proteolytic activation by thrombin, FVIII is activated to a heterotrimer of 2 heavy chain fragments (A1, 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 A1-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 A1 domain through an ion association. Eaton, D. et al., Biochemistry 25: 505 (1986); Lollar, 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 A2 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: A1, residues Ala1-Arg372; A2, residues Ser373-Arg740; A3, residues Ser1690-Asn2019; C1, residues Lys2020-Asn2172; C2, residues Ser2173-Tyr2332. The A3-C1-C2 sequence includes residues Ser1690-Tyr2332. The remaining sequence, residues Glu1649-Arg1689, is usually referred to as the a3 acidic region. The locations of the boundaries for all of the domains, including the B-domains, for porcine, mouse and canine 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 "BDD FVIII"). An example of a BDD FVIII is REFACTO® (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. 4,868,112; col. 2, line 1 to col. 3, line 19, col. 3, line 40 to col. 4, line 67, col. 7, line 43 to col. 8, line 26, and col. 11, line 5 to col. 13, line 39 of U.S. Patent no. 7,041,635; or col. 4, lines 25-53, of U.S. Patent No. 6,458,563. In some embodiments, a B-domain-deleted 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 VIII. Meulien 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., DNA* (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 FXIa 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 FVII, 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 11 glutamic acid residues at the N-terminus of the mature N-terminal chain. A further processing step occurs by cleavage between Arg182 and Ser183. This processing step also leads concomitantly to the deletion of the tripeptide Arg180-Lys181-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 covalently linked via a

disulfide bridge between Cys172 and Cys342. Further posttranslational processing steps include the .beta.-hydroxylation of Asp103 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 clotting 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 C, 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). Complexed 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 Gla-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 Gla 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 light 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). Exemplary clotting factors lacking a Gla domain are shown in the accompanying figures

[00204] 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 figures.

[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 GpIb of the GpIb/V/IX complex, and GpVI and nonactive form of GPIIb/IIIa.

[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 GpIIb/IIIa 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, OS1, 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 GPIIbIIIa. 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.04-1513fje; 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 GPIIbIIIa. 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 2010115866). 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 invention. 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 linked 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 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).

[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 can be produced by recombinant or biochemical methods that are well known in the art. Exemplary antigen-binding portions include Fv, Fab, Fab', and $(Fab')_2$ as well as scFv molecules.

[00217] In other embodiments, a chimeric clotting 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 *et al.*, *Proc. Natl. Acad. Sci. USA 85*:5879-5883 (1988); and Ward *et al.*, *Nature 334*:544-554 (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 al.*, *Science 242*:1038-1041 (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 V_L domain linked by a flexible linker to a V_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 V_H-linker-V_L orientation or V_L-linker-V_H orientation. The flexible linker that links the V_L and V_H 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; Pantoliano et al. 1991. *Biochemistry* 30:10117; 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 V_H domain and a V_L domain, wherein the V_H and V_L domains are linked by a disulfide bond between an amino acid in the V_H and an amino acid in the V_L domain. 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 V_H or V_L 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°C or 3°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 $(Gly_4Ser)_4$ scFv linker and a disulfide bond which links V_H amino acid 44 and V_L amino acid 100. Other exemplary stabilized scFv molecules which may be employed in the polypeptides of the invention are described in US Provisional 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) derived 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 splenocytes 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 *et 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 (ELISA). 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 59-103 (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., protein-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 assay is 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 SCID 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. Immun. 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 81:6851 (1984); EP 255,694; EP 266,663; and WO 88/03559. Reassorted 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 V_L and V_H domains on the same polypeptide chain cannot interact. Instead, the V_L and V_H domain of one polypeptide chain interact with the V_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).

[00229] 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 include an isolated heavy chain variable domain (V_H) of an antibody, i.e., a heavy chain variable domain, without a light chain variable domain, and an isolated light chain variable domain (V_L) of an antibody, i.e., a light chain variable domain, without a heavy chain variable domain,. Exemplary single-domain 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 (V-NAR) 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® (Ablynx 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.04-1513fjc; 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 GPIIbIIIa. 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 2010115866).

B. Non-Immunoglobulin Platelet Binding Molecules

[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 binding molecules" 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 PCR, 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 al. 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 antithrombin 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 cleavable 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. Exemplary factor FXIa cleavage sites include, e.g., TQSFNDFTR and SVSQTSKLTR. Exemplary thrombin cleavage sites include, e.g., DFLAEGGGVR, TTKIKPR, and a sequence comprising or consisting of ALRPR (e.g. ALRPRVVGGA)).

[00235] In one embodiment, the cleavable linker may be flanked on one or more sides (upstream, downstream or both) by a spacer moiety.

[00236] In one embodiment, the cleavable linker is interposed between the light chain and heavy chain of the clotting factor. In another embodiment, the cleavable linker is not interposed between the light chain and heavy chain of the clotting factor. In one embodiment, the cleavable 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 Fc (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.

[00240] 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 FcγR receptor (*e.g.*, FcγRIII), or a complement protein (e.g. C1q)) 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, F1 is a first Fc moiety or domain, P1 is an enzymatic cleavage site, L is an ScFc linker, P2 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. 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.

[00243] Exemplary polypeptides according to formula I include: A-F1-P1- L-P2-F2; F1-P1- L-P2-B-F2; A-F1-P1- L- F2; F1-P1- L- B-F2; A-F1- L-P2-F2; and F1-L-P2-B-F2.

[00244] In one embodiment, F1 and F2 each comprise a CH2 and CH3 moiety.

[00245] In one embodiment, after cleavage and substantial excision of the cscFc 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 F1 and F2 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

[00247] 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 IgM, IgG, IgD, IgA and IgE, and any immunoglobulin isotype, including IgG1, IgG2, IgG3 and IgG4. In a preferred embodiment, the human isotype IgG1 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 can be selected having a particular effector function (or lacking a particular effector function) or with a particular modification to reduce immunogenicity. Many sequences of antibodies and antibody-encoding genes have been published and suitable Fc moiety sequences (e.g. hinge, CH2, and/or CH3 sequences, or portions thereof) can be derived from these sequences using art recognized techniques. The genetic material obtained using any of the foregoing methods may then be altered or synthesized to obtain polypeptides of the present invention. It will further be appreciated that the scope of this invention encompasses alleles, variants and mutations of constant region DNA sequences.

[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 clone an 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 PCR. PCR amplification methods are described in detail in U.S. Pat. Nos.

4,683,195; 4,683,202; 4,800,159; 4,965,188; and in, e.g., "PCR Protocols: A Guide to Methods and Applications" Innis et al. eds., Academic Press, San Diego, CA (1990); Ho et al. 1989. Gene 77:51; Horton et al. 1993. Methods Enzymol. 217:270). PCR may be initiated by consensus constant region primers or by more specific primers based on the published heavy and light chain DNA and amino acid sequences. As discussed above, PCR also may be used to isolate DNA clones encoding the antibody light and heavy chains. In this case the libraries may be screened by consensus primers or larger homologous probes, such as mouse constant region probes. Numerous primer sets suitable for amplification of antibody genes are known in the art (e.g., 5' primers based on the N-terminal sequence of purified antibodies (Benhar and Pastan. 1994. Protein Engineering 7:1509); rapid amplification of cDNA ends (Ruberti, F. et al. 1994. J. Immunol. Methods 173:33); antibody leader sequences (Larrick et al. 1989 Biochem. Biophys. Res. Commun. 160:1250). The cloning of antibody sequences is further described in Newman *et al.*, U.S. Pat. No. 5,658,570, filed January 25, 1995, which is incorporated by reference herein.

[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 least 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 CH3 domain or portion thereof. In another embodiment, the polypeptide of the invention comprises at least one Fc moiety which comprises at least one CH4 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 CH2 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 CH2 domain or portion thereof and at least one CH3 domain or portion thereof (e.g, in the CH2-CH3 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 CH3 domain or portion thereof, for example in the orientation hinge-CH2-CH3, hinge-CH3-CH2, or CH2-CH3-hinge.

[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, CH2, and CH3 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 IgG1).

[00252] In 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 CH3 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 polypeptide of the invention comprises at least one Fc moiety comprising a hinge and a CH3 domain. In another embodiment, a polypeptide of the invention comprises at least one Fc moiety comprising a hinge, a CH_2, and a CH_3 domain. In preferred embodiments, the Fc moiety is derived from a human IgG immunoglobulin heavy chain (e.g., human IgG1). 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 M^{-1}, or more preferably higher than 10^8 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 al. 1994, J. Exp. Med. 180:2377). The FcRn receptor binds IgG (but not other immunoglobulin classes such as IgA, IgM, IgD, and IgE) at relatively low pH, actively transports the IgG transcellularly in a luminal to serosal direction, and then releases the IgG at relatively higher pH found in the interstitial fluids. It is expressed in adult epithelial tissue (U.S. Pat. Nos. 6,485,726, 6,030,613, 6,086,875; WO 03/077834; US2003-0235536A1) including lung and intestinal epithelium (Israel et al. 1997, Immunology 92:69) renal proximal tubular epithelium (Kobayashi et al. 2002, Am. J. Physiol. Renal Physiol. 282:F358) as well as nasal epithelium, vaginal surfaces, and biliary tree surfaces.

[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, and other fragments of IgG that include the complete binding region of FcRn. The major contact sites include amino acid residues 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. References made to amino acid numbering of 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 IgG1 Fc (Fc γ1) can be substituted without significant loss of Fc binding affinity for FcRn: P238A, S239A, K246A, K248A, D249A, M252A, T256A, E258A, T260A, D265A, S267A, H268A, E269A, D270A, E272A, L274A, N276A, Y278A, D280A, V282A, E283A, H285A, N286A, T289A, K290A, R292A, E293A, E294A, Q295A, Y296F, N297A, S298A, Y300F, R301A, V303A, V305A, T307A, L309A, Q311A, D312A, N315A, K317A, E318A, K320A, K322A, S324A, K326A, A327Q, P329A, A330Q, P331A, E333A, K334A, T335A, S337A, K338A, K340A, Q342A, R344A, E345A, Q347A, R355A, E356A, M358A, T359A, K360A, N361A, Q362A, Y373A, S375A, D376A, A378Q, E380A, E382A, S383A, N384A, Q386A, E388A, N389A, N390A, Y391F, K392A, L398A, S400A, D401A, D413A, K414A, R416A, Q418A, Q419A, N421A, V422A, S424A, E430A, N434A, T437A, Q438A, K439A, S440A, S444A, and K447A, where for example P238A represents 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 FcγRI, FcγRIIA, FcγRIIB, and FcγRIIIA, without compromising affinity for FcRn (Routledge et al. 1995, Transplantation 60:847; Friend et al. 1999, Transplantation 68:1632; Shields et

al. 1995, J. Biol. Chem. 276:6591). As a further example of new functionality arising from mutations described above affinity for FcRn may be increased beyond that of wild type in some instances. This increased affinity may reflect an increased "on" rate, a decreased "off" rate or both an increased "on" rate and a decreased "off" rate. Mutations believed to impart an increased affinity for FcRn include T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591).

[00258] Additionally, at least three human Fc gamma receptors appear to recognize a binding site on IgG within the lower hinge region, generally amino acids 234-237. Therefore, another example of new functionality and potential decreased immunogenicity may arise from mutations of this region, as for example by replacing amino acids 233-236 of human IgG1 "ELLG" to the corresponding sequence from IgG2 "PVA" (with one amino acid deletion). It has been shown that FcγRI, FcγRII, and FcγRIII, which mediate various effector functions will not bind to IgG1 when such mutations have been introduced. Ward and Ghetie 1995, Therapeutic Immunology 2:77 and Armour et al. 1999, Eur. J. Immunol. 29:2613.

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

[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 IgG1 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 IgG1 molecule and, in part, from an IgG3 molecule. As set forth herein, it will be understood by one of ordinary skill in 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 IgG1, EU numbering (with the primary contact sites being amino acids 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. Thus, an Fc 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 IgG1, IgG2, IgG3 and IgG4. In one embodiment, an FcRn binding portion from an antibody of the human isotype IgG1 is used. In another embodiment, an FcRn binding portion from an antibody of the human isotype IgG4 is used.

[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 (ΔCH2 constructs). Those skilled in the art will appreciate that such constructs may be preferred due to the regulatory properties of the CH2 domain on the catabolic rate of the antibody. In certain embodiments, polypeptides of the invention comprise CH2 domain-deleted Fc regions derived from a vector (e.g., from IDEC Pharmaceuticals, San Diego) encoding an IgG_1 human constant region domain (see, e.g., WO 02/060955A2 and WO02/096948A2). This exemplary vector is engineered to delete the CH2 domain and provide a synthetic vector expressing a domain-deleted IgG_1 constant region. It will be noted that these exemplary constructs are preferably engineered to fuse a binding CH3 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 CH2 domain and/or between a CH2 and a CH3 domains. For example, compatible constructs could be expressed wherein the CH2 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 may comprise 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-glycosylated. For example, the heteromeric scFc region may comprise a first, glycosylated, Fc moiety (e.g., a glycosylated CH2 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 IgG1 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 IgG1 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 CH2 domain or portion thereof. In another embodiment, the Fc variant comprises a substitution at an amino acid position located in a CH3 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. FcγRI, FcγRII, or FcγRIII) or complement proteins (e.g. C1q), or to trigger antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cytotoxicity (CDCC)). In other embodiments, the Fc variant provides an engineered cysteine residue

[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, WO96/14339A1, WO98/05787A1, WO98/23289A1, WO99/51642A1, WO99/58572A1, WO00/09560A2, WO00/32767A1, WO00/42072A2, WO02/44215A2, WO02/060919A2, WO03/074569A2, WO04/016750A2,

WO04/029207A2, WO04/035752A2, WO04/063351A2, WO04/074455A2, WO04/099249A2, WO05/040217A2, WO04/044859, WO05/070963A1, WO05/077981A2, WO05/092925A2, WO05/123780A2, WO06/019447A1, WO06/047350A2, and WO06/085967A2; US Patent Publication Nos. US2007/0231329, US2007/0231329, US2007/0237765, US2007/0237766, US2007/0237767, US2007/0243188, US20070248603, US20070286859, US20080057056 ; or US Patents 5,648,260; 5,739,277; 5,834,250; 5,869,046; 6,096,871; 6,121,022; 6,194,551; 6,242,195; 6,277,375; 6,528,624; 6,538,124; 6,737,056; 6,821,505; 6,998,253; 7,083,784; and 7,317,091, each of which is incorporated by reference herein. In one embodiment, the specific change (e.g., the specific substitution of one or more amino acids disclosed in the art) may be made at one or more of the disclosed amino acid positions. In another embodiment, a different change at one or more of the disclosed amino acid positions (e.g., the different substitution of one or more amino acid position disclosed in the art) may be made.

[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 Å FcRn "contact zone." As used herein, the term 15 Å FcRn "contact zone" includes residues at the following positions of a wild-type, full-length Fc moiety: 243-261, 275-280, 282-293, 302-319, 336- 348, 367, 369, 372-389, 391, 393, 408, 424, 425-440 (EU numbering). In preferred 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 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. FcγRI, FcγRII, or FcγRIII) or complement

proteins (e.g. C1q), 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., 1, 2, 3, 4, or 5 amino acid residues), about 10 residues, about 15 residues, about 20 residues, about 30 residues, about 40 residues, or about 50 residues). In yet other embodiments, the Fc 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 (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 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 arginines of the RRRR 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 F1 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 exemplary 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., non-naturally 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 heteromeric 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 gly/ser linker comprises an amino acid sequence of the formula $(Gly_4Ser)n$ (SEQ ID NO: 4), wherein is a positive integer (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10). A preferred gly/ser linker is $(Gly_4Ser)_2$ (SEQ ID NO:29), $(Gly_4Ser)_4$ (SEQ ID NO:6), or $(Gly_4Ser)_6$. (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 IgG1, IgG2, IgG3, or IgG4 molecule), at least a portion of a middle hinge region (e.g., derived from an IgG1, IgG2, IgG3, or IgG4 molecule) and a series of gly/ser amino acid residues (e.g., a gly/ser linker such as $(Gly_4Ser)n$) (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 15 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.

VIII. 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 terminus wherein A, if present, is a clotting factor or portion thereof, F1 is a first Fc moiety or domain, P1 is an enzymatic cleavage site, L is a cscFc linker, P2 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. 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 XIa or Xa cleavage site may be incorporated into a construct of the invention, e.g., in a cleavable linker. 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 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] Oligonucleotide-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 PCR 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 VII 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 lysing 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 germline 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; Baldassarre et al. 2003, Theriogenology 59 : 831 ; Robl et al. 2003, Theriogenology 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.

[00299] 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.*, ampicillin-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 globin major promoter, the SV40 origin of replication, the bovine growth hormone polyadenylation 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 G418 containing medium and methotrexate amplification. Vector systems are also taught in U.S. Pat. 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 flourescence-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 DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CV1 (monkey kidney line), COS (a derivative of CV1 with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), HAK (hamster kidney line), SP2/O (mouse myeloma), P3.times.63-Ag3.653 (mouse myeloma), BFA-1c1BPT (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/subtilisin/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 PC5 also include a Cys-rich domain, the function of which is unknown. In addition, PC5 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 PC5; 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 gp160 to gp120 and gp41.

[00313] Nucleotide and amino acid sequences of rodent PC5 were first described as PC5 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 trp1 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 3-phosphoglycerate 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-)affinity 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 scFc 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 precipitation, affinity column chromatography, HPLC 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 in 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, sublingually, buccally, sublingually, nasally, rectally, vaginally or via pulmonary route. The chimeric protein can be implanted within or linked to a biopolymer 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 propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoromethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[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, rectal 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, 0.01-0.1M and preferably 0.05M 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 replenishers, electrolyte replenishers, 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, propylene 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 prophylactic 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 IU/kg, e.g., 0.417 mg/kg to 1.67 mg/kg. In another embodiment, the dose of a biologically active moiety (e.g., comprising FVIII) can range from about 25 to 65 IU/kg, e.g., 0.003125 mg/kg to 0.008125 mg/kg. In another embodiment, the dose of a biologically active moiety (e.g., comprising FVII), can range from about 90 to 270 ug/kg or 0.090 to 0.270 mg/kg.

[00337] Dosages can range from 1000 ug/kg to 0.1 ng/kg body weight. In one embodiment, the dosing range is 1ug/kg to 100 ug/kg. The protein can be administered continuously or at specific timed intervals. In vitro assays may be employed to determine optimal dose ranges and/or schedules for administration. In vitro assays that measure clotting factor activity are known in the art, e. g. , STA-CLOT VIIa-rTF clotting assay. 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 receive 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 & GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS 1233-1287 ((Joel G. Hardman *et al.*, eds., 9th 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 clotting 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 inherited disorder. In one embodiment, the subject has hemophilia A, and the chimeric protein comprises Factor VII or Factor VIIIa. 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 VIIIa and the chimeric clotting factor comprises Factor VII or Factor VIIa. In yet another embodiment, the subject has inhibitory antibodies against Factor IX or Factor IXa and the chimeric protein comprises Factor VII or Factor VIIa.

[00353] The chimeric clotting factor of the invention can be used to prophylactically 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 invention employs, unless otherwise indicated, conventional techniques of chemistry, biophysics, molecular biology, recombinant DNA technology, immunology (especially, *e.g.*, antibody technology), and standard techniques in electrophoresis. See, *e.g.*, Sambrook, Fritsch and Maniatis, Molecular Cloning: Cold Spring Harbor Laboratory Press (1989); Antibody Engineering Protocols (Methods in Molecular Biology), 510, Paul, S., Humana Pr (1996); Antibody Engineering: A Practical Approach (Practical Approach Series, 169), McCafferty, Ed., Irl Pr (1996); Antibodies: A Laboratory Manual, Harlow *et al.*, CS.H.L. Press, Pub. (1999); and Current Protocols in Molecular Biology, eds. Ausubel *et al.*, John Wiley & Sons (1992).

Example 1. Heterodimeric Constructs comprising FVII-Fc and MB9-Fc at the amino terminus of the second Fc chain

Cloning of pSYN-FVII-027

[00361] The FVII-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 GPIIbIIIa, MB9.

[00362] Plasmid (pSYN-FVII-027) was generated for the expression FVII-Fc and MB9-Fc heterodimer, where MB9 is a scFv previously shown to bind to receptor GPIIb/IIIa on activated platelets. Protein from pSYN-FVII-027 is expressed in the cell as a single polypeptide where the C-terminus of the FVII-Fc subunit is linked to the N-terminus of the MB9-Fc subunit by a $(GGGGS)_{6x}$ polypeptide linker. Furthermore, RRRRS and RKRRKR sequences were inserted at the 5' and 3' end of the polypeptide linker, respectively, for intracellular cleavage by proprotein convertases following the last Arg at each sequence. Consequently, cells will express a 2 chain FVII-Fc/MB9-Fc 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:

HindIII-SalI-BpsEI-Fc-F

AGTCAAGCTTGTCGACTCCGGAACTCCTGGGCGGACC

BamHI-linker-Fc-R

CATCGGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCCGCCACCTTTAC

CCGGAGACAGGGAGAGG

BclI-Fc-F

CAGTCTTGATCAGACAAAACTCACACATGCCCACC

scFc-EcoRI-R

ACTGACGAATTCTCATTTACCCGGAGACAGGGAG

HindIII-Kozak-FVII-F:

CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG

FVII-HC-BspEI-R:

ACGACTTCCCGAGCTCGGCACCCTCGGCATCTGTCAGTTTTCTCCGATCCCCCCCCACCCGAACCTCCA

CCGCCTGATCCACCCCCACCTGATCCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCGGGA

AATGGGGCTCGCAGGAGG

A 50 ul PCR reaction was carried out with 25 pmol of HindIII-SalI-BpEI-Fc-F and BamHI-linker-Fc-R and template pSYN-Fc-001 using the following cycle: 95° C 2 minutes; 30 cycles of (95° C 30 seconds, 54° C 30 seconds, 72° C 1 minute). The expected sized band (~700 bp) was gel purified with a Gel Extraction kit (Qiagen, Valencia, Calif.) and cloned into the HindIII and BamHI restriction sites of

pBUDCE4 (Invitrogen, Carlsbad, Calif.) to generate intermediate pSYN-FVII-007. Primers HindIII-SalI-BpEI-Fc-F and BamHI-linker-Fc-R amplify the Fc region starting at amino acid 221 (EU numbering) and add a HindIII and a SalI restriction enzyme site immediately upstream of site Fc region, as well as a DNA fragment encoding a $(GGGGS)_{4x}$ linker followed by a BamHI site immediately downstream of the Fc coding region. Next, a 50 ul reaction was carried out with 25 pmol of BclI-Fc-F and scFc-EcoRI-R, and template pSYN-Fc-011 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 BclI/EcoRI restriction sites of pSYN-FVII-007 to generate the intermediate plasmid pSYN-FVII-008. The primer pair BclI-Fc-F and scFc-EcoRI-R amplifies the Fc region while adding a BclI and EcoRI restriction sites immediately upstream and downstream of the Fc coding region, respectively. To generate the last intermediate plasmid, a 50 ul PCR reaction was carried out with 25 pmol of HindIII-Kozak-FVII-F and FVII-HC-BspEI-R and template pSYN-FVII-001 using the following cycle: 95° C 2 minutes; 30 cycles of (95° C 30 seconds, 55° C 30 seconds, 72° 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 $(GGGGS)_{6x}$ polypeptide linker followed by a fragment of the Fc region ending at amino acid 221 (EU numbering). Primer HindIII-Kozak-FVII-F generates a HindIII restriction site at the 5' 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 (~1500 bp) was gel purified as above and cloned into the HindIII/BspEI sites of pSYN-FVII-008 to generate pSYN-FVII-011.

[00364] Next, 2 DNA fragments were synthesized: Genescript-FVII-027-1 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-$(GGGGS)_{6x}$-RKRRKR followed by a portion of the MB9 scFv (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 Genescript-FVII-027-1. In addition, the first 6 bases at the 5' include a SapI site found within the Fc region. Genscript-FVII-026-2 consists of a DNA fragment encoding a portion of the MB9 (residues 143-273) followed by a

$(GGGGS)_{6x}$ polypeptide linker followed by the Fc region and an EcoR1 site. 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 first 6 bases of Genescript-FVII-026-2.

[00365] Genescript-FVII-027-1 was cloned into the SapI and EcoRI sites of pSYN-FVII-011 to generate pSYN-FVII-036. Next, Genscript-FVII-026-2 was cloned into the EcoRI site of pSYN-FVII-036 to generate pSYN-FVII-027. Correct orientation of the last cloning step was confirmed by restriction enzyme analysis and DNA sequencing.

Example 2. Heterodimeric Constructs comprising FVII-Fc and MB9-Fc, MB9 at the carboxy terminus of the second Fc chain

Cloning of FVII-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 MB9 scFv which binds to GPIIbIIIa is attached to the c-terminus of the second Fc moiety.

[00367] Synthesis of DNA fragment Genscript-FVII-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-FVII-037 was subcloned into the SapI/EcoRI of pSYN-FVII-011 (refer to P0830) to generate and intermediate construct. A SapI fragment from pSYN-FVII-011 was subcloned into the SapI sites of the intermediate construct to generate pSYNFVII-037.

Example 3. Heterodimeric Constructs comprising FVII-Fc and a peptide against GPIb at the carboxy terminus of the second Fc chain

Cloning of THE pSYN-FVII-041 intermediate construct.

[00368] In order to make this construct, the FVII-041 construct was first made as an intermediate. Synthesis of DNA molecule Genscript-FVII-041 was outsourced (Genscript). This fragment was digested with SapI and cloned into SapI sites of pSYN-FVII-011 to generate pSYN-FVII-041. This process introduces a unique SalI 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 to 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 SalI/EcoRI and subcloned into the SalI/EcoRI sites of pSYN-FVII-041 to generate pSYN-FVII-044, -045 and -046.

Example 4. Heterodimeric Constructs comprising FVII-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-FVII-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 RsrII and subcloned into the BspEI/RsrII sites of pSYN-FVII-042 to generate pSYN-FVII-050. This process introduces a unique SalI site (residues 412-413 EU numbering, GTG GAC to GTC GAC) in the first Fc. A HindIII/EcoRI fragment of pSYNFVII-050 was subcloned into the HindIII/EcoRI sites of pSYN-FVII-011 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 OS1 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 Sal/RsrII fragment from Genscript-FVII-047, -048 and -049 was subcloned into SalI/RsrII sites of pSYN-FVII-043 to generate pSYN-FVII-047, -048 and 049, respectively.

Example 5. Heterodimeric Constructs comprising a Gla-deleted FVII-Fc and a targeting molecule

Cloning of the FVII-028 intermediate

[00374] In order to make this construct, the FVII-028 construct was first made as an intermediate. Synthesis of DNA fragment Genscript-FVII-028 was outsourced (Genscript). This fragment was cut with HindIII/XbaI and subcloned into pSYN-FVII-011 to generate pSYN-FVII-028.

Cloning of FVII-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 FVII 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-FVII-025 was outsourced and an XbaI/BsiWI fragment from this molecule was subcloned into XbaI/BsiWI sites of pSYN-FVII-028 to generate pSYN-FVII-053

Example 6. Heterodimeric Constructs comprising a Factor VII heavy and light chains as two separate polypeptides.

Cloning of pSYN-FVII-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 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-F1

GGGAATGTCAACAGGCAGGG

FVII-R1

CTTGGCTTTCTCTCCACAGGC

[00379] A 50 µl 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° C for 30 minutes for the reverse transcription followed by

denaturing at 94° C for 2 minutes and 30 cycles of (94° C 30 seconds, 53° C 30 seconds, 72° C 90 seconds) followed by 10 minutes at 72° 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 FVII-Fc and Fc heterodimer, the FVII coding sequence was PCR-amplified using the following primers:

HindIII-Kozak-FVII-F

CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG

BspeI-Fc-FVII-R

CGACTCCGGAGCTGGGCACGGTGGGCATGIGTGAGTTTTGTCGGGAAATGGGGCTCGCAGG

[00380] The forward primer HindIII-Kozak-FVII-F adds a HindIII restriction site followed by a Kozak sequence immediately upstream of the FVII coding region. The reverse primer BspeI-Fc-FVII-R adds a fragment of the constant region of IgG1 (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 ul reaction was carried out with 15 pmol of each primer and template pSYN-FVII-001 using Platinum Pfx DNA Polymerase system according to manufacturer's protocol in a MJ Thermocycler using the following cycles: 95° C 2 minutes; 30 cycles of (95° C 15 seconds, 49° C 30 seconds, 68° C 90 seconds); 68° C 10 minutes. Plasmid pSYN-FIX-027 (pBUD FIXFc/Fc) was digested with HindIII 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 HindIII 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-linker-F:

GACAAAACTCACACATGCCCACC

Fc-linker-R:

GCAGAATTCTCATTTACCCGGAG

[00381] Two 12 µl PCR reactions were carried out with either 12 pmol of FVII-linker-F and FVII-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 1 µg of pSYN-FVII-002 as template using the following cycle: 94 °C. 2 minutes; 14 cycles of (94°C. 30 seconds, 55°C. 30 seconds, 72°C. 2 minutes); 72°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 FVII-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 KpnI/EcoRI sites of pSYN-FVII-002 to generate pSYN-FVII-003 (pBUD FVIIFc/6x(GGGGS)/Fc).

Cloning of pSYN-FVII-024 to express a two-chain heterodimer

[00382] Plasmid (pSYN-FVII-024) was generated for the expression of a two-chain heterodimer where one chain consists of the FVII light chain (residues 1-152) followed by a $(GGGGS)_{6x}$ linker followed by the Fc region, while the other chain contains a FVII heavy chain (residues 153 to 406) followed by a $(GGGGS)_{6x}$ linker followed by the Fc region. The plasmid is designed to express the heterodimer as a single polypeptide where the C-terminus 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-$(GGGGS)_{6x}$-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-FVII-024 and several intermediate plasmids required the use of the following primers:

HindIII-SalI-BpEI-Fc-F

AGTCAAGCTTGTCGACTCCGGAACTCCTGGGCGGACC

BamHI-linker(PACE1)-Fc-R

CATCGGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCCGCCACCGCTCC

GGCGGCGCCGTTTACCCGGAGACAGGGAGAGG

HindIII-Kozak-FVII-F

CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG

BspEI-Fc-linker-FVIILC-R

GAGTTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTCTGATCCCCCGCCACCGGAACCTCCACC

GCCTGATCCACCCCCACCTGATCCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCTCGGCC

TTGGGGTTTGCTGG

BamHI-2xlink-pace-HC-F

CAGTCTGGATCCGGCGGTGGAGGTTCCGGTGGGGGTGGATCAAGGAAGAGGAGGAAGAGGATTGTGGGG

GGCAAGGTGTGCC

Fc-EcoRI-R

ATGTCTGAATTCTCATTTACCCGGAGACAGGGAGAGG

[00383] To generate the first intermediate plasmid, a PCR reaction was performed with 25 pmol of primers HindIII-SalI-BpEI-Fc-F and BamHI-linker(PACE1)-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° C 2 minutes; 30 cycles of (95° C 30 seconds, 58° C 30 seconds, and 72° C 1 minute); 72° C 10 minutes. The correct sized band (approximately 730 bp) was gel purified as above and cloned into the HindIII/BamHI sites of pBUDCE4 vector (Invitrogen, Carlsbad, Calif.), generating pSYN-FVII-014. PCR amplification with primers HindIII-SalI-BpEI-Fc-F and BamHI-linker(PACE1)-Fc-R generated a DNA fragment encoding a portion of the Fc region (Amino A X-Y) followed by an RRRRS sequence and $(GGGGS)_{2x}$ polypeptide linker. Primer HindIII-SalI-BpEI-Fc-F introduces a HindIII and SalI restriction site at the 5' end of the molecule, while primer BamHI-linker(PACE1)-Fc-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 PCR reaction was performed as above with primers HindIII-Kozak-FVII-F and BspEI-Fc-linker-FVIILC-R and template pSYN-FVII-002 using the same conditions described for cloning of pSYN-FVII-014, but with an annealing temperature of 57° C The expected sized band (approximately 700 bp) was gel purified and cloned into the HindIII and BspEI sites of pSYN-FVII-014 to generate pSYN-FVII-023. Primers HindIII-Kozak-FVII-F and BspEI-Fc-linker-FVIILC-R

amplified a DNA fragment encoding the FVII light chain followed by a $(GGGGS)_{6x}$ polypeptide linker and a portion of the Fc region up to amino acid 232 (EU numbering). Primer HindIII-Kozak-FVII-F introduces a HindIII restriction site at the 5' end of the molecule followed by a Kozak sequence while primer BspEI-Fc-linker-FVIILC-R adds a BspeI site at the 3' end of the molecule.

In the final step a PCR 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° C 2 minutes; 30 cycles of (95° C 30 seconds, 55° C 30 seconds, and 72° C 2 minute); 72 ° C 7 minutes. This PCR reaction generated a DNA molecule encoding a $(GGGGS)_{2x}$ polypeptide linker followed by a RKRRKR sequence followed by the FVII heavy chain. Primers BamHI-2xlink-pace-HC-F and Fc-EcoRI-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 of pSYN-FVII-023 to generate pSYN-FVII-024.

Cloning of intermediate pSYN-FVII-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 SalI 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 SalI to BsiWI sites of pSYN-FVII-057 was outsourced. This DNA was subcloned into the SalI/BsiWI sites of pSYN-FVII-073 to generate pSYN-FVII-057

Cloning of pSYN-FVII-058, pSYN-FVII-059, pSYN-FVII-060, pSYN-FVII-061 and pSYN-FVII-062

These constructs were cloned as described for pSYN-FVII-057 (outsourced synthesis of DNA from SalI to BsiWI and subcloned into pSYN-FVII-073)

Cloning of pSYN-FVII-066

The synthesis of the DNA sequence comprising nucleotides from the SalI to RsrII sites of pSYN-FVII-066 was outsourced. This DNA was subcloned into the SalI/RsrII sites of pSYN-FVII-043 to generate pSYN-FVII-066

Cloning of pSYN-FVII-067

The synthesis of the DNA sequence comprising nucleotides from the SalI to EcoRI sites of pSYN-FVII-067 was outsourced. This DNA was subcloned into the SalI/EcoRI sites of pSYN-FVII-041 to generate pSYN-FVII-067

Cloning of pSYN-FVII-090

The synthesis of the DNA sequence comprising nucleotides from the BamHI to BsiWI sites of pSYN-FVII-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/NotI) 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 PCR methods using the pSYN-FVII-090 as template and backbone structure to generate pSYN-FVII-100

Cloning of pSYN-FVII-115

A triple point mutation (V158D, E296V and M298Q; mature FVII sequence numbering) was introduced into the FVII coding region of pSYN-FVII-090 by PCR-based site-directed mutagenesis to generate pSYN-FVII-115

Cloning of pSYN-FVII-118

The synthesis of the DNA sequence comprising nucleotides from the XbaI to BsiWI sites of pSYN-FVII-118 was outsourced. This DNA was subcloned into the XbaI/BsiWI sites of pSYN-FVII-011 to generate pSYN-FVII-118

Cloning of pSYN-FVII-119

The synthesis of the DNA sequence comprising nucleotides from the XbaI to BsiWI sites of pSYN-FVII-119 was outsourced. This DNA was subcloned into the XbaI/BsiWI sites of pSYN-FVII-011 to generate pSYN-FVII-119

Cloning of pSYN-FVII-127

A DNA fragment comprising the 170 loop of trypsin was generated by PCR using pSYN-FVII-100 as template. This PCR reaction generated BsiWI and BspEI restriction sites at the 5' and 3', respectively. The DNA fragment was subcloned into the BsiWI/BspEI sites of pSYN-FVII-118 to generate pSYN-FVII-127.

Cloning of pSYN-FIX-042

A HindIII/BspEI fragment from pSYN-FIX-030 (as described in US Patent 7566565) was subcloned into the HindIII/BspEI sites of pSYN-FVII-011 to generate pSYN-FIX-042

Cloning of pSYN-FIX-068

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

Cloning of pSYN-FIX-088

A BspEI-EcoRI fragment from pSYN-FIX-067 was subcloned into BspEI-EcoRI sites of pSYN-FIX-053 to generate pSYN-FIX-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 XbaI site to the C-terminus of the protein followed by a 6x(GGGGGS) linker, the SCE5 coding sequence and an EcoRI site was outsourced for synthesis and subcloned into the XbaI/EcoRI sites of pSYN-FIX-053 to generate pSYN-FIX-090. The SCE5 sequence is set forth below:

AQVQLQESGGGLVQPGGSLRLSCAASGFMFSRYAMSWVRQAPGKGPEWVSGISGSGGSTYYADSVKGRF
TVSRDNSKNTLYLQMNSLRAEDTAVYYCARGATYTSRSDVPDQTSFDYWGQGTLVTVSSGSASAPKLEE
GEFSEARVSELTQDPAVSVALGQTVRITCQGDSLRNFYASWYQQKPGQAPTLVIYGLSKRPSGIPDRFS
ASSSGNTASLTITGAQAEDEADYYCLLYYGGGQQGVFGGGTKLTVLRQPKAAPSVTLFPPSSAA

Cloning of pSYN-FVII-094

A DNA fragment comprising a sequence encoding a 6x(GGGGS) linker followed by the SCE5 coding sequence was synthesized (outsourced) and cloned into the EcoRV/EcoRI sites of a pSYN-FVII-011 variant that had been previously modified to generate an EcoRV site at the C-terminus of the FVII coding region

Cloning of pSYN-FVII-088

The synthesis of the DNA sequence comprising nucleotides from the SalI to RsrII sites of pSYN-FVII-088 was outsourced. This DNA was subcloned into the SalI/RsrII sites of pSYN-FVII-066 to generate pSYN-FVII-088

Cloning of pSYN-FVII-125

A DNA fragment was PCR amplified from pSYN-FVII-088, comprising the AP3 region and part of the linker. This PCR reaction generated BamHI and EcoRI sites at

the 5' and 3' of the DNA fragment, respectively. This DNA fragment was subcloned into the BamHI/EcoRI sites of pSYN-FVII-011 to generate pSYN-FVII-125

Cloning of pSYN-FVIII-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 FVIII-specific primers. The FVIII 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 pBUDCE4.1 (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, EF1α, in the expression vector pBUDCE4.1. This final construct was designated pSYN-FVIII-013.

A second plasmid was created from similar constructs using PCR 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 monomer-dimer hybrid in transient transfection. This construct was designated pSYN-FVIII-041.

Cloning of pSYN-FVIII-049

Generated intermediate pSYN-FVIII-048 by cloning NheI/XhoI fragment from pBUD-CE4.1 into pSYN-FVIII-013. The synthesis of a DNA fragment comprising the region from RsrII to XbaI sites of pSYN-FVIII-049 was outsourced. This fragment was subcloned into the RsrII/XbaI sites of pSYN-FVIII-048 to generate pSYN-FVIII-049

Cloning of pSYN-FVIII-108

A SalI/RsrII fragment from pSYN-FVII-066 was subcloned into pSYN-FVIII-049 to generate pSYN-FVIII-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-FVII-010

[00385] The FVII-010 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 BspEI/HindIII sites of pSYN-FVII-008 (see supra), generating pSYN-FVII-009.

[00387] PCR amplify FVII light chain from pSYN-FVII-003 (refer to P0830) with primers FVII-LC-NotI-F/ FVII-LC-XhoI-R and clone in pSYN-FVII-009 to generate pSYN-FVII-010

Primers

FVII-HC-BspEI-R
AGGAGTTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTCGGATCC
CCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCCGCC
ACCGGACCCACCTCCGCCGGAGCCACCGCCACCGGGAAATGGGGCTCGCA
GGAGG

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

FVII-LC-NotI-F
ACTGACGCGGCCGCGCCGCCACCATGGTCTCCCAGG

FVII-LC-XhoI-R
ACTGACCTCGAGTTATCGGCCTTGGGGTTTGCTGG

Cloning of pSYN-FVII-013

[00388] The FVII-013 construct is one in which the light chain was expressed in the context of an scFc scaffold and the heavy chain was expressed separately.

[00389] PCR-amplify with primer pair FVII-LC-linker-BamHI-R/ HindIII-Kozak-FVII-F from pSYN-FVII-001 (refer to P0830) and clone in BamHI/HindIII

sites of pSYN-FVII-011, generating pSYN-FVII-012. PCR-amplify FVII-HC from pSYN-FVII-009 using primer pair FVII-HC-NotI-F/FVII-HC-XhoI-R ad subclone in pSYN-FVII-012 to generate pSYN-FVII-013

Primers
FVII-LC-6xlinker-BamHI-
RACTGACGGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCA
CCTGATCCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCTCG
GCCTTGGGGTTTGCTGGC
HindIII-Kozak-FVII-F
CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG
FVII-HC-NotI-F
ACTGACGCGGCCGCGCCGCCACCATGGAGACAGAC
FVII-HC-XhoI-R
ACTGACCTCGAGTTAGGGAAATGGGGCTCGCAGGAG

Cloning of pSYN-FVII-018

[00390] For the FVII-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.

[00391] Primers FVII-HC-Hind3-IggKss-F/scFc-EcoRI-R were used to PCR amplify HCFVII-linker-Fc, using pSYN-FVII-010 as template. Subclone in HindIII/EcoRI sites of pBUDCE4. This makes pSYN-FVII-017. Next, PCR-amplify from pSYN-FVII-013 with primers FVII-LC-NotI-F/FC-XHOI-R and subclone in XhoI/NotI sites of FVII-017. This makes PSYN-FVII-018

Primers
scFc-EcoRI-R
ACTGACGAATTCTCATTTACCCGGAGACAGGGAG

Fc-XhoI-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 XIa 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 I153V 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 I153V mutation in linear sequence attached to the N-terminus 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 I152V mutation was introduced in pSYN-FVII-039, -040 to increase FXIa cleavage efficiency

[00394] Synthesis of DNA molecule Genscript-FVII-039 and -040 was outsourced (Genscript). An XbaI/BsiWI fragment from Genscript-FVII-039 and -040 was subcloned into XbaI/BsiWI sites of pSYN-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 μg/liter (growth media) as suspension cells at 37° C/10% CO_2. Cells we subcultured every three to four days by seeding at cell density of $5x10^5$ cells/ml.

[00396] Twenty-four hours prior to transfection cells were seeded at a density of $7x10^5$ cells/ml in growth media supplemented with LONG™R3IGF-1 (Sigma Aldrich, St. Louis, MO) to 20 μg/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 mg/L) to a freshly made solution of PEI (60 mg/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™R3IGF-1 and 200 mM 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 10. Protein Purification of FVIIIFc Molecules (except FVII-028 and FVII-053) and FIXFc molecules

[00397] FVIIIFc 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 $CaCl_2$ to selectively elute the most active species), followed by 2) shFcRn (soluble human FcRn) 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 FVII-028 and FVII-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 immunoaffinity 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 FVIII-specific 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 Fc-containing 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.

Example 14. Activation of FVII constructs

[00401] Fractions eluted from the FcRn column containing FVIIFc were pooled, and total protein was concentrated to 4 mg/ml. The $CaCl_2$ concentration was raised to 5 mM and the sample was incubated at 4°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

FVIIaFc	IU/nM
FVII-011	991
FVII-024	929
FVII-027	790
FVII-037	1131
FVII-044	1300
FVII-045	906
FVII-046	1145
FVII-047	924
FVII-048	973
FVII-049	1130
FVII-053	929

Example 18. FACs assays to study binding of FVIIaFc and platelets

[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 mg/ml (6.7 mM), working concentration 50 ug/ml (67 uM)

FVII antibody-FITC-labeled: Affinity Biologicals SAFVII-APFTC

Platelet buffer: 15 mM HEPES, 138 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 5 mM CaCl2, 5.5 mM dextrose and mg/ml BSA, pH 7.4

Method

- Count platelets
- Add 20 ul of ~2-4x10^8 cells/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 HBS/5 mM $CaCl_2$/1.5% formaldehyde, incubate 20' at RT
- Spin 15' at 3000g
- Wash in HBS/5mM CaCl2/1 mg/ml BSA, spin again and resuspend in 100 ul of platelet buffer.
- Add 2.5 ul of FVII antibody-FITC-labeled and incubate for 30 ' at room temperature.
- Analyze by FACs

Example 17. Thrombin Generation assay

[00404] In this example, the following reagents and methods were used:

Reagents

FV: HTI, cat#HCV-0100, lot#Z0413, 5.1 mg/ml

Prothrombin: HTI, cat#HCP-0010, lot# Z0128, 4.8 mg/ml

FX: HTI, cat# HCX-0050, lot#X0401, 5.4 mg/ml

ATIII: HTI, cat# HCATIII-0120, lot#Y0401, 8.2 mg/ml

TFPI: American Diagnostica, cat# 4900PC, lot# 081031, 100 ug/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 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 5.5 mM Dextrose, supplemented with 1 mg/ml BSA before using

ADP: Sigma Aldrich, cat# A2754, stock 1 mM, working concentration 10 uM

SFLLRN peptide: in-house synthesis, stock concentration 5 mg/ml (6.7 mM), working concentration 50 ug/ml (67 uM)

	Primary stock (mg/ml)	Working solution (ug/ml)	[final] ug/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	2-10E8	1.74E8	0.6E8
FVIIaFc	1 mg/ml (10 uM)	1200 nM	200 nM
FVIIaFc	1 mg/ml (10 uM)	200 nM	62.5 nM
FVIIaFc	1 mg/ml (10 uM)	62.5 nM	12.5 nM

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, FII, FX, ATIII and TFPI to make working solution. Need 5 ul/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 FVIIaFc 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/well) 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-FVII-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°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 100°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 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 1 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 PC5 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 PC5 on cleavage site processing can not be detected because the FVII light chain-Fc and FVII 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 PC5 (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 FVII-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 μM solution of FVII-039, FVII-040 or FVII-011 (non activated) containing 15 nM FXIa in 50 mM Tris-HCl, 100 mM NaCl, 10 mM $CaCl_2$, 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, FVII-040 and nonactivated FVII-011, 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-linker-Fc-scFclinker-FXIa/thrombin cleavage site-heavy chain-linker-Fc). In some embodiment, the heavy chain will comprise the I152V mutation. Once the best cleavage site is determined, a 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.

[00418] 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 constructs (See Figure 31). Constructs 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 XIa 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, FVII-089, FVII-062, or plasma-derived FVII (FVII) increasing concentrations of thrombin were added and incubated for 10 minutes at 37˚C. The mixture was run on SDS-PAGE 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 FVIII-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 FVII-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 (LQQSRKVGDSPN, 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-115, 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-115 are illustrated in Figure 41

[00423] In a soluble tissue factor assay, the specific activity of FVII-011(wild type FVIIaFc) is 10,000 IU/mg. FVII-090 has a specific activity of 0.32 IU/mg, FVII-100 has a specific activity of 0.25 IU/mg, and FVII-115 has a specific activity of 14 IU/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 FVIIa 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 chain-Fc 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 HC-Fc band which therefore migrates faster on the gel and comigrates with the FVII LC-Fc band.. Thrombin generation assays were used to measure activity of activatable 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 nM 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 FVII-100 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

[00427] 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 FVIIa. 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 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 FVIIa 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 (10uM) was added and incubated for 15 minutes at 37C, 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 can 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 inserted between the light chain and heavy chain. In FVII-119, the sequence GGGGS-ALRPR was inserted between the light chain and heavy chain. For FVII-127, the construct was made like FVII-118, 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-011 (wild type FVIIaFc which had an activity of 10,000 IU/mg). FVII-118 had an activity of 4.5 IU/mg and FVII-127 had an activity of 1.8 IU/mg, demonstrating that these molecules had essentially no activity in their activatable form.

[00433] Thrombin cleavage reactions of FVII-118, FVII-119 and FVII-090 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 monomeric construct,FVII-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 FVII-118 lacking the high specific activity amino acid substitution. High activity with longer initiation time for FVII-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 GPIIbIIIa

In this example the constructs illustrated in Figure 44A were cloned, transiently expressed, purified and activated as previously described. FVII-066 was cotrasfected with PC5 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 GPIIbIIIa. 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 (FVII-067) of the second Fc moiety of FVIIaFc. In addition, the SCE5 was placed at the C-terminus 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 GPIIbIIIa receptor, FVII-066 was tested in thrombin generation assays using mouse FVIII-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 FVII-066 relative to the controls in both systems.

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

In this example, construct FVII-027 illustrated in Figure 12A was cloned, expressed (with PC5 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 GPIIbIIIa. FACS assays were performed as previously described to assess binding to activated platelets, and FVII-027 was shown to bind to activated platelets with higher affinity than the FVII-011 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-011 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 GPIIbIIIa, demonstrating the effects are mediated by the interaction of MB9 with the activated form of GPIIbIIIa. The MB9 targeting moiety was also placed at the C-terminus of the second Fc moiety of FVIIaFc to generate FVII-037 illustrated in Figure 15. Thombin generation assays in a FVIII-deficient 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 GPIIbIIIa. 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 FVIIaFc or FVIIa to the active and nonactive conformation of GPIIbIIIa results in increased activity. Binding of FVII-088 and FVIIaFc to activated human platelets was tested by FACS (Figure 45C). These data reveal that FVII-088 binds to platelets with higher affinity than FVIIaFc (FVII-011), showing that the AP3 targeting moiety can increase the affinity of FVIIaFc 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 FVIIaFc (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 nM. The NATEM reaction was initiated by the addition of $CaCl_2$. 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 FVIIaFc 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, OS1, 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 FVII-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 construct. In contrast, the FVII-047 construct employed the PS4 peptide attached to the N terminus of the second Fc moiety of the construct; the FVII-048 molecule employed the OS1 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 FVIIa, 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-terminal fusion constructs. Figure 47B shows that the FVII-045 construct 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-011 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-011 control

Example 29. An FVIIIFc variant targeted to the active form of GPIIbIIIa

[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, FVIII-108 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.

These proteins were tested in thrombin generation assays using FVIII 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 version of FVII targeted to platelets that lacks a Gla domain

[00438] In this example a version of FVIIaFc illustrated in Figure 21was 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 PC5 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 GPIIbIIIa 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-011. 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-011 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 PC5 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-glycyl-arginyl-chloromethylketone (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 inhibitor. 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-088, 3.5 IU/nmol; FIX-090, 13.8 IU/nmol, and BeneFIX, 12 IU/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 OS1 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 FIX-089 construct was 2.4 IU/nmol as compared to 6 IU/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 FVII or MB9 to Fc region is underlined, and linker with proprotein convertase processing sites is shown in bold

MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
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KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
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DNA sequence of FVII-027

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FVII-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 Fcs sites is shown in bold

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FIX-037 DNA sequence

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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 Fcs sites is shown in bold, and MB9 is italicized

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SDHTGTKRSC RCHEGYSLLA DGVSCTPIVE YPCGKIPILE KRNASKPQGR RKRRKRIVGG
KVCPKGECPW QVLLLVNGAQ LCGGTLINTI WVVSAAHCFD KIKNWRNLIA VLGEHDLSEH
DGDEQSRRVA QVIIPSTYVP GTINHDIALL RLHQPVVLTD HVVPLCLPER TFSERTLAFV
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KDSCKGDSGG PHATHYRGTW YLTGIVSWGQ GCATVGHFGV YTRVSQYIEW LQKLMRSEPR
PGVLLRAPFP GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
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LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP

SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
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FVII-053 DNA sequence

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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 Fcs sites is shown in bold, and PS4 peptide is italicized

MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
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THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
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YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K**GGGGSGGGG SGGGGSGGGG**
SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
DGVEVHNAKT KPREEQYNST YRVVSVLIVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGSGGGGSGG
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FVII-044 DNA sequence

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FVII-045 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or OS1 to Fc region is underlined, linker connecting both Fcs sites is shown in bold, and OS1 peptide is italicized

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MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
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KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
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PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
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FVII-045 DNA sequence

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FVII-046 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 Fcs sites is shown in bold, and OS2 peptide is italicized

MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
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KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
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YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K**GGGGSGGGG SGGGGSGGGG**
SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
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FVII-046 DNA sequence

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

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MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
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KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
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PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
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FVII-047 DNA sequence

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tga

FVII-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

MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
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KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
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LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGGSGGGG
SGGGGSGGGG *SACTERMALH NLC*GGGGSGG GGSGGGGSGG GGSGGGGSGG GGSDKTHTCP
PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
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DNA sequence of FVII-048

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tga

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

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THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTINHD IALLRLHQPV VLTDHVVPLC
LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
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YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
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DNA sequence of FVII-049

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atgcatgagg ctctgcacaa ccactacacg cagaagagcc tctccctgtc tccgggtaaa
tga

FVII-011 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, Gla domain is italicized, linker region connecting FVII to Fc region is underlined, and linker connecting both Fcs sites is shown in bold

MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN *AFLEELRPGS LERECKEEQC*
SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCIPTVE YPCGKIPILE
KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
ITEYMFCAGY SDGSKDSCKG DSGGPHAIHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K**GGGGSGGGG SGGGGSGGGG**
SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK

FVII-011 DNA sequence

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tccgacggct ccttcttcct ctacagcaag ctcaccgtgg acaagagcag gtggcagcag
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agcctctccc tgtctccggg taaatga
```

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

```
   1  MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
  51  PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101  DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151  GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201  GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251  HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301  RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351  EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401  WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451  TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 501  DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551  YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601  NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651  HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701  MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751  SKNNAIEPRS FSQNPPVLKR HQREITRTTL QSDQEEIDYD DTISVEMKKE
 801  DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLRNRAQSG
 851  SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
 901  RNQASRPYSF YSSLISYEED QRQGAEPRKN FVKPNETKTY FWKVQHHMAP
 951  TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1001  FALFFTIFDE TKSWYFTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI
```

```
1051  MDTLPGLVMA QDQRIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
1101  YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
1151  GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1201  LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
1251  FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1301  MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1351  PKEWLQVDFQ KTMKVTGVTT QGVKSLLTSM YVKEFLISSS QDGHQWTLFF
1401  QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
1451  CEAQDLY
```

Full length FVIII amino acid sequence: Signal peptide underlined

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   1  MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
  51  PRVPKSFPFN TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101  DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151  GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201  GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251  HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301  RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351  EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401  WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451  TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIYPHGIT
 501  DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551  YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601  NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651  HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701  MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751  SKNNAIEPRS FSQNSRHPST RQKQFNATTI PENDIEKTDP WFAHRTPMPK
 801  IQNVSSSDLL MLLRQSPTPH GLSLSDLQEA KYETFSDDPS PGAIDSNNSL
 851  SEMTHFRPQL HHSGDMVFTP ESGLQLRLNE KLGTTAATEL KKLDFKVSST
 901  SNNLISTIPS DNLAAGTDNT SSLGPPSMPV HYDSQLDTTL FGKKSSPLTE
 951  SGGPLSLSEE NNDSKLLESG LMNSQESSWG KNVSSTESGR LFKGKRAHGP
1001  ALLTKDNALF KVSISLLKTN KTSNNSATNR KTHIDGPSLL IENSPSVWQN
1051  ILESDTEFKK VTPLIHDRML MDKNATALRL NHMSNKTTSS KNMEMVQQKK
1101  EGPIPPDAQN PDMSFFKMLF LPESARWIQR THGKNSLNSG QGPSPKQLVS
1151  LGPEKSVEGQ NFLSEKNKVV VGKGEFTKDV GLKEMVFPSS RNLFLTNLDN
1201  LHENNTHNQE KKIQEEIEKK ETLIQENVVL PQIHTVTGTK NFMKNLFLLS
1251  TRQNVEGSYD GAYAPVLQDF RSLNDSTNRT KKHTAHFSKK GEEENLEGLG
1301  NQTKQIVEKY ACTTRISPNT SQQNFVTQRS KRALKQFRLP LEETELEKRI
1351  IVDDTSTQWS KNMKHLTPST LTQIDYNEKE KGAITQSPLS DCLTRSHSIP
1401  QANRSPLPIA KVSSFPSIRP IYLTRVLFQD NSSHLPAASY RKKDSGVQES
1451  SHFLQGAKKN NLSLAILTLE MTGDQREVGS LGTSATNSVT YKKVENTVLP
1501  KPDLPKTSGK VELLPKVHIY QKDLFPTETS NGSPGHLDLV EGSLLQGTEG
1551  AIKWNEANRP GKVPFLRVAT ESSAKTPSKL LDPLAWDNHY GTQIPKEEWK
1601  SQEKSPEKTA FKKKDTILSL NACESNHAIA AINEGQNKPE IEVTWAKQGR
1651  TERLCSQNPP VLKRHQREIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
1701  EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
1751  KVVFQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1801  PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1851  CKAWAYFSDV DLEKDVHSGL IGPLLVCHTN TLNPAHGRQV TVQEFALFFT
1901  IFDETKSWYF TENMERNCRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1951  LVMAQDQRIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
2001  VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
2051  IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
2101  HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
2151  SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
2201  SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
2251  VDFQKTMKVT GVTTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
2301  VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
2351  Y
```

FIX amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined

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MQRVNMIMAE SPGLITICLL GYLLSAECTV FLDHENANKI LNRPKRYNSG KLEEFVQGNL
ERECMEEKCS FEEAREVFEN TERTTEFWKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP
FGFEGKNCEL DVTCNIKNGR CEQFCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPFPCGR
VSVSQTSKLT RAETVFPDVD YVNSTEAETI LDNITQSTQS FNDFTRVVGG EDAKPGQFPW
```

QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETEHT EQKRNVIRII
PHHNYNAAIN KYNHDIALLE LDEPLVLNSY VTPICIADKE YTNIFLKFGS GYVSGWGRVF
HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
GTSFLTGIIS WGEECAMKGK YGIYTKVSRY VNWIKEKTKL T

FIX DNA sequence

atgcagcgcg tgaacatgat catggcagaa tcaccaggcc tcatcaccat ctgccttta
ggatatctac tcagtgctga atgtacagtt tttcttgatc atgaaaacgc acaaaatt
ctgaatcggc caaagaggta taattcaggt aaattggaag agtttgttca agggaatcta
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acttga

FX amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined

MGRPLHLVLL SASLAGLLLL GESLFIREEQ ANNILARVTR ANSFLEEMKK GHLERECMEE
TCSYEEAREV FEDSDKTNEF WNKYKDGDQC ETSPCQNQGK CKDGLGEYTC TCLEGFEGKN
CELFTRKLCS LDNGDCDQFC HEEQNSVVCS CARGYTLADN GKACIPTGPY PCGKQTLERR
KRSVAQATSS SGEAPDSITW KPYDAADLDP TENPFDLLDF NQTQPERGDN NLTRIVGGQE
CKDGECPWQA LLINEENEGF CGGTILSEFY ILTAAHCLYQ AKRFKVRVGD RNTEQEEGGE
AVHEVEVVIK HNRFTKETYD FDIAVLRLKT PITFRMNVAP ACLPERDWAE STLMTQKTGI
VSGFGRTHEK GRQSTRLKML EVPYVDRNSC KLSSSFIITQ NMFCAGYDTK QEDACQGDSG
GPHVTRFKDT YFVTGIVSWG EGCARKGKYG IYTKVTAFLK WIDRSMKTRG LPKAKSHAPE
VITSSPLK

FX DNA sequence

atggggcgcc cactgcacct cgtcctgctc agtgcctccc tggctggcct cctgctgctc
ggggaaagtc tgttcatccg cagggagcag gccaacaaca tcctggcgag ggtcacgagg
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gagggctgtg cccgtaaggg gaagtacggg atctacacca aggtcaccgc cttcctcaag
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gtcataacgt cctctccatt aaagtga

DNA sequence of FVII-066

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   1      ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG
      CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCCCAAAGGG
 601 GAGTGTCCAT GGCAGGTCCT GTTGTTGGTG AATGGAGCTC AGTTGTGTGG GGGGACCCTG
 661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAAAATCAA GAACTGGAGG
 721 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
 781 CGGCGGGTGG CGCAGGTCAT CATCCCCAGC ACGTACGTCC CGGGCACCAC CAACCACGAC
 841 ATCGCGCTGC TCCGCCTGCA CCAGCCCGTG GTCCTCACTG ACCATGTGGT GCCCCTCTGC
 901 CTGCCCGAAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TGCGCTTCTC ATTGGTCAGC
 961 GGCTGGGGCC AGCTGCTGGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
1021 CCCCGGCTGA TGACCCAGGA CTGCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
1081 ATCACGGAGT ACATGTTCTG TGCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAGTGGAG GCCCACATGC CACCCACTAC CGGGGCACGT GGTACCTGAC GGGCATCGTC
1201 AGCTGGGGCC AGGGCTGCGC AACCGTGGGC CACTTTGGGG TGTACACCAG GGTCTCCCAG
1261 TACATCGAGT GGCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCCAGGAGT CCTCCTGCGA
1321 GCCCCATTTC CCGGTGGCGG TGGCTCCGGC GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
1381 GGGGGTGGAT CAGGCGGTGG AGGTTCCGGT GGCGGGGGAT CCGACAAAAC TCACACATGC
1441 CCACCGTGCC CAGCTCCGGA ACTCCTGGGA GGACCGTCAG TCTTCCTCTT CCCCCCAAAA
1501 CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG
1561 AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
1621 GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCCTC
1681 ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA
1741 GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
1801 CAGGTGTACA CCCTGCCCCC ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGACC
1861 TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
1921 CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGTTGGACT CCGACGGCTC CTTCTTCCTC
1981 TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
2041 GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT
2101 AAACGGCGCC GCCGGAGCGG TGGCGGCGGA TCAGGTGGCG GTGGATCAGG CGGTGGAGGT
2161 TCCGGTGGCG GGGGATCCGG CGGTGGAGGT TCCGGTGGGG GTGGATCAAG GAAGAGGAGG
2221 AAGAGGGCGC AGGTGCAGCT GCAGGAGTCT GGGGGAGGCT TGGTACAGCC TGGGGGGTCC
2281 CTGAGACTCT CCTGTGCAGC CTCTGGATTC ATGTTTAGCA GGTATGCCAT GAGCTGGGTC
2341 CGCCAGGCTC CAGGGAAGGG GCCAGAGTGG GTCTCAGGTA TTAGTGGTAG TGGTGGTAGT
2401 ACATACTACG CAGACTCCGT GAAGGGCCGG TTCACCGTCT CCAGAGACAA TTCCAAGAAC
2461 ACGCTGTATC TGCAAATGAA CAGCCTGAGA GCCGAGGACA CGGCTGTATA TTACTGCGCC
2521 CGGGGCGCCA CCTACACCAG CCGGAGCGAC GTGCCCGACC AGACCAGCTT CGACTACTGG
2581 GGCCAGGGAA CCCTGGTCAC CGTCTCCTCA GGGAGTGCAT CCGCCCCAAA GCTTGAAGAA
2641 GGTGAATTTT CAGAAGCACG CGTATCTGAA CTGACTCAGG ACCCTGCTGT GTCTGTGGCC
2701 TTGGGACAGA CAGTCAGGAT CACATGCCAA GGAGACAGCC TCAGAAACTT TTATGCAAGC
2761 TGGTACCAGC AGAAGCCAGG ACAGGCCCCT ACTCTTGTCA TCTATGGTTT AAGTAAAAGG
2821 CCCTCAGGGA TCCCAGACCG ATTCTCTGCC TCCAGCTCAG GAAACACAGC TTCCTTGACC
2881 ATCACTGGGG CTCAGGCGGA AGATGAGGCT GACTATTACT GCCTGCTGTA CTACGGCGGC
2941 GGCCAGCAGG GCGTGTTCGG CGGCGGCACC AAGCTGACCG TCCTACGTCA GCCCAAGGCT
3001 GCCCCCTCGG TCACTCTGTT CCCGCCCTCT TCTGCGGCCG GTGGCGGTGG CTCCGGCGGA
3061 GGTGGGTCCG GTGGCGGCGG ATCAGGTGGG GGTGGATCAG GCGGTGGAGG TTCCGGTGGC
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3121 GGGGGATCAG ACAAAACTCA CACATGCCCA CCGTGCCCAG CACCGGAACT CCTGGGCGGA
3181 CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
3241 GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG
3301 TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA GCAGTACAAC
3361 AGCACGTACC GTGTGGTCAG CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG
3421 GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
3481 AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGATGAG
3541 CTGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC CAGCGACATC
3601 GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT ACAAGACCAC GCCTCCCGTG
3661 TTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA CCGTGGACAA GAGCAGGTGG
3721 CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
3781 CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA TGA
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FVII-066 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVII or SCE5 to Fc region is underlined, and linker with proprotein convertase processing sites is shown in bold

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   1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
 241 NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
 301 LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
 361 ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
 421 YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
 481 PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
 541 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
 601 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
 661 YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KRRRRSGGGG SGGGGSGGGG
 721 SGGGGSGGGG SGGGGSRKRR KRAQVQLQES GGGLVQPGGS LRLSCAASGF MFSRYAMSWV
 781 RQAPGKGPEW VSGISGSGGS TYYADSVKGR FTVSRDNSKN TLYLQMNSLR AEDTAVYYCA
 841 RGATYTSRSD VPDQLSFDYW GQGTLVTVSS GSASAPKLEE GEFSEARVSE LTQDPAVSVA
 901 LGQTVRITCQ GDSLRNFYAS WYQQKPGQAP VLVIYGLSKR PSGIPDRFSA SSSGNTASLT
 961 ITGAQAEDEA DYYCLLYYGG QQQGVFGGGT KLTVLRQPKA APSVTLFPPS SAAGGGGSGG
1021 GGSGGGGSGG GGSGGGGSGG GGSDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP
1081 EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
1141 EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI
1201 AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
1261 QKSLSLSPGK *
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DNA sequence for FVII-057

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   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTCCG GCCCGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
 601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCC
 661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGAGG ACCGTCAGTC
 721 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 901 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTTCCGGTG GCGGGGGATC CGGCGGTGGA GGTTCCGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAAGCG TGAGCCAGAC CAGCAAGCTG ACCCGGATTG TGGGGGGCAA GGTGTGCCCC
1501 AAAGGGGAGT GTCCATGGCA GGTCCTGTTG TTGGTGAATG GAGCTCAGTT GTGTGGGGGG
1561 ACCCTGATCA ACACCATCTG GGTGGTCTCC GCGGCCCACT GTTTCGACAA AATCAAGAAC
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1621 TGGAGGAACC TGATCGCGGT GCTGGGCGAG CACGACCTCA GCGAGCACGA CGGGGATGAG
1681 CAGAGCCGGC GGGTGGCGCA GGTCATCATC CCCAGCACGT ACGTCCCGGG CACCACCAAC
1741 CACGACATCG CGCTGCTCCG CCTGCACCAG CCCGTGGTCC TCACTGACCA TGTGGTGCCC
1801 CTCTGCCTGC CCGAACGGAC GTTCTCTGAG AGGACGCTGG CCTTCGTGCG CTTCTCATTG
1861 GTCAGCGGCT GGGGCCAGCT GCTGGACCGT GGCGCCACGG CCCTGGAGCT CATGGTCCTC
1921 AACGTGCCCC GGCTGATGAC CCAGGACTGC CTGCAGCAGT CACGGAAGGT GGGAGACTCC
1981 CCAAATATCA CGGAGTACAT GTTCTGTGCC GGCTACTCGG ATGGCAGCAA GGACTCCTGC
2041 AAGGGGACA GTGGAGGCCC ACATGCCACC CACTACCGGG GCACGTGGTA CCTGACGGGC
2101 ATCGTCAGCT GGGGCCAGGG CTGCGCAACC GTGGGCCACT TTGGGGTGTA CACCAGGGTC
2161 TCCCAGTACA TCGAGTGGCT GCAAAAGCTC ATGCGCTCAG AGCCACGCCC AGGAGTCCTC
2221 CTGCGAGCCC CATTTCCCGG TGGCGGTGGC TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA
2281 TCAGGTGGGG GTGGATCAGG CGGTGGAGGT TCCGGTGGCG GGGGATCAGA CAAAACTCAC
2341 ACATGCCCAC CGTGCCCAGC ACCTGAACTC CTGGGAGGAC CGTCAGTCTT CCTCTTCCCC
2401 CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG
2461 GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
2521 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
2581 GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC
2641 AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
2701 GAACCACAGG TGTACACCCT GCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC
2761 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
2821 GGGCAGCCGG AGAACAACTA CAAGACCACG CCTCCCGTGT TGGACTCCGA CGGCTCCTTC
2881 TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA
2941 TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
3001 CCGGGTAAAT GA
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FVII-057 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 Factor XIa cleavage site is shown in bold, and the Factor XIa cleavage site is shown in dashed underline

```
   1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
 241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
 361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
 421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
 481 GSSVSQTSKL TRTVGGKVCP KGTCPWQVLL LVNGAQLCGG TLINTIWVVS AAHCFDKIKN
 541 WRNLIAVLGE HDLSEHDGDE QSRRVAQVII PSTYVPGTTN HDIALLRLHQ PVVLTDHVVP
 601 LCLPERTFSE RTLAFVRFSL VSGWGQLLDR GATALELMVL NVPRLMTQDC LQQSRKVGDS
 661 PNITEYMFCA GYSDGSKDSC KGDSGGPHAT HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV
 721 SQYIEWLQKL MRSEPRPGVL LRAPFPGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSDKTH
 781 TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 841 HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 901 EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
 961 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK*
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DNA sequence for FVII-058

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   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACCCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGCCCGA GGTGCGGTG GCTCCGGCGG AGGTGGGTCC
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 661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGAGG ACCGTCAGTC
 721 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 781 TGCGTGGTGG TGGACGTCAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 901 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
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1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTCGACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTTCCGGTG GCGGGGGATC CGGCGGTGGA GGTTCCGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGACT TCCTGGCCGA GGGCGGCGGC GTGCGGATTG TGGGGGGCAA GGTGTGCCCC
1501 AAAGGGGAGT GTCCATGGCA GGTCCTGTTG TTGGTGAATG GAGCTCAGTT GTGTGGGGGG
1561 ACCCTGATCA ACACCATCTG GGTGGTCTCC GCGGCCCACT GTTTCGACAA AATCAAGAAC
1621 TGGAGGAACC TGATCGCGGT GCTGGGCGAG CACGACCTCA GCGAGCACGA CGGGGATGAG
1681 CAGAGCCGGC GGGTGGCGCA GGTCATCATC CCCAGCACGT ACGTCCCGGG CACCACCAAC
1741 CACGACATCG CGCTGCTCCG CCTGCACCAG CCCGTGGTCC TCACTGACCA TGTGGTGCCC
1801 CTCTGCCTGC CCGAACGGAC GTTCTCTGAG AGGACGCTGG CCTTCGTGCG CTTCTCATTG
1861 GTCAGCGGCT GGGGCCAGCT GCTGGACCGC GGCGCCACGG CCCTGGAGCT CATGGTCCTC
1921 AACGTGCCCC GGCTGATGAC CCAGGACTGC CTGCAGCAGT CACGGAAGGT GGGAGACTCC
1981 CCAAATATCA CGGAGTACAT GTTCTGTGCC GGCTACTCGG ATGGCAGCAA GGACTCCTGC
2041 AAGGGGGACA GTGGAGGCCC ACATGCCACC CACTACCGGG GCACGTGGTA CCTGACGGGC
2101 ATCGTCAGCT GGGGCCAGGG CTGCGCAACC GTGGGCCACT TTGGGGTGTA CACCAGGGTC
2161 TCCCAGTACA TCGAGTGGCT GCAAAAGCTC ATGCGCTCAG AGCCACGCCC AGGAGTCCTC
2221 CTGCGAGCCC CATTTCCCGG TGGCGGTGGC TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA
2281 TCAGGTGGGG GTGGATCAGG CGGTGGAGGT TCCGGTGGCG GGGGATCAGA CAAAACTCAC
2341 ACATGCCCAC CGTGCCCAGC ACCTGAACTC CTGGGAGGAC CGTCAGTCTT CCTCTTCCCC
2401 CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG
2461 GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
2521 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
2581 GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC
2641 AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
2701 GAACCACAGG TGTACACCCT GCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC
2761 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
2821 GGGCAGCCGG AGAACAACTA CAAGACCACG CCTCCCGTGT TGGACTCCGA CGGCTCCTTC
2881 TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA
2941 TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
3001 CCGGGTAAAT GA
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FVII-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 AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
 241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
 361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
 421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
 481 GSDFLAEGGG VRIVGGKVCP KGECPWQVLL LVNGAQLCGG TLINTIWVVS AAHCFDKIKN
 541 WRNLIAVLGE HDLSEHDGDE QSRRVAQVII PSTYVPGTTN HDIALLRLHQ PVVLTDHVVP
 601 LCLPERTFSE RTLAFVRFSL VSGWGQLLDR GATALELMVL NVPRLMTQDC LQQSRKVGDS
 661 PNITEYMFCA GYSDGSKDSC KGDSGGPHAT HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV
 721 SQYIEWLQKL MRSEPRPGVL LRAPFPGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSDKTH
 781 TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
 841 HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 901 EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
 961 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK*
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DNA sequence for FVII-059

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   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
 601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCC
 661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGAGG ACCGTCAGTC
 721 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
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 781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 901 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTTCCGGTG GCGGGGGATC CGGCGGTGGA GGTTCCGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAACCA CCAAGATCAA GCCCCGGATT GTGGGGGGCA AGGTGTGCCC CAAAGGGGAG
1501 TGTCCATGGC AGGTCCTGTT GTTGGTGAAT GGAGCTCAGT TGTGTGGGGG GACCCTGATC
1561 AACACCATCT GGGTGGTGTC CGCGGCCCAC TGTTTCGACA AAATCAAGAA CTGGAGGAAC
1621 CTGATCGCGG TGCTGGGCGA GCACGACCTC AGCGAGCACG ACGGGGATGA GCAGAGCCGG
1681 CGGGTGGCGC AGGTCATCAT CCCCAGCACG TACGTCCCGG GCACCACCAA CCACGACATC
1741 GCGCTGCTCC GCCTGCACCA GCCCGTGGTC CTCACTGACC ATGTGGTGCC CCTCTGCCTG
1801 CCCGAACGGA CGTTCTCTGA GAGGACGCTG GCCTTCGTGC GCTTCTCATT GGTCAGCGGC
1861 TGGGGCCAGC TGCTGGACCG TGGCGCCACG GCCCTGGAGC TCATGGTCCT CAACGTGCCC
1921 CGGCTGATGA CCCAGGACTG CCTGCAGCAG TCACGGAAGG TGGGAGACTC CCCAAATATC
1981 ACGGAGTACA TGTTCTGTGC CGGCTACTCG GATGGCAGCA AGGACTCCTG CAAGGGGGAC
2041 AGTGGAGGCC CACATGCCAC CCACTACCGG GGCACGTGGT ACCTGACGGG CATCGTCAGC
2101 TGGGGCCAGG GCTGCGCAAC CGTGGGCCAC TTTGGGGTGT ACACCAGGGT CTCCCAGTAC
2161 ATCGAGTGGC TGCAAAAGCT CATGCGCTCA GAGCCACGCC CAGGAGTCCT CCTGCGAGCC
2221 CCATTTCCCG GTGGCGGTGG CTCCGGCGGA GGTGGGTCCG GTGGCGGCGG ATCAGGTGGG
2281 GGTGGATCAG GCGGTGGAGG TTCCGGTGGC GGGGGATCAG ACAAAACTCA CACATGCCCA
2341 CCGTGCCCAG CACCTGAACT CCTGGGAGGA CCGTCAGTCT TCCTCTTCCC CCCAAAACCC
2401 AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC
2461 CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC
2521 AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC
2581 GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC
2641 CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG
2701 GTGTACACCC TGCCCCCATC CCGGGATGAG CTGACCAAGA ACCAGGTCAG CCTGACCTGC
2761 CTGGTCAAAG GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
2821 GAGAACAACT ACAAGACCAC GCCTCCCGTG TTGGACTCCG ACGGCTCCTT CTTCCTCTAC
2881 AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG
2941 ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA
3001 TGA
```

FVII-059 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 AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
 241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
 361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
 421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
 481 GSTTKIKPRI VGGKVCPKGE CPWQVLLLVN GAQLCGGTLI NTIWVVSAAH CFDKIKNWRN
 541 LIAVLGEHDL SEHDGDEQSR RVAQVIIPST YVPGTTNHDI ALLRLHQPVV LTDHVVPLCL
 601 PERTFSERTL AFVRFSLVSG WGQLLDRGAT ALELMVLNVP RLMTQDCLQQ SRKVGDSPNI
 661 TEYMFCAGYS DGSKDSCKGD SGGPHATHYR GTWYLTGIVS WGQGCATVGH FGVYTRVSQY
 721 IEWLQKLMRS EPRPGVLLRA PFPGGGGSGG GGSGGGGSGG GGSGGGGSGG GGSDKTHTCP
 781 PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
 841 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
 901 VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY
 961 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK *
```

DNA sequence for FVII-060

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   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
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   361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
   421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
   481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
   541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
   601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCC
   661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGAGG ACCGTCAGTC
   721 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
   781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
   841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
   901 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
   961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
  1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
  1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
  1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
  1201 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
  1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
  1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
  1381 GGTTCCGGTG GCGGGGGATC CGGCGGTGGA GGTTCCGGTG GGGGTGGATC AGGAGGAGGT
  1441 GGTTCAGCCC TGCGGCCCCG GGTGGTGGGC GGCGCCGTGG TGGGGGGCAA GGTGTGCCCC
  1501 AAAGGGGAGT GTCCATGGCA GGTCCTGTTG TTGGTGAATG GAGCTCAGTT GTGTGGGGGG
  1561 ACCCTGATCA ACACCATCTG GGTGGTCTCC GCGGCCCACT GTTTCGACAA AATCAAGAAC
  1621 TGGAGGAACC TGATCGCCGT GCTGGGCCAG CACGACCTCA GCGAGCACGA CGGGGATGAG
  1681 CAGAGCCGGC GGGTGGCGCA GGTCATCATC CCCAGCACGT ACGTCCCGGG CACCACCAAC
  1741 CACGACATCG CGCTGCTCCG CCTGCACCAG CCCGTGGTCC TCACTGACCA TGTGGTGCCC
  1801 CTCTGCCTGC CCGAACGGAC GTTCTCTGAG AGGACGCTGG CCTTCGTGCG CTTCTCATTG
  1861 GTCAGCGGCT GGGGCCAGCT GCTGGACCGT GGCGCCACGG CCCTGGAGCT CATGGTCCTC
  1921 AACGTGCCCC GGCTGATGAC CCAGGACTGC CTGCAGCAGT CACGGAAGGT GGGAGACTCC
  1981 CCAAATATCA CGGAGTACAT GTTCTGTGCC GGCTACTCGG ATGGCAGCAA GGACTCCTGC
  2041 AAGGGGGACA GTGGAGGCCC ACATGCCACC CACTACCGGG GCACGTGGTA CCTGACGGGC
  2101 ATCGTCAGCT GGGGCCAGGG CTGCGCAACC GTGGGCCACT TTGGGGTGTA CACCAGGGTC
  2161 TCCCAGTACA TCGAGTGGCT GCAAAAGCTC ATGCGCTCAG AGCCACGCCC AGGAGTCCTC
  2221 CTGCGAGCCC CATTTCCCGG TGGCGGTGGC TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA
  2281 TCAGGTGGGG GTGGATCAGG CGGTGGAGGT TCCGGTGGCG GGGGATCAGA CAAAACTCAC
  2341 ACATGCCCAC CGTGCCCAGC ACCTGAACTC CTGGGAGGAC CGTCAGTCTT CCTCTTCCCC
  2401 CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG
  2461 GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
  2521 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
  2581 GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC
  2641 AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
  2701 GAACCACAGG TGTACACCCT GCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC
  2761 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
  2821 GGGCAGCCGG AGAACAACTA CAAGACCACG CCTCCCGTGT TGGACTCCGA CGGCTCCTTC
  2881 TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA
  2941 TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
  3001 CCGGGTAAAT GA
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FVII-060 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 AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
   61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
  121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
  181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
  241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
  301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
  361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
  421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
  481 GSALRPRVVG GAVVGGKVCP KGECPWQVLL LVNGAQLCGG TLINTIWVVS AAHCFDKIKN
  541 WRNLIAVLGE HDLSEHDGDE QSRRVAQVII PSTYVPGTTN HDIALLRLHQ PVVLTDHVVP
  601 LCLPERTFSE RTLAFVRFSL VSGWGQLLDR GATALELMVL NVPRLMTQDC LQQSRKVGDS
  661 PNITEYMFCA GYSDGSKDSC KGDSGGPHAT HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV
  721 SQYIEWLQKL MRSEPRPGVL LRAPFPGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSDKTH
  781 TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
  841 HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
  901 EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
  961 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK*
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DNA sequence for FVII-061

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   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
 601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCC
 661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGAGG ACCGTCAGTC
 721 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 901 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTTCCGGTG GCGGGGGATC CGGCGGTGGA GGTTCCGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGCCC TGCGGCCCCG GGTGGTGGGC GGCGCCATTG TGGGGGGCAA GGTGTGCCCC
1501 AAAGGGGAGT GTCCATGGCA GGTCCTGTTG TTGGTGAATG GAGCTCAGTT GTGTGGGGGG
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1621 TGGAGGAACC TGATCGCGGT GCTGGGCGAG CACGACCTCA GCGAGCACGA CGGGGATGAG
1681 CAGAGCCGGC GGGTGGCGCA GGTCATCATC CCCAGCACGT ACGTCCCGGG CACCACCAAC
1741 CACGACATCG CGCTGCTCCG CCTGCACCAG CCCGTGGTCC TCACTGACCA TGTGGTGCCC
1801 CTCTGCCTGC CCGAACGGAC GTTCTCTGAG AGGACGCTGG CCTTCGTGCG CTTCTCATTG
1861 GTCAGCGGCT GGGGCCAGCT GCTGGACCGT GGCGCCACGG CCCTGGAGCT CATGGTCCTC
1921 AACGTGCCCC GGCTGATGAC CCAGGACTGC CTGCAGCAGT CACGGAAGGT GGGAGACTCC
1981 CCAAATATCA CGGAGTACAT GTTCTGTGCC GGCTACTCGG ATGGCAGCAA GGACTCCTGC
2041 AAGGGGGACA GTGGAGGCCC ACATGCCACC CACTACCGGG GCACGTGGTA CCTGACGGGC
2101 ATCGTCAGCT GGGGCCAGGG CTGCGCAACC GTGGGCCACT TTGGGGTGTA CACCAGGGTC
2161 TCCCAGTACA TCGAGTGGCT GCAAAAGCTC ATGCGCTCAG AGCCACGCCC AGGAGTCCTC
2221 CTGCGAGCCC CATTTCCCGG TGGCGGTGGC TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA
2281 TCAGGTGGGG GTGGATCAGG CGGTGGAGGT TCCGGTGGCG GGGGATCAGA CAAAACTCAC
2341 ACATGCCCAC CGTGCCCAGC ACCTGAACTC CTGGGAGGAC CGTCAGTCTT CCTCTTCCCC
2401 CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG
2461 GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
2521 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
2581 GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC
2641 AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
2701 GAACCACAGG TGTACACCCT GCCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC
2761 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
2821 GGGCAGCCGG AGAACAACTA CAAGACCACG CCTCCCGTGT TGGACTCCGA CGGCTCCTTC
2881 TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA
2941 TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
3001 CCGGGTAAAT GA
```

FVII-061 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 AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
 241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
 361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
 421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
 481 GSALRPRVVG GAIVGGKVCP KGECPWQVLL LVNGAQLCGG TLINTIWVVS AAHCFDKIKN
 541 WRNLIAVLGE HDLSEHDGDE QSRRVAQVII PSTYVPGTTN HDIALLRLHQ PVVLTDHVVP
 601 LCLPERTFSE RTLAFVRFSL VSGWGQLLDR GATALELMVL NVPRLMTQDC LQQSRKVGDS
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661 PNITEYMFCA GYSDGSKDSC KGDSGGPHAT HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV
721 SQYIEWLQKL MRSEPRPGVL LRAPFPGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSDKTH
781 TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
841 HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
901 EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
961 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK*

DNA sequence for FVII-062

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCACGAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCA
661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGCGG ACCGTCAGTC
721 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
901 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTTCCGGTG GCGGGGGATC CGGCGGTGGA GGTTCCGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGGTG GTGGAGGATC CATTGTGGGG GGCAAGGTGT GCCCCAAAGG GGAGTGTCCA
1501 TGGCAGGTCC TGTTGTTGGT GAATGGAGCT CAGTTGTGTG GGGGGACCCT GATCAACACC
1561 ATCTGGGTGG TCTCCGCGGC CCACTGTTTC GACAAAATCA AGAACTGGAG GAACCTGATC
1621 GCGGTGCTGG GCGAGCACGA CCTCAGCGAG CACGACGGGG ATGAGCAGAG CCGGCGGGTG
1681 GCGCAGGTCA TCATCCCCAG CACGTACGTC CCGGGCACCA CCAACCACGA CATCGCGCTG
1741 CTCCGCCTGC ACCAGCCCGT GGTCCTCACT GACCATGTGG TGCCCCTCTG CCTGCCCGAA
1801 CGGACGTTCT CTGAGAGGAC GCTGGCCTTC GTGCGCTTCT CATTGGTCAG CGGCTGGGGC
1861 CAGCTGCTGG ACCGTGGCGC CACGGCCCTG GAGCTCATGG TCCTCAACGT GCCCCGGCTG
1921 ATGACCCAGG ACTGCCTGCA GCAGTCACGG AAGGTGGGAG ACTCCCCAAA TATCACGGAG
1981 TACATGTTCT GTGCCGGCTA CTCGGATGGC AGCAAGGACT CCTGCAAGGG GGACAGTGGA
2041 GGCCCACATG CCACCCACTA CCGGGGCACG TGGTACCTGA CGGGCATCGT CAGCTGGGGC
2101 CAGGGCTGCG CAACCGTGGG CCACTTTGGG GTGTACACCA GGGTCTCCCA GTACATCGAG
2161 TGGCTGCAAA AGCTCATGCG CTCAGAGCCA CGCCCAGGAG TCCTCCTGCG AGCCCCATTT
2221 CCCGGTGGCG GTGGCTCCGG CGGAGGTGGG TCCGGTGGCG GCGGATCAGG TGGGGGTGGA
2281 TCAGGCGGTG GAGGTTCCGG TGGCGGGGGA TCAGACAAAA CTCACACATG CCCACCGTGC
2341 CCAGCACCTG AACTCCTGGG AGGACCGTCA GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC
2401 ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA
2461 GACCCTGAGG TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA
2521 AAGCCGCGGG AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG
2581 CACCAGGACT GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
2641 GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC
2701 ACCCTGCCCC CATCCCGGGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC
2761 AAAGGCTTCT ATCCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC
2821 AACTACAAGA CCACGCCTCC CGTGTTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG
2881 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
2941 GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG TAAATGA

FVII-062 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker region connecting FVIIa 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 AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK

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  361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
  421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
  481 GSGGGGSIVG GKVCPKGECP WQVLLLVNGA QLCGGTLINT IWVVSAAHCF DKIKNWRNLI
  541 AVLGEHDLSE HDGDEQSRRV AQVIIPSTYV PGTTNHDIAL LRLHQPVVLT DHVVPLCLPE
  601 RTFSERTLAF VRFSLVSGWG QLLDRGATAL ELMVLNVPRL MTQDCLQQSR KVGDSPNITE
  661 YMFCAGYSDG SKDSCKGDSG GPHATHYRGT WYLTGIVSWG QGCATVGHFG VYTRVSQYIE
  721 WLQKLMRSEP RPGVLLRAPF PGGGGSGGGG SGGGGSGGGG SGGGGSGGGG SDKTHTCPPC
  781 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT
  841 KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY
  901 TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK
  961 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK*
```

DNA sequence for FVII-090

```
    1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
   61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
  121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
  181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
  241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
  301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
  361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
  421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
  481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
  541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
  601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCA
  661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGCGG ACCGTCAGTC
  721 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
  781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
  841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
  901 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
  961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
 1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
 1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
 1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
 1201 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
 1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
 1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
 1381 GGTTCCGGTG GCGGGGGATC CGGCGGTGGA GGTTCCGGTG GGGGTGGATC AGGAGGAGGT
 1441 GGTTCAGCCC TGCGGCCCCG GATTGTGGGG GGCAAGGTGT GCCCCAAAGG GGAGTGTCCA
 1501 TGGCAGGTCC TGTTGTTGGT GAATGGAGCT CAGTTGTGTG GGGGGACCCT GATCAACACC
 1561 ATCTGGGTGG TCTCCGCGGC CCACTGTTTC GACAAAATCA AGAACTGGAG GAACCTGATC
 1621 GCGGTGCTGG GCGAGCACGA CCTCAGCGAG CACGACGGGG ATGAGCAGAG CCGGCGGGTG
 1681 GCGCAGGTCA TCATCCCCAG CACGTACGTC CCGGGCACCA CCAACCACGA CATCGCGCTG
 1741 CTCCGCCTGC ACCAGCCCGT GGTCCTCACT GACCATGTGG TGCCCCTCTG CCTGCCCGAA
 1801 CGGACGTTCT CTGAGAGGAC GCTGGCCTTC GTGCGCTTCT CATTGGTCAG CGGCTGGGGC
 1861 CAGCTGCTGG ACCGTGGCGC CACGGCCCTG GAGCTCATGG TCCTCAACGT GCCCCGGCTG
 1921 ATGACCCAGG ACTGCCTGCA GCAGTCACGG AAGGTGGGAG ACTCCCCAAA TATCACGGAG
 1981 TACATGTTCT GTGCCGGCTA CTCGGATGGC AGCAAGGACT CCTGCAAGGG GGACAGTGGA
 2041 GGCCCACATG CCACCCACTA CCGGGGCACG TGGTACCTGA CGGGCATCGT CAGCTGGGGC
 2101 CAGGGCTGCG CAACCGTGGG CCACTTTGGG GTGTACACCA GGGTCTCCCA GTACATCGAG
 2161 TGGCTGCAAA AGCTCATGCG CTCAGAGCCA CGCCCAGGAG TCCTCCTGCG AGCCCCATTT
 2221 CCCGGTGGCG GTGGCTCCGG CGGAGGTGGG TCCGGTGGCG GCGGATCAGG TGGGGGTGGA
 2281 TCAGGCGGTG GAGGTTCCGG TGGCGGGGGA TCAGACAAAA CTCACACATG CCCACCGTGC
 2341 CCAGCACCTG AACTCCTGGG AGGACCGTCA GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC
 2401 ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA
 2461 GACCCTGAGG TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA
 2521 AAGCCGCGGG AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG
 2581 CACCAGGACT GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
 2641 GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC
 2701 ACCCTGCCCC CATCCCGGGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC
 2761 AAAGGCTTCT ATCCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC
 2821 AACTACAAGA CCACGCCTCC CGTGTTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG
 2881 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
 2941 GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG TAAATGA
```

FVII-090 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 AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
```

```
     61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
    121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
    181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
    241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
    301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
    361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
    421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
    481 GSALRPRIVG GKVCPKGECP WQVLLLVNGA QLCGGTLINT IWVVSAAHCF DKIKNWRNLI
    541 AVLGEHDLSE HDGDEQSRRV AQVIIPSTYV PGTTNHDIAL LRLHQPVVLT DHVVPLCLPE
    601 RTFSERTLAF VRFSLVSGWG QLLDRGATAL ELMVLNVPRL MTQDCLQQSR KVGDSPNITE
    661 YMFCAGYSDG SKDSCKGDSG GPHATHYRGT WYLTGIVSWG QGCATVGHFG VYTRVSQYIE
    721 WLQKLMRSEP RPGVLLRAPF PGGGGSGGGG SGGGGSGGGG SGGGGSGGGG SDKTHTCPPC
    781 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT
    841 KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY
    901 TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK
    961 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK*

DNA sequence for FVII-100
     1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
    61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
   121 GCGTTCCTGG AGGAGCTCCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
   181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
   241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
   301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
   361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
   421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
   481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
   541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
   601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCC
   661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGAGG ACCGTCAGTC
   721 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
   781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
   841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
   901 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
   961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
  1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
  1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
  1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
  1201 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
  1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
  1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
  1381 GGTTCCGGTG GCGGGGGATC CGGCGGTGGA GGTTCCGGTG GGGGTGGATC AGGAGGAGGT
  1441 GGTTCAGCCC TGCGGCCCCG GATTGTGGGG GGCAAGGTGT GCCCCAAAGG GGAGTGTCCA
  1501 TGGCAGGTCC TGTTGTTGGT GAATGGAGCT CAGTTGTGTG GGGGGACCCT GATCAACACC
  1561 ATCTGGGTGG TCTCCGCGGC CCACTGTTTC GACAAAATCA AGAACTGGAG GAACCTGATC
  1621 GCGGTGCTGG GCGAGCACGA CCTCAGCGAG CACGACGGGG ATGAGCAGAG CCGGCGGGTG
  1681 GCGCAGGTCA TCATCCCCAG CACGTACGTC CCGGGCACCA CCAACCACGA CATCGCGCTG
  1741 CTCCGCCTGC ACCAGCCCGT GGTCCTCACT GACCATGTGG TGCCCCTCTG CCTGCCCGAA
  1801 CGGACGTTCT CTGAGAGGAC GCTGGCCTTC GTGCGCTTCT CATTGGTCAG CGGCTGGGGC
  1861 CAGCTGCTGG ACCGTGGCGC CACGGCCCTG GAGCTCATGG TCCTCAACGT GCCCCGGCTG
  1921 ATGACCCAGG ACTGCGAGGC CAGCTACCCC GGCAAGATCA CGGAGTACAT GTTCTGTGCC
  1981 GGCTACTCGG ATGGCAGCAA GGACTCCTGC AAGGGGGACA GTGGAGGCCC ACATGCCACC
  2041 CACTACCGGG GCACGTGGTA CCTGACGGGC ATCGTCAGCT GGGGCCAGGG CTGCGCAACC
  2101 GTGGGCCACT TTGGGGTGTA CACCAGGGTC TCCCAGTACA TCGAGTGGCT GCAAAAGCTC
  2161 ATGCGCTCAG AGCCACGCCC AGGAGTCCTC CTGCGAGCCC CATTTCCCGG TGGCGGTGGC
  2221 TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA TCAGGTGGGG GTGGATCAGG CGGTGGAGGT
  2281 TCCGGTGGCG GGGGATCAGA CAAAACTCAC ACATGCCCAC CGTGCCCAGC ACCTGAACTC
  2341 CTGGGAGGAC CGTCAGTCTT CCTCTTCCCC CCAAAACCCA AGGACACCCT CATGATCTCC
  2401 CGGACCCCTG AGGTCACATG CGTGGTGGTG GACGTGAGCC ACGAAGACCC TGAGGTCAAG
  2461 TTCAACTGGT ACGTGGACGG CGTGGAGGTG CATAATGCCA AGACAAAGCC GCGGGAGGAG
  2521 CAGTACAACA GCACGTACCG TGTGGTCAGC GTCCTCACCG TCCTGCACCA GGACTGGCTG
  2581 AATGGCAAGG AGTACAAGTG CAAGGTCTCC AACAAAGCCC TCCCAGCCCC CATCGAGAAA
  2641 ACCATCTCCA AAGCCAAAGG GCAGCCCCGA GAACCACAGG TGTACACCCT GCCCCCATCC
  2701 CGGGATGAGC TGACCAAGAA CCAGGTCAGC CTGACCTGCC TGGTCAAAGG CTTCTATCCC
  2761 AGCGACATCG CCGTGGAGTG GGAGAGCAAT GGGCAGCCGG AGAACAACTA CAAGACCACG
  2821 CCTCCCGTGT TGGACTCCGA CGGCTCCTTC TTCCTCTACA GCAAGCTCAC CGTGGACAAG
  2881 AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAAC
  2941 CACTACACGC AGAAGAGCCT CTCCCTGTCT CCGGGTAAAT GA
```

FVII-100 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, the thrombin cleavage site is shown in dashed underline, and the trypsin 170 loop region is wave underlined

```
   1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
 241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
 361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
 421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
 481 GSALRPRIVG GKVCPKGECP WQVLLLVNGA QLCGGTLINT IWVVSAAHCF DKIKNWRNLI
 541 AVLGEHDLSE HDGDEQSPRV AQVIIPSTYV PGTTNHDIAL LRLHQPVVLT DHVVPLCLPE
 601 RTFSERTLAF VRFSLVSGWG QLLDRGATAL ELMVLNVPRL MTQDCLQASY PGKITENMFCA
 661 GYSDGSKDSC KGDSGGPHAT HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV SQYIEWLQKL
 721 MRSEPRPGVL LRAPFPGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSDKTH TCPPCPAPEL
 781 LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
 841 QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS
 901 RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF FLYSKLTVDK
 961 SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK*
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DNA sequence for FVII-115

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   1      ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG
       CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGC CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
 601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCA
 661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGCGG ACCGTCAGTC
 721 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGCGGAG AGCAGTACAA CAGCACCTAC
 901 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
 961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTTCCGGTG GCGGGGGATC CGGCGGTGGA GGTTCCGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGCCC TGCGGCCCCG GATTGTGGGG GGCAAGGACT GCCCCAAAGG GGAGTGTCCA
1501 TGGCAGGTCC TGTTGTTGGT GAATGGAGCT CAGTTGTGTG GGGGGACCCT GATCAACACC
1561 ATCTGGGTGG TCTCCGCGGC CCACTGTTTC GACAAAATCA AGAACTGGAG GAACCTGATC
1621 GCGGTGCTGG GCGAGCACGA CCTCAGCGAG CACGACGGGG ATGAGCAGAG CCGGCGGGTG
1681 GCGCAGGTCA TCATCCCCAG CACGTACGTC CCGGGCACCA CCAACCACGA CATCGCGCTG
1741 CTCCGCCTGC ACCAGCCCGT GGTCCTCACC GACCATGTGG TGCCCCTCTG CCTGCCCGAA
1801 CGGACGTTCT CTGAGAGGAC GCTGGCCTTC GTGCGCTTCT CATTGGTCAG CGGCTGGGGC
1861 CAGCTGCTGG ACCGTGGCGC CACGGCCCTG GTACTCCAAG TCCTCAACGT GCCCCGGCTG
1921 ATGACCCAGG ACTGCCTGCA GCAGTCACGG AAGGTGGGAG ACTCCCCAAA TATCACGGAG
1981 TACATGTTCT GTGCCGGCTA CTCGGATGGC AGCAAGGACT CCTGCAAGGG GGACAGTGGA
2041 GGCCCACATG CCACCCACTA CCGGGGCACG TGGTACCTGA CGGGCATCGT CAGCTGGGGC
2101 CAGGGCTGCG CAACCGTGGG CCACTTTGGG GTGTACACCA GGGTCTCCCA GTACATCGAG
2161 TGGCTGCAAA AGCTCATGCG CTCAGAGCCA CGCCCAGGAG TCCTCCTGCG AGCCCCATTT
2221 CCCGGTGGCG GTGGCTCCGG CGGAGGTGGG TCCGGTGGCG GCGGATCAGG TGGGGGTGGA
2281 TCAGGCGGTG GAGGTTCCGG TGGCGGGGGA TCAGACAAAA CTCACACATG CCCACCGTGC
2341 CCAGCACCTG AACTCCTGGG AGGACCGTCA GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC
2401 ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA
2461 GACCCTGAGG TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA
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2521 AAGCCGCGGG AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG
2581 CACCAGGACT GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
2641 GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC
2701 ACCCTGCCCC CATCCCGGGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC
2761 AAAGGCTTCT ATCCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC
2821 AACTACAAGA CCACGCCTCC CGTGTTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG
2881 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
2941 GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG TAAATGA
```

FVII-115 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, the thrombin cleavage site is shown in dashed underline, and the three point mutations in FVIIa (V158D, E296V and M298Q) are in bold and underlined

```
1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61 SFEEARETFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
241 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG GSGGGGSGGG GSGGGGSGGG
481 GSALRPRIVG GKDCPKGECP WQVLLLVNGA QLCGGTLINT IWVVSAAHCF DKIKNWRNLI
541 AVLGEHDLSE HDGDEQSRRV AQVIIPSTYV PGTTNHDIAL LRLHQPVVLT DHVVPLCLPE
601 RTFSERTLAF VRFSLVSGWG QLLDRGATAL VLQVLNVPRL MTQDCLQQSR KVGDSPNITE
661 YMFCAGYSDG SKDSCKGDSG GPHATHYRGT WYLTGIVSWG QGCATVGHFG VYTRVSQYIE
721 WLQKLMRSEP RPGVLLRAPF PGGGGSGGGG SGGGGSGGGG SGGGGSGGGG SDKTHTCPPC
781 PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT
841 KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY
901 TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK
961 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK*
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DNA sequence for FVII-118

```
1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCGCC CTGCGGCCCC GGATTGTGGG GGGCAAGGTG
601 TGCCCCAAAG GGGAGTGTCC ATGGCAGGTC CTGTTGTTGG TGAATGGAGC TCAGTTGTGT
661 GGGGGGACCC TGATCAACAC CATCTGGGTG GTCTCCGCGG CCCACTGTTT CGACAAAATC
721 AAGAACTGGA GGAACCTGAT CGCGGTGCTG GGCGAGCACG ACCTCAGCGA GCACGACGGG
781 GATGAGCAGA GCCGGCGGGT GGCGCAGGTC ATCATCCCCA GCACGTACGT CCCGGGCACC
841 ACCAACCACG ACATCGCGCT GCTCCGCCTG CACCAGCCCG TGGTCCTCAC TGACCATGTG
901 GTGCCCCTCT GCCTGCCCGA ACGGACGTTC TCTGAGAGGA CGCTGGCCTT CGTGCGCTTC
961 TCATTGGTCA GCGGCTGGGG CCAGCTGCTG GACCGTGGCG CCACGGCCCT GGAGCTCATG
1021 GTCCTCAACG TGCCCCGGCT GATGACCCAG GACTGCCTGC AGCAGTCACG GAAGGTGGGA
1081 GACTCCCCAA ATATCACGGA GTACATGTTC TGTGCCGGCT ACTCGGATGG CAGCAAGGAC
1141 TCCTGCAAGG GGGACAGTGG AGGCCCACAT GCCACCCACT ACCGGGGCAC GTGGTACCTG
1201 ACGGGCATCG TCAGCTGGGG CCAGGGCTGC GCAACCGTGG GCCACTTTGG GGTGTACACC
1261 AGGGTCTCCC AGTACATCGA GTGGCTGCAA AAGCTCATGC GCTCAGAGCC ACGCCCAGGA
1321 GTCCTCCTGC GAGCCCCATT TCCCGGTGGC GGTGGCTCCG GCGGAGGTGG GTCCGGTGGC
1381 GGCGGATCAG GTGGGGGTGG ATCAGGCGGT GGAGGTTCCG GTGGCGGGGG ATCCGACAAA
1441 ACTCACACAT GCCCACCGTG CCCAGCTCCG GAACTCCTGG GCGGACCGTC AGTCTTCCTC
1501 TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG
1561 GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA ACTGGTACGT GGACGGCGTG
1621 GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG
1681 GTCAGCGTCC TCACCGTCCT GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG
1741 GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
1801 CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG ATGAGCTGAC CAAGAACCAG
1861 GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG ACATCGCCGT GGAGTGGGAG
1921 AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGTTGGA CTCCGACGGC
1981 TCCTTCTTCC TCTACAGCAA GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC
2041 TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC
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2101 CTGTCTCCGG GTAAAGGTGG CGGCGGATCA GGTGGGGGTG GATCAGGCGG TGGAGGTTCC
2161 GGTGGCGGGG GATCAGACAA AACTCACACA TGCCCACCGT GCCCAGCACC TGAACTCCTG
2221 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-118 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 FVII heavy chain to Fc region is underlined, and the linker region connecting the Fc regions is shown in bold

```
   1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGA LRPRIVGGKV CPKGECPWQV LLLVNGAQLC GGTLINTIWV VSAAHCFDKI
 241 KNWRNLIAVL GEHDLSEHDG DEQSRRVAQV IIPSTYVPGT TNHDIALLRL HQPVVLTDHV
 301 VPLCLPERTF SERTLAFVRF SLVSGWGQLL DRGATALELM VLNVPRLMTQ DCLQQSRKVG
 361 DSPNITEYMF CAGYSDGSKD SCKGDSGGPH ATHYRGTWYL TGIVSWGQGC ATVGHFGVYT
 421 RVSQYIEWLQ KLMRSEPRPG VLLRAPFPGG GGSGGGGSGG GGSGGGGSGG GGSGGGGSDK
 481 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
 541 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ
 601 PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
 661 SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
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GGGGS GGGGSGGGGS GGGGS

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 721 DKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF
 781 NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT
 841 ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP
 901 PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK*
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DNA sequence for FVII-119

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   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGC CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCGGA GGAGGTGGTT CAGCCCTGCG GCCCCGGATT
 601 GTGGGGGGCA AGGTGTGCCC CAAAGGGCAG TGTCCATGCC AGGTCCTGTT GTTGGTGAAT
 661 GGAGCTCAGT TGTGTGGGGG GACCCTGATC AACACCATCT GGGTGGTCTC CGCGGCCCAC
 721 TGTTTCGACA AAATCAAGAA CTGGAGGAAC CTGATCGCGG TGCTGGGCGA GCACGACCTC
 781 AGCGAGCACG ACGGGGATGA GCAGAGCCGG CGGGTGGCGC AGGTCATCAT CCCCAGCACG
 841 TACGTCCCGG GCACCACCAA CCACGACATC GCGCTGCTCC GCCTGCACCA GCCCGTGGTC
 901 CTCACTGACC ATGTGGTGCC CCTCTGCCTG CCCGAACGGA CGTTCTCTGA GAGGACGCTG
 961 GCCTTCGTGC GCTTCTCATT GGTCAGCGGC TGGGGCCAGC TGCTGGACGG TGGCGCCACG
1021 GCCCTGGAGC TCATGGTCCT CAACGTGCCC CGGCTGATGA CCCAGGACTG CCTGCAGCAG
1081 TCACGGAAGG TGGGAGACTC CCCAAATATC ACGGAGTACA TGTTCTGTGC CGGCTACTCG
1141 GATGGCAGCA AGGACTCCTG CAAGGGGGAC AGTGGAGGCC CACATGCCAC CCACTACCGG
1201 GGCACGTGGT ACCTGACGGG CATCGTCAGC TGGGGCCAGG GCTGCGCAAC CGTGGGCCAC
1261 TTTGGGGTGT ACACCAGGGT CTCCCAGTAC ATCGAGTGGC TGCAAAAGCT CATGCGCTCA
1321 GAGCCACGCC CAGGAGTCCT CCTGCGAGCC CCATTTCCCG GTGGCGGTGG CTCCGGCGGA
1381 GGTGGGTCCG GTGGCGGCGG ATCAGGTGGG GGTGGATCAG GCGGTGGAGG TTCCGGTGGC
1441 GGGGGATCCG ACAAAACTCA CACATGCCCA CCGTGCCCAG CTCCGGAACT CCTGGGCGGA
1501 CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
1561 GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG
1621 TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA GCAGTACAAC
1681 AGCACGTACC GTGTGGTCAG CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG
1741 GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
1801 AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGATGAG
1861 CTGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG GCTTCTATCC CAGCGACATC
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1921 GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT ACAAGACCAC GCCTCCCGTG
1981 TTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA CCGTGGACAA GAGCAGGTGG
2041 CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
2101 CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA GGTGGCGGCG GATCAGGTGG GGGTGGATCA
2161 GGCGGTGGAG GTTCCGGTGG CGGGGGATCA GACAAAACTC ACACATGCCC ACCGTGCCCA
2221 GCACCTGAAC TCCTGGGAGG ACCGTCAGTC TTCCTCTTCC CCCCAAAACC CAAGGACACC
2281 CTCATGATCT CCCGGACCCC TGAGGTCACA TGCGTGGTGG TGGACGTGAG CCACGAAGAC
2341 CCTGAGGTCA AGTTCAACTG GTACGTGGAC GGCGTGGAGG TGCATAATGC CAAGACAAAG
2401 CCGCGGGAGG AGCAGTACAA CAGCACGTAC CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC
2461 CAGGACTGGC TGAATGGCAA GGAGTACAAG TGCAAGGTCT CCAACAAAGC CCTCCCAGCC
2521 CCCATCGAGA AAACCATCTC CAAAGCCAAA GGGCAGCCCC GAGAACCACA GGTGTACACC
2581 CTGCCCCCAT CCCGCGATGA GCTGACCAAG AACCAGGTCA GCCTGACCTG CCTGGTCAAA
2641 GGCTTCTATC CCAGCGACAT CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAAC
2701 TACAAGACCA CGCCTCCCGT GTTGGACTCC GACGGCTCCT TCTTCCTCTA CAGCAAGCTC
2761 ACCGTGGACA AGAGCAGGTG GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGCATGAG
2821 GCTCTGCACA ACCACTACAC GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA ATGA
```

FVH-119 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, the thrombin cleavage site with GGGS linker is shown in dashed underline, the linker region connecting FVH heavy chain to Fc region is underlined, and the linker region connecting the Fc regions is shown in bold

```
   1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGG GGGSALRFRI VGGKVCPKGE CPWQVLLLVN GAQLCGGTLI NTIWVVSAAH
 241 CFDKIKNWRN LIAVLGEHDL SEHDGDEQSR RVAQVIIPST YVPGTTNHDI ALLRLHQPVV
 301 LTDHVVPLCL PERTFSERTL AFVRFSLVSG WGQLLDRGAT ALELMVLNVP RLMTQDCLQQ
 361 SRKVGDSPNI TEYMFCAGYS DGSKDSCKGD SGGPHATHYR GTWYLTGIVS WGQGCATVGH
 421 FGVYTRVSQY IEWLQKLMRS EPRPGVLLRA PFPGGGGSGG GGSGGGGSGG GGSGGGGSGG
 481 GGSDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW
 541 YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
 601 KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
 661 LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK GGGGSGGGGS
 721 GGGGSGGGGS DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
 781 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 841 PIEKTISKAK GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN
 901 YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*
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DNA sequence for FVH-127

```
   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTCCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGCCCCGCGA GATCTTCAAG GACGCGGACA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTCAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCGCC CTGCGGCCCC GGATTGTGGG GGGCAAGGTG
 601 TGCCCCAAAG GGGAGTGTCC ATGGCAGGTC CTGTTGTTGG TGAATGGAGC TCAGTTGTGT
 661 GGGGGGACCC TGATCAACAC CATCTGGGTG GTCTCCGCGG CCCACTGTTT CGACAAAATC
 721 AAGAACTGGA GGAACCTGAT CGCGGTGCTG GGCGAGCACG ACCTCAGCGA GCACGACGGG
 781 GATGAGCAGA GCCGGCGGGT GGCGCAGGTC ATCATCCCCA GCACGTACGT CCCGGGCACC
 841 ACCAACCACG ACATCGCGCT GCTCCGCCTG CACCAGCCCG TGGTCCTCAC TGACCATGTG
 901 GTGCCCCTCT GCCTGCCCGA ACGGACGTTC TCTGAGAGGA CGCTGGCCTT CGTGCGCTTC
 961 TCATTGGTCA GCGGCTGGGG CCAGCTGCTG GACCGTGGCG CCACGGCCCT GGAGCTCATG
1021 GTCCTCAACG TGCCCCGGCT GATGACCCAG GACTGCGAGG CCAGCTACCC CGGCAAGATC
1081 ACGGAGTACA TGTTCTGTGC CGGCTACTCG GATGGCAGCA AGGACTCCTG CAAGGGGGAC
1141 AGTGGAGGCC CACATGCCAC CCACTACCGG GGCACGTGGT ACCTGACGGG CATCGTCAGC
1201 TGGGGCCAGG GCTGCGCAAC CGTGGGCCAC TTTGGGGTGT ACACCAGGGT CTCCCAGTAC
1261 ATCGAGTGGC TGCAAAAGCT CATGCGCTCA GAGCCACGCC CAGGAGTCCT CCTGCGAGCC
1321 CCATTTCCCG GTGGCGGTGG CTCCGGCGGA GGTGGGTCCG GTGGCGGCGG ATCAGGTGGG
1381 GGTGGATCAG GCGGTGGAGG TTCCGGTGGC GGGGGATCAG ACAAAACTCA CACATGCCCA
1441 CCGTGCCCAG CTCCGGAACT CCTGGGCGGA CCGTCAGTCT TCCTCTTCCC CCCAAAACCC
1501 AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC
1561 CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC
1621 AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC
1681 GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC
1741 CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG
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1801 GTGTACACCC TGCCCCCATC CGGGGATGAG 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 CTCTGCACAA 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 shown in bold

```
   1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGA LRPRIVGGKV CPKGECPWQV LLLVNGAQLC GGTLINTIWV VSAAHCFDKI
 241 KNWRNLIAVL GEHDLSEHDG DEQSRRVAQV IIPSTYVPGT TNHDIALLRL HQPVVLTDHV
 301 VPLCLPERTF SERTLAFVRF SLVSGWGQLL DRGATALELM VLNVPRLMTQ DCLQASYPGK
 361 TEYMFCAGYS DGSKDSCKGD SGGPHATHYR GTWYLTGIVS WGQGCATVGH FGVYTRVSQY
 421 IEWLQKLMRS EPRPGVLLRA PFPGGGGSGG GGSGGGGSGG GGSGGGGSGG GGSDKTHTCP
 481 PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
 541 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
 601 VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY
 661 SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK GGGGSGGGGS GGGGSGGGGS
 721 DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
 781 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
 841 GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
 901 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQRS LSLSPGK*
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DNA sequence for FVII-125

```
   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTCCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCCCAAAGGG
 601 GAGTGTCCAT GGCAGGTCCT GTTGTTGGTG AATGGAGCTC AGTTGTGTGG GGGGACCCTG
 661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAAAATCAA GAACTGGAGG
 721 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
 781 CGGCGGGTGG CGCAGGTCAT CATCCCCAGC ACGTACGTCC CGGGCACCAC CAACCACGAC
 841 ATCGCGCTGC TCCGCCTGCA CCAGCCCGTG GTCCTCACTG ACCATGTGGT GCCCCTCTGC
 901 CTGCCCGAAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TGCGCTTCTC ATTGGTCAGC
 961 GGCTGGGGCC AGCTGCTGGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
1021 CCCCGGCTGA TGACCCAGGA CTGCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
1081 ATCACGGAGT ACATGTTCTG TGCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAGTGGAG GCCCACATGC CACCCACTAC CGGGGCACGT GGTACCTGAC GGGCATCGTC
1201 AGCTGGGGCC AGGGCTGCGC AACCGTGGGC CACTTTGGGG TGTACACCAG GGTCTCCCAG
1261 TACATCGAGT GGCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCCAGGAGT CCTCCTGCGA
1321 GCCCCATTTC CCGGTGGCGG TGGCTCCGGC GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
1381 GGGGGTGGAT CAGGCGGTGG AGGTTCCGGT GGCGGGGGAT CCGACATCGT GATGACCCAG
1441 GCCGCCCCCA GCGTGCCCGT GACCCCCGGC GAGAGCGTGA GCATCAGCTG CCGGAGCAGC
1501 CGGAGCCTGC TGCACAGCAA CGGCAACACC TACCTGTGCT GGTTCCTGCA GCGGCCCGGC
1561 CAGAGCCCCC AGCTGCTGAT CTACCGGATG AGCAACCTGG CCAGCGGCGT GCCCGACCGG
1621 TTCAGCGGCA GCGGCAGCGG CACCGCCTTC ACCCTGCGGA TCAGCCGGGT GGAGGCCGAG
1681 GACGTGGGCG TGTACTACTG CATGCAGCAC CTGGAGTACC CCTTCACCTT CGGCAGCGGC
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1741 ACCAAGCTGG AGATCAAGCG GGGCGGCGGC GGCAGCGGCG GCGGCGGCAG CGGCGGCGGC
1801 GGCAGCCAGG TGCAGCTGCA GCAGAGCGGC GCCGAGCTGG TGCGGCCCGG CACCAGCGTG
1861 AAGATCAGCT GCAAGGCCAG CGGCTACACC TTCACCAACT ACTGGCTGGG CTGGGTGAAG
1921 CAGCGGCCCG GCCACGGCCT GGAGTGGATC GGCGACATCT ACCCCGGCGG CGGCTACAAC
1981 AAGTACAACG AGAACTTCAA GGGCAAGGCC ACCCTGACCG CCGACACCAG CAGCAGCACC
2041 GCCTACATGC AGCTGAGCAG CCTGACCAGC GAGGACAGCG CCGTGTACTT CTGCGCCCGG
2101 GAGTACGGCA ACTACGACTA CGCCATGGAC AGCTGGGGCC AGGGCACCAG CGTGACCGTG
2161 AGCAGCTGA
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FVII-125 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, and linker region connecting FVII to AP3 is bold, and AP3 scFv is italicized

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   1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
 241 NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
 301 LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
 361 ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
 421 YIEWLQKLMR SEPRPGVLLR APFP*GGGGSG GGGSGGGGSG GGGSGGGGSG GGGS*DIVMTQ
 481 AAPSVPVTPG ESVSISCRSS RSLLHSNGNT YLCWFLQRPG QSPQLLIYRM SNLASGVPDR
 541 FSGSGSGTAF TLRISRVEAE DVGVYYCMQH LEYPFTFGSG TKLEIKRGGG GSGGGGSGGG
 601 GSQVQLQQSG AELVRPGTSV KISCKASGYT FTNYWLGWVK QRPGHGLEWI GDIYPGGGYN
 661 KYNENFKGKA TLTADTSSST AYMQLSSLTS EDSAVYFCAR EYGNYDYAMD SWGQGTSVTV
 721 SS*
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DNA sequence for FVII-067

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   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCCCAAAGGG
 601 GAGTGTCCAT GGCAGGTCCT GTTGTTGGTG AATGGAGCTC AGTTGTGTGG GGGGACCCTG
 661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAAAATCAA GAACTGGAGG
 721 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
 781 CGGCGGGTGG CGCAGGTCAT CATCCCCAGC ACGTACGTCC CGGGCACCAC CAACCACGAC
 841 ATCGCGCTGC TCCGCCTGCA CCAGCCCGTG GTCCTCACTG ACCATGTGGT GCCCCTCTGC
 901 CTGCCCGAAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TGCGCTTCTC ATTGGTCAGC
 961 GGCTGGGGCC AGCTGCTGGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
1021 CCCCGGCTGA TGACCCAGGA CTGCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
1081 ATCACGGAGT ACATGTTCTG TGCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAGTGGAG GCCCACATGC CACCCACTAC CGGGGCACGT GGTACCTGAC GGGCATCGTC
1201 AGCTGGGGCC AGGGCTGCGC AACCGTGGGC CACTTTGGGG TGTACACCAG GGTCTCCCAG
1261 TACATCGAGT GGCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCCAGGAGT CCTCCTGCGA
1321 GCCCCATTTC CCGGTGGCGG TGGCTCCGGC GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
1381 GGGGGTGGAT CAGGCGGTGG AGGTTCCGGT GGCGGGGGAT CCGACAAAAC TCACACATGC
1441 CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG TCTTCCTCTT CCCCCCAAAA
1501 CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG
1561 AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
1621 GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCCTC
1681 ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA
1741 GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
1801 CAGGTGTACA CCCTGCCCCC ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGACC
1861 TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
1921 CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGTTGGACT CCGACGGCTC CTTCTTCCTC
1981 TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
2041 GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT
2101 AAAGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGCGGTG GAGGTTCCGG TGGCGGGGGA
2161 TCAGACAAAA CTCACACATG CCCACCGTGC CCAGCACCTG AACTCCTGGG AGGACCGTCA
2221 GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
2281 ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
2341 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA CAACAGCACG
2401 TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT GGCTGAATGG CAAGGAGTAC
2461 AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAGCC
2521 AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGGGA TGAGCTGACC
2581 AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
2641 GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC CGTGTTGGAC
2701 TCCGACGGCT CCTTCTTCCT CTACAGCAAG CTCACCGTCG ACAAGAGCAG GTGGCAGCAG
2761 GGGAACGTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG
2821 AGCCTCTCCC TGTCTCCGGG TAAAGGTGGC GGTGGCTCCG GCGGAGGTGG GTCCGGTGGC
2881 GGCGGATCAG GTGGGGGTGG ATCAGGCGGT GGAGGTTCCG GTGGCGGGGG ATCAGCGCAG
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2941 GTGCAGCTGC AGGAGTCTGG GGGAGGCTTG GTACAGCCTG GGGGGTCCCT GAGACTCTCC
3001 TGTGCAGCCT CTGGATTCAT GTTTAGCAGG TATGCCATGA GCTGGGTCCG CCAGGCTCCA
3061 GGGAAGGGGC CAGAGTGGGT CTCAGGTATT AGTGGTAGTG GTGGTAGTAC ATACTACGCA
3121 GACTCCGTGA AGGGCCGGTT CACCGTCTCC AGAGACAATT CCAAGAACAC GCTGTATCTG
3181 CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTATATT ACTGCGCCCG GGGCGCCACC
3241 TACACCAGCC GGAGCGACGT GCCCGACCAG ACCAGCTTCG ACTACTGGGG CCAGGGAACC
3301 CTGGTCACCG TCTCCTCAGG GAGTGCATCC GCCCCAAAGC TTGAAGAAGG TGAATTTTCA
3361 GAAGCACGCG TATCTGAACT GACTCAGGAC CCTGCTGTGT CTGTGGCCTT GGGACAGACA
3421 GTCAGGATCA CATGCCAAGG AGACAGCCTC AGAAACTTTT ATGCAAGCTG GTACCAGCAG
3481 AAGCCAGGAC AGGCCCCTAC TCTTGTCATC TATGGTTTAA GTAAAAGGCC CTCAGGGATC
3541 CCAGACCGAT TCTCTGCCTC CAGCTCAGGA AACACAGCTT CCTTGACCAT CACTGGGGCT
3601 CAGGCGGAAG ATGAGGCTGA CTATTACTGC CTGCTGTACT ACGGCGGCGG CCAGCAGGGC
3661 GTGTTCGGCG GCGGCACCAA GCTGACCGTC CTACGTCAGC CCAAGGCTGC CCCCTCGGTC
3721 ACTCTGTTCC CGCCCTCTTC TGCGGCCTGA
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FVII-067 amino 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 Fc regions is dashed underlined and linker connecting the Fc region to SCE5 is in bold

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   1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
 241 NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
 301 LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
 361 ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
 421 YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGGGGGGSG GGGGGGGGSG GGGSDKTHTC
 481 PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
 541 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
 601 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
 661 YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGGSGGGG
 721 SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
 781 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
 841 KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD
 901 SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGSGGGGSGG
 961 GGSGGGGSGG GGSGGGGSAQ VQLQESGGGL VQPGGSLRLS CAASGFMFSR YAMSWVRQAP
1021 GKGPEWVSGI SGSGGSTYYA DSVKGRFTVS RDNSKNTLYL QMNSLRAEDT AVYYCARGAI
1081 YTSRSDVPDQ TSFDYWGQGT LVTVSSGSAS APKLEEGEFS EARVSELTQD PAVSVALGQT
1141 VRITCQGDSL RNFYASWYQQ KPGQAPTLVI YGLSKRPSGI PDRFSASSSG NTASLTITGA
1201 QAEDEADYYC LLYYGGGQQG VFGGGTKLTV LRQPKAAPSV TLFPPSSAA*
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DNA sequence for FVII-094

```
   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGGAGAAC GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCCCAAAGGG
 601 GAGTGTCCAT GGCAGGTCCT GTTGTTGGTG AATGGAGCTC AGTTGTGTGG GGGGACCCTG
 661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAAAATCAA GAACTGGAGG
 721 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
 781 CGGCGGGTGG CGCAGGTCAT CATCCCCAGC ACGTACGTCC CGGGCACCAC CAACCACGAC
 841 ATCGCGCTGC TCCGCCTGCA CCAGCCCGTG GTCCTCACTG ACCATGTGGT GCCCCTCTGC
 901 CTGCCCGAAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TGCGCTTCTC ATTGGTCAGC
 961 GGCTGGGGCC AGCTGCTGGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
1021 CCCCGGCTGA TGACCCAGGA CTGCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
1081 ATCACGGAGT ACATGTTCTG TGCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAGTGGAG GCCCACATGC CACCCACTAC CGGGGCACGT GGTACCTGAC GGGCATCGTC
1201 AGCTGGGGCC AGGGCTGCGC AACCGTGGGC CACTTTGGGG TGTACACCAG GGTCTCCCAG
1261 TACATCGAGT GGCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCCAGGAGT CCTCCTGCGA
1321 GCCCCATTTC CCGATATCGG TGGCGGTGGC TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA
1381 TCAGGTGGGG GTGGATCAGG CGGTGGAGGT TCCGGTGGCG GGGGATCAGC GCAGGTGCAG
1441 CTGCAGGAGT CTGGGGGAGG CTTGGTACAG CCTGGGGGGT CCCTGAGACT CTCCTGTGCA
1501 GCCTCTGGAT TCATGTTTAG CAGGTATGCC ATGAGCTGGG TCCGCCAGGC TCCAGGGAAG
1561 GGGCCAGAGT GGGTCTCAGG TATTAGTGGT AGTGGTGGTA GTACATACTA CGCAGACTCC
1621 GTGAAGGGCC GGTTCACCGT CTCCAGAGAC AATTCCAAGA ACACGCTGTA TCTGCAAATG
1681 AACAGCCTGA GAGCCGAGGA CACGGCTGTA TATTACTGCG CCGGGGGCGC CACCTACACC
1741 AGCCGGAGCG ACGTGCCCGA CCAGACCAGC TTCGACTACT GGGGCCAGGG AACCCTGGTC
1801 ACCGTCTCCT CAGGGAGTGC ATCCGCCCCA AAGCTTGAAG AAGGTGAATT TTCAGAAGCA
1861 CGCGTATCTG AACTGACTCA GGACCCTGCT GTGTCTGTGG CCTTGGGACA GACAGTCAGG
1921 ATCACATGCC AAGGAGACAG CCTCAGAAAC TTTTATGCAA GCTGGTACCA GCAGAAGCCA
1981 GGACAGGCCC CTACTCTTGT CATCTATGGT TTAAGTAAAA GGCCCTCAGG GATCCCAGAC
2041 CGATTCTCTG CCTCCAGCTC AGGAAACACA GCTTCCTTGA CCATCACTGG GGCTCAGGCG
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2101 GAAGAIGAGG CTGACTATTA CTGCCTGCTG TACTACGGCG GCGGCCAGCA GGGCGTGTTC
2161 GGCGGCGGCA CCAAGCTGAC CGTCCTACGT CAGCCCAAGG CTGCCCCCTC GGTCACTCTG
2221 TTCCCGCCCT CTTCTGCGGC CTGA

FVII-094 amino acid sequence. Signal sequence is shown in dotted underline,
propeptide is double underlined, and linker region connecting FVII to SCE5
is underlined
   1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
 241 NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
 301 LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
 361 ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
 421 YIEWLQKLMR SEPRPGVLLR APFPDIGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSAQVQ
 481 LQESGGGLVQ PGGSLRLSCA ASGFMFSRYA MSWVRQAPGK GPEWVSGISG SGGSTYYADS
 541 VKGRFTVSRD NSKNTLYLQM NSLRAEDTAV YYCARGATYT SRSDVPDQTS FDYWGQGTLV
 601 TVSSGSASAP KLEEGEFSEA RVSELTQDPA VSVALGQTVR ITCQGDSLRN FYASWYQQKP
 661 GQAPTLVIYG LSKRPSGIPD RFSASSSGNT ASLTITGAQA EDEADYYCLL YYGGGQQGVF
 721 GGGTKLTVLR QPKAAPSVTL FPPSSAA*

DNA sequence for FVII-028
   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCGGAAGTGC AGCTGGTGCA GTCTGGAGCT GAGGTGAATA AGCCTGGGGC CTCAGTGAAG
 121 GTCTCCTGCA AGGCTTCTGG ATACACCTTC ACCGGCTACT ATATGCACTG GGTGCGACAG
 181 GCCCCTGGAC AAGGGCTTGA GTGGATGGGA TGGATCAACC CTAACAGTGG TGGCACAAAC
 241 TATGCACAGA AGTTTCAGGG CTGGGTCACC ATGACCAGGG ACACGTCCAT CAGCACCGCC
 301 TACATGGAGC TGAGCAGGCT GAGATCTGAC GACACGGCCG TGTATTACTG TGCGAGAGGC
 361 CGTGCTTTGT ATAACGGAA CGACCGGTCC CCCAACTGGT TCGACCCCTG GGGCCAGGGA
 421 ACCCTGGTCA CCGTCTCCTC AGGGAGTGCA TCCGCCCCAA CCCTTAAACT TGAAGAAGGT
 481 GAATTTTCAG AAGCACGCGT ACAGGCTGTG CTGACTCAGC CGCCCTCGGT GTCAGTGGCC
 541 CCAGGACAGA CGGCCAGGAT TACCTGTGGG GGAAACAACA TTGGAAGTAA AAGTGTGCAG
 601 TGGTACCAGC AGAAGCCAGG CCAGGCCCCT GTGCTGGTCG TCTATGATGA TAGCGACCGG
 661 CCCTCAGGGA TCCCTGAGCG ATTCTCTGGC TCCAACTCTG GGAACATGGC CACCCTGACC
 721 ATCAGCAGGG TCGAAGCCGG GGATGAGGCC GACTATTACT GTCAGGTGTG GGATAGTAGT
 781 AGTGATCATG TGGTATTCGG CGGAGGGACC AAGCTGACCG TCCTAGGTCA GCCCAAGGCT
 841 GCCCCCTCGG TCACTCTGTT CCCGCCGTCC GCGGCCGCTA GGACGAAGCT GTTCTGGATT
 901 TCTTACAGTG ATGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 961 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
1021 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
1081 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
1141 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
1201 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCCCAAAGGG
1261 GAGTGTCCAT GGCAGGTCCT GTTGTTGGTG AATGGAGCTC AGTTGTGTGG GGGGACCCTG
1321 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAAAATCAA GAACTGGAGG
1381 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
1441 CGGCGGGTGG CGCAGGTCAT CATCCCCAGC ACGTACGTCC CGGGCACCAC CAACCACGAC
1501 ATCGCGCTGC TCCGCCTGCA CCAGCCCGTG GTCCTCACTG ACCATGTGGT GCCCCTCTGC
1561 CTGCCCGAAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TGCGCTTCTC ATTGGTCAGC
1621 GGCTGGGGCC AGCTGCTGGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
1681 CCCCGGCTGA TGACCCAGGA CTGCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
1741 ATCACGGAGT ACATGTTCTG TGCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1801 GACAGTGGAG GCCCACATGC CACCCACTAC CGGGGCACGT GGTACCTGAC GGGCATCGTC
1861 AGCTGGGGCC AGGGCTGCGC AACCGTGGGC CACTTTGGGG TGTACACCAG GGTCTCCCAG
1921 TACATCGAGT GGCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCCAGGAGT CCTCCTGCGA
1981 GCCCCATTTC CCGGTGGCGG TGGCTCCGGC GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
2041 GGGGGTGGAT CAGGCGGTGG AGGTTCCGGT GGCGGGGGAT CCGACAAAAC TCACACATGC
2101 CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG TCTTCCTCTT CCCCCCAAAA
2161 CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG
2221 AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
2281 GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCCTC
2341 ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA
2401 GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
2461 CAGGTGTACA CCCTGCCCCC ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGACC
2521 TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
2581 CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGTTGGACT CCGACGGCTC CTTCTTCCTC
2641 TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
2701 GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT
2761 AAAGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGCGGTG GAGGTTCCGG TGGCGGGGGA
2821 TCCGACAAAA CTCACACATG CCCACCGTGC CCAGCACCTG AACTCCTGGG AGGACCGTCA
2881 GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
2941 ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
3001 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA CAACAGCACG
3061 TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT GGCTGAATGG CAAGGAGTAC
3121 AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAGCC
3181 AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGCGA TGAGCTGACC
3241 AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
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3301 GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC CGTGTTGGAC
3361 TCCGACGGCT CCTTCTTCCT CTACAGCAAG CTCACCGTGG ACAAGAGCAG GTGGCAGCAG
3421 GGGAACGTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG
3481 AGCCTCTCCC TGTCTCCGGG TAAATGA
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FVII-028 amino acid sequence. Signal sequence is shown in dotted underline, linker region connecting FVII to Fc region is underlined, linker connecting both Fcs sites is shown in bold, and MB9 is italicized

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   1 MVSQALRLLC LLLGLQGCLA AEVQLVQSGA EVNKPGASVK VSCKASGYTF TGYYMHWVRQ
  61 APGQGLEWMG WINPNSGGTN YAQKFQGWVT MTRDTSISTA YMELSRLRSD DTAVYYCARG
 121 RALYNRNDRS PNWFDPWGQG TLVTVSSGSA SAPTLKLEBG EFSEARVQAV LTQPPSVSVA
 181 PGQTARITCG GNNIGSKSVQ WYQQKPGQAP VLVVYDDSDR PSGIPERFSG SNSGNMATLT
 241 ISRVEAGDEA DYYCQVWDSS SDHVVFGGGT KLTVLGQPKA APSVTLFPPS AAARTKLFWI
 301 SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE THKDDQLICV NENGGCEQYC
 361 SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE KRNASKPQGR IVGGKVCPKG
 421 ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR NLIAVLGEHD LSEHDGDEQS
 481 RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC LPERTFSERT LAFVRFSLVS
 541 GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN ITEYMFCAGY SDGSKDSCKG
 601 DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ YIEWLQKLMR SEPRPGVLLR
 661 APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC PPCPAPELLG GPSVFLFPPK
 721 PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
 781 TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTKNQVSLT
 841 CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS
 901 VMHEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGGSGGGG SDKTHTCPPC PAPELLGGPS
 961 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
1021 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
1081 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
1141 GNVFSCSVMH EALHNHYTQK SLSLSPGK*
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DNA sequence FVII-039

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   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
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 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGC CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGCGGAGGAG ACTTCACTCG GGTTGTGGGG
 601 GGCAAGGTGT GCCCCAAAGG GGAGTGTCCA TGGCAGGTCC TGTTGTTGGT GAATGGAGCT
 661 CAGTTGTGTG GGGGGACCCT GATCAACACC ATCTGGGTGG TCTCCGCGGC CCACTGTTTC
 721 GACAAAATCA AGAACTGGAG GAACCTGATC GCGGTGCTGG GCGAGCACGA CCTCAGCGAG
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 841 CCGGGCACCA CCAACCACGA CATCGCGCTG CTCCGCCTGC ACCAGCCCGT GGTCCTCACT
 901 GACCATGTGG TGCCCCTCTG CCTGCCCGAA CGGACGTTCT CTGAGAGGAC GCTGGCCTTC
 961 GTGCGCTTCT CATTGGTCAG CGGCTGGGGC CAGCTGCTGG ACCGTGGCGC CACGGCCCTG
1021 GAGCTCATGG TCCTCAACGT GCCCCGGCTG ATGACCCAGG ACTGCCTGCA GCAGTCACGG
1081 AAGGTGGGAG ACTCCCCAAA TATCACGGAG TACATGTTCT GTGCCGGCTA CTCGGATGGC
1141 AGCAAGGACT CCTGCAAGGG GGACAGTGGA GGCCCACATG CCACCCACTA CCGGGGCACG
1201 TGGTACCTGA CGGGCATCGT CAGCTGGGGC CAGGGCTGCG CAACCGTGGG CCACTTTGGG
1261 GTGTACACCA GGGTCTCCCA GTACATCGAG TGGCTGCAAA AGCTCATGCG CTCAGAGCCA
1321 CGCCCAGGAG TCCTCCTGCG AGCCCCATTT CCCGGTGGCG GTGGCTCCGG CGGAGGTGGG
1381 TCCGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGCGGTG GAGGTTCCGG TGGCGGGGGA
1441 TCCGACAAAA CTCACACATG CCCACCGTGC CCAGCTCCGG AACTCCTGGG CGGACCGTCA
1501 GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
1561 ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
1621 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA CAACAGCACG
1681 TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT GGCTGAATGG CAAGGAGTAC
1741 AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAGCC
1801 AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGGGA TGAGCTGACC
1861 AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
1921 GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC CGTGTTGGAC
1981 TCCGACGGCT CCTTCTTCCT CTACAGCAAG CTCACCGTGG ACAAGAGCAG GTGGCAGCAG
2041 GGGAACGTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG
2101 AGCCTCTCCC TGTCTCCGGG TAAAGGTGGC GGCGGATCAG GTGGGGGTGG ATCAGGCGGT
2161 GGAGGTTCCG GTGGCGGGGG ACTCAGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT
2221 GAACTCCTGG GAGGACCGTC AGTCTTCCTC TTCCCCCCAA AACCCAAGGA CACCCTCATG
2281 ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG
2341 GTCAAGTTCA ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG
2401 GAGGAGCAGT ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT GCACCAGGAC
2461 TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC
2521 GAGAAAACCA TCTCCAAAGC CAAAGGGCAG CCCCGAGAAC CACAGGTGTA CACCCTGCCC
2581 CCATCCCGCG ATGAGCTGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC
2641 TATCCCAGCG ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG
2701 ACCACGCCTC CCGTGTTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA GCTCACCGTG
2761 GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG
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2821 CACAACCACT ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAATGA

FVII-039 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, the FXIa cleavage site is shown in dashed underline, the linker region connecting FVII heavy chain to Fc region is underlined, and the linker region connecting the Fc regions is shown in bold

1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
181 KRNASKPQGR GGSDFTRVVG GKVCPKGECP WQVLLLVNGA QLCGGTLINT IWVVSAAHCF
241 DKIKNWRNLI AVLGEHDLSE HDGDEQSRRV AQVIIPSTYV PGTTNHDIAL LRLHQPVVLT
301 DHVVPLCLPE RTFSERTLAF VRFSLVSGWG QLLDRGATAL ELMVLNVPRL MTQDCLQQSR
361 KVGDSPNITE YMFCAGYSDG SKDSCKGDSG GPHATHYRGT WYLTGIVSWG QGCATVGHFG
421 VYTRVSQYIE WLQKLMRSEP RPGVLLRAPF PGGGGSGGGG SGGGGSGGGG SGGGGSGGGG
481 SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
541 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
601 KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD
661 SDGSFFLYSR LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGSGGGGSGG
721 GGSGGGGSDK THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
781 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI
841 EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK
901 TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK*

DNA sequence for FVII-040

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCGGC GGAGGAGACT TCACTCGGGT TGTGGGGGGC
601 AAGGTGTGCC CCAAAGGGGA GTGTCCATGG CAGGTCCTGT TGTTGGTGAA TGGAGCTCAG
661 TTGTGTGGGG GGACCCTGAT CAACACCATC TGGGTGGTCT CCGCGGCCCA CTGTTTCGAC
721 AAAATCAAGA ACTGGAGGAA CCTGATCGCG GTGCTGGGCG AGCACGACCT CAGCGAGCAC
781 GACGGGGATG AGCAGAGCCG GCGGGTGGCG CAGGTCATCA TCCCCAGCAC GTACGTCCCG
841 GGCACCACCA ACCACGACAT CGCGCTGCTC CGCCTGCACC AGCCCGTGGT CCTCACTGAC
901 CATGTGGTGC CCCTCTGCCT GCCCGAACGG ACGTTCTCTG AGAGGACGCT GGCCTTCGTG
961 CGCTTCTCAT TGGTCAGCGG CTGGGGCCAG CTGCTGGACC GTGGCGCCAC GGCCCTGGAG
1021 CTCATGGTCC TCAACGTGCC CCGGCTGATG ACCCAGGACT GCCTGCAGCA GTCACGGAAG
1081 GTGGGAGACT CCCCAAATAT CACGGAGTAC ATGTTCTGTG CCGGCTACTC GGATGGCAGC
1141 AAGGACTCCT GCAAGGGGGA CAGTGGAGGC CCACATGCCA CCCACTACCG GGGCACGTGG
1201 TACCTGACGG GCATCGTCAG CTGGGGCCAG GGCTGCGCAA CCGTGGGCCA CTTTGGGGTG
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1321 CCAGGAGTCC TCCTGCGAGC CCCATTTCCC GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
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1441 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGCGG ACCGTCAGTC
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1561 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
1621 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
1681 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1741 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1801 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1861 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1921 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1981 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
2041 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
2101 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
2161 GGTTCCGGTG GCGGGGGATC AGACAAAACT CACACATGCC CACCGTGCCC AGCACCTGAA
2221 CTCCTGGGAG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC CCAAGGACAC CCTCATGATC
2281 TCCCGGACCC CTGAGGTCAC ATGCGTGGTG GTGGACGTGA GCCACGAAGA CCCTGAGGTC
2341 AAGTTCAACT GGTACGTGGA CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG
2401 GAGCAGTACA ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG
2461 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC CCCCATCGAG
2521 AAAACCATCT CCAAAGCCAC AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCCA
2581 TCCCGCGATG AGCTGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAT
2641 CCCAGCGACA TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC
2701 ACGCCTCCCG TGTTGGACTC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC
2761 AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC
2821 AACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA 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 underline, the linker region connecting FVII heavy chain to Fc region is underlined, and the linker region connecting the Fc regions is shown in bold

```
      1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
     61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
    121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
    181 KRNASKPQGR GGDFTRVVGG KVCPKGECPW QVLLLVNGAQ LCGGTLINTI WVVSAAHCFD
    241 KIKNWRNLIA VLGEHDLSEH DGDEQSRRVA QVIIPSTYVP GTTNHDIALL RLHQPVVLTD
    301 HVVPLCLPER TFSERTLAFV RFSLVSGWGQ LLDRGATALE LMVLNVPRLM TQDCLQQSRK
    361 VGDSPNITEY MFCAGYSDGS KDSCKGDSGG PHATHYRGTW YLTGIVSWGQ GCATVGHFGV
    421 YTRVSQYIEW LQKLMRSEPR PGVLLRAPFP GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS
    481 DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
    541 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
    601 GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
    661 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG GSGGGGSGGG
    721 GSGGGGSDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV
    781 KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE
    841 KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT
    901 TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*
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DNA sequence for FIX-042

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      1 ATGCAGCGCG TGAACATGAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCTTTTA
     61 GGATATCTAC TCAGTGCTGA ATGTACAGGT TTGTTTCCTT TTTTAAAATA CATTGAGTAT
    121 GCTTGCCTTT TAGATATAGA AATATCTGAT GCTGTCTTCT TCACTAAATT TTGATTACAT
    181 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 AATTGGAAGA GTTTGTTCAA GGGAATCTAG
    481 AGAGAGAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA
    541 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
    781 ATCGACTTGC AGAAAACCAG AAGTCCTGTG AACCAGCAGT GCCATTTCCA TGTGGAAGAG
    841 TTTCTGTTTC ACAAACTTCT AAGCTCACCC GTGCTGAGAC TGTTTTTCCT GATGTGGACT
    901 ATGTAAATTC TACTGAAGCT GAAACCATTT TGGATAACAT CACTCAAAGC ACCCAATCAT
    961 TTAATGACTT CACTCGGGTT GTTGGTGGAG AAGATGCCAA ACCAGGTCAA TTCCCTTGGC
   1021 AGGTTGTTTT GAATGGTAAA GTTGATGCAT TCTGTGGAGG CTCTATCGTT AATGAAAAAT
   1081 GGATTGTAAC TGCTGCCCAC TGTGTTGAAA CTGGTGTTAA AATTACAGTT GTCGCAGGTG
   1141 AACATAATAT TGAGGAGACA GAACATACAG AGCAAAAGCG AAATGTGATT CGAATTATTC
   1201 CTCACCACAA CTACAATGCA GCTATTAATA AGTACAACCA TGACATTGCC CTTCTGGAAC
   1261 TGGACGAACC CTTAGTGCTA AACAGCTACG TTACACCTAT TTGCATTGCT GACAAGGAAT
   1321 ACACGAACAT CTTCCTCAAA TTTGGATCTG GCTATGTAAG TGGCTGGGGA AGAGTCTTCC
   1381 ACAAAGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTT GACCGAGCCA
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   1501 AAGGAGGTAG AGATTCATGT CAAGGAGATA GTGGGGGACC CCATGTTACT GAAGTGGAAG
   1561 GGACCAGTTT CTTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCAAAT
   1621 ATGGAATATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAGGAAAAA ACAAAGCTCA
   1681 CTGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG
   1741 TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA
   1801 CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
   1861 ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT
   1921 ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA
   1981 AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA
   2041 AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT GAGCTGACCA
   2101 AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG
   2161 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
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   2461 AACTCCTGGG AGGACCGTCA GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA
   2521 TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG
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   2641 AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
   2701 GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG
   2761 AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC
   2821 CATCCCGCGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT
   2881 ATCCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA
   2941 CCACGCCTCC CGTGTTGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG CTCACCGTGG
   3001 ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC
   3061 ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG TAAATGA
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FIX-042 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, and linker region connecting the Fc regions is underlined

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      1 MQRVNMIMAE SPGLITICLL GYLLSAECTV FLDHENANKI LNRPKRYNSG KLEEFVQGNL
     61 ERECMEEKCS FEEAREVFEN TERTTEFWKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP
    121 FGFEGKNCEL DVTCNIKNGR CEQFCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPFPCGR
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       181 VSVSQISKLT RAETVFPDVD YVNSTEAETI LDNITQSTQS FNDFTRVVGG EDAKPGQFPW
       241 QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETEHT EQKRNVIRII
       301 PHHNYNAAIN KYNHDIALLE LDEPLVLNSY VTPICIADKE YTNIFLKFGS GYVSGWGRVF
       361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
       421 GTSFLTGIIS WGEECAMKGK YGIYTKVSRY VNWIKEKTKL TDKTHTCPPC PAPELLGGPS
       481 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
       541 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
       601 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LIVDKSRWQQ
       661 GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGSGGGGSGG GGSGGGGSDK THTCPPCPAP
       721 ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR
       781 EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP
       841 PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV
       901 DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK*

DNA sequence for FIX-068
         1 ATGCAGCGCG TGAACATGAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCTTTTA
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       121 GCTTGCCTTT TAGATATAGA AATATCTGAT GCTGTCTTCT TCACTAAATT TTGATTACAT
       181 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 AATTGGAAGA GTTTGTTCAA GGGAATCTAG
       481 AGAGAGAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA
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       661 TTGGATTTGA AGGAAAGAAC TGTGAATTAG ATGTAACATG TAACATTAAG AATGGCAGAT
       721 GCGAGCAGTT TTGTAAAAAT AGTGCTGATA ACAAGGTGGT TTGCTCCTGT ACTGAGGGAT
       781 ATCGACTTGC AGAAAACCAG AAGTCCTGTG AACCAGCAGT GCCATTTCCA TGTGGAAGAG
       841 TTTCTGTTTC ACAAACTTCT AAGCTCACCC GTGCTGAGAC TGTTTTTCCT GATGTGGACT
       901 ATGTAAATTC TACTGAAGCT GAAACCATTT TGGATAACAT CACTCAAAGC ACCCAATCAT
       961 TTAATGACTT CACTCGGGTT GTTGGTGGAG AAGATGCCAA ACCAGGTCAA TTCCCTTGGC
      1021 AGGTTGTTTT GAATGGTAAA GTTGATGCAT TCTGTGGAGG CTCTATCGTT AATGAAAAAT
      1081 GGATTGTAAC TGCTGCCCAC TGTGTTGAAA CTGGTGTTAA AATTACAGTT GTCGCAGGTG
      1141 AACATAATAT TGAGGAGACA GAACATACAG AGCAAAAGCG AAATGTGATT CGAATTATTC
      1201 CTCACCACAA CTACAATGCA GCTATTAATA AGTACAACCA TGACATTGCC CTTCTGGAAC
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      1321 ACACGAACAT CTTCCTCAAA TTTGGATCTG GCTATGTAAG TGGCTGGGGA AGAGTCTTCC
      1381 ACAAAGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTT GACCGAGCCA
      1441 CATGTCTTCG ATCTACAAAG TTCACCATCT ATAACAACAT GTTCTGTGCT GGCTTCCATG
      1501 AAGGAGGTAG AGATTCATGT CAAGGAGATA GTGGGGGACC CCATGTTACT GAAGTGGAAG
      1561 GGACCAGTTT CTTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCAAAT
      1621 ATGGAATATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAGGAAAAA ACAAAGCTCA
      1681 CTGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG
      1741 TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA
      1801 CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
      1861 ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT
      1921 ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA
      1981 AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA
      2041 AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT GAGCTGACCA
      2101 AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG
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      2221 CCGACGGCTC CTTCTTCCTC TACAGCAAGC TCACCGTCGA CAAGAGCAGG TGGCAGCAGG
      2281 GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA
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      2401 GTGGATCAGG CGGTGGAGGT TCCGGTGGCG GGGGATCCGG CGGTGGAGGT TCCGGTGGGG
      2461 GTGGATCAAG GAAGAGGAGG AAGAGGGCGC AGGTGCAGCT GCAGGAGTCT GGGGGAGGCT
      2521 TGGTACAGCC TGGGGGGTCC CTGAGACTCT CCTGTGCAGC CTCTGGATTC ATGTTTAGCA
      2581 GGTATGCCAT GAGCTGGGTC CGCCAGGCTC CAGGGAAGGG GCCAGAGTGG GTCTCAGGTA
      2641 TTAGTGGTAG TGGTGGTAGT ACATACTACG CAGACTCCGT GAAGGGCCGG TTCACCGTCT
      2701 CCAGAGACAA TTCCAAGAAC ACGCTGTATC TGCAAATGAA CAGCCTGAGA GCCGAGGACA
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      2821 AGACCAGCTT CGACTACTGG GGCCAGGGAA CCCTGGTCAC CGTCTCCTCA GGGAGTGCAT
      2881 CGGCCCCAAA GCTTGAAGAA GGTGAATTTT CAGAAGCACG CGTATCTGAA CTGACTCAGG
      2941 ACCCTGCTGT GTCTGTGGCC TTGGGACAGA CAGTCAGGAT CACATGCCAA GGAGACAGCC
      3001 TCAGAAACTT TTATGCAAGC TGGTACCAGC AGAAGCCAGG ACAGGCCCCT ACTCTTGTCA
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      3121 GAAACACAGC TTCCTTGACC ATCACTGGGG CTCAGGCGGA AGATGAGGCT GACTATTACT
      3181 GCCTGCTGTA CTACGGCGGC GGCCAGCAGG GCGTGTTCGG CGGCGGCACC AAGCTGACCG
      3241 TCCTACGTCA GCCCAAGGCT GCCCCCTCGG TCACTCTGTT CCCGCCCTCT TCTGCGGCCG
      3301 GTGGCGGTGG CTCCGGCGGA GGTGGGTCCG GTGGCGGCGG ATCAGGTGGG GGTGGATCAG
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      3421 CACCGGAACT CCTGGGCGGA CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC
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      3541 CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC
      3601 CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG CGTCCTCACC GTCCTGCACC
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3721 CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC
3781 TGCCCCCATC CCGCGATGAG CTGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
3841 GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAACT
3901 ACAAGACCAC GCCTCCCGTG TTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA
3961 CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAGG
4021 CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA TGA

FIX-068 amino acid sequence. Signal sequence is shown in dotted underline,
propeptide is double underlined, linker region connecting SCE5 to Fc region
is underlined, and linker with proprotein convertase processing sites is
shown in bold
   1 MQRVNMIMAE SPGLITICLL GYLLSAECTV FLDHENANKI LNRPKRYNSG KLEEFVQGNL
  61 ERECMEEKCS FEEAREVFEN TERTTEFWKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP
 121 FGFEGKNCEL DVTCNIKNGR CEQFCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPFPCGR
 181 VSVSQTSKLT RAETVFPDVD YVNSTEAETI LDNITQSTQS FNDFTRVVGG EDAKPGQFPW
 241 QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETEHT EQKRNVIRII
 301 PHHNYNAAIN KYNHDIALLE LDEPLVLNSY VTPICIADKE YTNIFLKFGS GYVSGWGRVF
 361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
 421 GTSFLTGIIS WGEECAMKGK YGIYTKVSRY VNWIKEKTKL TDKTHTCPPC PAPELLGGPS
 481 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
 541 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
 601 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
 661 GNVFSCSVMH EALHNHYTQK SLSLSPGKRR RRSGGGGSGG GGSGGGGSGG GGSGGGGSGG
 721 GGSRRRRRRA QVQLQESGGG LVQPGGSLRL SCAASGFMFS RYAMSWVRQA PGKGPEWVSG
 781 ISGSGGSTYY ADSVKGRFTV SRDNSKNTLY LQMNSLRAED TAVYYCARGA TYTSRSDVPD
 841 QTSPDYWGQG TLVTVSSGSA SAPKLEEGEF SEARVSELTQ DPAVSVALGQ TVRITCQGDS
 901 LRNFYASWYQ QKPGQAPTLV IYGLSKRPSG IPDRFSASSS GNTASLTITG AQAEDEADYY
 961 CLLYYGGQQQ GVFGGGTKLT VLRQPKAAPS VTLFPPSSAA GGGSGGGGGS GGGGSGGGGS
1021 GGGGSGGGGS DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
1081 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
1141 PIEKTISKAK GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN
1201 YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*

DNA sequence for FIX-088
   1 ATGCAGCGCG TGAACATGAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCTTTTA
  61 GGATATCTAC TCAGTGCTGA ATGTACAGGT TTGTTTCTTT TTTTAAAATA CATTGAGTAT
 121 GCTTGCCTTT TAGATATAGA AATATCTGAT GCTGTCTTCT TCACTAAATT TTGATTACAT
 181 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 AATTGGAAGA GTTTGTTCAA GGGAATCTAG
 481 AGAGAGAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA
 541 CTGAAAGAAC AACTGAATTT TGGAAGCAGT ATGTTGATGG AGATCAGTGT GAGTCCAATC
 601 CATGTTTAAA TGGCGGCAGT TGCAAGGATG ACATTAATTC CTATGAATGT TGGTGTCCCT
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 721 GCGAGCAGTT TTGTAAAAAT AGTGCTGATA ACAAGGTGGT TTGCTCCTGT ACTGAGGGAT
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 841 TTTCTGTTTC ACAAACTTCT AAGCTCACCC GTGCTGAGAC TGTTTTTCCT GATGTGGACT
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1021 AGGTTGTTTT GAATGGTAAA GTTGATGCAT TCTGTGGAGG CTCTATCGTT AATGAAAAAT
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1381 ACAAAGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTT GACCGAGCCA
1441 CATGTCTTCG ATCTACAAAG TTCACCATCT ATAACAACAT GTTCTGTGCT GGCTTCCATG
1501 AAGGAGGTAG AGATTCATGT CAAGGAGATA GTGGGGGACC CCATGTTACT GAAGTGGAAG
1561 GGACCAGTTT CTTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCAAAT
1621 ATGGAATATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAGGAAAAA ACAAAGCTCA
1681 CTGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG
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1801 CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
1861 ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT
1921 ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA
1981 AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA
2041 AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT GAGCTGACCA
2101 AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG
2161 AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGTTGGACT
2221 CCGACGGCTC CTTCTTCCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG
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2461 AACTCCTGGG AGGACCGTCA GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA
2521 TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG
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2581 TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG
2641 AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
2701 GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG
2761 AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC ACAGGTGTAC ACCCTGCCCC
2821 CATCCCGCGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT
2881 ATCCCAGCGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA
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3121 GCGGAGGTGG GTCCGGTGGC GGCGGATCAG GTGGGGGTGG ATCAGGCGGT GGAGGTTCCG
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3541 ACTACTGGGG CCAGGGAACC CTGGTCACCG TCTCCTCAGG GAGTGCATCC GCCCCAAAGC
3601 TTGAAGAAGG TGAATTTTCA GAAGCACGCG TATCTGAACT GACTCAGGAC CCTGCTGTGT
3661 CTGTGGCCTT GGGACAGACA GTCAGGATCA CATGCCAAGG AGACAGCCTC AGAAACTTTT
3721 ATGCAAGCTG GTACCAGCAG AAGCCAGGAC AGGCCCCTAC TCTTGTCATC TATGGTTTAA
3781 GTAAAAGGCC CTCAGGGATC CCAGACCGAT TCTCTGCCTC CAGCTCAGGA AACACAGCTT
3841 CCTTGACCAT CACTGGGGCT CAGGCGGAAG ATGAGGCTGA CTATTACTGC CTGCTGTACT
3901 ACGGCGGCGG CCAGCAGGGC GTGTTCGGCG GCGGCACCAA GCTGACCGTC CTACGTCAGC
3961 CCAAGGCTGC CCCCTCGGTC ACTCTGTTCC CGCCCTCTTC TGCGGCCTGA
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FIX-088 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker connecting both Fc regions is underlined and linker connecting the Fc region to SCE5 is in bold

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   1 MQRVNMIMAE SPGLITICLL GYLLSAECTV FLDHENANKI LNRPKRYNSG KLEEFVQGNL
  61 ERECMEEKCS FEEAREVFEN TERTTEFWKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP
 121 FGFEGKNCEL DVTCNIKNGR CEQFCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPFPCGR
 181 VSVSQTSKLT RAETVFPDVD YVNSTEAETI LDNITQSTQS FNDFTRVVGG EDAKPGQFPW
 241 QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETEHT EQKRNVIRII
 301 PHHNYNAAIN KYNHDIALLE LDEPLVLNSY VTPICIADKE YTNIFLKFGS GYVSGWGRVF
 361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
 421 GTSFLTGIIS WGEECAMKGK YGIYTKVSRY VNWIKEKTKL TDKTHTCPPC PAPELLGGPS
 481 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
 541 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
 601 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
 661 GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGSGGGGSGG GGSGGGGSDK THTCPPCPAP
 721 ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR
 781 EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP
 841 PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV
 901 DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGKGGGGS GGGGSGGGGS GGGGSGGGGS
 961 GGGGSAQVQL QESGGGLVQP GGSLRLSCAA SGFMFSRYAM SWVRQAPGKG PEWVSGISGS
1021 GGSTYYADSV KGRFTVSRDN SKNTLYLQMN SLRAEDTAVY YCARGATYTS RSDVPDQTSF
1081 DYWGQGTLVT VSSGSASAPK LEEGEFSEAR VSELTQDPAV SVALGQTVRI TCQGDSLRNF
1141 YASWYQQKPG QAPTLVIYGL SKRPSGIPDR FSASSSGNTA SLTITGAQAE DEADYYCLLY
1201 YGGGQQGVFG GGTKLTVLRQ PKAAPSVTLF PPSSAA*
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DNA sequence for FIX-089

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 121 GCTTGCCTTT TAGATATAGA AATATCTGAT GCTGTCTTCT TCACTAAATT TTGATTACAT
 181 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 AATTGGAAGA GTTTGTTCAA GGGAATCTAG
 481 AGAGAGAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA
 541 CTGAAAGAAC AACTGAATTT TGGAAGCAGT ATGTTGATGG AGATCAGTGT GAGTCCAATC
 601 CATGTTTAAA TGGCGGCAGT TGCAAGGATG ACATTAATTC CTATGAATGT TGGTGTCCCT
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 961 TTAATGACTT CACTCGGGTT GTTGGTGGAG AAGATGCCAA ACCAGGTCAA TTCCCTTGGC
1021 AGGTTGTTTT GAATGGTAAA GTTGATGCAT TCTGTGGAGG CTCTATCGTT AATGAAAAAT
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1321 ACACGAACAT CTTCCTCAAA TTTGGATCTG GCTATGTAAG TGGCTGGGGA AGAGTCTTCC
1381 ACAAAGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTT GACCGAGCCA
1441 CATGTCTTCG ATCTACAAAG TTCACCATCT ATAACAACAT GTTCTGTGCT GGCTTCCATG
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1561 GGACCAGTTT CTTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCAAAT
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1801 CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
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1981 AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA
2041 AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT GAGCTGACCA
2101 AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG
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2461 CCGAGCGGGAT GGCCCTGCAC AACCTGTGCG GTGGCGGTGG CTCCGGCGGA GGTGGGTCCG
2521 GTGGCGGCGG ATCAGGTGGG GGTGGATCAG GCGGTGGAGG TTCCGGTGGC GGGGGATCCG
2581 ACAAAACTCA CACATGCCCA CCGTGCCCAG CACCGGAACT CCTGGGCGGA CCGTCAGTCT
2641 TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTCACAT
2701 GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGGACG
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2821 GTGTGGTCAG CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
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3121 ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
3181 ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC
3241 TCTCCCTGTC TCCGGGTAAA TGA
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FIX-089 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underlined, linker regions connecting OS1 to Fc regions are underlined, and OS1 peptide is italicized

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   1 MQRVNMIMAE SPGLITICLL GYLLSAECTV FLDHENANKI LNRPKRYNSG KLEEFVQGNL
  61 ERECMEEKCS FEEAREVFEN TERTTEFWKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP
 121 FGFEGKNCEL DVTCNIKNGR CEQFCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPFPCGR
 181 VSVSQTSKLT RAETVFPDVD YVNSTEAETI LDNITQSTQS FNDFTRVVGG EDAKPGQFPW
 241 QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETEHT EQKRNVIRII
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 361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
 421 GTSFLTGIIS WGEECAMKGK YGIYTKVSRY VNWIKEKTKL TDKTHTCPPC PAPELLGGPS
 481 VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
 541 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
 601 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
 661 GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGSGGGGSGG GGSGGGGSGG GGSGGGGSAC
 721 TERMALHNLC GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
 781 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
 841 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
 901 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
 961 NVFSCSVMHE ALHNHYTQKS LSLSPGK*
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DNA sequence for FIX-090

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   1 ATGCAGCGGG TGAACATGAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCTTTTA
  61 GGATATCTAC TCAGTGCTGA ATGTACAGGT TTGTTTCCTT TTTTAAAATA CATTGAGTAT
 121 GCTTGCCTTT TAGATATAGA AATATCTGAT GCTGTCTTCT TCACTAAATT TTGATTACAT
 181 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 AATTGGAAGA GTTTGTTCAA GGGAATCTAG
 481 AGAGAGAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA
 541 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
 781 ATCGACTTGC AGAAAACCAG AAGTCCTGTG AACCAGCAGT GCCATTTCCA TGTGGAAGAG
 841 TTTCTGTTTC ACAAACTTCT AAGCTCACCC GTGCTGAGAC TGTTTTTCCT GATGTGGACT
 901 ATGTAAATTC TACTGAAGCT GAAACCATTT TGGATAACAT CACTCAAAGC ACCCAATCAT
 961 TTAATGACTT CACTCGGGTT GTTGGTGGAG AAGATGCCAA ACCAGGTCAA TTCCCTTGGC
1021 AGGTTGTTTT GAATGGTAAA GTTGATGCAT TCTGTGGAGG CTCTATCGTT AATGAAAAAT
1081 GGATTGTAAC TGCTGCCCAC TGTGTTGAAA CTGGTGTTAA AATTACAGTT GTCGCAGGTG
1141 AACATAATAT TGAGGAGACA GAACATACAG AGCAAAAGCG AAATGTGATT CGAATTATTC
1201 CTCACCACAA CTACAATGCA GCTATTAATA AGTACAACCA TGACATTGCC CTTCTGGAAC
1261 TGGACGAACC CTTAGTGCTA AACAGCTACG TTACACCTAT TTGCATTGCT GACAAGGAAT
1321 ACACGAACAT CTTCCTCAAA TTTGGATCTG GCTATGTAAG TGGCTGGGGA AGAGTCTTCC
1381 ACAAAGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTT GACCGAGCCA
1441 CATGTCTTCG ATCTACAAAG TTCACCATCT ATAACAACAT GTTCTGTGCT GGCTTCCATG
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1501 AAGGAGGTAG AGATTCATGT CAAGGAGATA GTGGGGGACC CCATGTTACT GAAGTGGAAG
1561 GGACCAGTTT CTTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCAAAT
1621 ATGGAATATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAGGAAAAA ACAAAGCTCA
1681 CTGGTGGCGG TGGCTCCGGC GGAGGTGGGT CCGGTGGCGG CGGATCAGGT GGGGGTGGAT
1741 CAGGCGGTGG AGGTTCCGGT GGCGGGGGAT CAGCGCAGGT GCAGCTGCAG GAGTCTGGGG
1801 GAGGCTTGGT ACAGCCTGGG GGGTCCCTGA GACTCTCCTG TGCAGCCTCT GGATTCATGT
1861 TTAGCAGGTA TGCCATGAGC TGGGTCCGCC AGGCTCCAGG GAAGGGGCCA GAGTGGGTCT
1921 CAGGTATTAG TGGTAGTGGT GGTAGTACAT ACTACGCAGA CTCCGTGAAG GGCCGGTTCA
1981 CCGTCTCCAG AGACAATTCC AAGAACACGC TGTATCTGCA AATGAACAGC CTGAGAGCCG
2041 AGGACACGGC TGTATATTAC TGCGCCCGGG GCGCCACCTA CACCAGCCGG AGCGACGTGC
2101 CCGACCAGAC CAGCTTCGAC TACTGGGGCC AGGGAACCCT GGTCACCGTC TCCTCAGGGA
2161 GTGCATCCGC CCCAAAGCTT GAAGAAGGTG AATTTTCAGA AGCACGCGTA TCTGAACTGA
2221 CTCAGGACCC TGCTGTGTCT GTGGCCTTGG GACAGACAGT CAGGATCACA TGCCAAGGAG
2281 ACAGCCTCAG AAACTTTTAT GCAAGCTGGT ACCAGCAGAA GCCAGGACAG GCCCCTACTC
2341 TTGTCATCTA TGGTTTAAGT AAAAGGCCCT CAGGGATCCC AGACCGATTC TCTGCCTCCA
2401 GCTCAGGAAA CACAGCTTCC TTGACCATCA CTGGGGCTCA GGCGGAAGAT GAGGCTGACT
2461 ATTACTGCCT GCTGTACTAC GGCGGCGGCC AGCAGGGCGT GTTCGGCGGC GGCACCAAGC
2521 TGACCGTCCT ACGTCAGCCC AAGGCTGCCC CCTCGGTCAC TCTGTTCCCG CCCTCTTCTG
2581 CGGCCTGA
```

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

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   1 MQRVNMIMAE SPGLITICLL GYLLSAECTV FLDHENANKI LNRPKRYNSG KLEEFVQGNL
  61 ERECMEEKCS FEEAREVFEN TERTTEFWKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP
 121 FGFEGKNCEL DVTCNIKNGR CEQFCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPFPCGR
 181 VSVSQTSKLT RAETVFPDVD YVNSTEAETI LDNITQSTQS FNDFTRVVGG EDAKPGQFPW
 241 QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETEHT EQKRNVIRII
 301 PHHNYNAAIN KYNHDIALLE LDEPLVLNSY VTPICIADKE YTNIFLKFGS GYVSGWGRVF
 361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
 421 GTSFLTGIIS WGEECAMKGK YGIYTKVSRY VNWIKEKTKL TGGGGSGGGG SGGGGSGGGG
 481 SGGGGSGGGG SAQVQLQESG GGLVQPGGSL RLSCAASGFM FSRYAMSWVR QAPGKGPEWV
 541 SGISGSGGST YYADSVKGRF TVSRDNSKNT LYLQMNSLRA EDTAVYYCAR GATYTSRSDV
 601 PDQTSFDYWG QGTLVTVSSG SASAPKLEEG EFSEARVSEL TQDPAVSVAL GQTVRITCQG
 661 DSLRNFYASW YQQKPGQAPT LVIYGLSKRP SGIPDRFSAS SSGNTASLTI TGAQAEDEAD
 721 YYCLLYYGGG QQGVFGGGTK LTVLRQPKAA PSVTLFPPSS AA*
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DNA sequence for FVII-088

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   1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
  61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
 121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
 181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
 241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
 301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGGCCG GAACTGTGAG
 361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTCTGA GCAGTACTGC
 421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
 481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
 541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCCCAAAGGG
 601 GAGTGTCCAT GGCAGGTCCT GTTGTTGGTG AATGGAGCTC AGTTGTGTGG GGGGACCCTG
 661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAAAATCAA GAACTGGAGG
 721 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
 781 CGGCGGGTGG CGCAGGTCAT CATCCCCAGC ACGTACGTCC CGGGCACCAC CAACCACGAC
 841 ATCGCGCTGC TCCGCCTGCA CCAGCCCGTG GTCCTCACTG ACCATGTGGT GCCCCTCTGC
 901 CTGCCCGAAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TGCGCTTCTC ATTGGTCAGC
 961 GGCTGGGGCC AGCTGCTGGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
1021 CCCCGGCTGA TGACCCAGGA CTGCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
1081 ATCACGGAGT ACATGTTCTG TGCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAGTGGAG GCCCACATGC CACCCACTAC CGGGGCACGT GGTACCTGAC GGGCATCGTC
1201 AGCTGGGGCC AGGGCTGCGC AACCGTGGGC CACTTTGGGG TGTACACCAG GGTCTCCCAG
1261 TACATCGAGT GGCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCCAGGAGT CCTCCTGCGA
1321 GCCCCATTTC CCGGTGGCGG TGGCTCCGGC GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
1381 GGGGGTGGAT CAGGCGGTGG AGGTTCCGGT GGCGGGGGAT CCGACAAAAC TCACACATGC
1441 CCACCGTGCC CAGCTCCGGA ACTCCTGGGA GGACCGTCAG TCTTCCTCTT CCCCCCAAAA
1501 CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG
1561 AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
1621 GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCCTC
1681 ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA
1741 GCCCTCCCAG CCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
1801 CAGGTGTACA CCCTGCCCCC ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGACC
1861 TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
1921 CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGTTGGACT CCGACGGCTC CTTCTTCCTC
1981 TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
2041 GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT
2101 AAACGGCGCC GCCGGAGCGG TGGCGGCGGA TCAGGTGGGG GTGGATCAGG CGGTGGAGGT
2161 TCCGGTGGCG GGGGATCCGG CGGTGGAGGT TCCGGTGGGG GTGGATCAAG GAAGAGGAGG
2221 AAGAGGGACA TCGTGATGAC CCAGGCCGCC CCCAGCGTGC CCGTGACCCC CGGCGAGAGC
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2281 GTGAGCATCA GCTGCCGGAG CAGCCGGAGC CTGCTGCACA GCAACGGCAA CACCTACCTG
2341 TGCTGGTTCC TGCAGCGGCC CGGCCAGAGC CCCCAGCTGC TGATCTACCG GATGAGCAAC
2401 CTGGCCAGCG GCGTGCCCGA CCGGTTCAGC GGCAGCGGCA GCGGCACCGC CTTCACCCTG
2461 CGGATCAGCC GGGTGGAGGC CGAGGACGTG GGCGTGTACT ACTGCATGCA GCACCTGGAG
2521 TACCCCTTCA CCTTCGGCAG CGGCACCAAG CTGGAGATCA AGCGGGGCGG CGGCGGCAGC
2581 GGCGGCGGCG GCAGCGGCGG CGGCGGCAGC CAGGTGCAGC TGCAGCAGAG CGGCGCCGAG
2641 CTGGTGCGGC CCGGCACCAG CGTGAAGATC AGCTGCAAGG CCAGCGGCTA CACCTTCACC
2701 AACTACTGGC TGGGCTGGGT GAAGCAGCGG CCCGGCCACG GCCTGGAGTG GATCGGCGAC
2761 ATCTACCCCG GCGGCGGCTA CAACAAGTAC AACGAGAACT TCAAGGGCAA GGCCACCCTG
2821 ACCGCCGACA CCAGCAGCAG CACCGCCTAC ATGCAGCTGA GCAGCCTGAC CAGCGAGGAC
2881 AGCGCCGTGT ACTTCTGCGC CGGGGAGTAC GGCAACTACG ACTACGCCAT GGACAGCTGG
2941 GGCCAGGGCA CCAGCGTGAC CGTGAGCAGC GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
3001 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GGCGGTGGAG GTTCCGGTGG CGGGGGATCA
3061 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCGGAAC TCCTGGGCGG ACCGTCAGTC
3121 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
3181 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
3241 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
3301 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
3361 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
3421 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
3481 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
3541 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
3601 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
3661 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
3721 CTCTCCCTGT CTCCGGGTAA ATGA
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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 scFv italicized, and linker with proprotein convertase processing sites is shown in bold

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   1 MVSQALRLLC LLLGLQGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
  61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
 121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
 181 KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
 241 NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
 301 LPERTFSERT LAFVRFSLVS GWGQLLDRGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
 361 ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
 421 YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
 481 PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
 541 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
 601 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSDGSFFL
 661 YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KRRRRSGGGG SGGGGSGGGG
 721 SGGGGSGGGG SGGGGSRKRR KRDIVMTQAA PSVPVTPGES VSISCRSSRS LLHSNGNTYL
 781 CWFLQRPGQS PQLLIYRMSN LASGVPDRFS GSGSGTAFTL RISRVEAEDV GVYYCMQHLE
 841 YPFTFGSGTK LEIKRGGGGS GGGGSGGGGS QVQLQQSGAE LVRPGTSVKI SCKASGYTFT
 901 NYWLGWVKQR PGHGLEWIGD IYPGGGYNKY NENFKGKATL TADTSSSTAY MQLSSLTSED
 961 SAVYFCAREY GNYDYAMDSW GQGTSVTVSS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS
1021 DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
1081 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
1141 GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS
1201 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*
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DNA sequence for FVIII-041

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   1 ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG CTTTAGTGCC
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 121 GGTGAGCTGC CTGTGGACGC AAGATTTCCT CCTAGAGTGC CAAAATCTTT TCCATTCAAC
 181 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
 421 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCCTG
 481 AAAGAGAATG GTCCAATGGC CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT
 541 GTGGACCTGG TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
 601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
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 781 CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAATGGGC
 841 ACCACTCCTG AAGTGCACTC AATATTCCTC GAAGGTCACA CATTTCTTGT GAGGAACCAT
 901 CGCCAGGCGT CCTTGGAAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC ACTCTTGATG
 961 GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
1021 GCTTATGTCA AAGTAGACAG CTGTCCAGAG GAACCCCAAC TACGAATGAA AAATAATGAA
1081 GAAGCGGAAG ACTATGATGA TGATCTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
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1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT AGTCCTCGCC
1261 CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG GCCCTCAGCG GATTGGTAGG
1321 AAGTACAAAA AAGTCCGATT TATGGCATAC ACAGATGAAA CCTTTAAGAC TCGTGAAGCT
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1381 ATTCAGCATG AATCAGGAAT CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG
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1561 CCAATTCTGC CAGGAGAAAT ATTCAAATAT AAATGGACAG TGACTGTAGA AGATGGGCCA
1621 ACTAAATCAG ATCCTCGGTG CCTGACCCGC TATTACTCTA GTTTCGTTAA TATGGAGAGA
1681 GATCTAGCTT CAGGACTCAT TGGCCCTCTC CTCATCTGCT ACAAAGAATC TGTAGATCAA
1741 AGAGGAAACC AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTTCTGT ATTTGATGAG
1801 AACCGAAGCT GGTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC AGCTGGAGTG
1861 CAGCTTGAGG ATCCAGAGTT CCAAGCCTCC AACATCATGC ACAGCATCAA TGGCTATGTT
1921 TTTGATAGTT TGCAGTTGTC AGTTTGTTTG CATGAGGTGG CATACTGGTA CATTCTAAGC
1981 ATTGGAGCAC AGACTGACTT CCTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA
2041 ATGGTCTATG AAGACACACT CACCCTATTC CCATTCTCAG GAGAAACTGT CTTCATGTCG
2101 ATGGAAAACC CAGGTCTATG GATTCTGGGG TGCCACAACT CAGACTTTCG GAACAGAGGC
2161 ATGACCGCCT TACTGAAGGT TTCTAGTTGT GACAAGAACA CTGGTGATTA TTACGAGGAC
2221 AGTTATGAAG ATATTTCAGC ATACTTGCTG AGTAAAAACA ATGCCATTGA ACCAAGAAGC
2281 TTCTCTCAAA ACCCACCAGT CTTGAAACGC CATCAACGGG AAATAACTCG TACTACTCTT
2341 CAGTCAGATC AAGAGGAAAT TGACTATGAT GATACCATAT CAGTTGAAAT GAAGAAGGAA
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2461 CGACACTATT TTATTGCTGC AGTGGAGAGG CTCTGGGATT ATGGGATGAG TAGCTCCCCA
2521 CATGTTCTAA GAAACAGGGC TCAGAGTGGC AGTGTCCCTC AGTTCAAGAA AGTTGTTTTC
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2641 TTGGGACTCC TGGGGCCATA TATAAGAGCA GAAGTTGAAG ATAATATCAT GGTAACTTTC
2701 AGAAATCAGG CCTCTCGTCC CTATTCCTTC TATTCTAGCC TTATTTCTTA TGAGGAAGAT
2761 CAGAGGCAAG GAGCAGAACC TAGAAAAAAC TTTGTCAAGC CTAATGAAAC CAAAACTTAC
2821 TTTTGGAAAG TGCAACATCA TATGGCACCC ACTAAAGATG AGTTTGACTG CAAAGCCTGG
2881 GCTTATTTCT CTGATGTTGA CCTGGAAAAA GATGTGCACT CAGGCCTGAT TGGACCCCTT
2941 CTGGTCTGCC ACACTAACAC ACTGAACCCT GCTCATGGGA GACAAGTGAC AGTACAGGAA
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3121 TATCGCTTCC ATGCAATCAA TGGCTACATA ATGGATACAC TACCTGGCTT AGTAATGGCT
3181 CAGGATCAAA GGATTCGATG GTATCTGCTC AGCATGGGCA GCAATGAAAA CATCCATTCT
3241 ATTCATTTCA GTGGACATGT GTTCACTGTA CGAAAAAAAG AGGAGTATAA AATGGCACTG
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3541 TCCGGATCAA TCAATGCCTG GAGCACCAAG GAGCCCTTTT CTTGGATCAA GGTGGATCTG
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3841 TATAGCATTC GCAGCACTCT TCGCATGGAG TTGATGGGCT GTGATTTAAA TAGTTGCAGC
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3961 TTTACCAATA TGTTTGCCAC CTGGTCTCCT TCAAAAGCTC GACTTCACCT CCAAGGGAGG
4021 AGTAATGCCT GGAGACCTCA GGTGAATAAT CCAAAAGAGT GGCTGCAAGT GGACTTCCAG
4081 AAGACAATGA AAGTCACAGG AGTAACTACT CAGGGAGTAA AATCTCTGCT TACCAGCATG
4141 TATGTGAAGG AGTTCCTCAT CTCCAGCAGT CAAGATGGCC ATCAGTGGAC TCTCTTTTTT
4201 CAGAATGGCA AAGTAAAGGT TTTTCAGGGA AATCAAGACT CCTTCACACC TGTGGTGAAC
4261 TCTCTAGACC CACCGTTACT GACTCGCTAC CTTCGAATTC ACCCCCAGAG TTGGGTGCAC
4321 CAGATTGCCC TGAGGATGGA GGTTCTGGGC TGCGAGGCAC AGGACCTCTA CGACAAAACT
4381 CACACATGCC CACCGTGCCC AGCTCCAGAA CTCCTGGGCG GACCGTCAGT CTTCCTCTTC
4441 CCCCCAAAAC CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG
4501 GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA CGGCGTGGAG
4561 GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA ACAGCACGTA CCGTGTGGTC
4621 AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA AGGAGTACAA GTGCAAGGTC
4681 TCCAACAAAG CCCTCCCAGC CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC
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4801 AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA GTGGGAGAGC
4861 AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG TGTTGGACTC CGACGGCTCC
4921 TTCTTCCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG GAACGTCTTC
4981 TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG
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5221 CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC
5281 TGGTACGTGG ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC
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5401 AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCCAG CCCCCATCGA GAAAACCATC
5461 TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGCGAT
5521 GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC
5581 ATCGCCGTGG AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC
5641 GTGTTGGACT CCGACGGCTC CTTCTTCCTC TACAGCAAGC TCACCGTGGA CAAGAGCAGG
5701 TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC
5761 ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAATGA
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FVIII-041 amino acid sequence. Signal sequence is shown in dotted underline, and linker region connecting the Fc regions is underlined

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   1 MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP PRVPKSFPFN
  61 TSVVYKKTLF VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY DTVVITLKNM ASHPVSLHAV
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 181 VDLVKDLNSG LIGALLVCRE GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD
 241 AASARAWPKM HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE EPQLRMKNNE
 361 EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT WVHYIAAEEE DWDYAPLVLA
 421 PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY TDETFKTREA IQHESGILGP LLYGEVGDTL
 481 LIIFKNQASR PYNIYPHGIT DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP
 541 TKSDPRCLTR YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL HEVAYWYILS
 661 IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS MENPGLWILG CHNSDFRNRG
 721 MTALLKVSSC DKNTGDYYED SYEDISAYLL SKNNAIEPRS FSQNPPVLKR HQREITRTTL
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 961 AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE FALFFTIFDE TKSWYFTENM
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1081 IHFSGHVFTV RKKEEYKMAL YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL
1141 VYSNKCQTPL GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1201 LAPMIIHGIK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV FFGNVDSSGI
1261 KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS MPLGMESKAI SDAQITASSY
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1381 YVKEFLISSS QDGHQWTLFF QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH
1441 QIALRMEVLG CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1501 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV
1561 SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES
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1681 SPGKGGGGSG GGGSGGGGSG GGGSDKTHTC PPCPAPELLG GPSVFLFPPK PKDTLMISRT
1741 PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG
1801 KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYILPPSRD ELTKNQVSLT CLVKGFYPSD
1861 IAVEWESNGQ PENNYKTTPP VLDSDGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
1921 TQKSLSLSPG K*
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DNA sequence for FVIII-108

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   1 ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG CTTTAGTGCC
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 421 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCCTG
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1381 ATTCAGCATG AATCAGGAAT CTTGGGACCT TTACTTTATG GGGAAGTTGG AGACACACTG
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2281 TTCTCTCAAA ACCCACCAGT CTTGAAACGC CATCAACGGG AAATAACTCG TACTACTCTT
2341 CAGTCAGATC AAGAGGAAAT TGACTATGAT GATACCATAT CAGTTGAAAT GAAGAAGGAA
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2401 GATTTTGACA TTTATGATGA GGATGAAAAT CAGAGCCCCC GCAGCTTTCA AAAGAAAACA
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5161 AAGAGGAGGA AGAGGGCGCA GGTGCAGCTG CAGGAGTCTG GGGGAGGCTT GGTACAGCCT
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5281 AGCTGGGTCC GCCAGGCTCC AGGGAAGGGG CCAGAGTGGG TCTCAGGTAT TAGTGGTAGT
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6721 CACTACACGC AGAAGAGCCT CTCCCTGTCT CCGGGTAAAT GA
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FVIII-108 amino acid sequence. Signal sequence is shown in dotted underline, linker region connecting SCE5 to Fc region is underlined, and linker with proprotein convertase processing sites is shown in bold

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   1 MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP PRVPKSFPFN
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     KGRFTVSRDN
1801     SKNTLYLQMN SLRAEDTAVY YCARGATYTS RSDVPDQTSF DYWGQGTLVT
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1861     LEEGEFSEAR VSELTQDPAV SVALGQTVRI TCQGDSLRNF YASWYQQKPG
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1921     SKRPSGIPDR FSASSSGNTA SLTITGAQAE DEADYYCLLY YGGGQQGVFG
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1981     PKAAPSVTLF PPSSAAGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSDKTH
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2041     LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
     HNAKTKPREE
2101     QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
     EPQVYTLPPS
2161     RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF
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2221     SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK*
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DNA sequence for pSYN-FVIII-049

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5821 TACACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGA

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

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CLAIMS

1. A chimeric clotting factor comprising:

(i) a clotting factor selected from the group consisting of factor VII (FVII), factor IX (FIX) and factor X (FX);

(ii) a targeting moiety which binds to platelets; and

(iii) a first Fc moiety and a second Fc moiety,

wherein the clotting factor is fused to the first Fc moiety, and

wherein the targeting moiety is fused to the second Fc moiety.

2. 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 a corresponding clotting factor lacking a targeting moiety.

3. The chimeric clotting factor of claim 1 or claim 2, further comprising a cleavable scFc (cscFc) linker interposed between the first Fc moiety and the second Fc moiety, wherein the cscFc linker comprises at least one enzymatic cleavage site which results in cleavage of the cscFc linker.

4. The chimeric clotting factor of claim 3, wherein the at least one enzymatic cleavage site is an intracellular processing site.

5. The chimeric clotting factor of claim 3 or claim 4, wherein the cscFc linker is flanked by two enzymatic cleavage sites which are recognized by the same or by different enzymes.

6. The chimeric clotting factor of any one of claims 3 to 5, wherein the cscFc linker has a length of about 10 to about 50 amino acids.

7. The chimeric clotting factor of any one of claims 3 to 6, wherein the cscFc linker has a length of about 20 to about 30 amino acids.

8. The chimeric clotting factor of any one of claims 3 to 7, wherein the cscFc linker comprises a gly/ser peptide.

9. The chimeric clotting factor of claim 8, wherein the gly/ser peptide is comprised of a formula $(Gly_4Ser)n$ or $Ser(Gly_4Ser)n$, wherein n is a positive integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

10. The chimeric clotting factor of claim 9, wherein the gly/ser peptide having the formula $(Gly_4\ Ser)n$ is selected from the group consisting of $(Gly_4\ Ser)_6$, $Ser(Gly_4\ Ser)_6$, $(Gly_4\ Ser)_4$ and $Ser(Gly_4\ Ser)_4$.

11. The chimeric clotting factor of claim 1 or claim 2, wherein the clotting factor comprises two polypeptide chains.

12. The chimeric clotting factor of claim 1 or claim 2, wherein the chimeric clotting factor has a structure selected from the group consisting of:

the clotting factor fused to the first Fc moiety and the targeting moiety fused to the second Fc moiety;

the clotting factor fused to the first Fc moiety via a spacer moiety and the targeting moiety fused to the second Fc moiety via a spacer moiety;

the clotting factor fused to the first Fc moiety and the targeting moiety fused to the second Fc moiety via a spacer moiety; and

the clotting factor fused to the first Fc moiety via a spacer moiety and the targeting moiety fused to the second Fc moiety.

13. The chimeric clotting factor of claim 12, which comprises two polypeptides wherein:

(i) the first polypeptide comprises the clotting factor, a spacer moiety and the first Fc moiety, and the second polypeptide comprises the targeting moiety and the second Fc moiety;

(ii) the first polypeptide comprises the clotting factor, a first spacer moiety and the first Fc moiety, and the second polypeptide comprises the targeting moiety, a second spacer moiety and the second Fc moiety;

(iii) the first polypeptide comprises the clotting factor and the first Fc moiety, and the second polypeptide comprises the targeting moiety and the second Fc moiety; or

(iv) the first polypeptide comprises the clotting factor and the first Fc moiety and the second polypeptide comprises the targeting moiety, a spacer moiety, and the second Fc moiety, and

wherein the two polypeptides form a covalent bond.

14. The chimeric clotting factor of claim 1 or claim 2, wherein the targeting moiety is fused to the second Fc moiety directly.

15. The chimeric clotting factor of claim 1 or claim 2, wherein the targeting moiety is fused to the second Fc moiety via a spacer moiety.

16. The chimeric clotting factor of claim 1 or claim 2, wherein the targeting moiety is fused to the second Fc moiety via a cleavable linker.

17. The chimeric clotting factor of any one of claims 1 to 16, 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 and a peptide.

18. The chimeric clotting factor of any one of claims 1 to 17, wherein the targeting moiety binds to resting platelets.

19. The chimeric clotting factor of any one of claims 1 to 17, wherein the targeting moiety selectively binds to activated platelets.

20. The chimeric clotting factor of claim 18, wherein the targeting moiety selectively binds to a target selected from the group consisting of: GPIba, GPVI, and the nonactive form of GPIIb/IIIa.

21. The chimeric clotting factor of claim 19, 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.

22. The chimeric clotting factor of claim 18, wherein the targeting moiety binds to the GPIb complex.

23. The chimeric clotting factor of claim 17, wherein the targeting moiety is a peptide selected from the group consisting of: PS4, OS1 and OS2.

24. The chimeric clotting factor of claim 17, wherein the targeting moiety comprises an antibody variable region from an antibody selected from the group consisting of: SCE5, MB9 and AP3.

25. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is Factor VII.

26. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is a high specific activity variant of Factor VII.

27. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is Factor IX.

28. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is a high specific activity variant of Factor IX.

29. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is Factor X.

30. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is a high specific activity variant of Factor X.

31. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is secreted by a cell in an active form.

32. The chimeric clotting factor of any one of claims 1 to 24, wherein the clotting factor is activated *in vivo*.

33. The chimeric clotting factor of any one of claims 1 to 32, wherein the chimeric clotting factor comprises a heterologous enzymatic cleavage site not naturally present in the clotting factor.

34. The chimeric clotting factor of claim 33, wherein the clotting factor comprises a heavy chain and a light chain, and the heterologous enzymatic cleavage site is genetically fused to the amino terminus of the heavy chain of the clotting factor.

35. A nucleic acid molecule encoding the chimeric clotting factor of any one of claims 1 to 34.

36. An expression vector comprising the nucleic acid molecule of claim 35.

37. The expression vector of claim 36, wherein the vector further comprises a nucleotide sequence encoding an enzyme which cleaves at least one of the enzymatic cleavage sites.

38. An isolated host cell comprising the expression vector of claim 36 or claim 37.

39. The host cell of claim 38, which expresses an enzyme capable of intracellular processing.

40. The host cell of claim 39, wherein the enzyme is endogenous to the cell.

41. The host cell of claim 39, wherein the enzyme is heterologous to the cell.

42. A method of producing a chimeric clotting factor, said method comprising culturing the host cell of any one of claims 38 to 41 in culture medium and recovering the chimeric clotting factor from the medium.

43. A processed, heterodimeric polypeptide comprising two polypeptide chains, wherein said processed, heterodimeric polypeptide is made by expressing the vector of claim 36 or claim 37 in a cell cultured in cell culture medium and isolating the heterodimeric polypeptide from the cell culture medium.

44. A composition comprising the chimeric clotting factor of any one of claims 1 to 34 and a pharmaceutically acceptable carrier.

45. A composition comprising the nucleic acid molecule of claim 35 and a pharmaceutically acceptable carrier.

46. A method of improving hemostasis in a subject, said method comprising administering to the subject the composition of claim 44 or claim 45.

Date: 30 November 2015

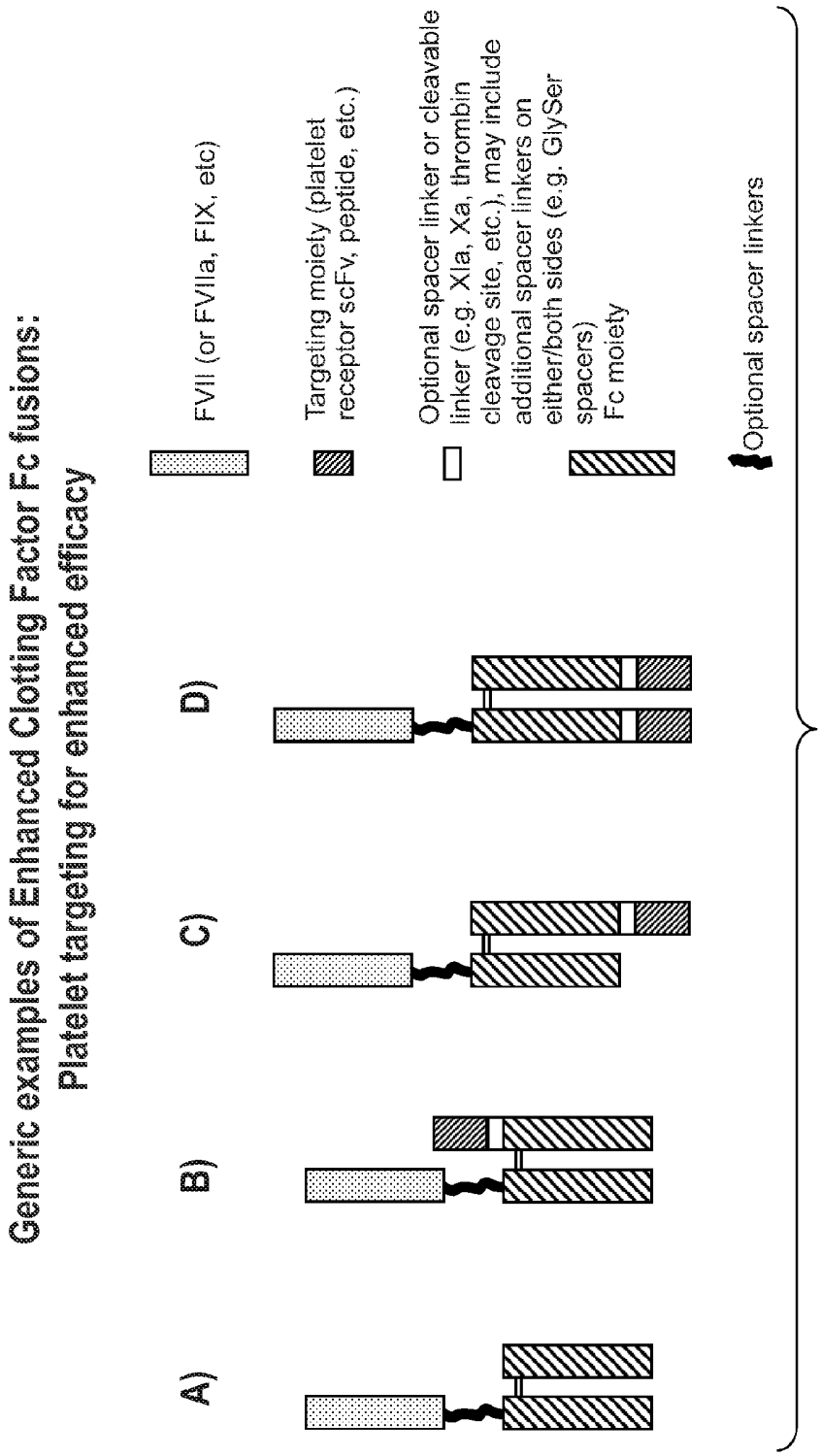

Generic examples of Enhanced Clotting Factor Fc fusions:
Platelet targeting for enhanced efficacy
A)
B)
C)
D)
FVII (or FVIIa, FIX, etc)
Targeting moiety (platelet receptor scFv, peptide, etc.)
Optional spacer linker or cleavable linker (e.g. XIa, Xa, thrombin cleavage site, etc.), may include additional spacer linkers on either/both sides (e.g. GlySer spacers)
Fc moiety
Optional spacer linkers

Fig. 1

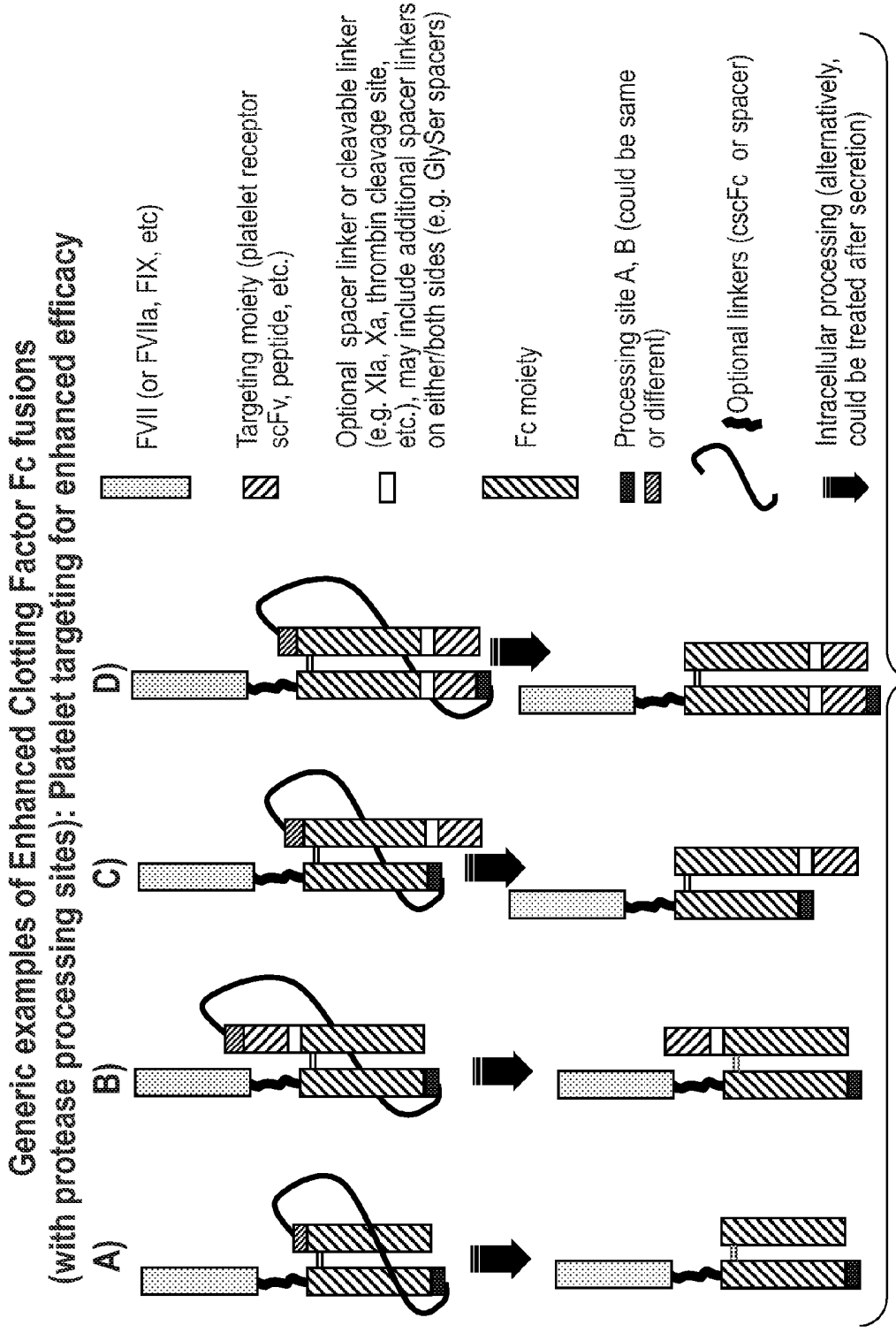

Generic examples of Enhanced Clotting Factor Fc fusions
(with protease processing sites): Platelet targeting for enhanced efficacy
A)
B)
C)
D)
FVII (or FVIIa, FIX, etc)
Targeting moiety (platelet receptor scFv, peptide, etc.)
Optional spacer linker or cleavable linker (e.g. XIa, Xa, thrombin cleavage site, etc.), may include additional spacer linkers on either/both sides (e.g. GlySer spacers)
Fc moiety
Processing site A, B (could be same or different)
Optional linkers (cscFc or spacer)
Intracellular processing (alternatively, could be treated after secretion)

Fig. 2

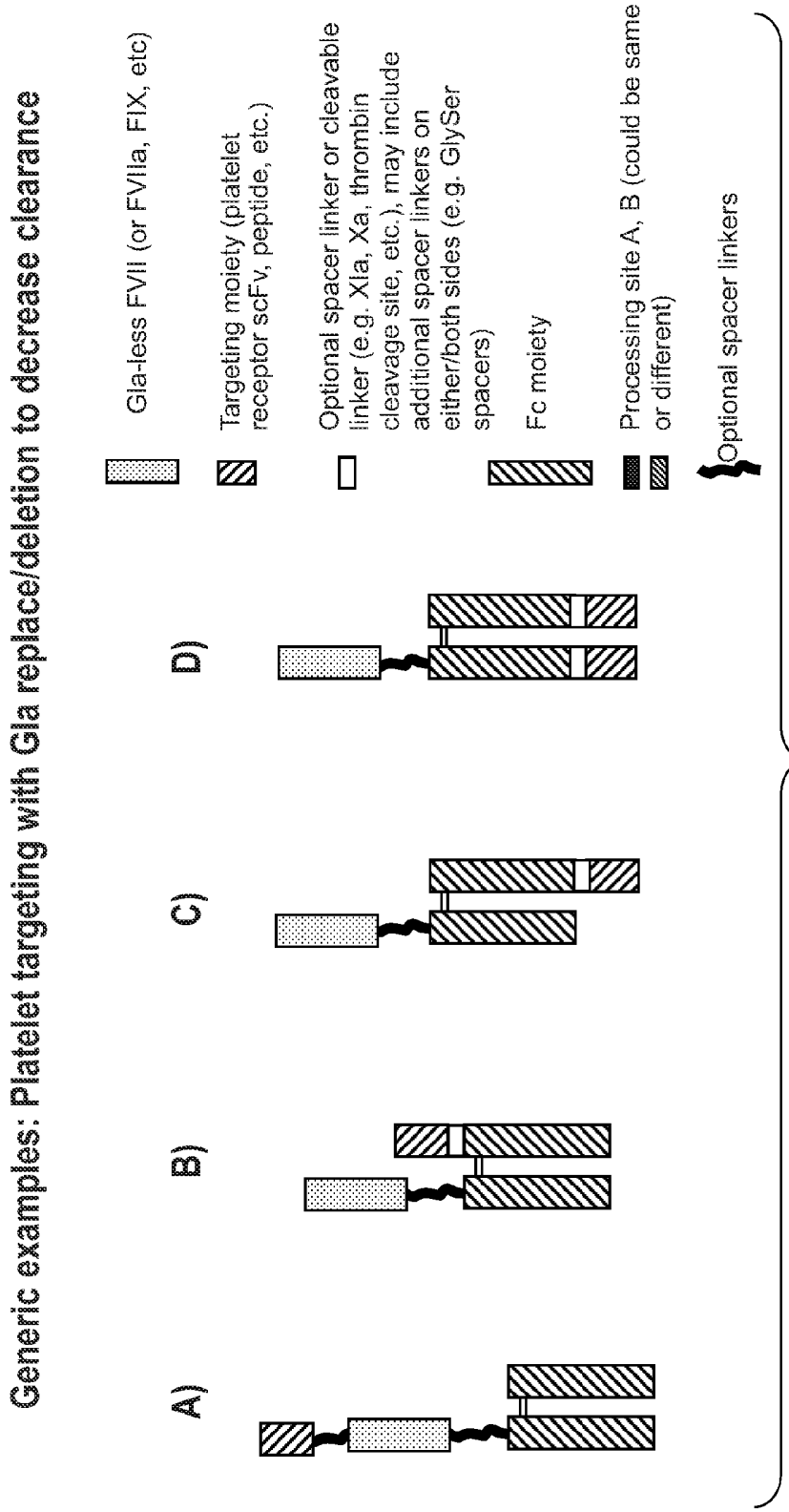

Generic examples: Platelet targeting with Gla replace/deletion to decrease clearance
A)
B)
C)
D)
Gla-less FVII (or FVIIa, FIX, etc)
Targeting moiety (platelet receptor scFv, peptide, etc.)
Optional spacer linker or cleavable linker (e.g. Xla, Xa, thrombin cleavage site, etc.), may include additional spacer linkers on either/both sides (e.g. GlySer spacers)
Fc moiety
Processing site A, B (could be same or different)
Optional spacer linkers

Fig. 3

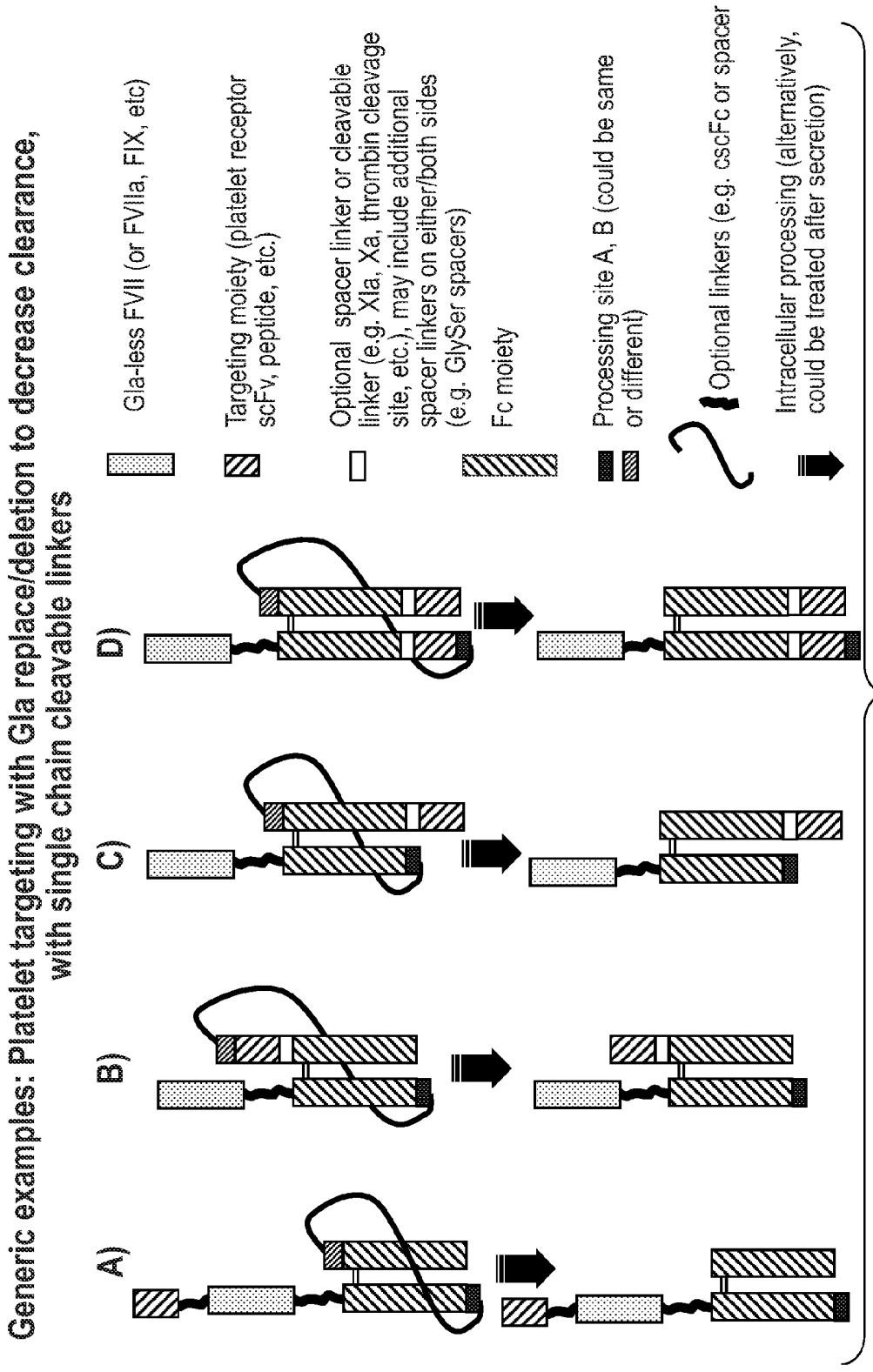

Generic examples: Platelet targeting with Gla replace/deletion to decrease clearance, with single chain cleavable linkers
A)
B)
C)
D)
Gla-less FVII (or FVIIa, FIX, etc)
Targeting moiety (platelet receptor scFv, peptide, etc.)
Optional spacer linker or cleavable linker (e.g. Xla, Xa, thrombin cleavage site, etc.), may include additional spacer linkers on either/both sides (e.g. GlySer spacers)
Fc moiety
Processing site A, B (could be same or different)
Optional linkers (e.g. cscFc or spacer
Intracellular processing (alternatively, could be treated after secretion)

Fig. 4

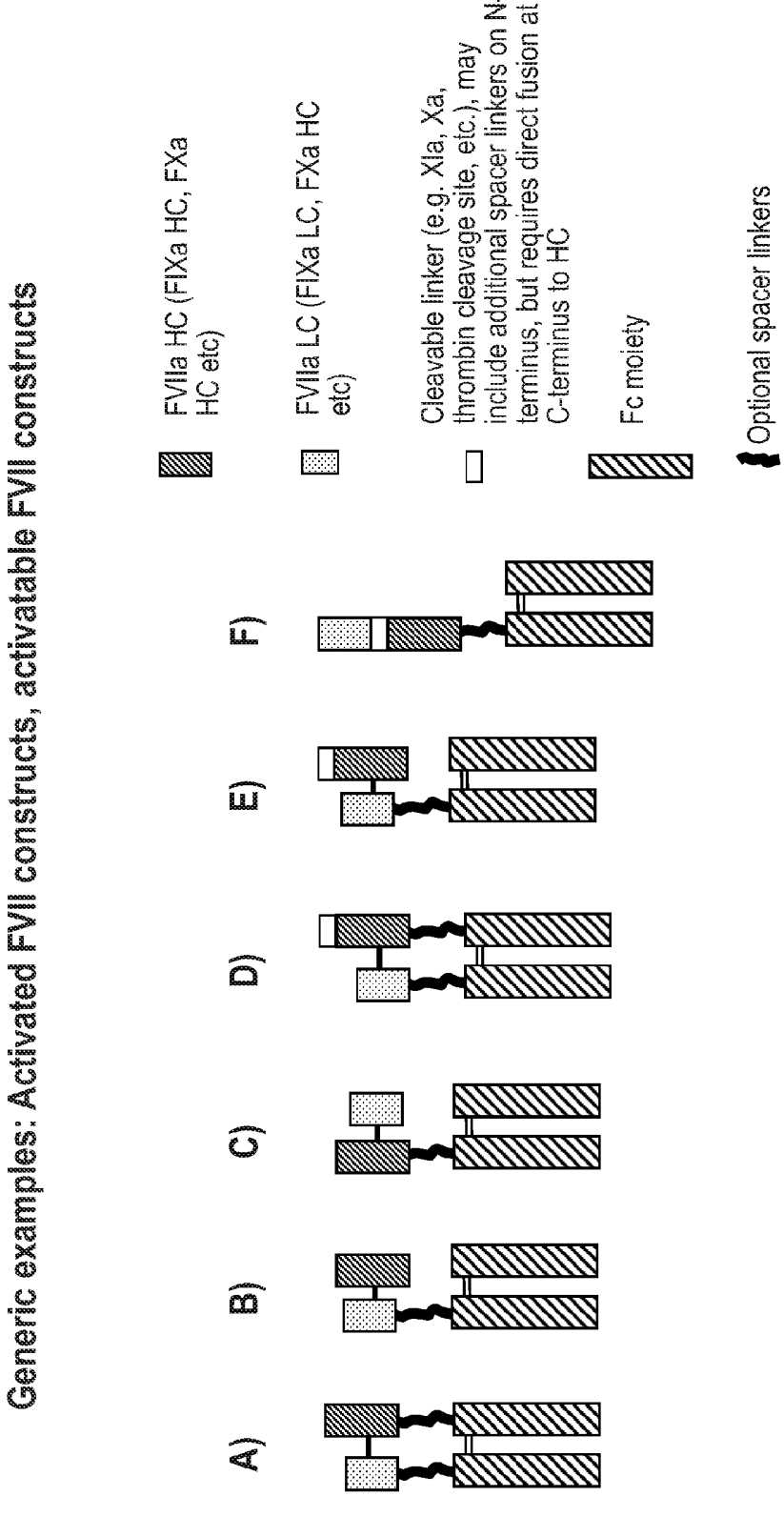

Generic examples: Activated FVII constructs, activatable FVII constructs
FVIIa HC (FIXa HC, FXa HC etc)
FVIIa LC (FIXa LC, FXa HC etc)
Cleavable linker (e.g. XIa, Xa, thrombin cleavage site, etc.), may include additional spacer linkers on N-terminus, but requires direct fusion at C-terminus to HC
Fc moiety
Optional spacer linkers
A)
B)
C)
D)
E)
F)

Fig. 5

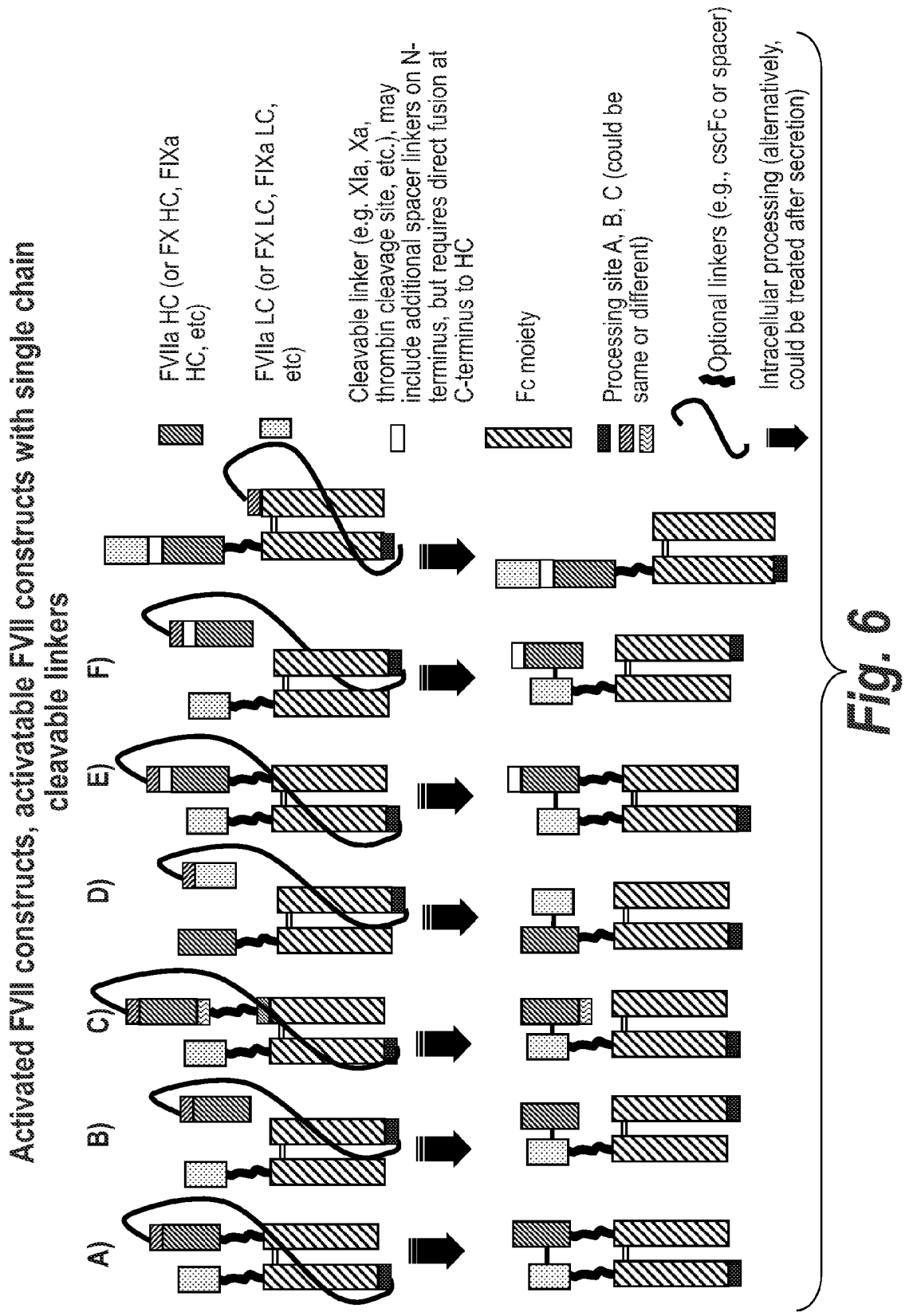

Activated FVIII constructs, activatable FVII constructs with single chain cleavable linkers
A)
B)
C)
D)
E)
F)
FVIIa HC (or FX HC, FIXa HC, etc)
FVIIa LC (or FX LC, FIXa LC, etc)
Cleavable linker (e.g. XIa, Xa, thrombin cleavage site, etc.), may include additional spacer linkers on N-terminus, but requires direct fusion at C-terminus to HC
Fc moiety
Processing site A, B, C (could be same or different)
Optional linkers (e.g., cscFc or spacer)
Intracellular processing (alternatively, could be treated after secretion)

Fig. 6

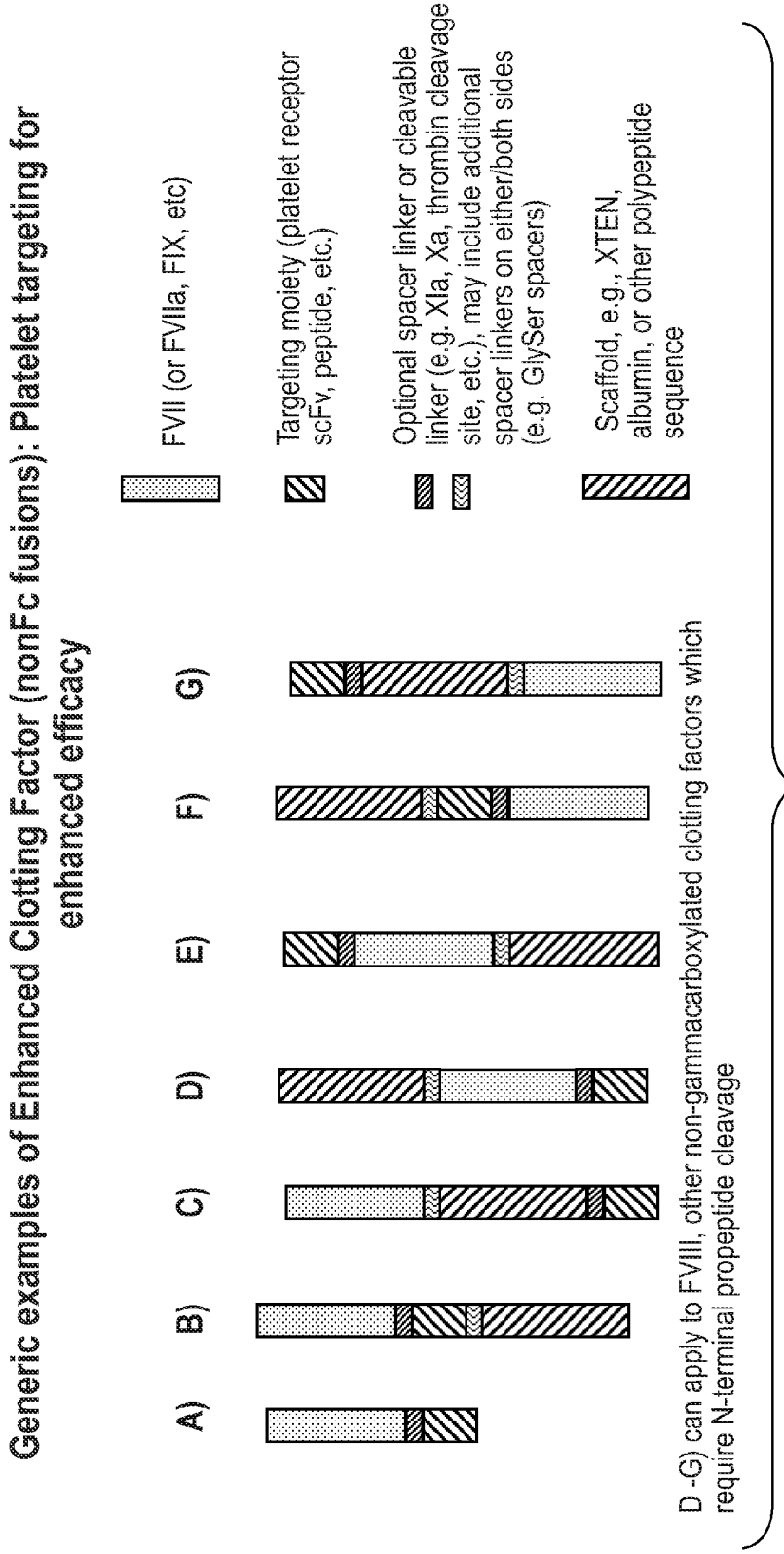

Generic examples of Enhanced Clotting Factor (nonFc fusions): Platelet targeting for enhanced efficacy
A)
B)
C)
D)
E)
F)
G)
FVII (or FVIIa, FIX, etc)
Targeting moiety (platelet receptor scFv, peptide, etc.)
Optional spacer linker or cleavable linker (e.g. XIa, Xa, thrombin cleavage site, etc.), may include additional spacer linkers on either/both sides (e.g. GlySer spacers)
Scaffold, e.g., XTEN, albumin, or other polypeptide sequence
D -G) can apply to FVIII, other non-gammacarboxylated clotting factors which require N-terminal propeptide cleavage

Fig. 7

Generic examples: Platelet targeting with Gla replace/deletion to decrease clearance, to FVII, FIX, FX
(non-Fc fusions)
A)
B)
C)
D)
E)
F)
G)
H)
Gla-less FVII (or FVIIa, FIX, etc)
Targeting moiety (platelet receptor scFv, peptide, etc.)
Optional spacer linker or cleavable linker (e.g. XIa, 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

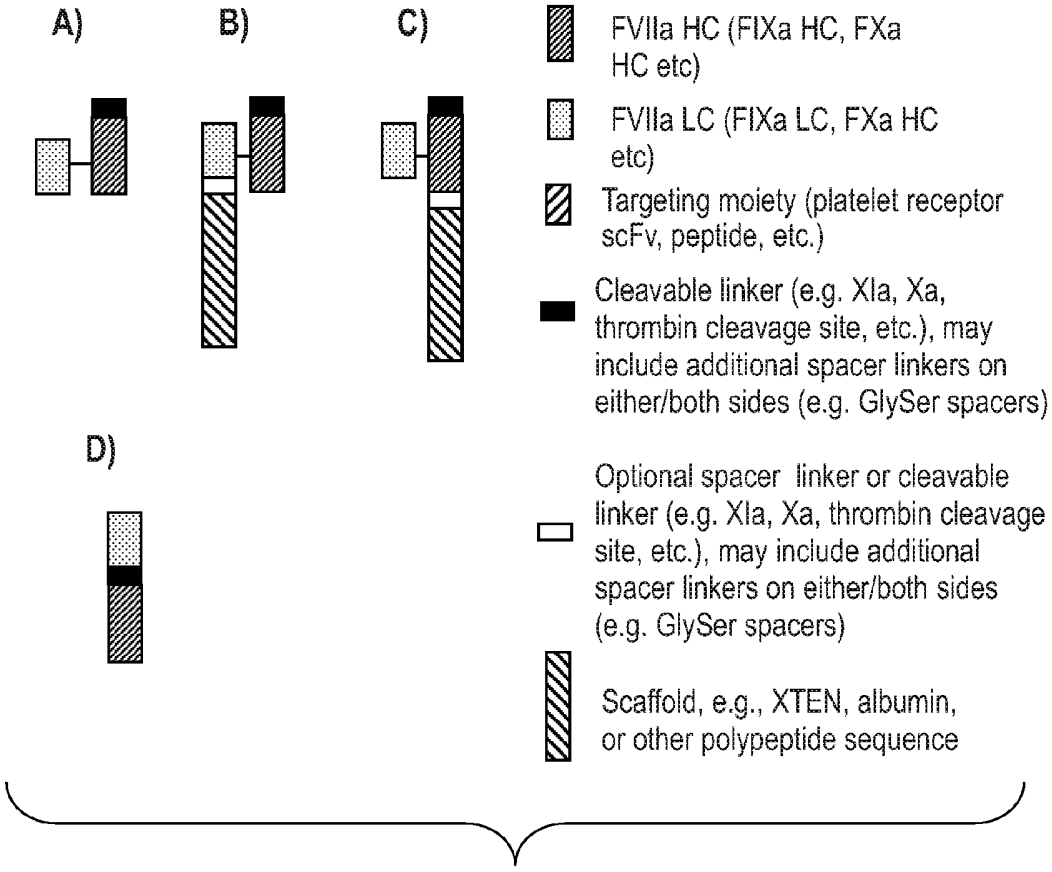

Activated FVII constructs, activatable FVII constructs with non-Fc fusions
A)
B)
C)
D)
FVIIa HC (FIXa HC, FXa HC etc)
FVIIa LC (FIXa LC, FXa HC etc)
Targeting moiety (platelet receptor scFv, peptide, etc.)
Cleavable linker (e.g. XIa, Xa, thrombin cleavage site, etc.), may include additional spacer linkers on either/both sides (e.g. GlySer spacers)
Optional spacer linker or cleavable linker (e.g. XIa, 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

Fig. 9

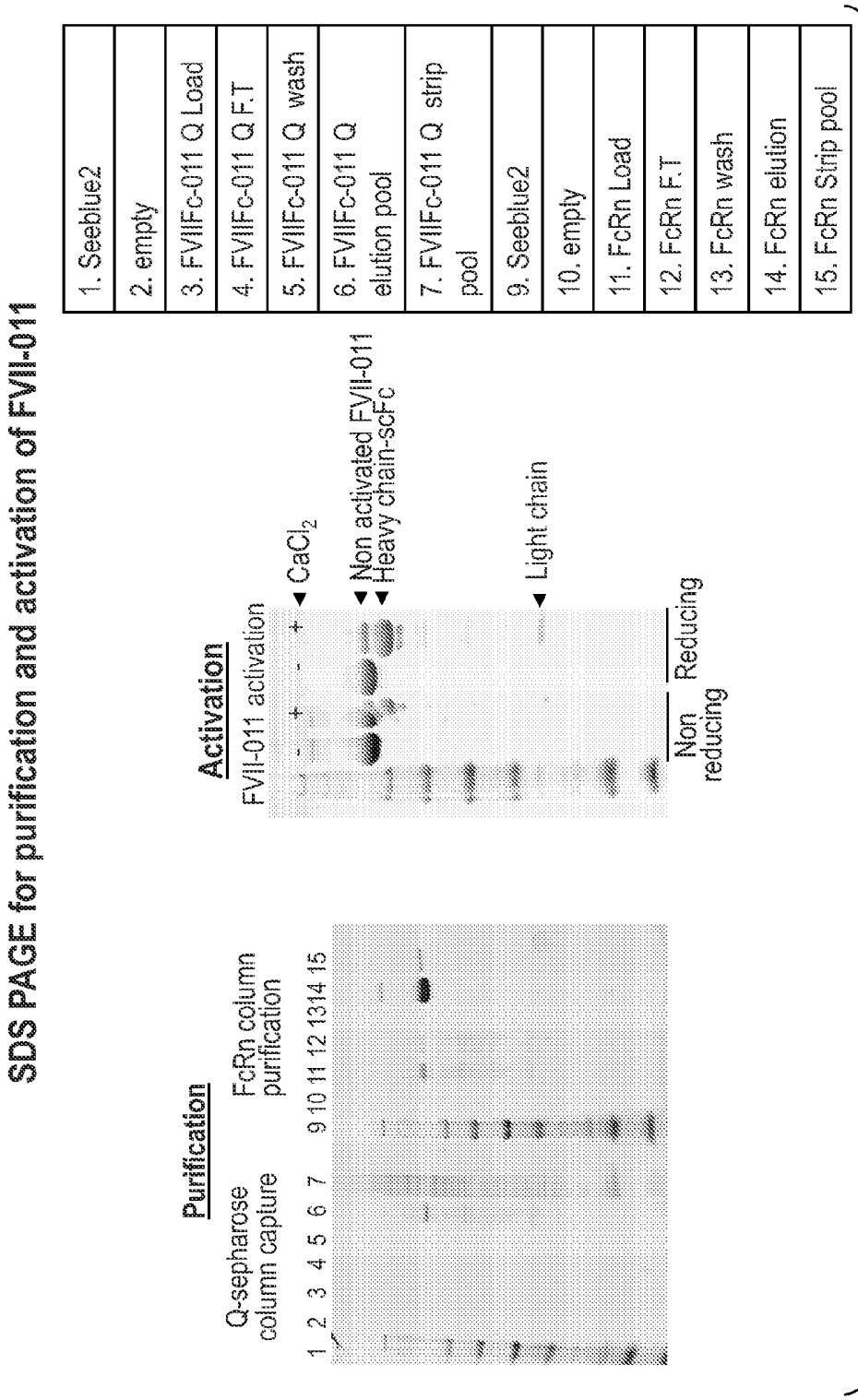
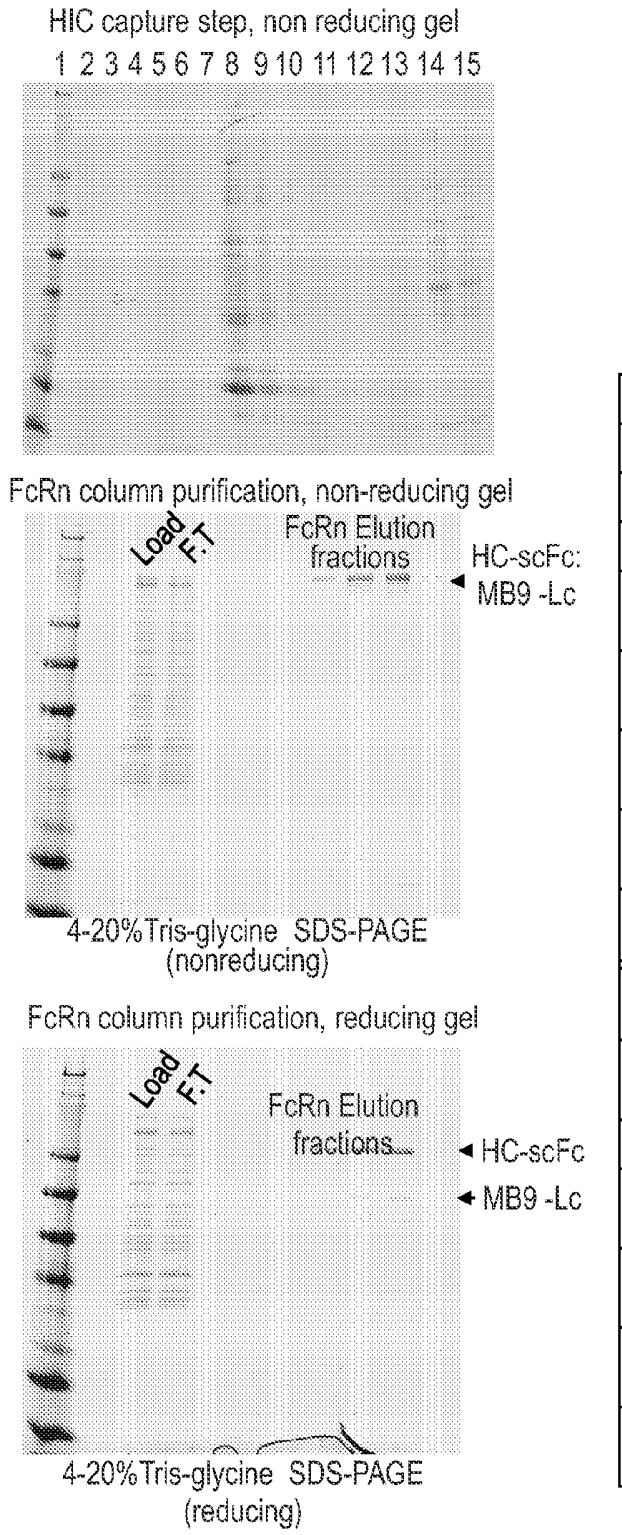

SDS PAGE for purification and activation of FVII-011
Purification
Q-sepharose column capture
FcRn column purification
1 2 3 4 5 6 7 9 10 11 12 13 14 15
Activation
FVII-011 activation
- + - +
CaCl2
Non activated FVII-011
Heavy chain-scFc
Light chain
Non reducing
Reducing
1. Seeblue2
2. empty
3. FVIIFc-011 Q Load
4. FVIIFc-011 Q F.T
5. FVIIFc-011 Q wash
6. FVIIFc-011 Q elution pool
7. FVIIFc-011 Q strip pool
9. Seeblue2
10. empty
11. FcRn Load
12. FcRn F.T
13. FcRn wash
14. FcRn elution
15. FcRn Strip pool

Fig. 10



1. Seeblue2
2. Harvest
3. FVIIFc-053 HIC Load
4. FVIIFc-053 HIC F.T
5. FVIIFc-053 HIC wash Fraction #A2
6. FVIIFc-053 HIC wash Fraction #A3
7. FVIIFc-053 HIC wash Fraction #A4
8. FVIIFc-053 HIC wash Fraction #A5
9. FVIIFc-053 HIC wash Fraction #A6
9. FVIIFc-053 HIC wash Fraction #A6
10. FVIIFc-053 HIC wash Fraction #B7
11. Empty
12. FVIIFc-053 HIC Elution Fraction #B6
13. FVIIFc-053 HIC Elution Fraction #B5
14. FVIIFc-053 HIC Elution Fraction #B4
15. FVIIFc-053 HIC Elution Fraction #B3

Fig. 11

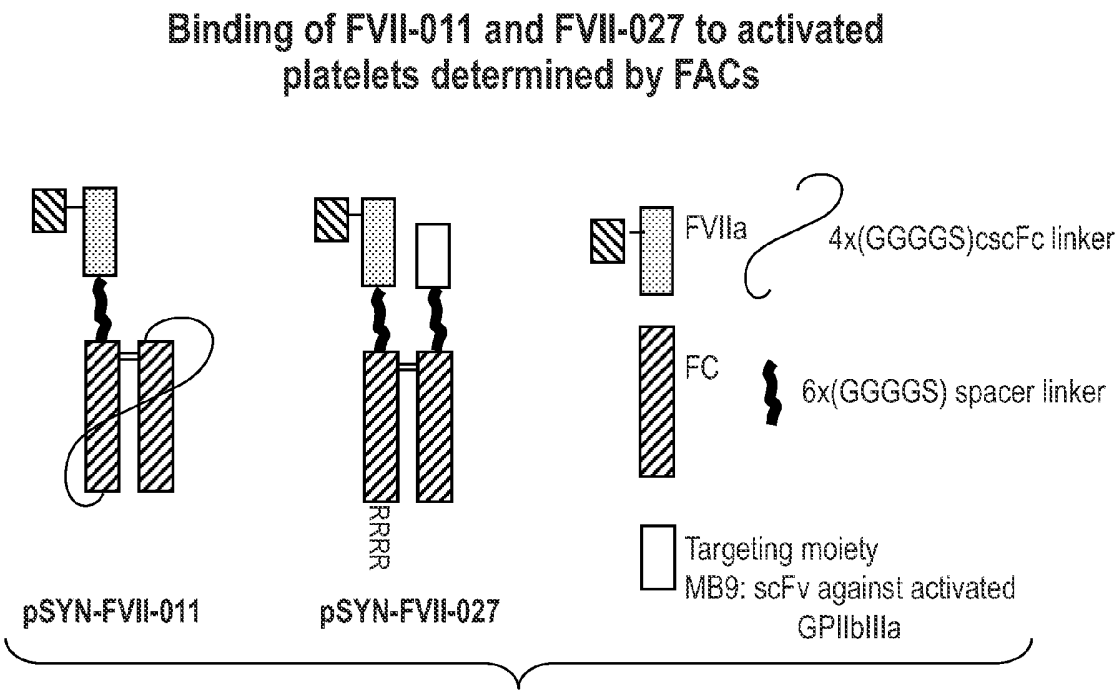


Fig. 12A

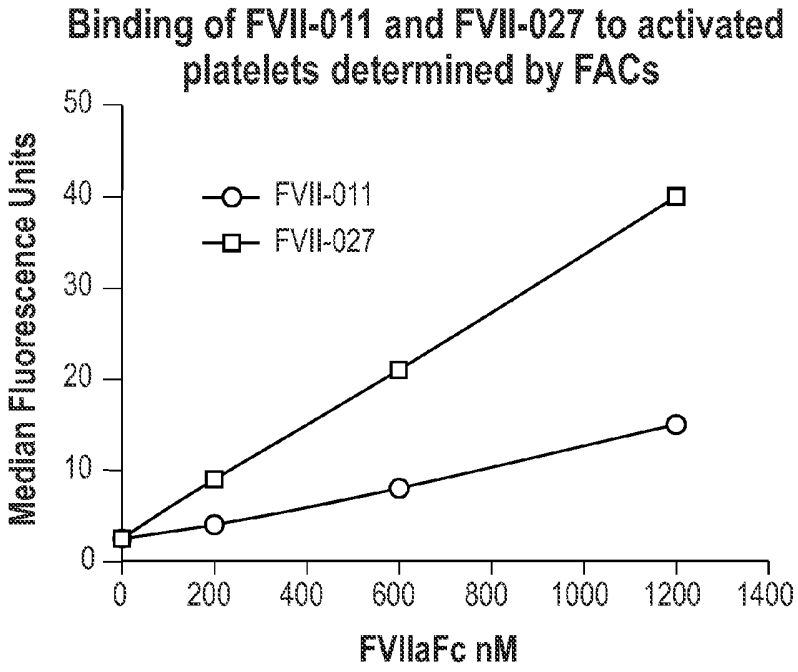


Fig. 12B

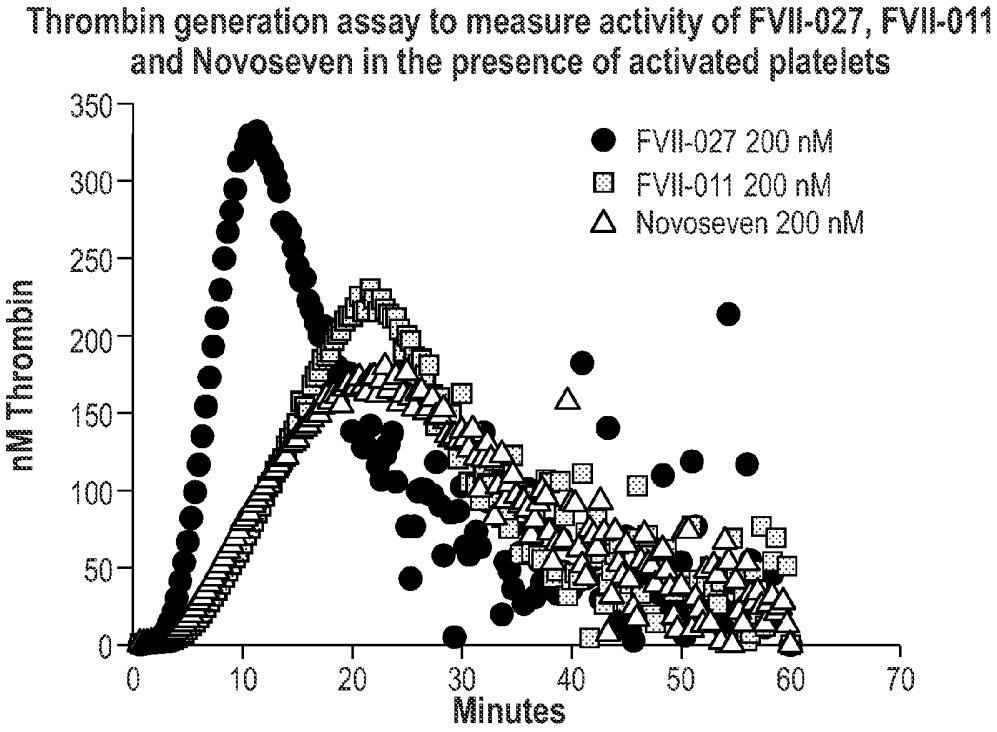


Fig. 13A

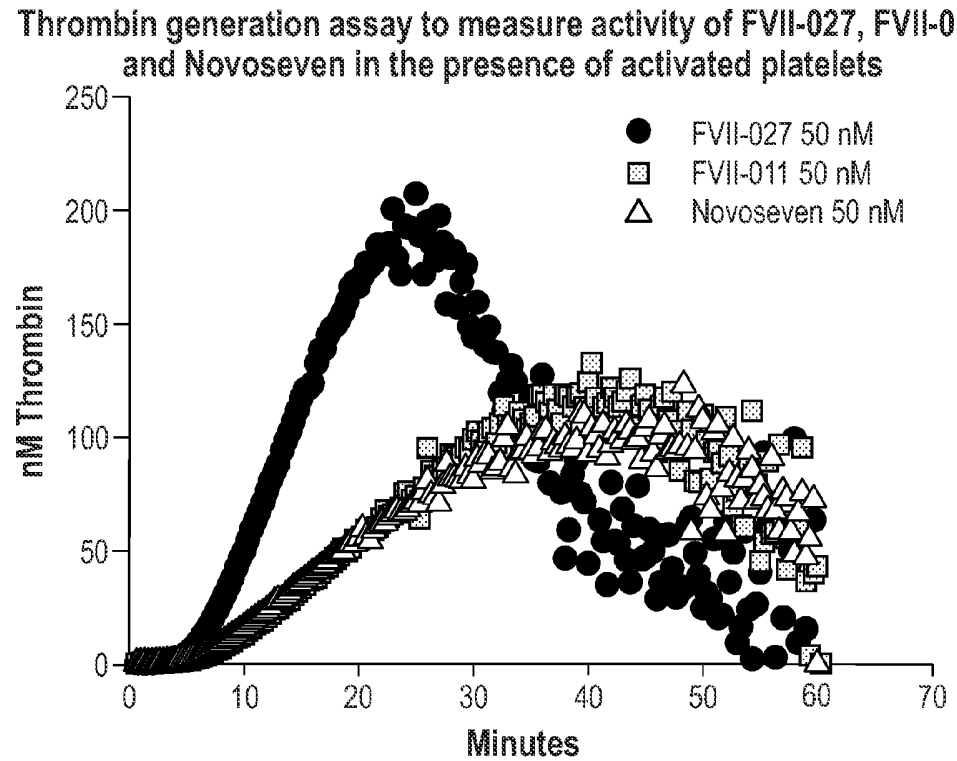


Fig. 13B

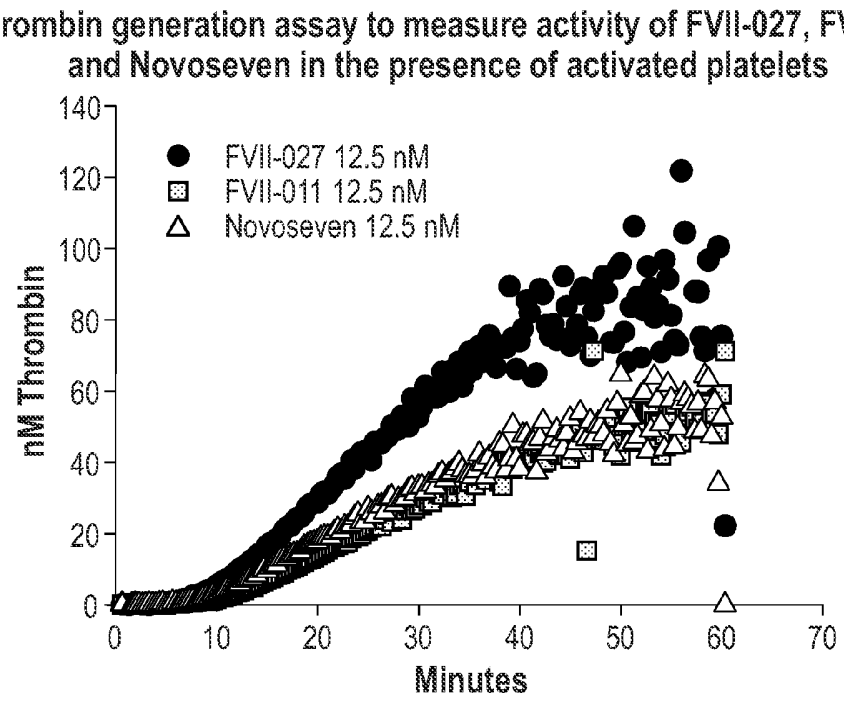


Fig. 13C

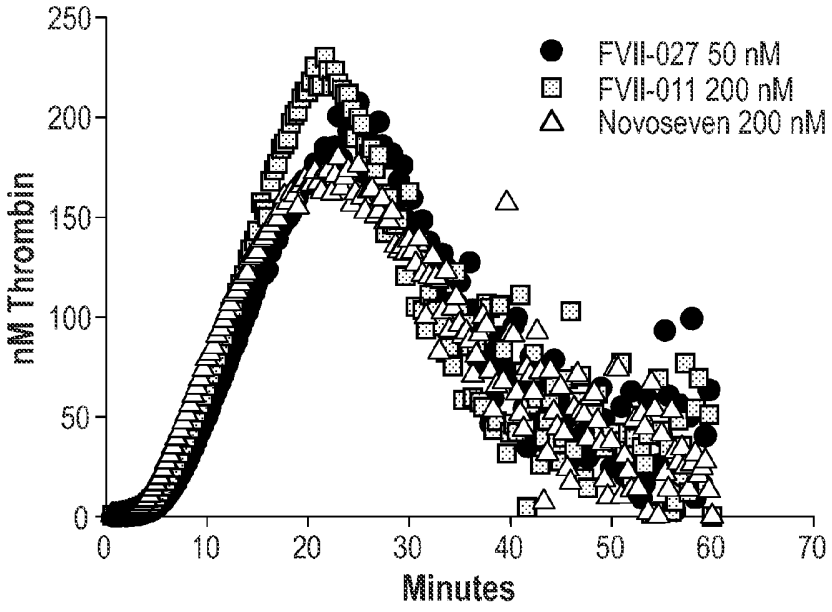


Fig. 13D

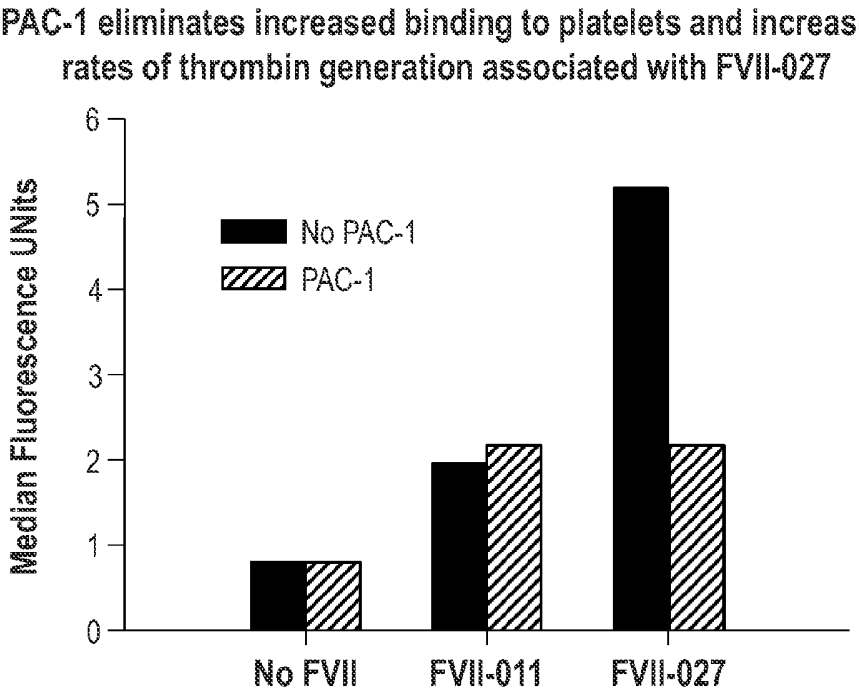


Fig. 14A

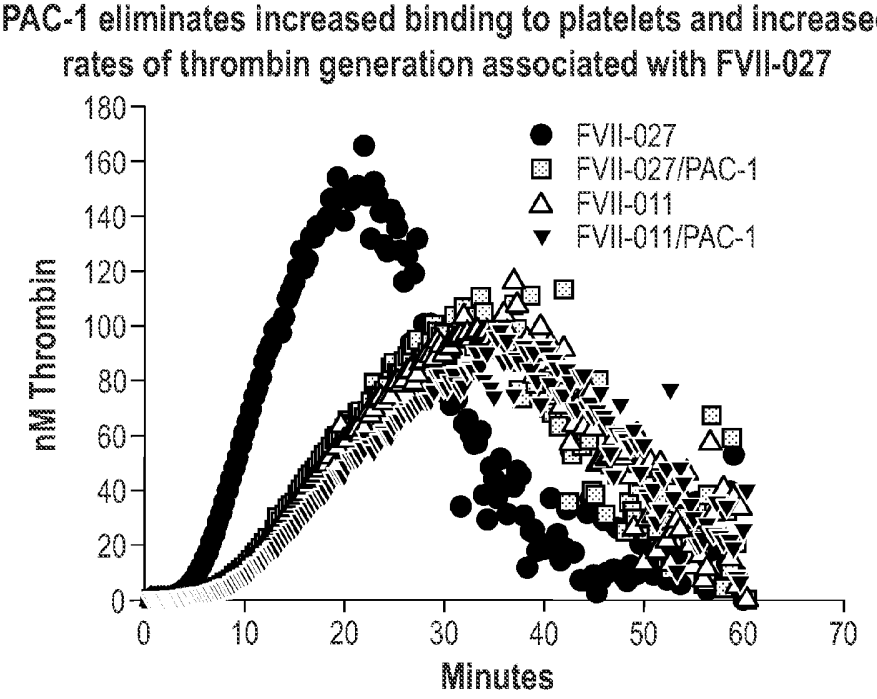


Fig. 14B

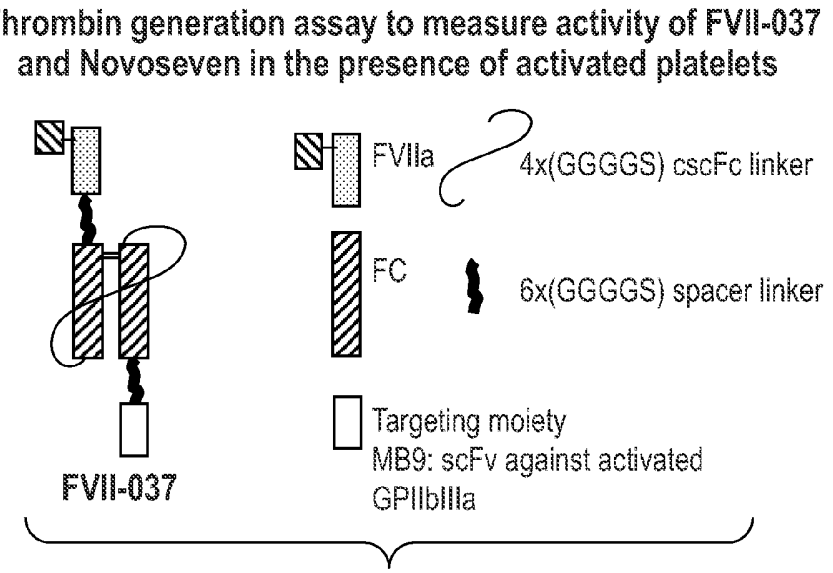


Fig. 15

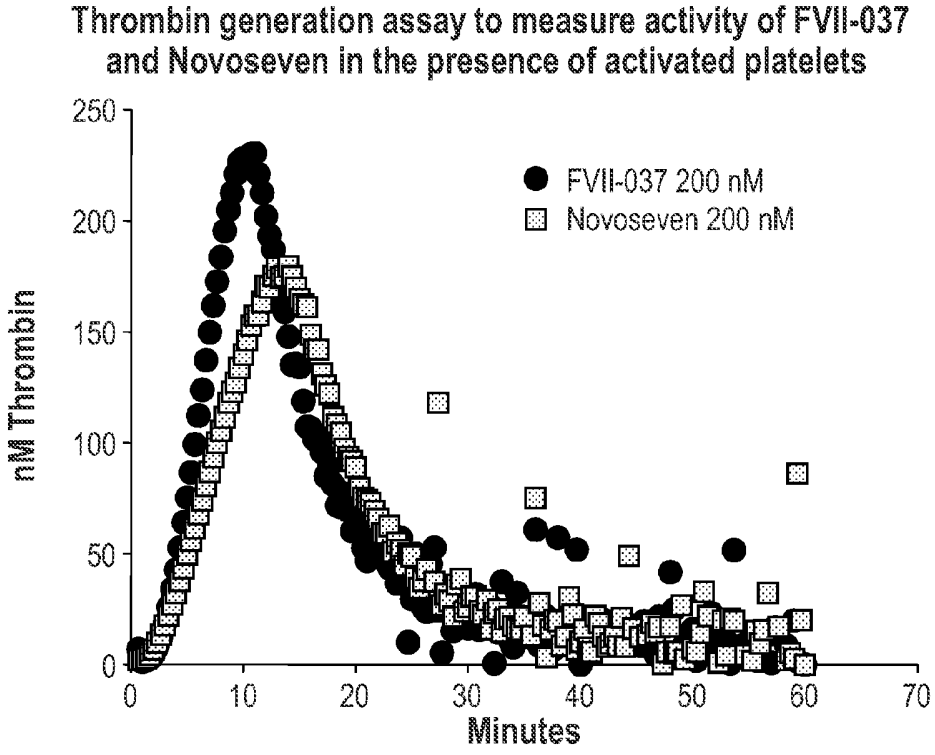


Fig. 16A

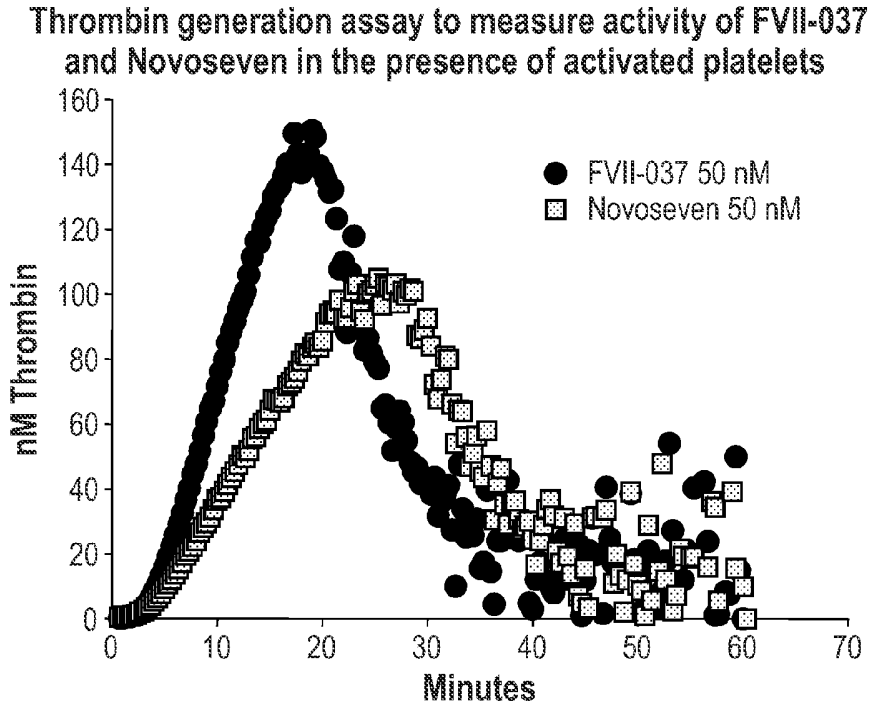


Fig. 16B

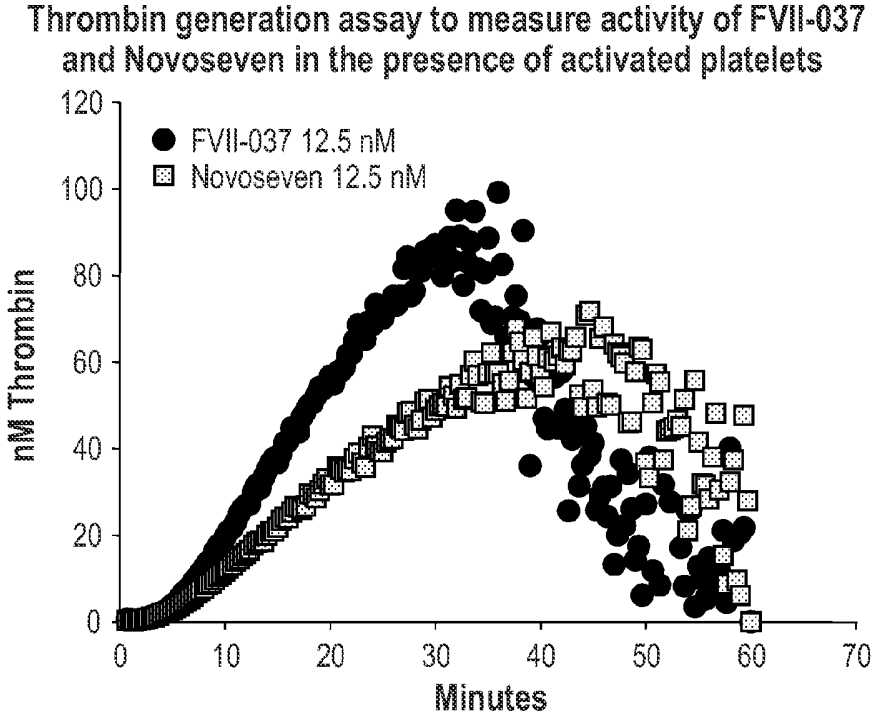
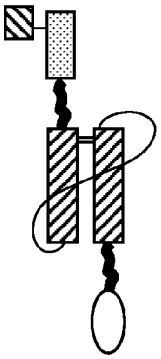
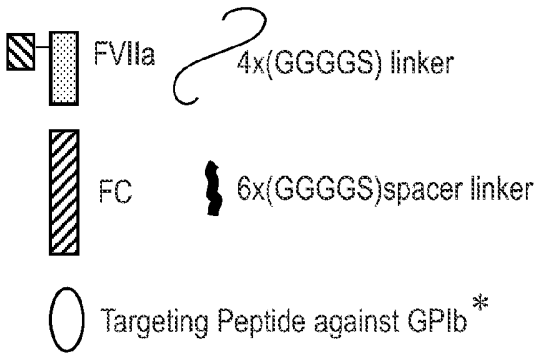


Fig. 16C

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





Peptide*	Affinity K_D, nM	FVIIFc
PS4	64	-044
OS1	0.74	-045
OS2	31	-046

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

Fig. 17

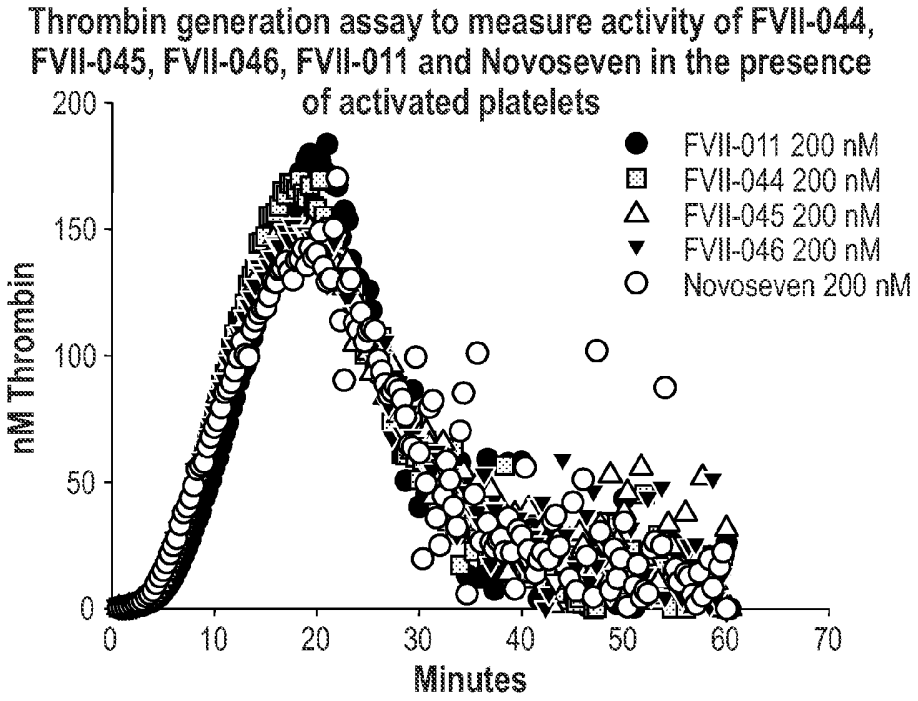


Fig. 18A

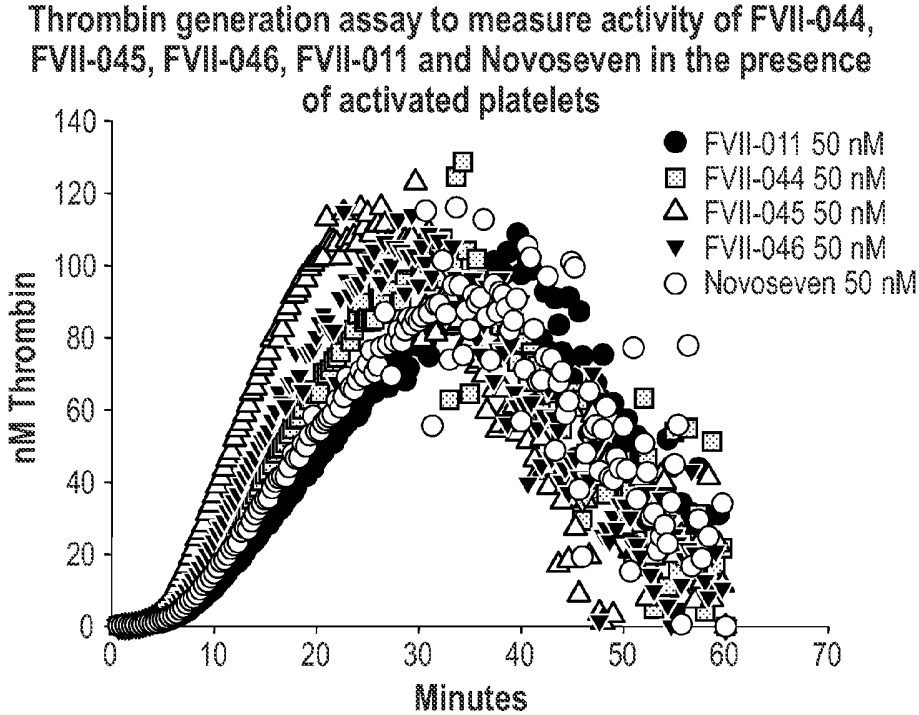


Fig. 18B

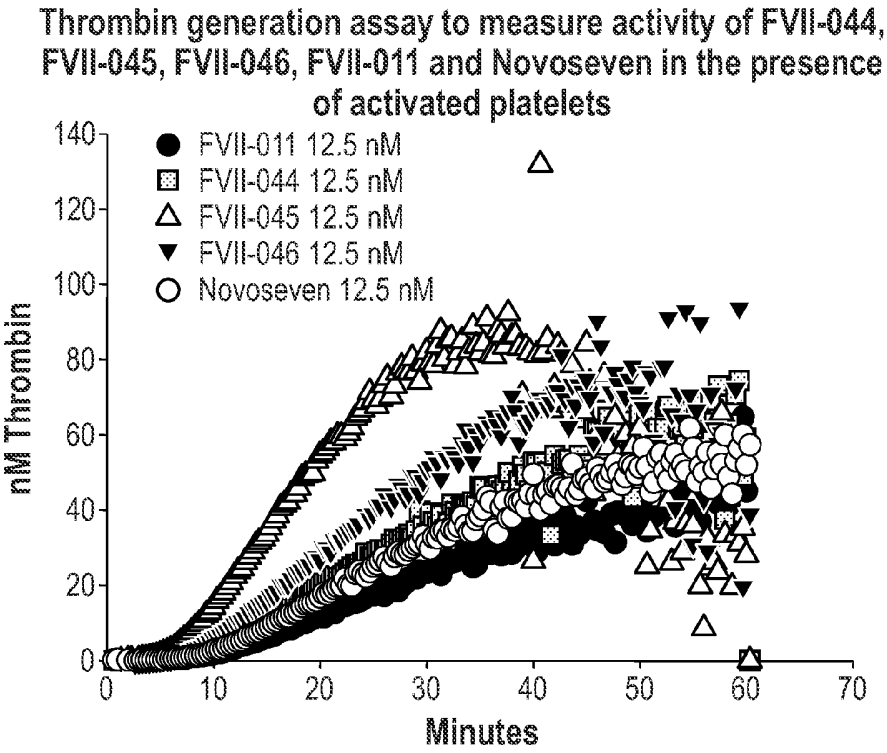


Fig. 18C

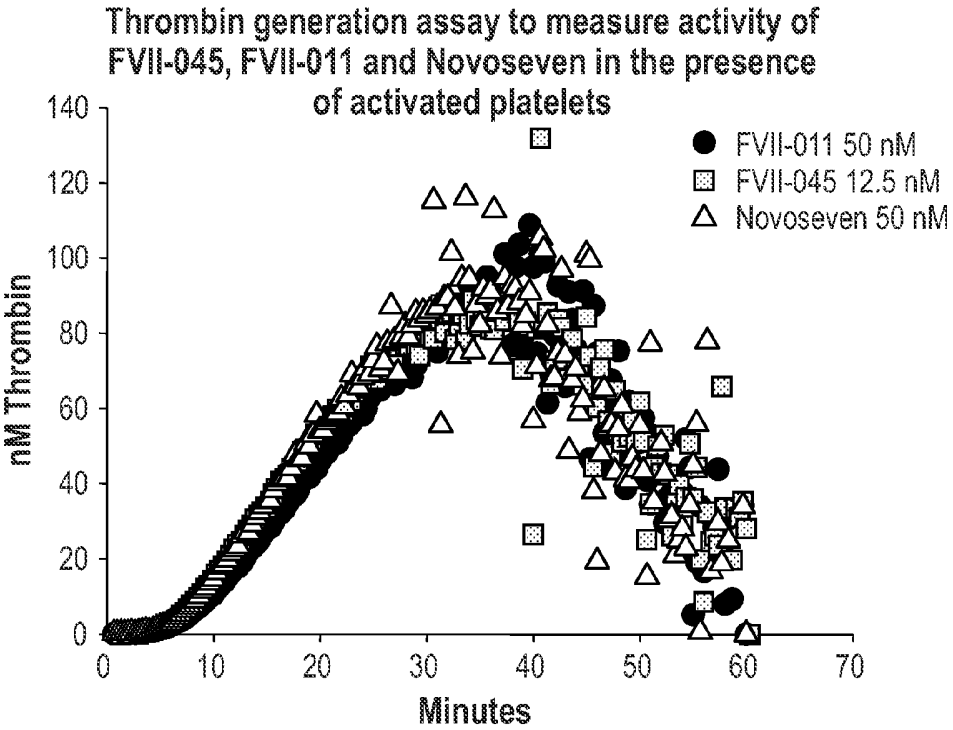


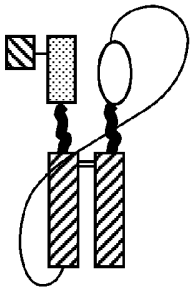
Fig. 18D

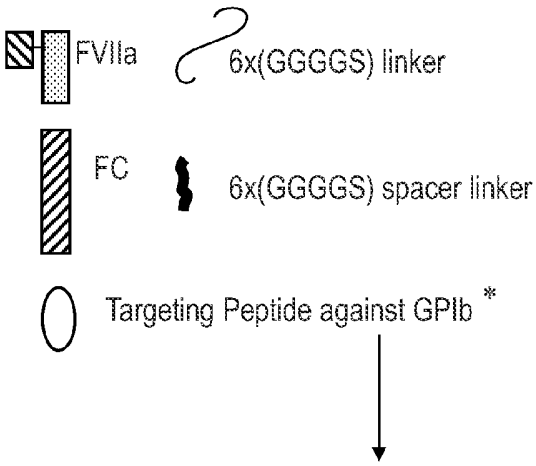
Thrombin generation assay to measure activity of FVII-047, FVII-048, FVII-049, FVII-011 and Novoseven in the presence of activated platelets

FVII-047

FVII-048

FVII-049





Peptide*	Affinity K_D, nM	FVIIFc
PS4	64	-047
OS1	0.74	-048
OS2	31	-049

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

Fig. 19

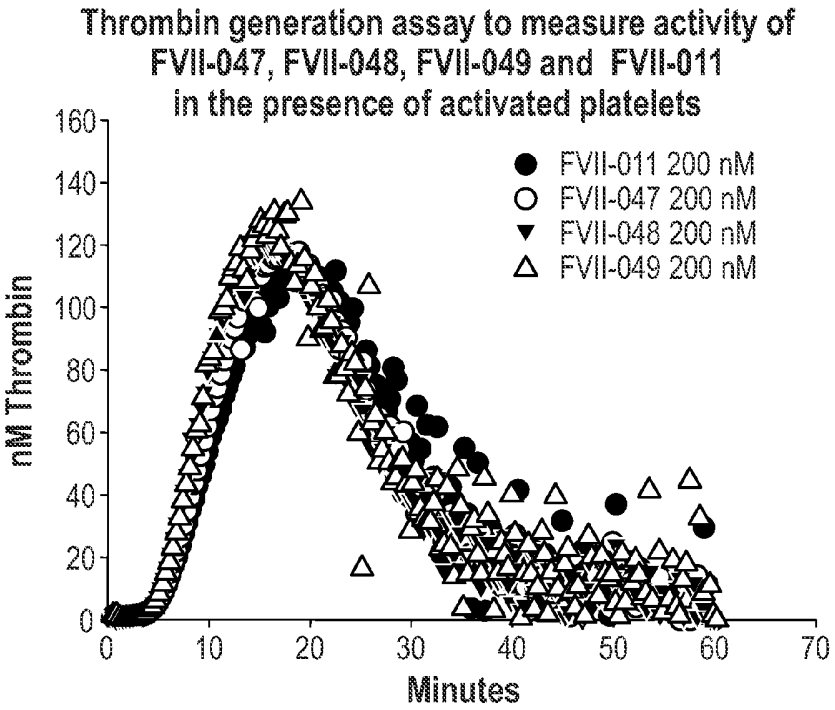


Fig. 20A

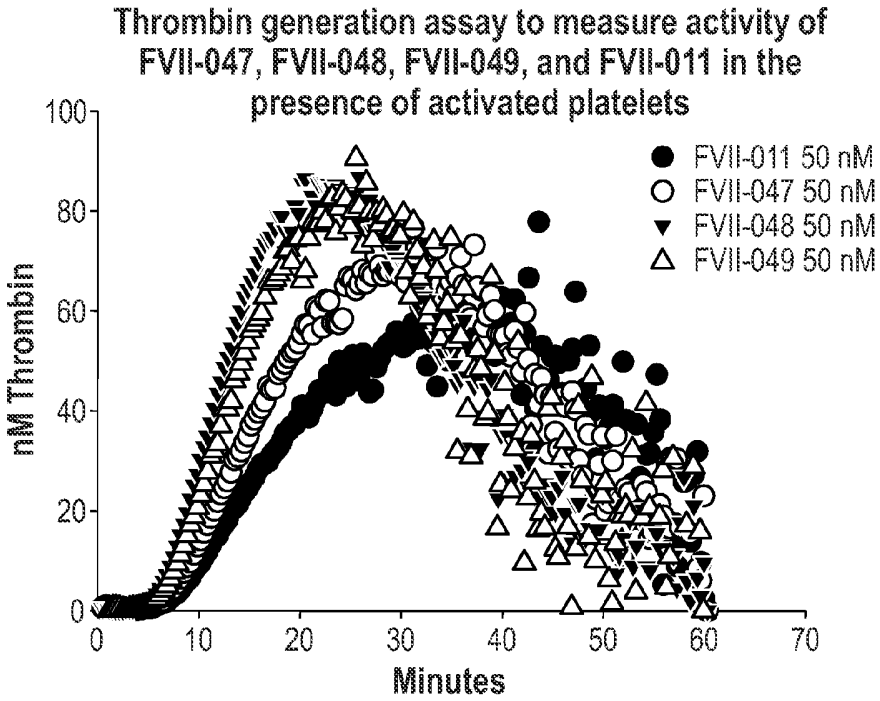


Fig. 20B

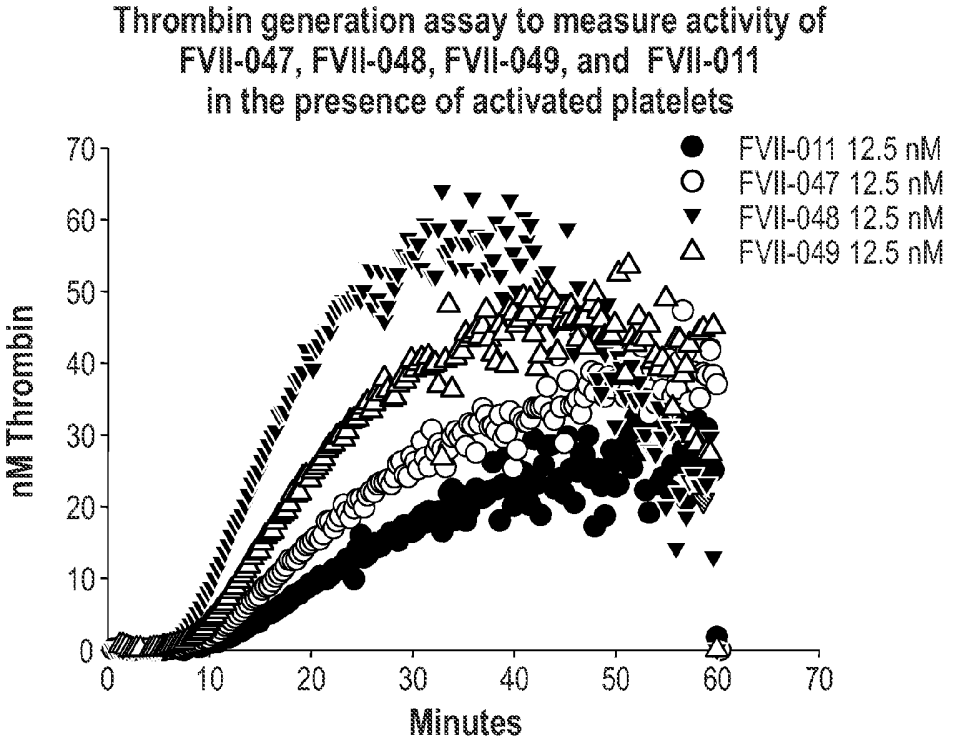


Fig. 20C

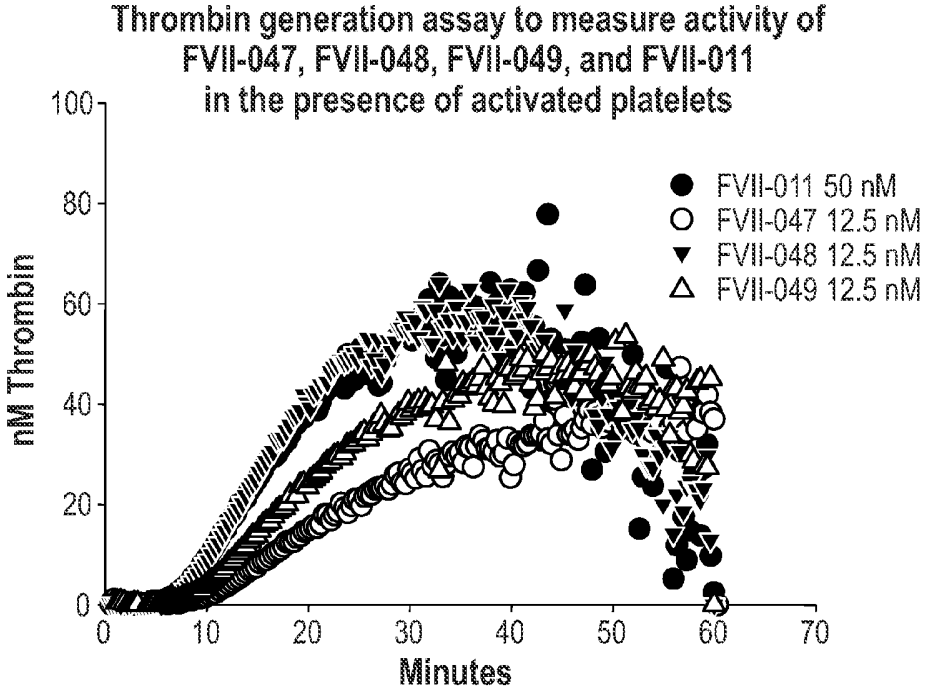


Fig. 20D

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

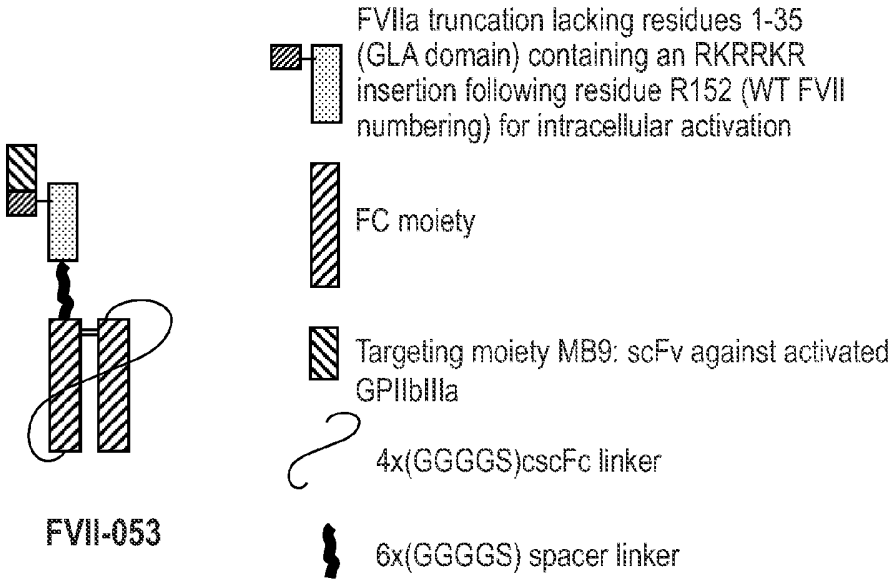


Fig. 21

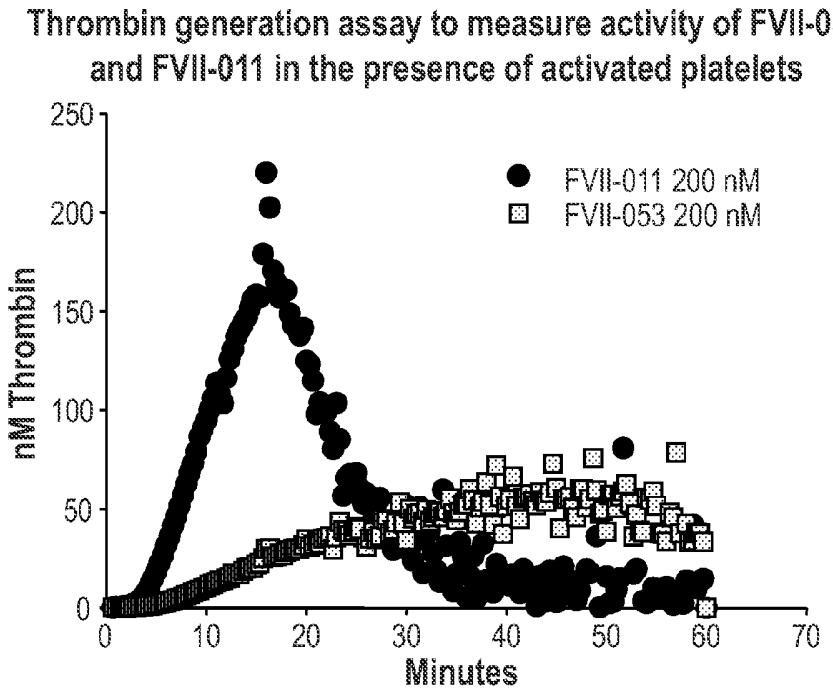


Fig. 22A

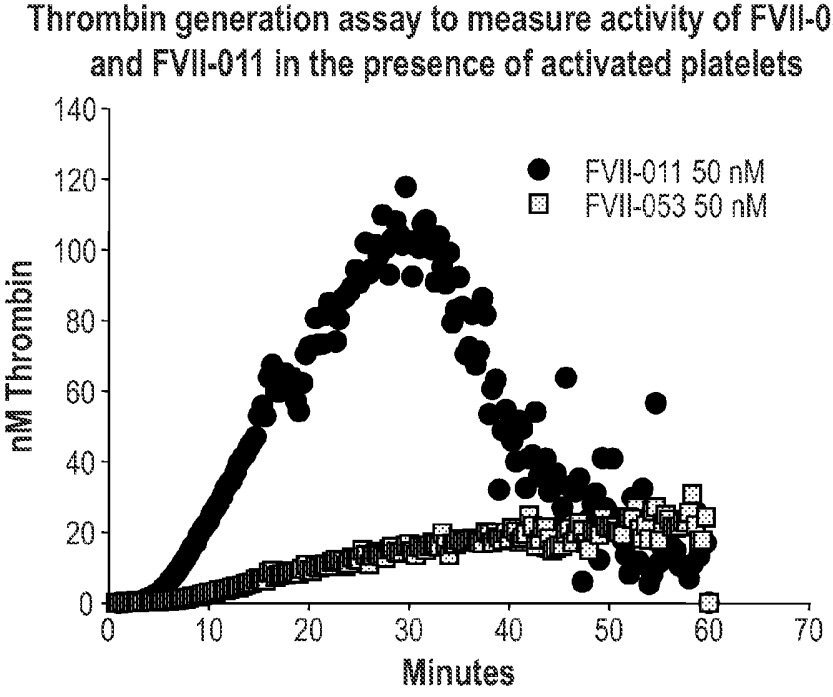


Fig.22B

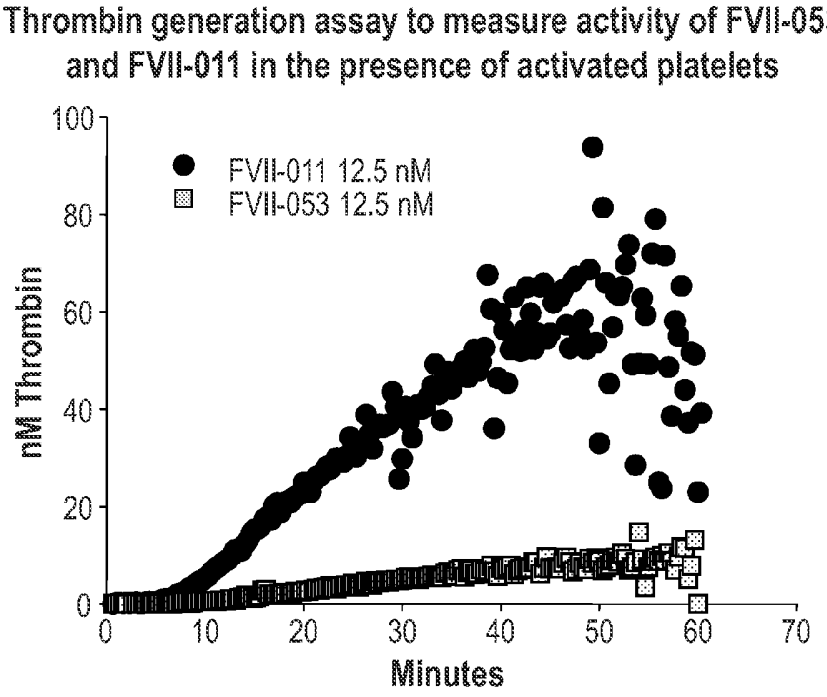


Fig. 22C

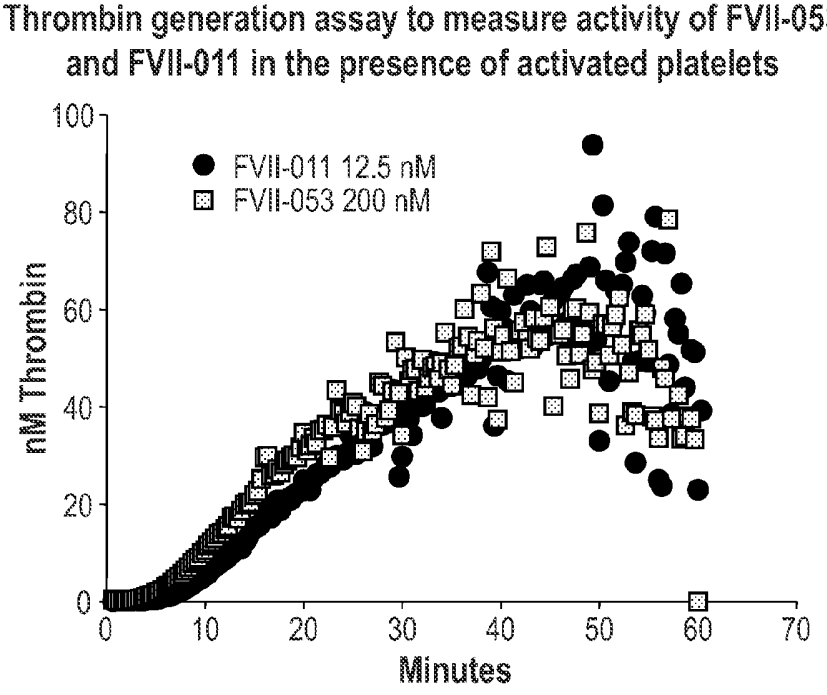


Fig. 22D

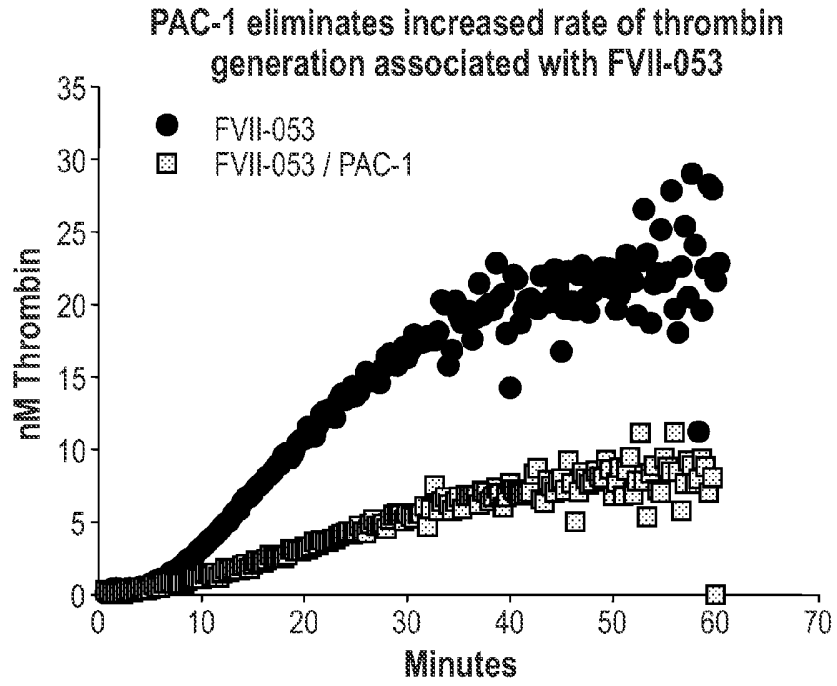


Fig. 23A

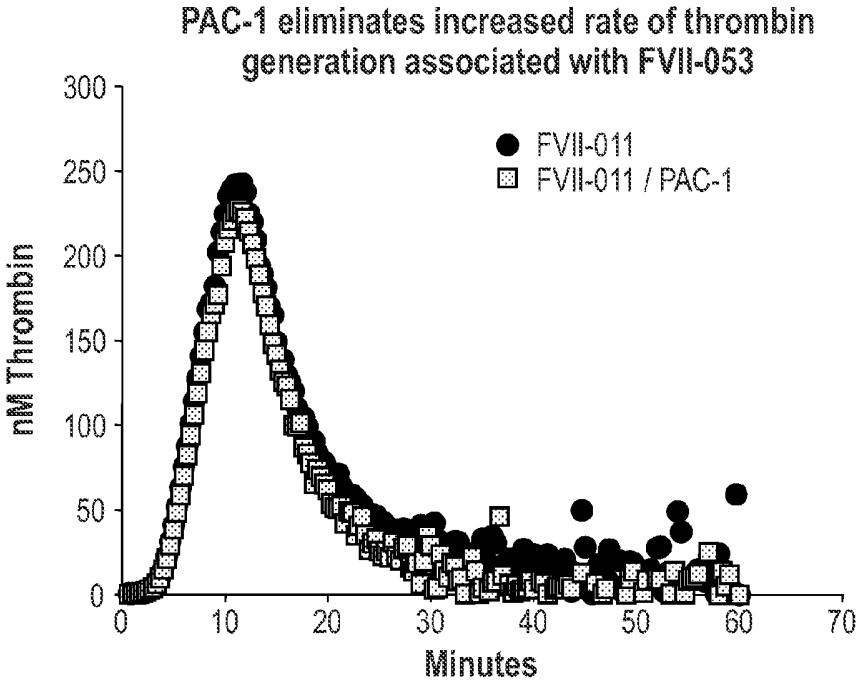


Fig. 23B

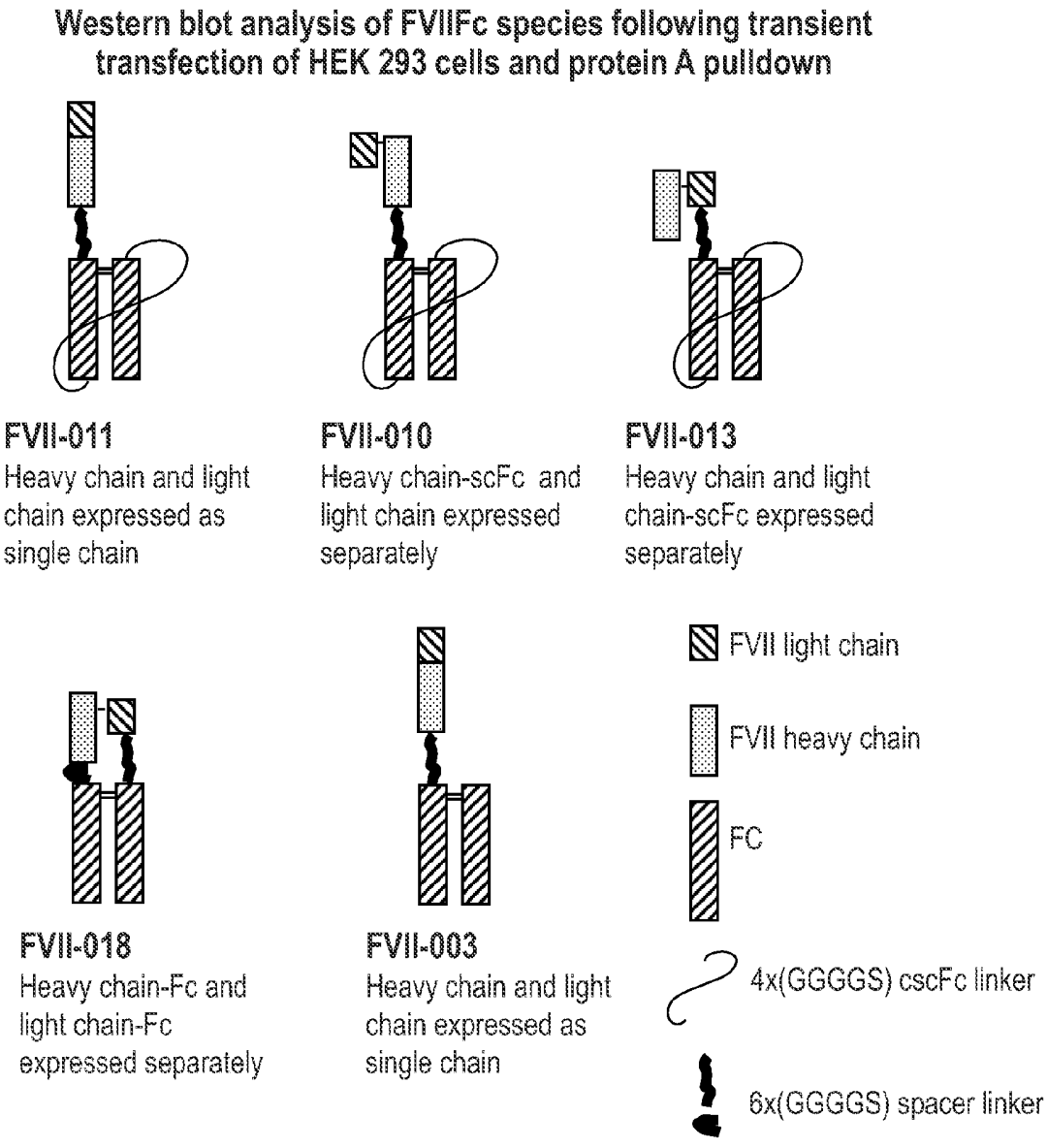
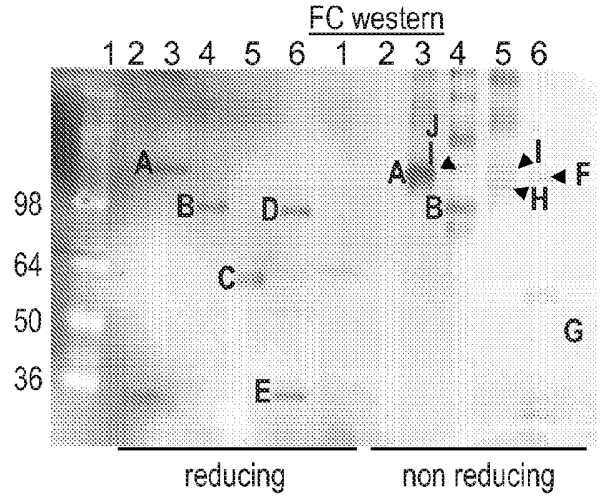
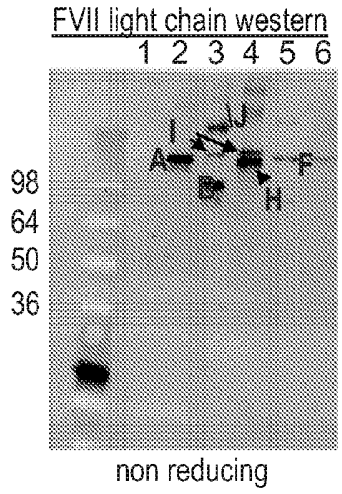

Western blot analysis of FVIIFc species following transient transfection of HEK 293 cells and protein A pulldown
FVII-011
Heavy chain and light chain expressed as single chain
FVII-010
Heavy chain-scFc and light chain expressed separately
FVII-013
Heavy chain and light chain-scFc expressed separately
FVII-018
Heavy chain-Fc and light chain-Fc expressed separately
FVII-003
Heavy chain and light chain expressed as single chain
FVII light chain
FVII heavy chain
FC
4x(GGGGS) cscFc linker
6x(GGGGS) spacer linker

Fig. 24

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





Interpretation of bands

A. FVII-scFc
B. Light chain-scFc
C. Light chain-Fc
D. FVII-Fc
E. Fc
F. FVII-Fc:Fc
G. Fc dimer
H. Light chain-Fc dimer
I. Light chain-Fc:HC-Fc
J. Light chain-scFc dimer

Proteins loaded in gel

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

Fig. 25

Western blot of protein A immunoprecipitation following transient transfection of pSYN-FVII-024 with or without pSYN-PC5-003. Lane 1, Seeblue Plus2 marker; lane 2, pSYN-FVII-024, non reducing; lane 3, pSYN-FVII-024+pSYN-PC5-003, non reducing; lane 4, pSYN-FVII-024, reducing; lane 5, pSYN-FVII-024+pSYN-PC5-003, reducing.

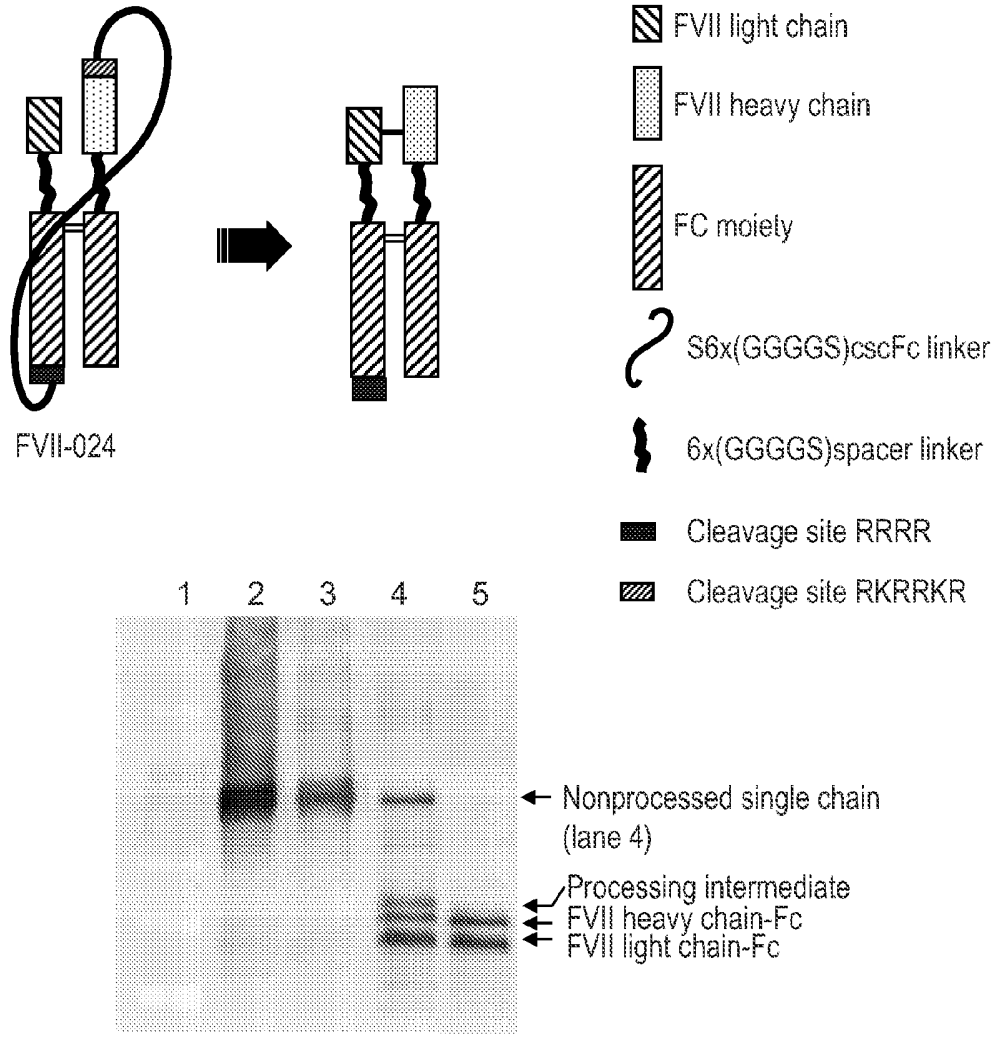


Fig. 26

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

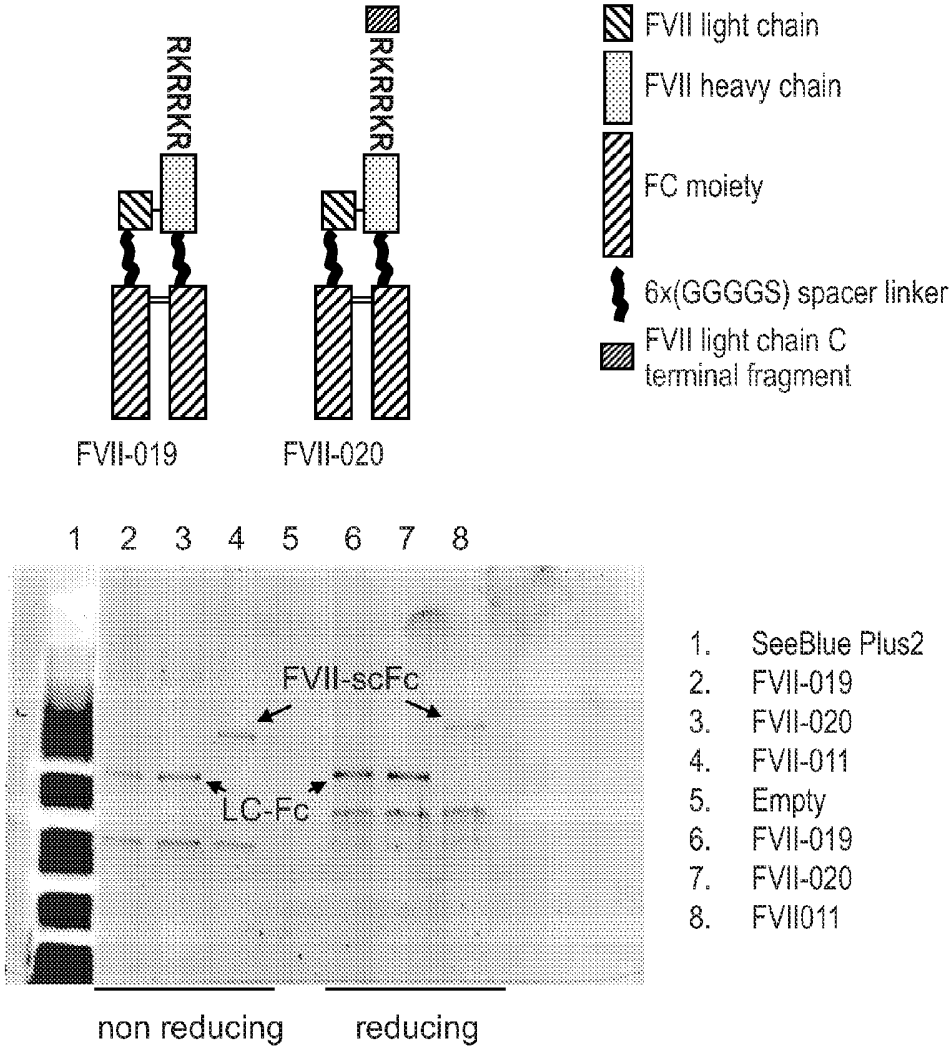
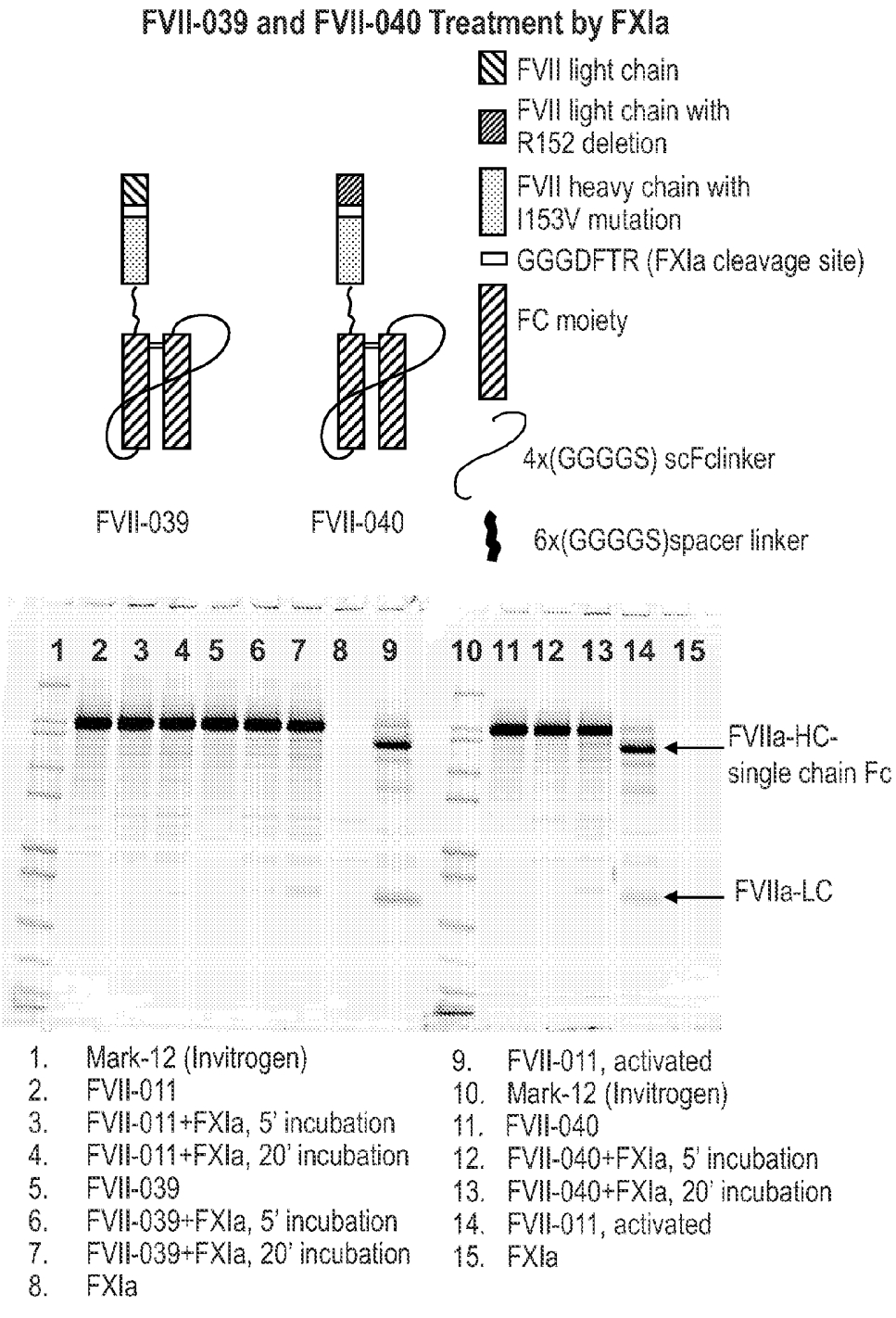
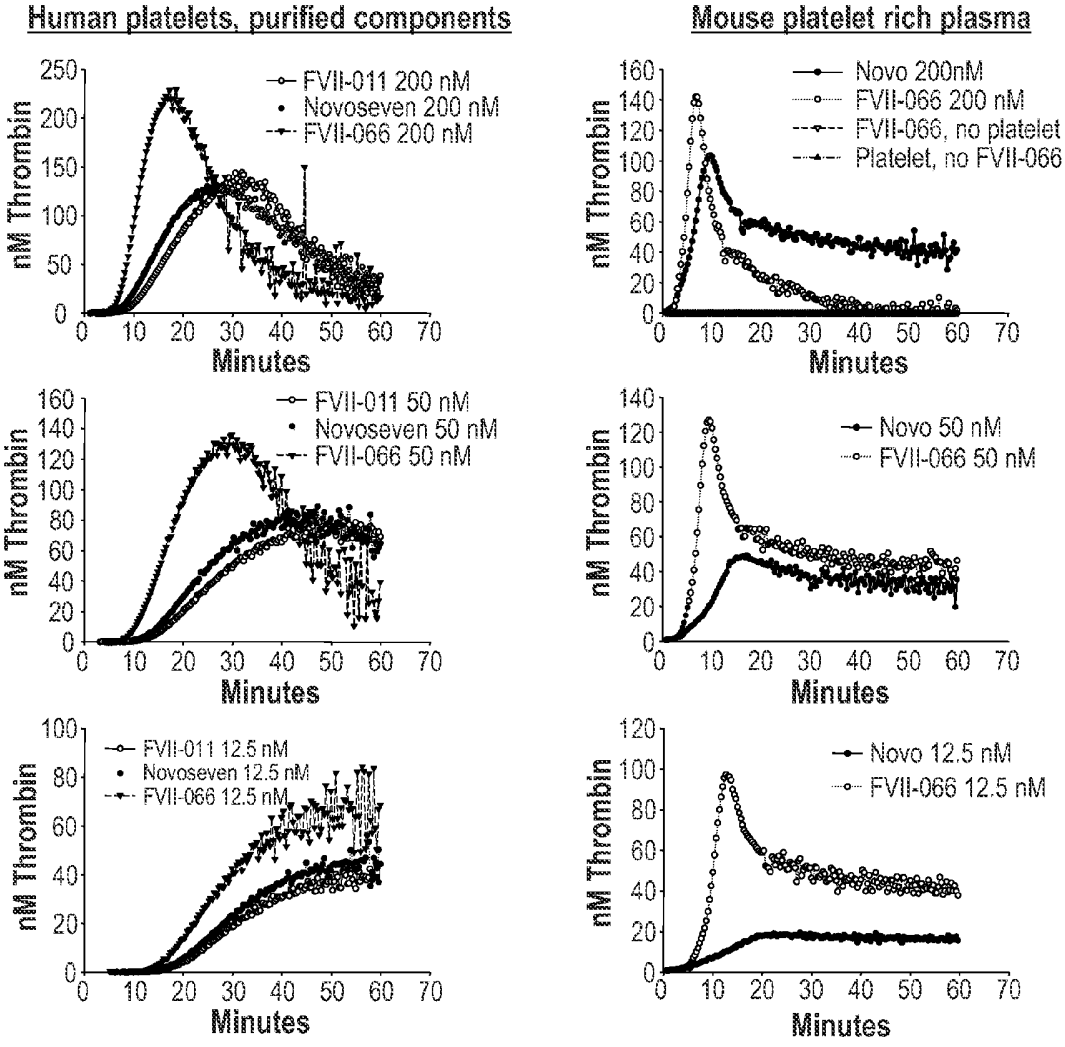


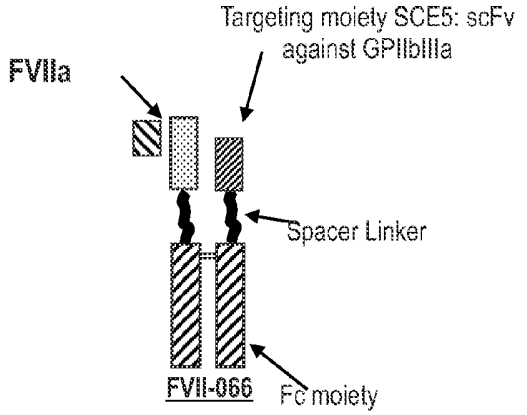
Fig. 27



1. Mark-12 (Invitrogen)
2. FVII-011
3. FVII-011+FXIa, 5' incubation
4. FVII-011+FXIa, 20' incubation
5. FVII-039
6. FVII-039+FXIa, 5' incubation
7. FVII-039+FXIa, 20' incubation
8. FXIa
9. FVII-011, activated
10. Mark-12 (Invitrogen)
11. FVII-040
12. FVII-040+FXIa, 5' incubation
13. FVII-040+FXIa, 20' incubation
14. FVII-011, activated
15. FXIa

Fig. 28





SCE5

-scFv against active GPIIbIIIa

-Crossreacts with mouse and human receptors

Fig. 29

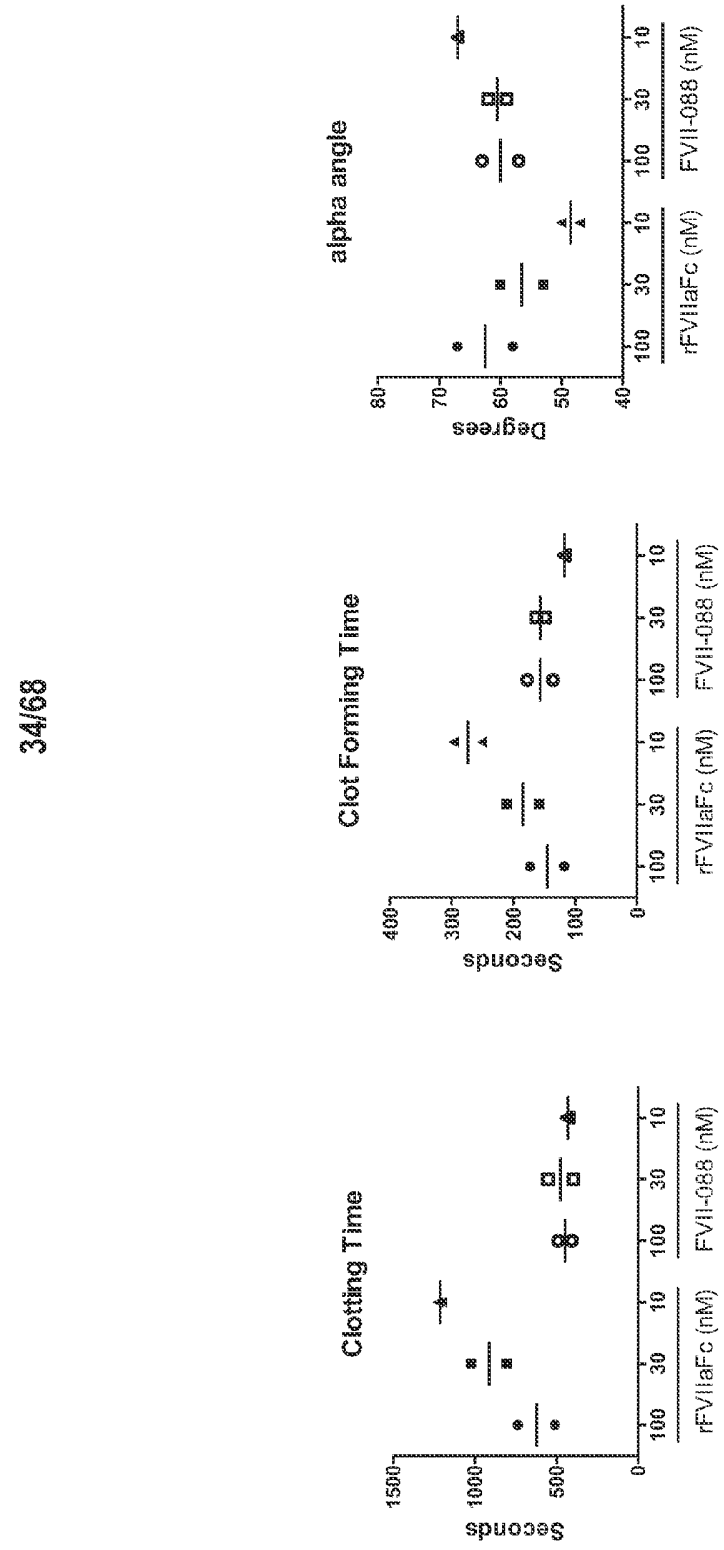

Clotting Time
Seconds
1500
1000
500
0
100 30 10 100 30 10
rFVIIaFc (nM)
FVII-088 (nM)
Clot Forming Time
Seconds
400
300
200
100
0
100 30 10 100 30 10
rFVIIaFc (nM)
FVII-088 (nM)
alpha angle
Degrees
80
70
60
50
40
100 30 10 100 30 10
rFVIIaFc (nM)
FVII-088 (nM)

Fig. 30

Activatable Constructs

FXIa cleavage sites

LC-7x(GGGGS)-**SVSQTSKLTR**-IVGG: **FVII-057**

Thrombin cleavage sites

LC-7x(GGGGS)-**DFLAEGGGVR**-IVGG :**FVII-058**
LC-7x(GGGGS)-**TTKIKPR**-IVGG : **FVII-059**
LC-7x(GGGGS)-**ALRPRVVGGA**-VVGG : **FVII-060**
LC-7x(GGGGS)-**ALRPRVVGGA**-IVGG : **FVII-061**

Negative control

LC-8x(GGGGS)-IVGG: **FVII-062**

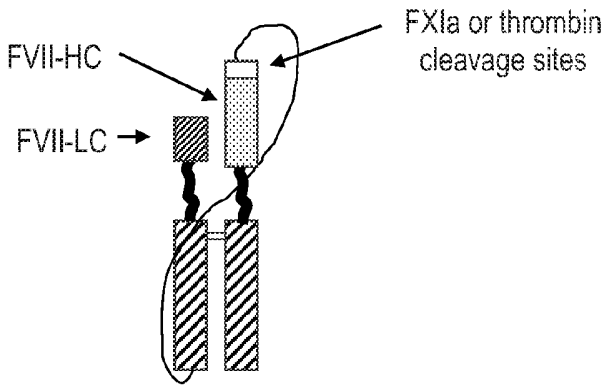


Fig. 31

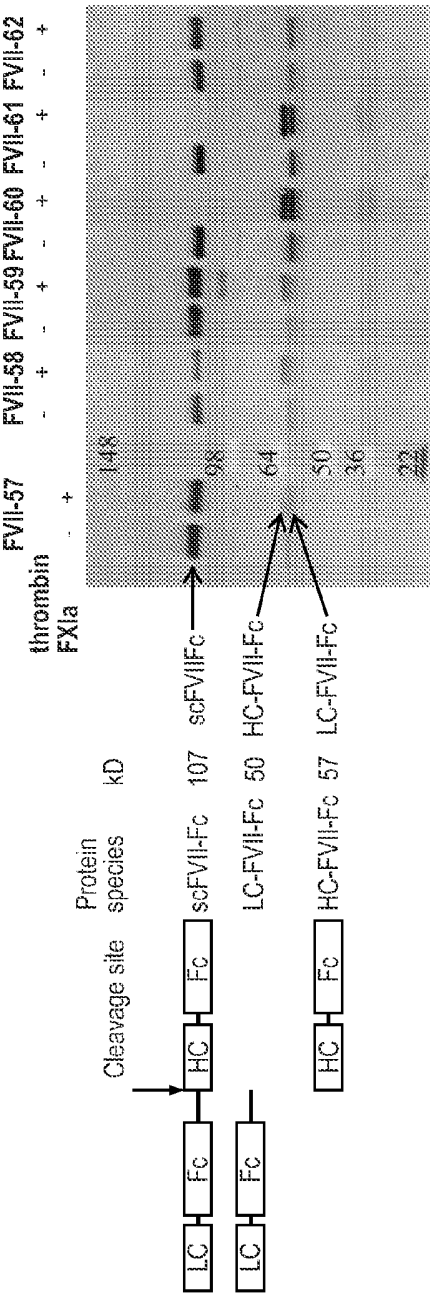

FVII-57
thrombin
FXIa
- +
FVII-58 FVII-59 FVII-60 FVII-61 FVII-62
- + - + - + - + - +
148
98
64
50
36
22
kD
Protein species
Cleavage site
scFVII-Fc
LC-FVII-Fc
HC-FVII-Fc
107
50
57
scFVIIFc
HC-FVII-Fc
LC-FVII-Fc
LC
HC
Fc

Fig. 32

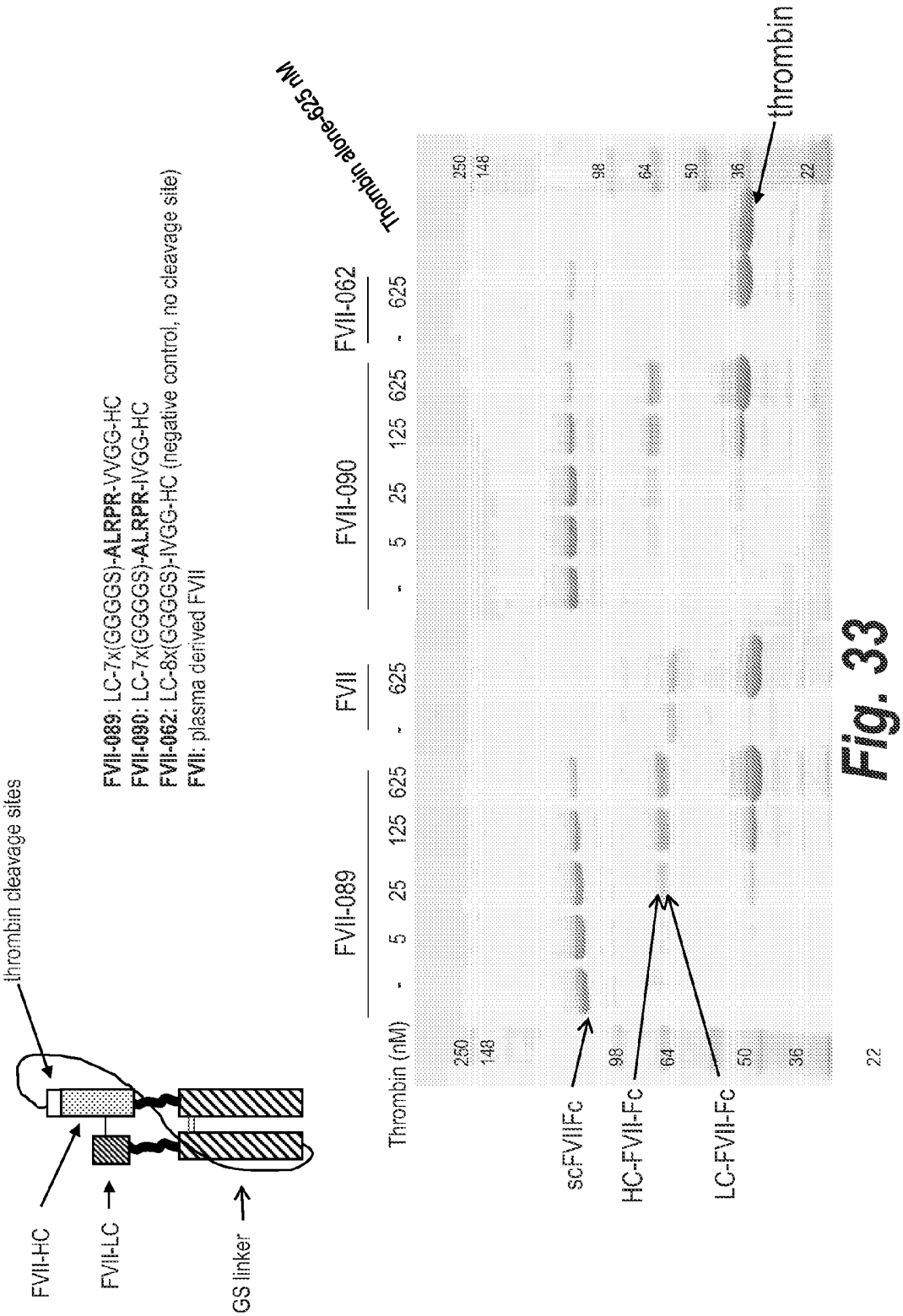

thrombin cleavage sites
FVII-HC
FVII-LC
GS linker
FVII-089: LC-7x(GGGGS)-ALRPR-VVGG-HC
FVII-090: LC-7x(GGGGS)-ALRPR-IVGG-HC
FVII-062: LC-8x(GGGGS)-IVGG-HC (negative control, no cleavage site)
FVII: plasma derived FVII
Thrombin alone-625 nM
FVII-089
FVII
FVII-090
FVII-062
Thrombin (nM) - 5 25 125 625 - 625 - 5 25 125 625 -
250
148
98
64
50
36
22
scFVIIFc
HC-FVII-Fc
LC-FVII-Fc
thrombin

Fig. 33

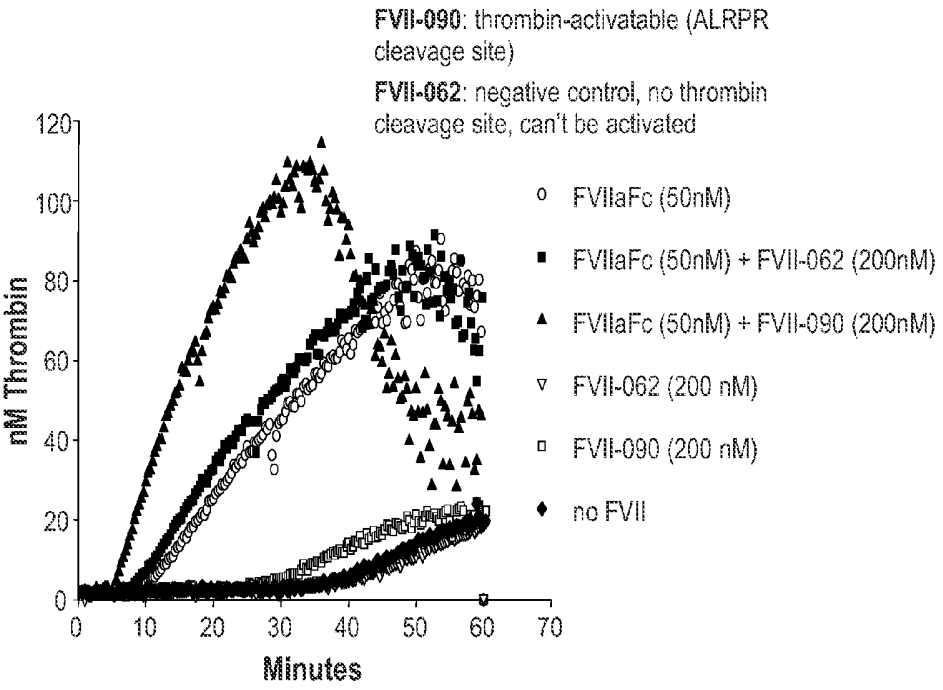

FVII-090: thrombin-activatable (ALRPR cleavage site)
FVII-062: negative control, no thrombin cleavage site, can't be activated
FVIIaFc (50nM)
FVIIaFc (50nM) + FVII-062 (200nM)
FVIIaFc (50nM) + FVII-090 (200nM)
FVII-062 (200 nM)
FVII-090 (200 nM)
no FVII
nM Thrombin
Minutes
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70

Fig. 34

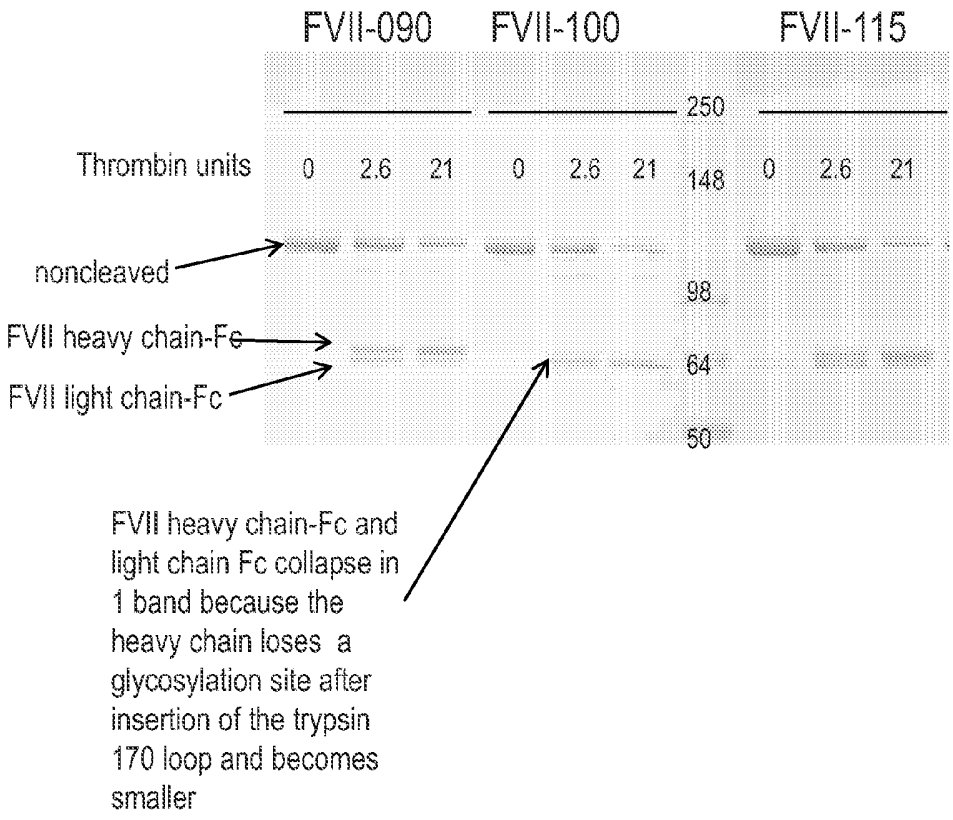

FVII-090
FVII-100
FVII-115
250
Thrombin units
0
2.6
21
0
2.6
21
148
0
2.6
21
noncleaved
98
FVII heavy chain-Fc
64
FVII light chain-Fc
50
FVII heavy chain-Fc and light chain Fc collapse in 1 band because the heavy chain loses a glycosylation site after insertion of the trypsin 170 loop and becomes smaller

Fig. 35

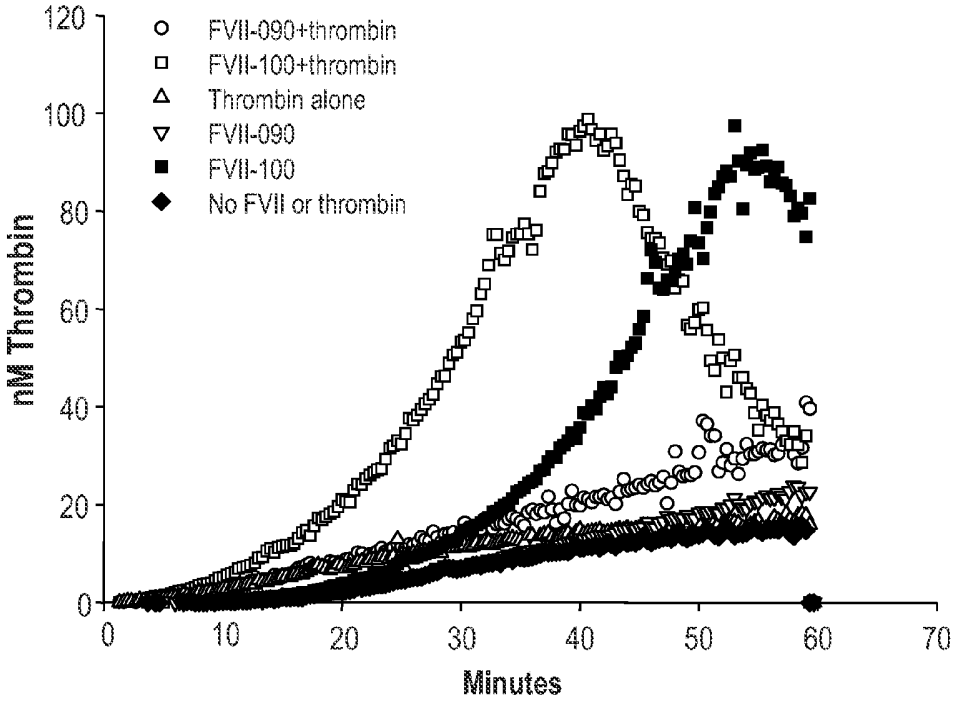

FVII-090+thrombin
FVII-100+thrombin
Thrombin alone
FVII-090
FVII-100
No FVII or thrombin
nM Thrombin
Minutes
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70

Fig. 36

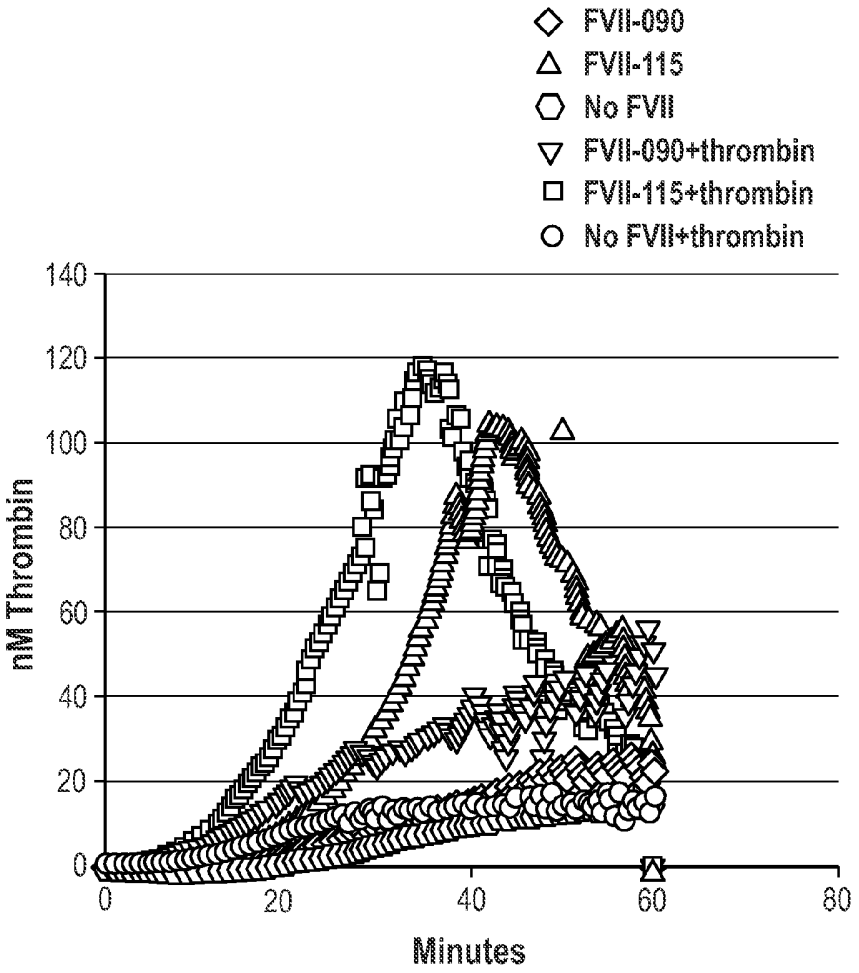

FVII-090
FVII-115
No FVII
FVII-090+thrombin
FVII-115+thrombin
No FVII+thrombin
140
120
100
80
60
40
20
0
nM Thrombin
0
20
40
60
80
Minutes

Fig. 37

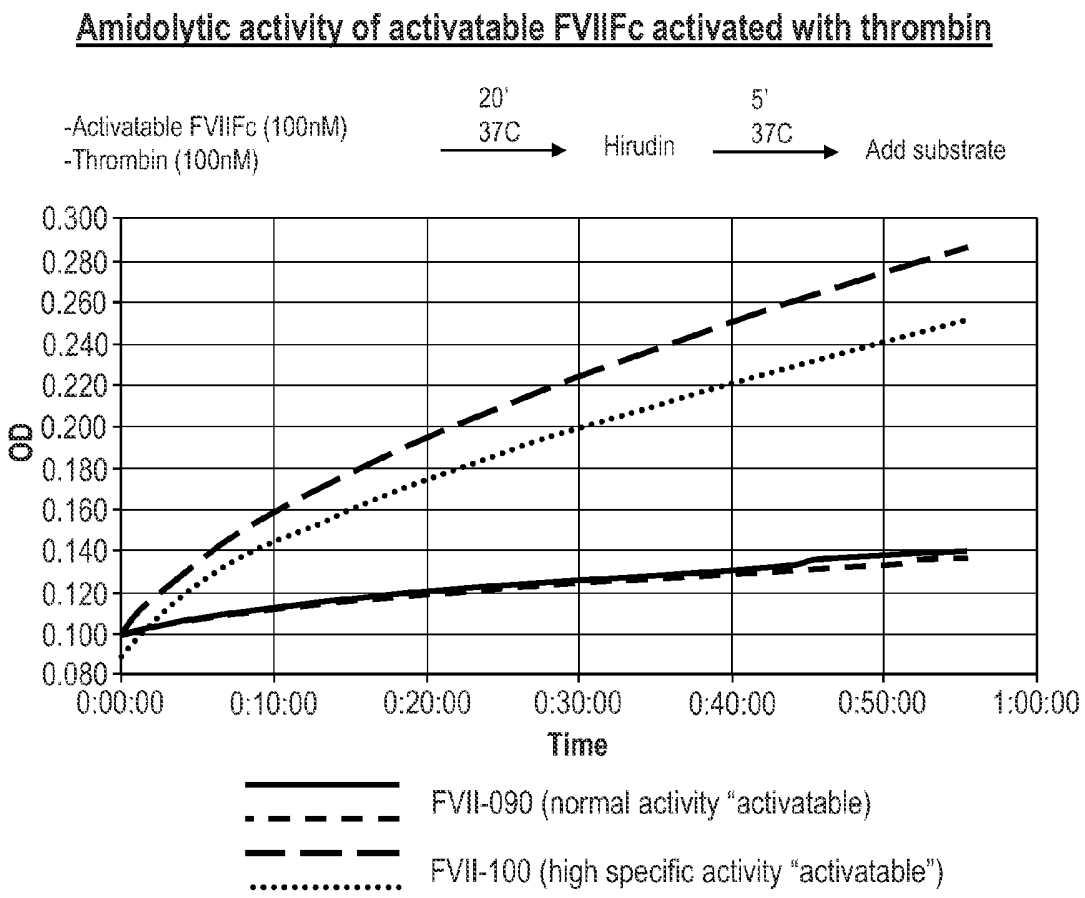

Amidolytic activity of activatable FVIIFc activated with thrombin
-Activatable FVIIFc (100nM)
-Thrombin (100nM)
20'
37C
Hirudin
5'
37C
Add substrate
0.300
0.280
0.260
0.240
0.220
0.200
0.180
0.160
0.140
0.120
0.100
0.080
OD
0:00:00
0:10:00
0:20:00
0:30:00
0:40:00
0:50:00
1:00:00
Time
FVII-090 (normal activity "activatable)
FVII-100 (high specific activity "activatable")

Fig. 38

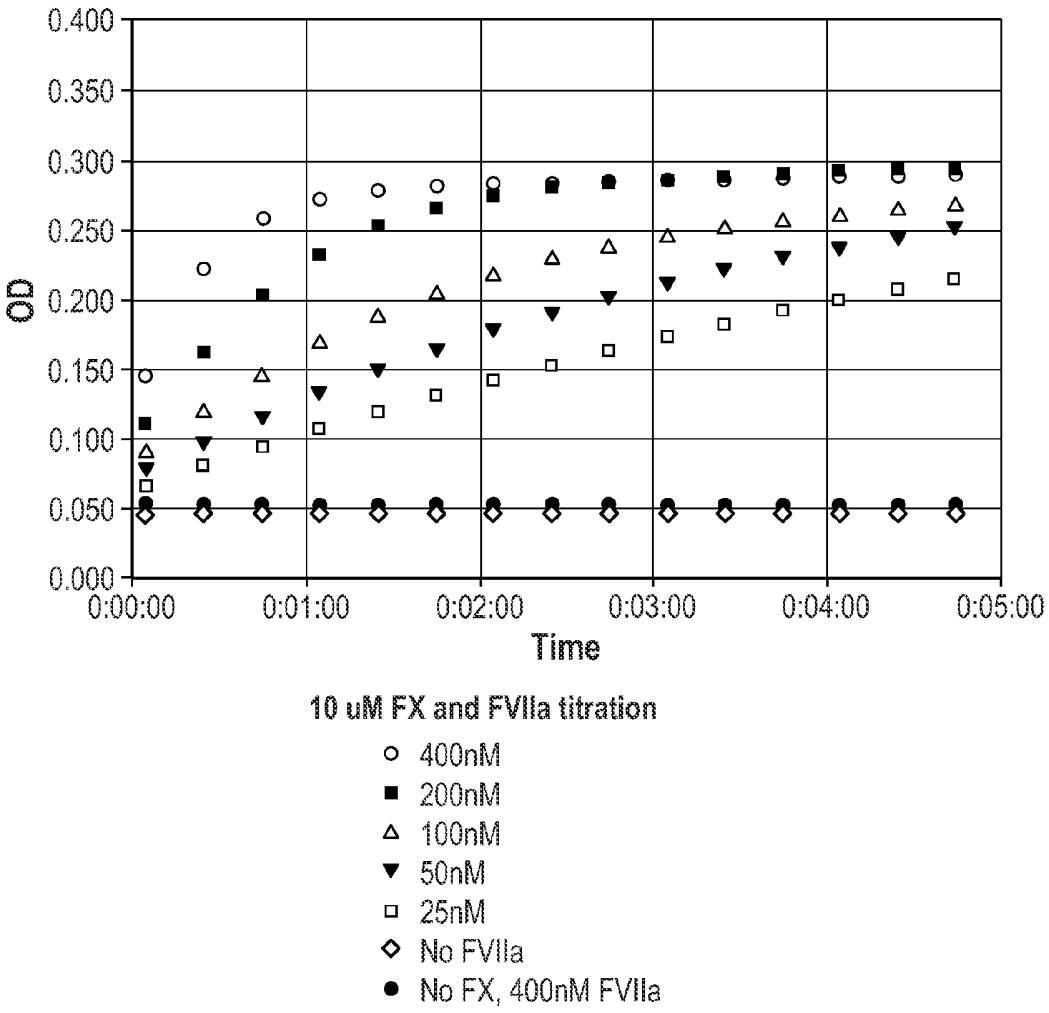

Activation of FX by FVIIa
OD
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
0:00:00
0:01:00
0:02:00
0:03:00
0:04:00
0:05:00
Time
10 uM FX and FVIIa titration
400nM
200nM
100nM
50nM
25nM
No FVIIa
No FX, 400nM FVIIa

Fig. 39

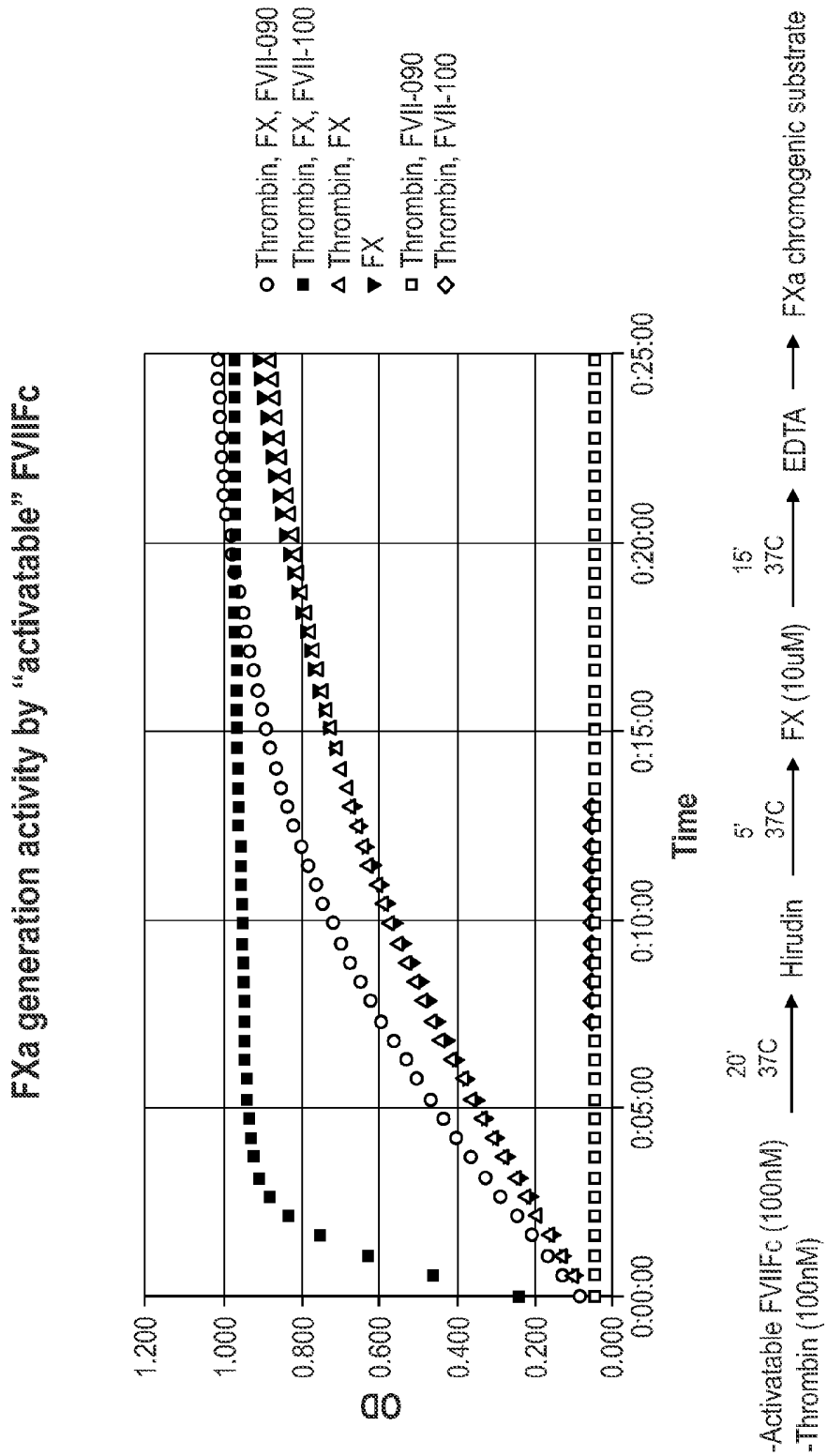

FXa generation activity by "activatable" FVIIFc
Thrombin, FX, FVII-090
Thrombin, FX, FVII-100
Thrombin, FX
FX
Thrombin, FVII-090
Thrombin, FVII-100
OD
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0:00:00
0:05:00
0:10:00
0:15:00
0:20:00
0:25:00
Time
-Activatable FVIIFc (100nM)
-Thrombin (100nM)
20'
37C
Hirudin
5'
37C
FX (10uM)
15'
37C
EDTA
FXa chromogenic substrate

Fig. 40

Thrombin cleavage site
(ALRPR)
7x(GGGGS) linker
rFVII-LC
rFVII-HC
Fc moiety
Activatable heterodimer structure
FVII-090
Activatable with high specific activity
FVII-100: 170 loop in the heavy chain replaced with 170 loop from trypsin. Same backbone as FVII-090
FVII-115: triple point mutation in heavy chain (V158D-E296V-M298Q). Same backbone as FVII-090
Activatable monomer structure
FVII-118 :R152 from light chain deleted, insert ALRPR between light chain and heavy chain
FVII-119 :R152 from light chain deleted, insert GGGGS-ALRPR between light chain and heavy chain
FVII-127: R152 from light chain deleted, insert ALRPR between light chain and heavy chain. Same high specific activity mutation found in FVII-100

Fig. 41

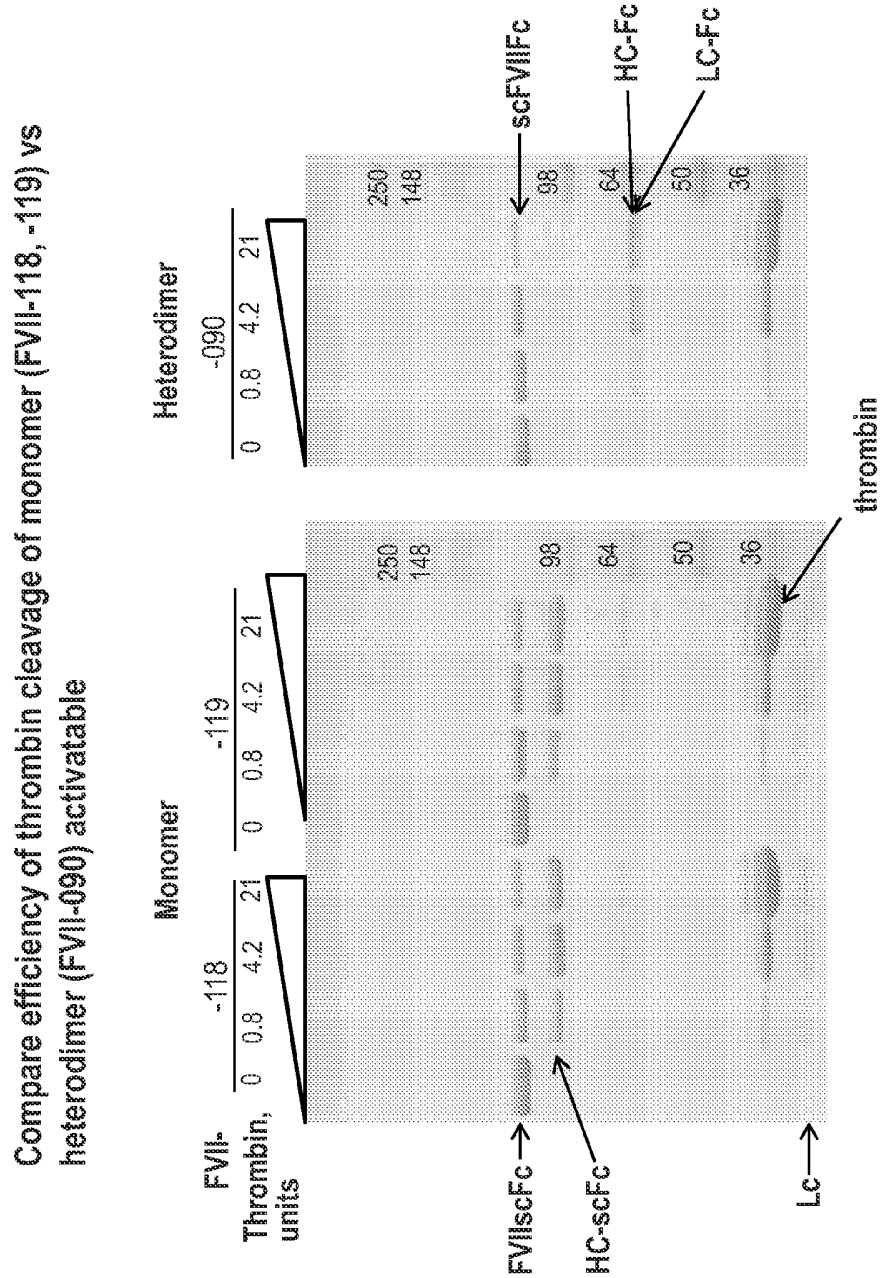

Compare efficiency of thrombin cleavage of monomer (FVII-118, -119) vs heterodimer (FVII-090) activatable
Monomer
Heterodimer
FVII-Thrombin, units
-118
-119
-090
0
0.8
4.2
21
250
148
98
64
50
36
scFVIIFc
HC-Fc
LC-Fc
thrombin
FVIIscFc
HC-scFc
Lc

Fig. 42

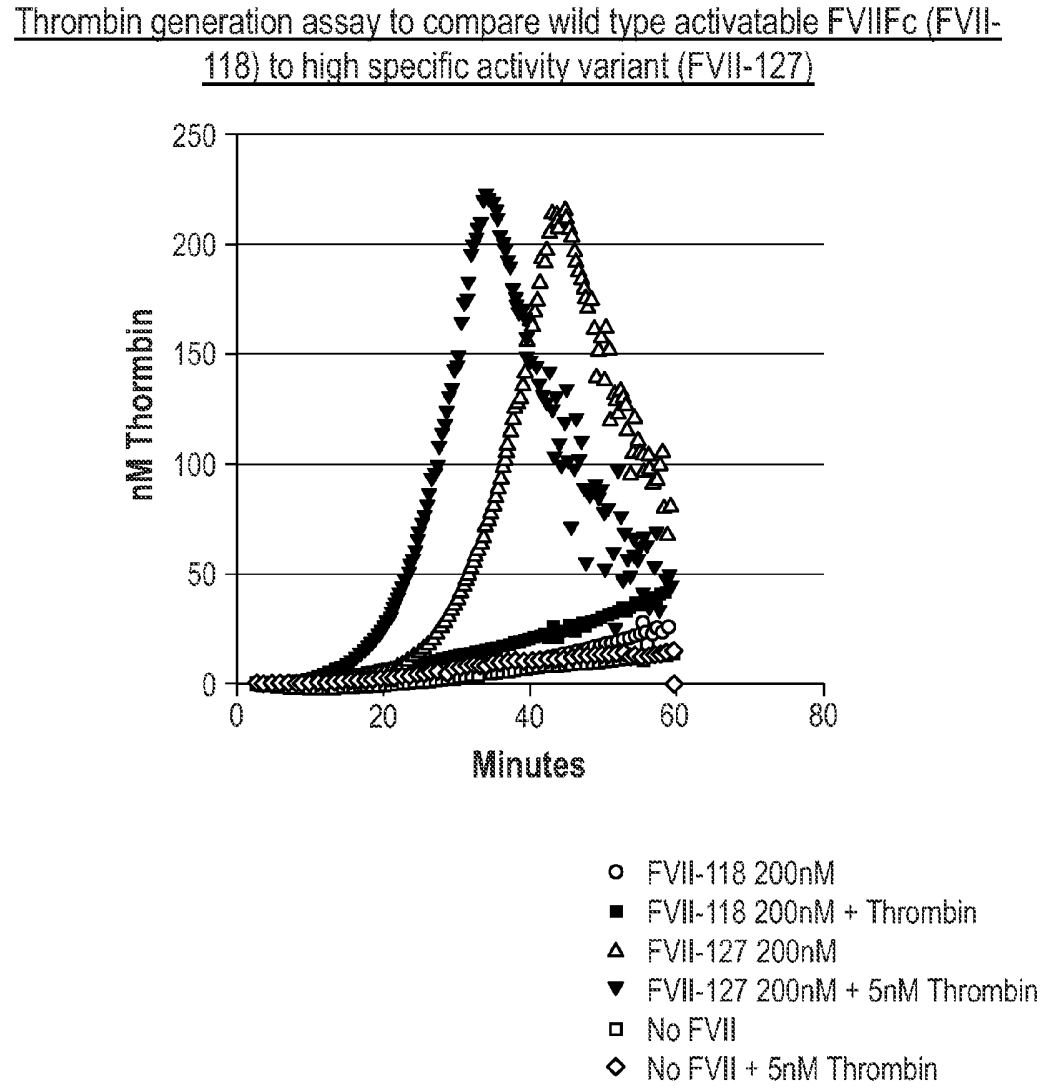

Thrombin generation assay to compare wild type activatable FVIIFc (FVII-118) to high specific activity variant (FVII-127)
250
200
150
100
50
0
nM Thrombin
0
20
40
60
80
Minutes
FVII-118 200nM
FVII-118 200nM + Thrombin
FVII-127 200nM
FVII-127 200nM + 5nM Thrombin
No FVII
No FVII + 5nM Thrombin

Fig. 43

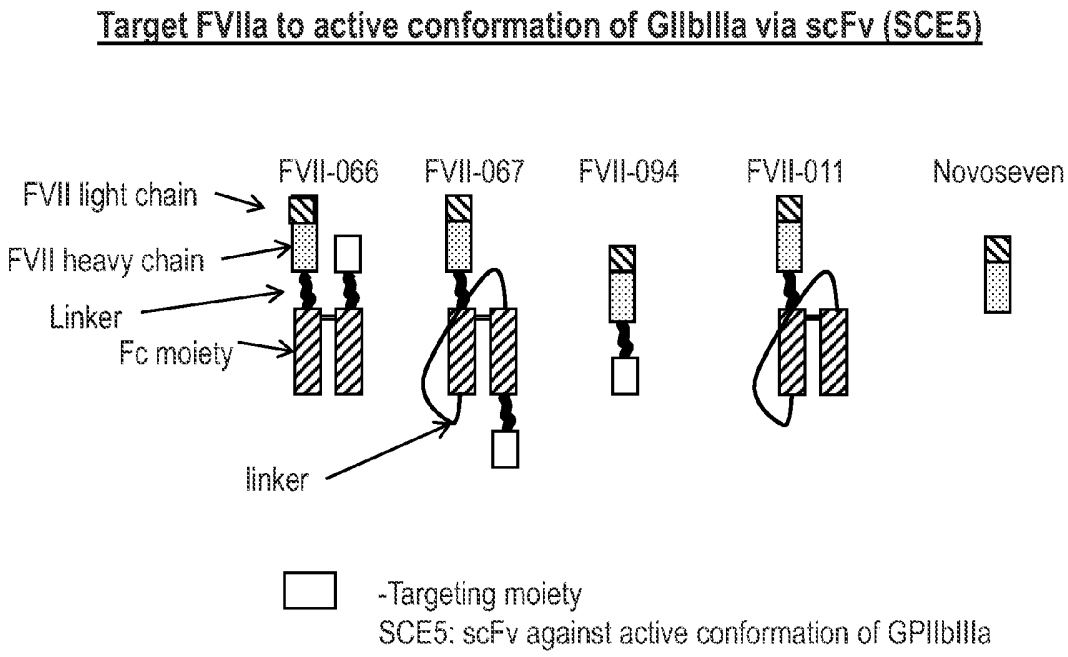

Target FVIIa to active conformation of GIIbIIIa via scFv (SCE5)
FVII-066
FVII-067
FVII-094
FVII-011
Novoseven
FVII light chain
FVII heavy chain
Linker
Fc moiety
linker
-Targeting moiety
SCE5: scFv against active conformation of GPIIbIIIa

Fig. 44A

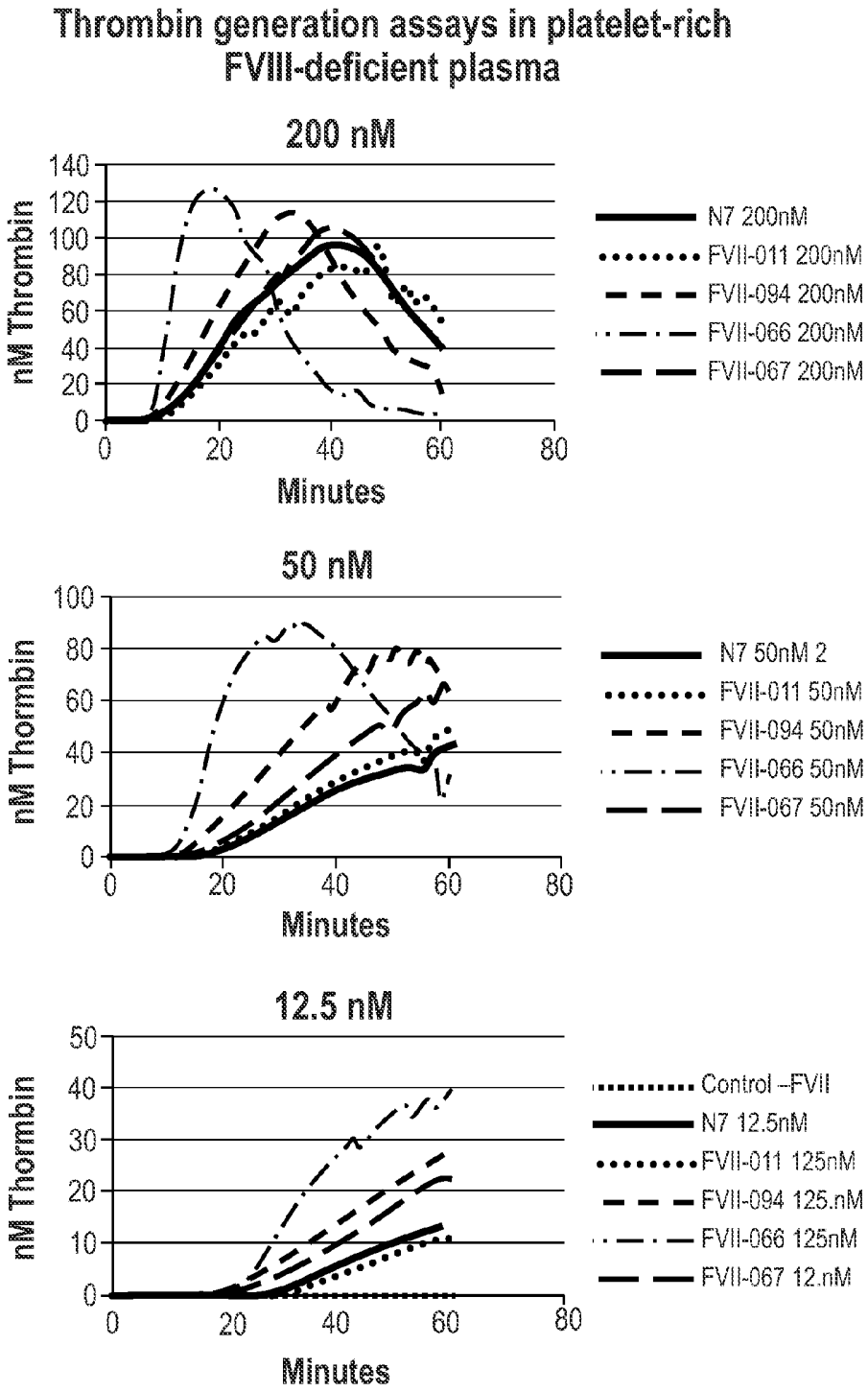

Thrombin generation assays in platelet-rich FVIII-deficient plasma
200 nM
nM Thrombin
Minutes
N7 200nM
FVII-011 200nM
FVII-094 200nM
FVII-066 200nM
FVII-067 200nM
50 nM
nM Thrombin
Minutes
N7 50nM 2
FVII-011 50nM
FVII-094 50nM
FVII-066 50nM
FVII-067 50nM
12.5 nM
nM Thrombin
Minutes
Control –FVII
N7 12.5nM
FVII-011 125nM
FVII-094 125.nM
FVII-066 125nM
FVII-067 12.nM

Fig. 44B

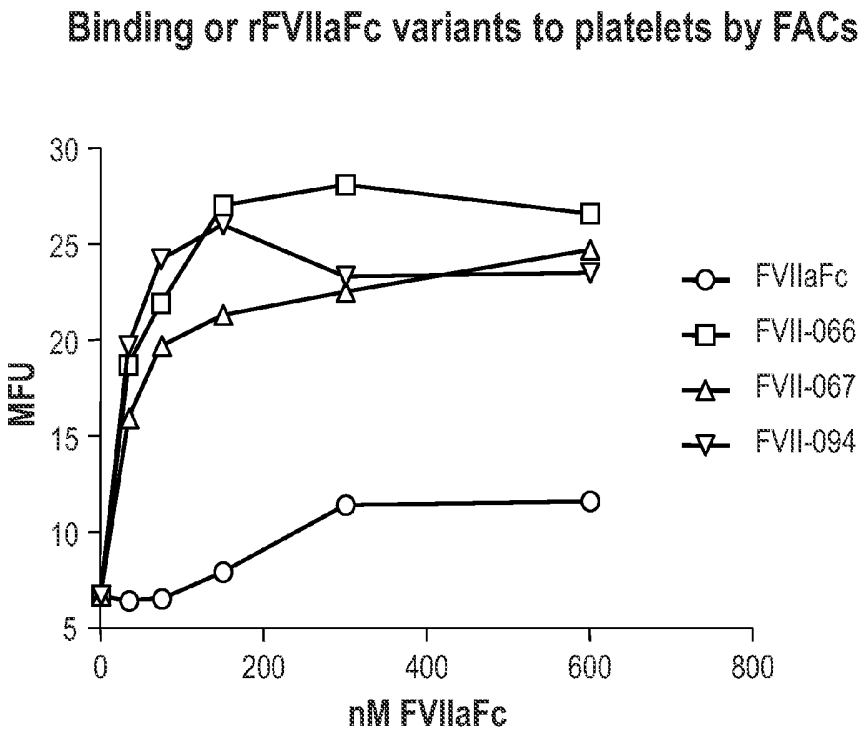

Binding or rFVIIaFc variants to platelets by FACs
MFU
30
25
20
15
10
5
0
200
400
600
800
nM FVIIaFc
FVIIaFc
FVII-066
FVII-067
FVII-094

Fig. 44C

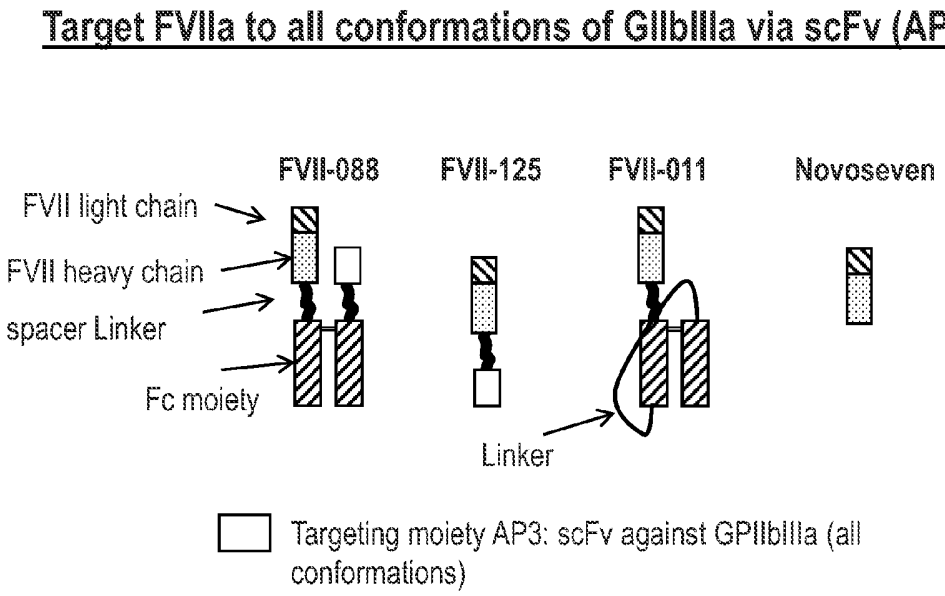

Target FVIIa to all conformations of GIIbIIIa via scFv (AP3)
FVII-088
FVII-125
FVII-011
Novoseven
FVII light chain
FVII heavy chain
spacer Linker
Fc moiety
Linker
Targeting moiety AP3: scFv against GPIIbIIIa (all conformations)

Fig. 45A

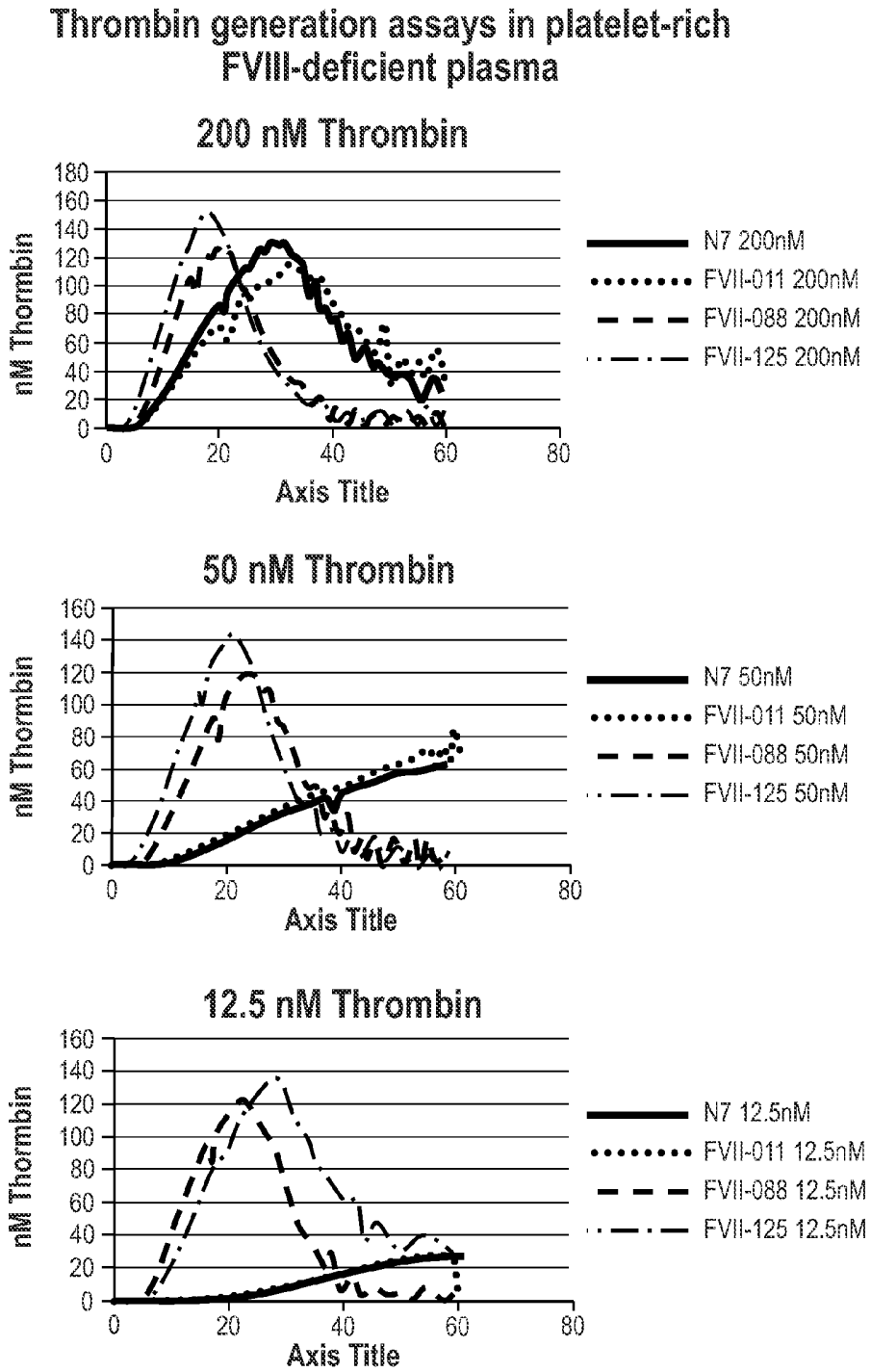

Thrombin generation assays in platelet-rich FVIII-deficient plasma
200 nM Thrombin
nM Thrombin
Axis Title
N7 200nM
FVII-011 200nM
FVII-088 200nM
FVII-125 200nM
50 nM Thrombin
nM Thrombin
Axis Title
N7 50nM
FVII-011 50nM
FVII-088 50nM
FVII-125 50nM
12.5 nM Thrombin
nM Thrombin
Axis Title
N7 12.5nM
FVII-011 12.5nM
FVII-088 12.5nM
FVII-125 12.5nM

Fig. 45B

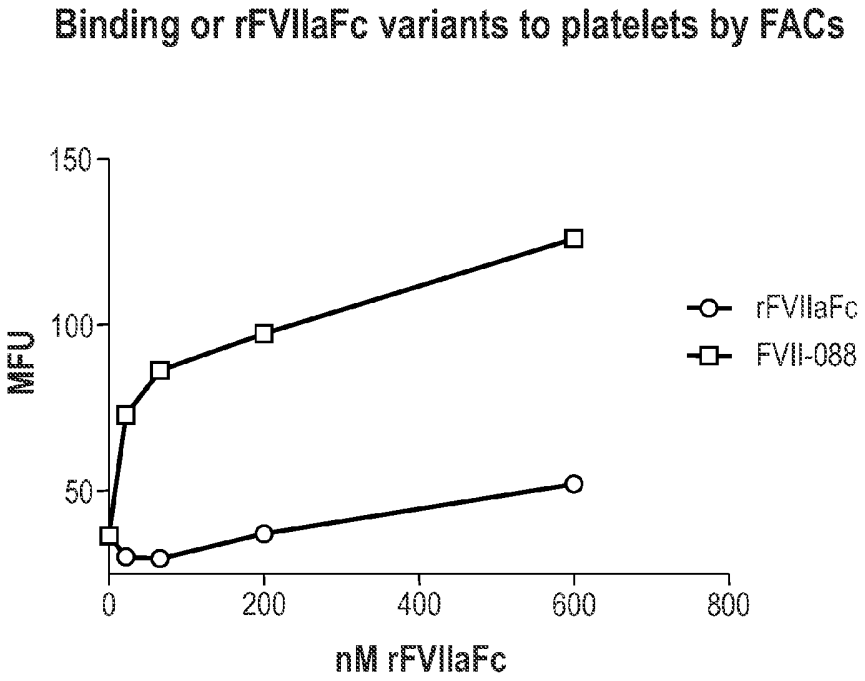
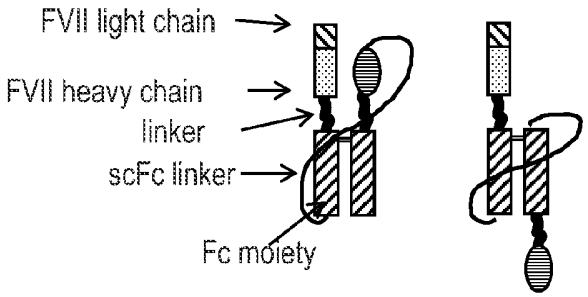

Binding or rFVIIaFc variants to platelets by FACs
150
100
50
MFU
0
200
400
600
800
nM rFVIIaFc
rFVIIaFc
FVII-088

Fig. 45C

Targeting FVIIaFc to GPIb with peptides



-Targeting moieties PS4, OS1 and OS2: peptides against GPIb-alpha

N-terminus	C-terminus
-FVII-047 (PS4)	-FVII-044 (PS4)
-FVII-048 (OS1)	-FVII-045 (OS1)
-FVII-049 (OS2)	-FVII-046 (OS2)

Fig. 46A

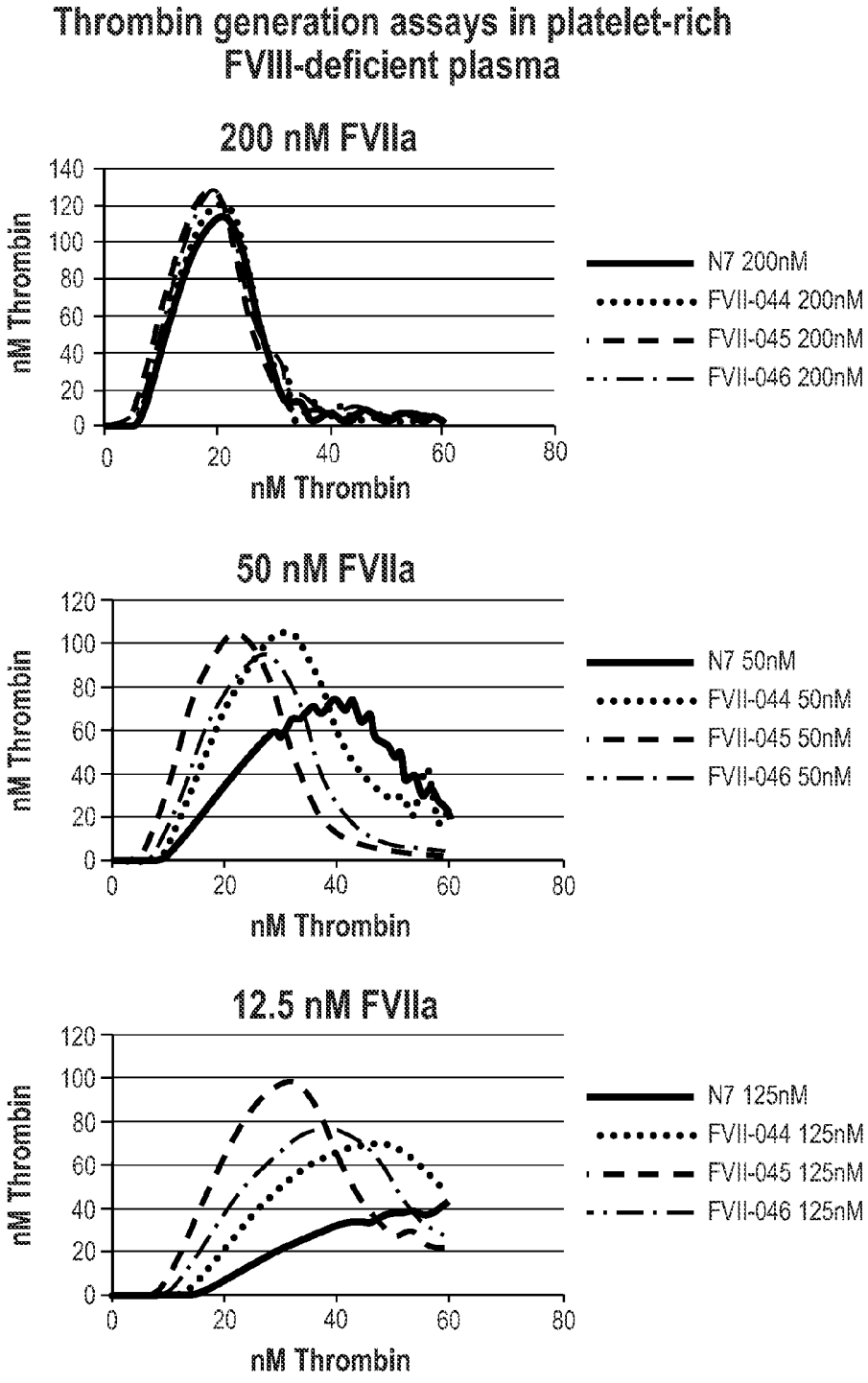

Thrombin generation assays in platelet-rich FVIII-deficient plasma
200 nM FVIIa
nM Thrombin
nM Thrombin
N7 200nM
FVII-044 200nM
FVII-045 200nM
FVII-046 200nM
50 nM FVIIa
nM Thrombin
nM Thrombin
N7 50nM
FVII-044 50nM
FVII-045 50nM
FVII-046 50nM
12.5 nM FVIIa
nM Thrombin
nM Thrombin
N7 125nM
FVII-044 125nM
FVII-045 125nM
FVII-046 125nM

Fig. 46B

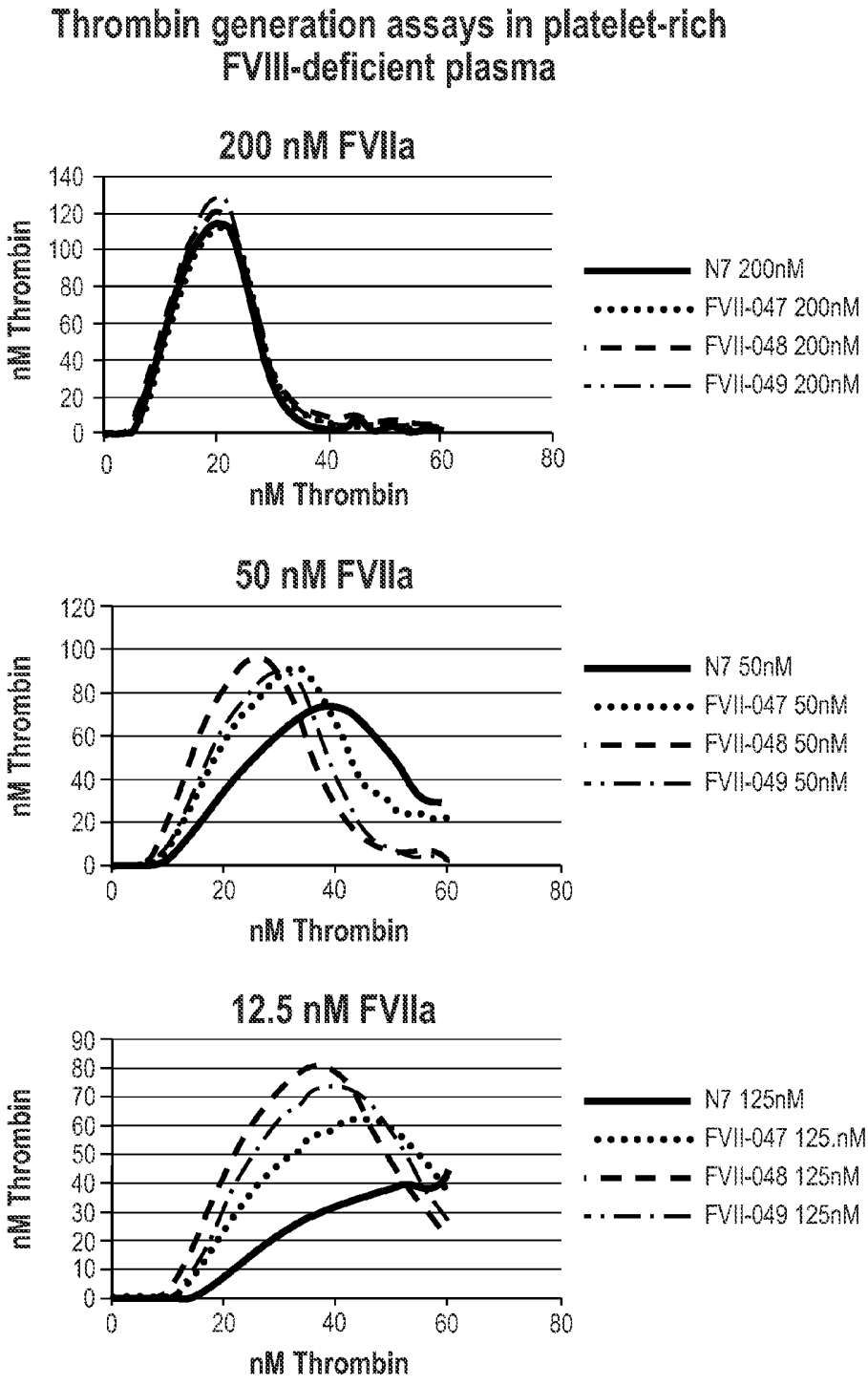

Thrombin generation assays in platelet-rich FVIII-deficient plasma
200 nM FVIIa
nM Thrombin
nM Thrombin
N7 200nM
FVII-047 200nM
FVII-048 200nM
FVII-049 200nM
50 nM FVIIa
nM Thrombin
nM Thrombin
N7 50nM
FVII-047 50nM
FVII-048 50nM
FVII-049 50nM
12.5 nM FVIIa
nM Thrombin
nM Thrombin
N7 125nM
FVII-047 125.nM
FVII-048 125nM
FVII-049 125nM

Fig. 47A

Thrombin generation assays in platelet-rich FVIII-deficient plasma
Comparison of FVII-045 and FVII-048

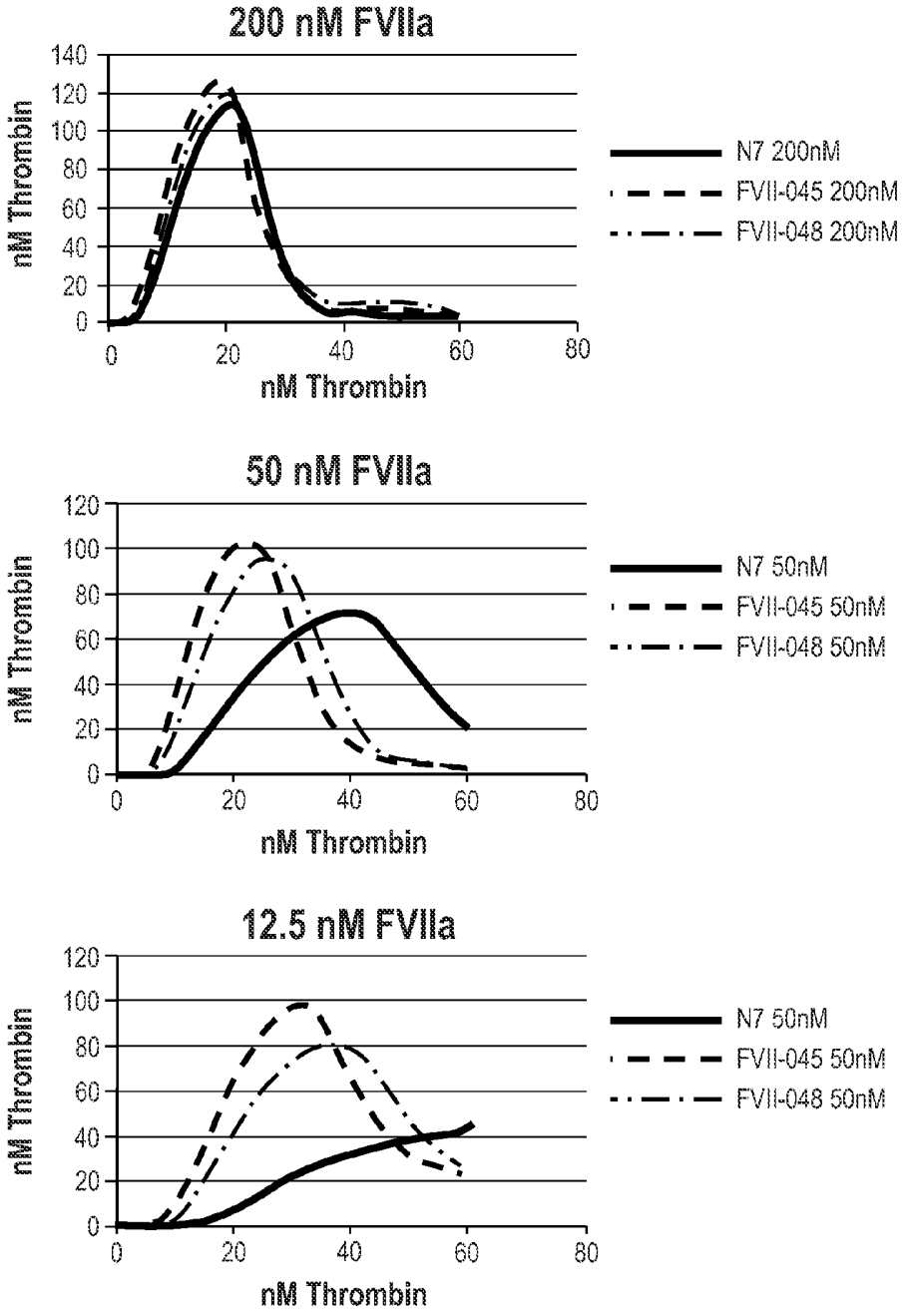
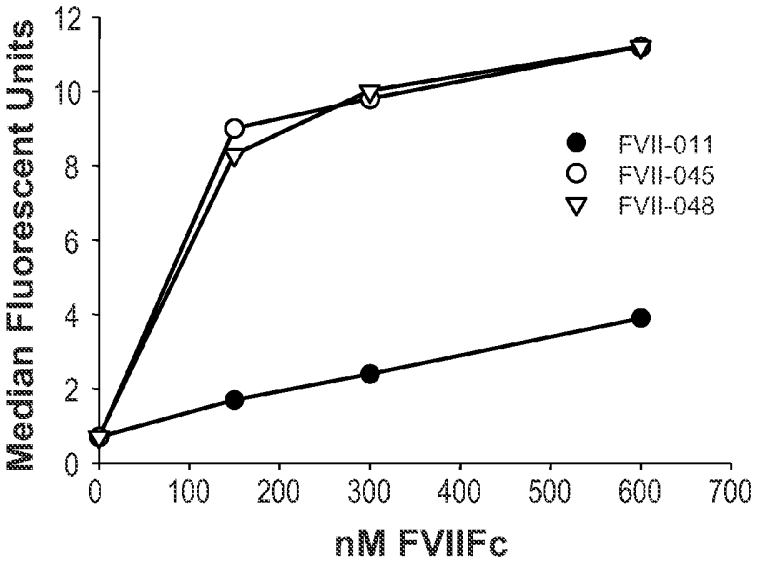


Fig. 47B

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



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

Peptide	Affinity K_D, nM
PS4	64
OS1	0.74
OS2	31

Fig. 48

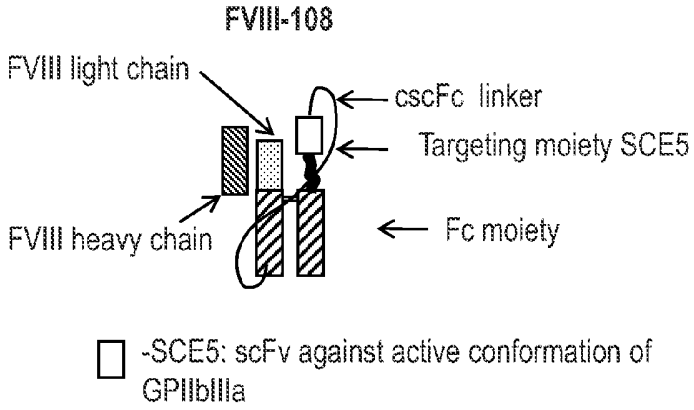

FVIII-108
FVIII light chain
cscFc linker
Targeting moiety SCE5
FVIII heavy chain
Fc moiety
-SCE5: scFv against active conformation of GPIIbIIIa
FVIII-041
FVIII light chain
scFc linker
FVIII heavy chain
Fc

Fig. 49A

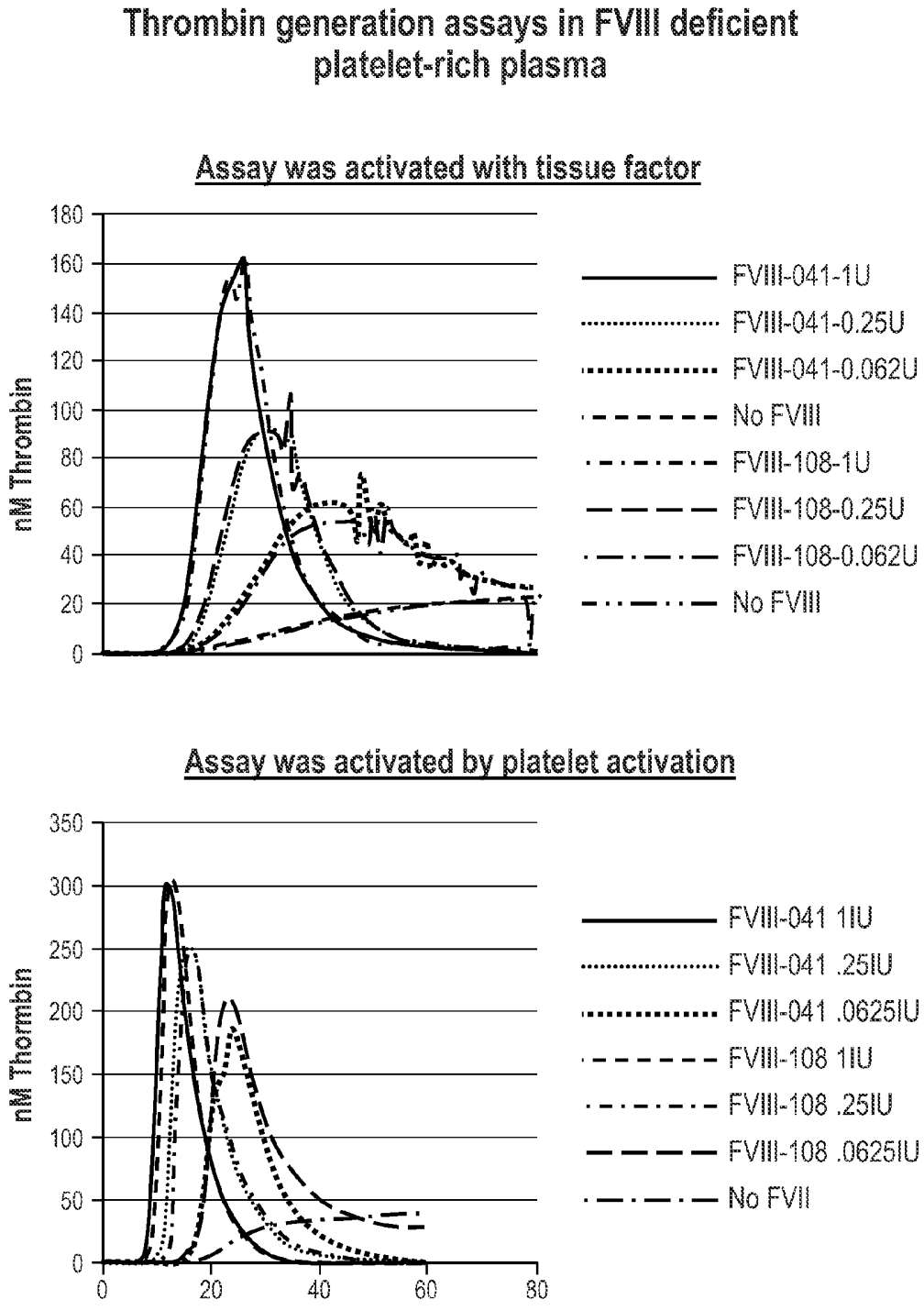

Thrombin generation assays in FVIII deficient platelet-rich plasma
Assay was activated with tissue factor
nM Thrombin
180
160
140
120
100
80
60
40
20
0
FVIII-041-1U
FVIII-041-0.25U
FVIII-041-0.062U
No FVIII
FVIII-108-1U
FVIII-108-0.25U
FVIII-108-0.062U
No FVIII
Assay was activated by platelet activation
nM Thrombin
350
300
250
200
150
100
50
0
0
20
40
60
80
FVIII-041 1IU
FVIII-041 .25IU
FVIII-041 .0625IU
FVIII-108 1IU
FVIII-108 .25IU
FVIII-108 .0625IU
No FVII

Fig. 49B

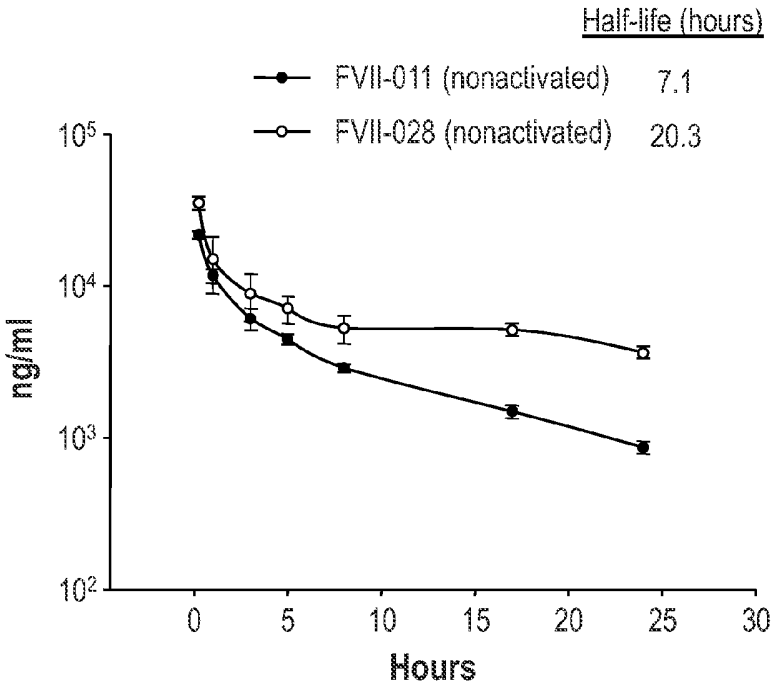

Half-life (hours)
FVII-011 (nonactivated) 7.1
FVII-028 (nonactivated) 20.3
10^5
10^4
10^3
10^2
ng/ml
0
5
10
15
20
25
30
Hours

Fig. 50

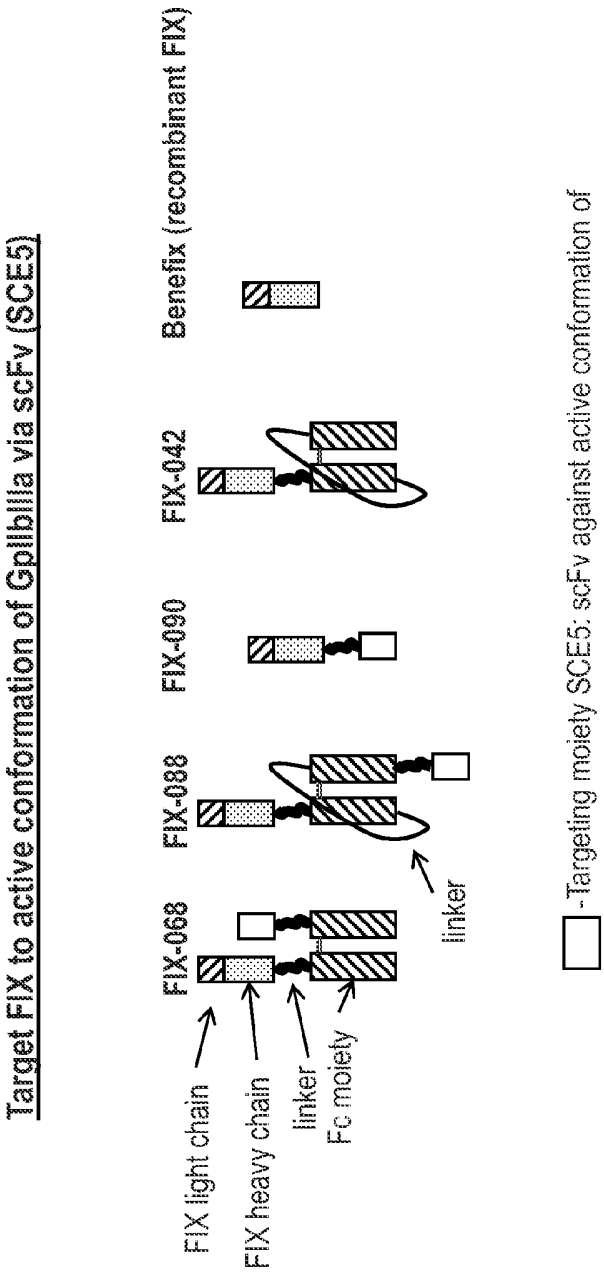

Target FIX to active conformation of GpIIbIIIa via scFv (SCE5)
Benefix (recombinant FIX)
FIX-042
FIX-090
FIX-088
FIX-068
FIX light chain
FIX heavy chain
linker
Fc moiety
linker
- Targeting moiety SCE5: scFv against active conformation of GPIIbIIIa

Fig. 51A

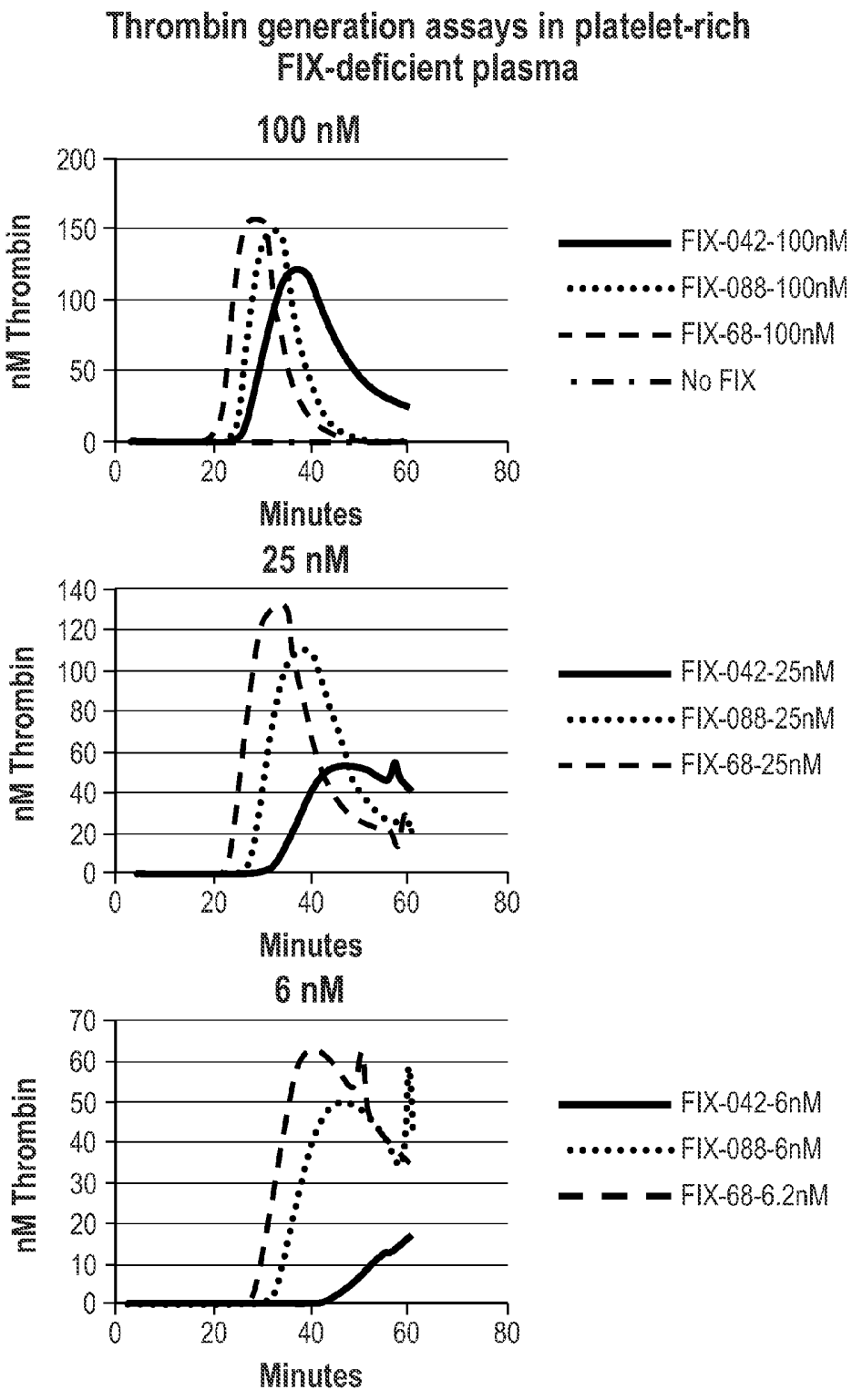

Thrombin generation assays in platelet-rich FIX-deficient plasma
100 nM
nM Thrombin
200
150
100
50
0
0
20
40
60
80
Minutes
FIX-042-100nM
FIX-088-100nM
FIX-68-100nM
No FIX
25 nM
nM Thrombin
140
120
100
80
60
40
20
0
0
20
40
60
80
Minutes
FIX-042-25nM
FIX-088-25nM
FIX-68-25nM
6 nM
nM Thrombin
70
60
50
40
30
20
10
0
0
20
40
60
80
Minutes
FIX-042-6nM
FIX-088-6nM
FIX-68-6.2nM

Fig. 51B

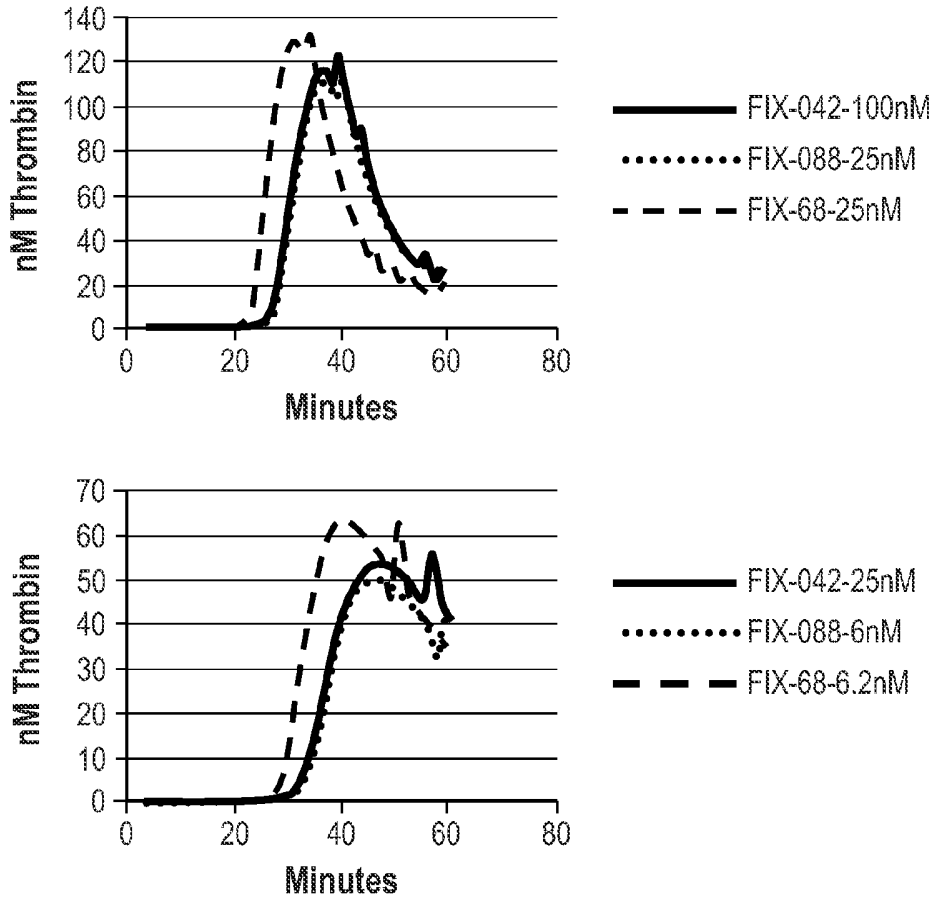

140
120
100
80
60
40
20
0
nM Thrombin
0
20
40
60
80
Minutes
FIX-042-100nM
FIX-088-25nM
FIX-68-25nM
70
60
50
40
30
20
10
0
nM Thrombin
0
20
40
60
80
Minutes
FIX-042-25nM
FIX-088-6nM
FIX-68-6.2nM

Fig. 51C

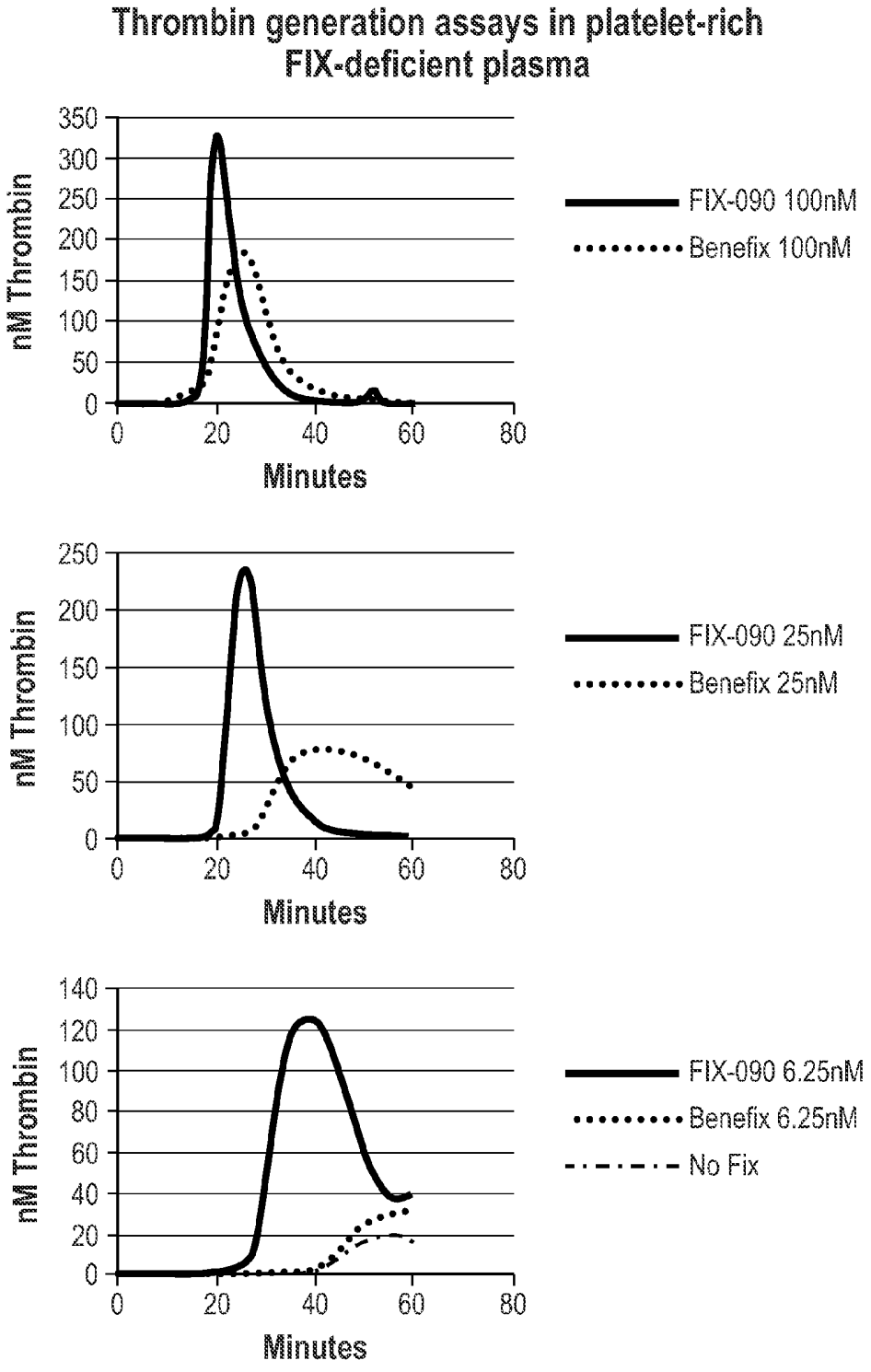

Thrombin generation assays in platelet-rich FIX-deficient plasma
nM Thrombin
Minutes
FIX-090 100nM
Benefix 100nM
FIX-090 25nM
Benefix 25nM
FIX-090 6.25nM
Benefix 6.25nM
No Fix

Fig. 52A

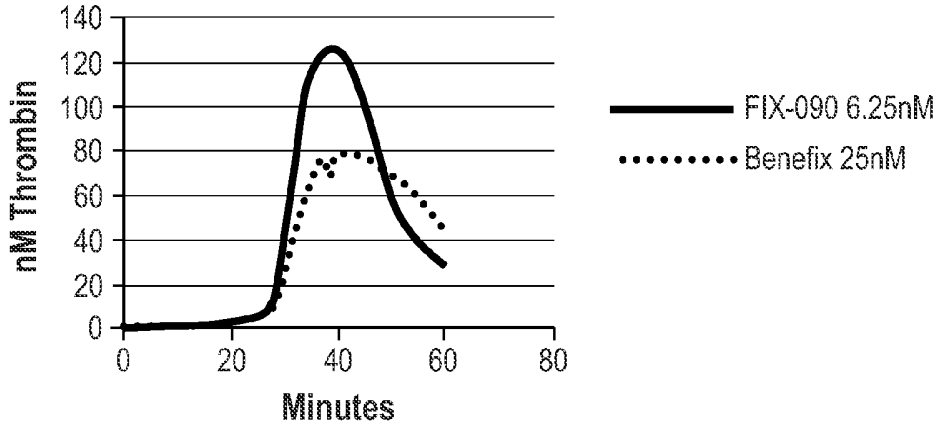


Fig. 52B

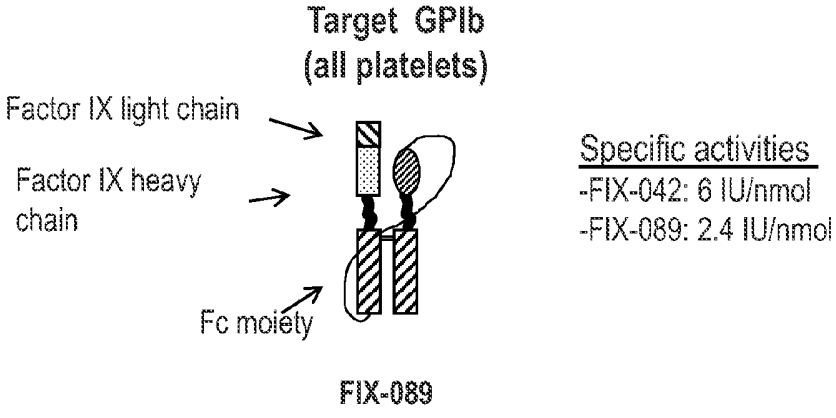


Fig. 53A

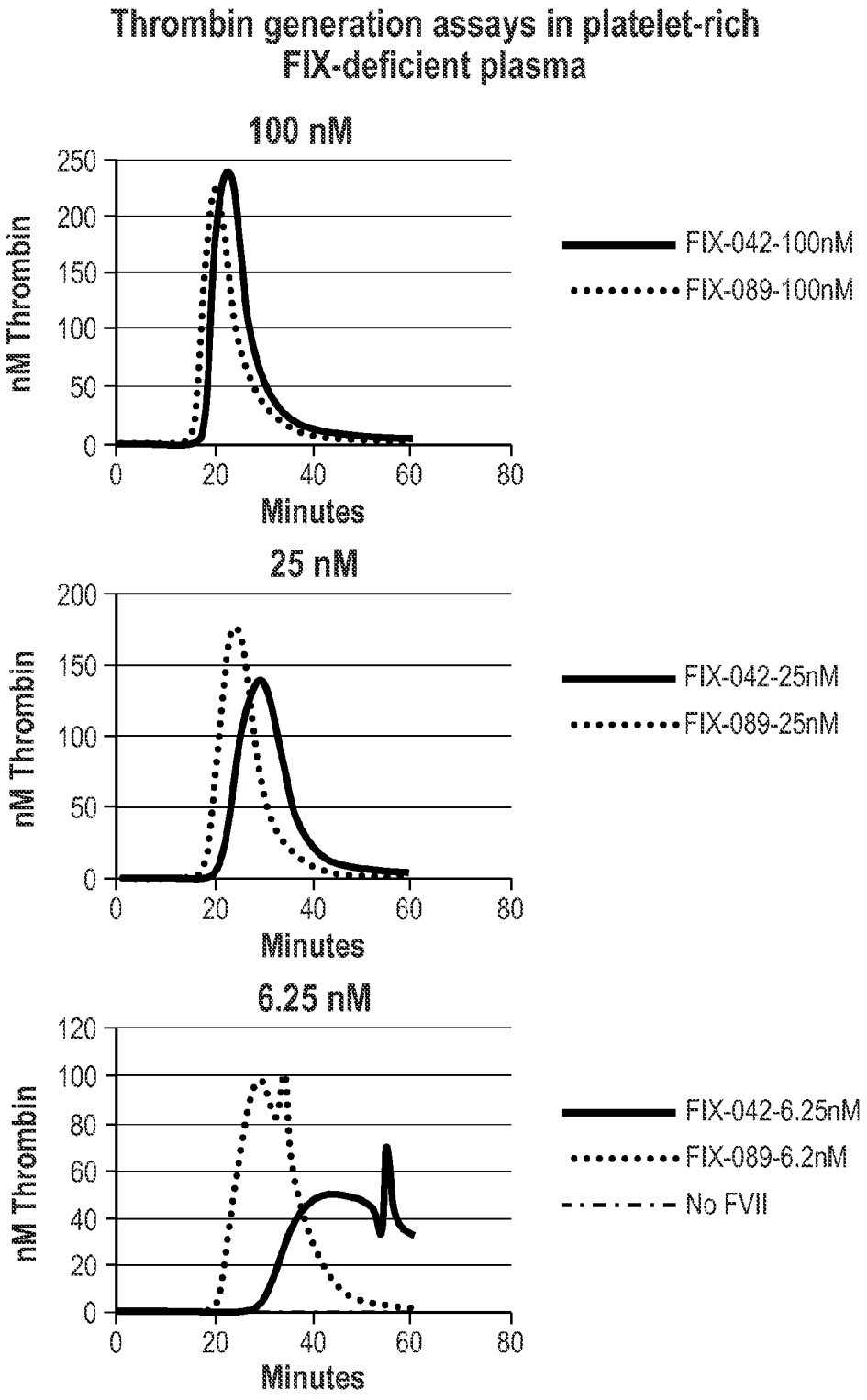

Thrombin generation assays in platelet-rich FIX-deficient plasma
100 nM
nM Thrombin
250
200
150
100
50
0
0
20
40
60
80
Minutes
FIX-042-100nM
FIX-089-100nM
25 nM
nM Thrombin
200
150
100
50
0
0
20
40
60
80
Minutes
FIX-042-25nM
FIX-089-25nM
6.25 nM
nM Thrombin
120
100
80
60
40
20
0
0
20
40
60
80
Minutes
FIX-042-6.25nM
FIX-089-6.2nM
No FVII

Fig. 53B

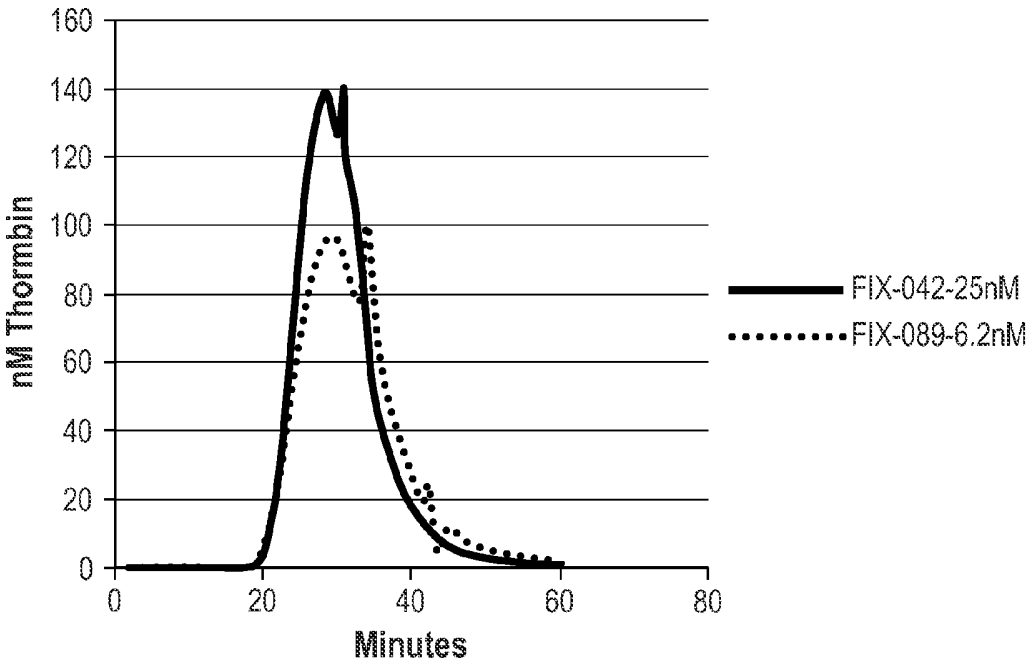

160
140
120
100
80
60
40
20
0
nM Thrombin
0
20
40
60
80
Minutes
FIX-042-25nM
FIX-089-6.2nM

Fig. 53C