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DESCRIPTION

BACKGROUND OF THE INVENTION

[0001] 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.

[0002] 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 to be tissue factor-independent.

[0003] 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 IXa β (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 IXa β (mol wt approximately equal to 46,000). Factor IXa β 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.

[0004] 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.

[0005] 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. WO2009/140598 A1 discloses targeted coagulation factors comprising a coagulation factor linked with at least one domain that specifically binds to a membrane protein on a blood cell. The disclosed targeted coagulation factors are suggested to increase the efficiency of coagulation factors and prolong their duration of action and thus, are taught to be an improvement for the treatment of hematological diseases such as hemophilia A. The contribution of the Ω -loop of the Gla (γ -carboxyglutamic acid) domain and the EGF2 (second epidermal growth factor) domain of FIXa (Factor IXa) in the assembly of the FX-activating complex on activated platelets and phospholipid membranes, was assessed in Yang X. et al., "An ordered sequential mechanism for Factor IX and Factor IXa binding to platelet receptors in the assembly of the Factor X-activating complex", Biochemical Journal, The Biochemical Society, London, GB, vol. 390, no. 1, doi:10.1042/BJ20050029, ISSN 0264-6021, (20050815), pages 157 - 167.

[0006] 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

[0007] The instant invention relates to chimeric clotting factors comprising a clotting factor comprising a FVII polypeptide, a targeting moiety which binds to platelets, and a scaffold moiety comprising an FcRn binding moiety, wherein the clotting factor is linked to the scaffold moiety directly or by a first linker, and wherein the targeting moiety is linked to the scaffold moiety directly or by a second linker, and wherein the scaffold moiety is a dimeric Fc region comprising a first Fc moiety, F1, and a second Fc moiety, F2. 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.

[0008] 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.

[0009] In one embodiment, the clotting factor comprises a scaffold moiety and, optionally, a second spacer moiety.

[0010] In one embodiment, the clotting factor comprises a clotting factor A, a spacer moiety B, a targeting moiety C, 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.

[0011] In one embodiment, E is a dimeric Fc region comprising a first Fc moiety, F1 and a second Fc moiety, F2.

[0012] 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.

[0013] In one embodiment, the cscFc linker is adjacent to at least one enzymatic cleavage site which results in cleavage of the cscFc linker.

[0014] In one embodiment the at least one enzymatic cleavage site is an intracellular processing site.

[0015] In one embodiment, the polypeptide linker is flanked by two enzymatic cleavage sites which are recognized by the same or by different enzymes.

[0016] In one embodiment, the polypeptide linker has a length of about 10 to about 50 amino acids.

[0017] In one embodiment, the polypeptide linker has a length of about 20 to about 30 amino acids.

[0018] In one embodiment, the polypeptide linker comprises a gly/ser peptide.

[0019] In one embodiment, the gly/ser peptide is of the formula (Gly₄Ser)_n, or Ser(Gly₄Ser)_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 (Gly₄ Ser)_n linker is selected from the group consisting of (Gly₄

Ser)₆, Ser(Gly₄ Ser)₆, (Gly₄ Ser)₄ and Ser(Gly₄ Ser)₄.

[0020] In one embodiment, the clotting factor comprises two polypeptide chains.

[0021] In one embodiment, the chimeric clotting factor has a structure 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.

[0022] In one embodiment, a chimeric clotting 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.

[0023] 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: GPIIb, 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.

[0024] The clotting factor is Factor VII.

[0025] In one embodiment, the clotting factor is secreted by a cell in active form. In one embodiment, the clotting factor is activated *in vivo*.

[0026] In one embodiment, the chimeric clotting factor comprises a heterologous enzymatic cleavage site not naturally present in the clotting factor.

[0027] In one embodiment, the enzymatic cleavage site is genetically fused to the amino terminus of the heavy chain moiety of the clotting factor.

[0028] In one embodiment, the clotting factor comprises a scaffold moiety that 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

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[0029] 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.

[0030] In one embodiment, the polypeptide comprises a scaffold moiety and, optionally, a spacer moiety.

[0031] The scaffold moiety is a dimeric Fc region comprising a first Fc moiety, F1 and a second Fc moiety, F2.

[0032] In one embodiment, the clotting factor comprises two polypeptide chains.

[0033] In one embodiment, the chimeric clotting factor has a structure 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.

[0034] The chimeric clotting factor further comprises a targeting moiety.

[0035] 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.

[0036] 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.

[0037] In one embodiment, the cscFc linker comprises a gly/ser peptide.

[0038] In one embodiment, wherein the gly/ser peptide is of the formula (Gly₄Ser)_n, or Ser(Gly₄Ser)_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 (Gly₄ Ser)_n linker is selected from the group consisting of (Gly₄ Ser)₆, Ser(Gly₄ Ser)₆, (Gly₄ Ser)₄ and Ser(Gly₄ Ser)₄.

[0039] It is disclosed that 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 cleavage 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.

[0040] In one embodiment, the targeting moiety binds to resting platelets. In one embodiment, the targeting moiety selectively binds to activated platelets.

[0041] 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.

[0042] 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®

[0043] 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.

[0044] 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.

[0045] 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.

[0046] 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.

[0047] 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.

[0048] 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.

[0049] In one embodiment, the invention pertains to a composition comprising a chimeric clotting factor and a pharmaceutically acceptable carrier.

[0050] In another embodiment, the invention pertains to a composition comprising the nucleic acid molecule of the invention and a pharmaceutically acceptable carrier.

[0051] In another embodiment, the invention pertains to a method for improving hemostasis in a subject, comprising administering the composition of the invention.

[0052] Disclosed is 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 (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.

[0053] 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.

[0054] In still another aspect, the invention pertains to an chimeric clotting factor, which comprises a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: 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.

[0055] In yet another aspect, the invention pertains to an chimeric clotting factor, which comprises a linear sequence of moieties from amino terminus to carboxy terminus selected from the group consisting of: ABF1:F2; 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.

[0056] Disclosed is 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0057]

Figure 1 illustrates exemplary chimeric clotting factor constructs comprising a targeting domain. These exemplary constructs comprise an Fc region.

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.

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.

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.

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.

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.

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

Figure 8 illustrates exemplary chimeric clotting factor constructs which comprise a targeting domain and wherein the clotting factor moiety lacks a Gla domain.

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.

Figure 10 shows SDS PAGE for purification and activation of FVII-011.

Figure 11 shows SDS PAGE for purification of active FVII-053.

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.

Figure 13 shows thrombin generation assay to measure activity of FVII-027, FVII-011 and Novoseven in the presence of activated platelets.

Figure 14 shows that PAC-1 eliminates increased binding to platelets and increased rates of thrombin generation associated with FVII-027.

Figure 15 shows constructs used in a thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets.

Figure 16 shows a thrombin generation assay to measure activity of FVII-037 and Novoseven in the presence of activated platelets.

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.

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.

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.

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.

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.

Figure 22 shows a thrombin generation assay to measure activity of FVII-053 and FVII-011 in the presence of activated platelets

Figure 23 shows that PAC-1 eliminates increased rate of thrombin generation associated with FVII-053

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.

Figure 25 shows Western blot analysis of FVIIFc species following transient transfection of HEK 293 cells and protein A pulldown.

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.

Figure 27 shows Western blot analysis (Fc western) of FVIIFc species following transient transfection of HEK 293 cells and protein A pulldown.

Figure 28 shows FVII-039 and FVII-040 treatment by FXIa.

Figure 29 shows that an FVIIaFc variant targeted to active form of GPIIb/IIIa shows an increased rate of thrombin generation.

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.

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.

Figure 32 shows cleavage of the constructs illustrated in Figure 31.

Figure 33 shows additional activatable constructs and a Western blot illustrating their cleavage.

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 construct cannot be activated. FVII-090 contains the ALRPR cleavage site and so is activatable by thrombin.

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.

Figure 36 illustrates the results of a thrombin generation assay using FVII-090 and FVII-100.

Figure 37 illustrates the results of a thrombin generation assay using FVII-090 and FVII-115.

Figure 38 illustrates amidolytic activity of activatable FVIIc 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.

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.

Figure 40 shows FXa generation activity by "activatable FVIIc." 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, FVIIc (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.

Figure 41 illustrates exemplary activatable construct formats, including an activatable monomer structure used in FVII-118, FVII-119, and FVII-127.

Figure 42 illustrates the efficiency of thrombin cleavage of activatable constructs, specifically monomeric (FVII-118 and -119) as compared to the heterodimeric (FVII-090).

Figure 43 illustrates the results of a thrombin generation assay to compare wild type activatable FVIIc (FVII-118) to the high specific activity variant (FVII-127).

Figure 44A illustrates several targeted constructs. In this instance, an SCE5 scFv which binds to the active conformation of GPIIb/IIIa was included at various sites in the construct. 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.

Figure 45A illustrates several targeted FVIIa constructs which include AP3, an scFv against GPIIb/IIIa 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.

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.

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.

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.

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).

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).

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.

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.

Figure 53A shows a targeted FIX construct comprising a peptide that binds to GPIb, present on resting and activated platelets. Figure 53B 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

[0058] The present invention relates to chimeric clotting factors comprising a clotting factor comprising a FVII polypeptide, a targeting moiety which binds to platelets, and a scaffold moiety comprising an FcRn binding moiety, wherein the clotting factor is linked to the scaffold moiety directly or by a first linker, and wherein the targeting moiety is linked to the scaffold moiety

directly or by a second linker, and wherein the scaffold moiety is a dimeric Fc region comprising a first Fc moiety, F1, and a second Fc moiety, F2. 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.

[0059] 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.

[0060] The targeting moiety can be positioned at a number of places in a chimeric clotting 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.

[0061] In another embodiment, a chimeric clotting factor of the invention is made in a form that is activatable at the site 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.

[0062] The heterologous cleavage site is present in a cleavable linker can be positioned at a number of places in a chimeric clotting 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.

[0063] The chimeric clotting factor 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. The scaffold is an FcRn binding moieties (i.e. complete Fc regions or portions thereof which bind to FcRn).

[0064] The chimeric clotting factor of the invention employs an FcRn binding portion 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.

[0065] 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.

[0066] 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.

[0067] 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.

[0068] 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.

[0069] 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.

[0070] 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.

[0071] In order to provide a clear understanding of the specification and claims, the following definitions are provided below.

I. Definitions

[0072] 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.

[0073] 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, omithine, 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.

[0074] 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.

[0075] 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

[0076] 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 contrast, 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.

[0077] 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.

[0078] 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 Xla, 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.

[0079] 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 $(\text{Gly}_4 \text{Ser})_n$. (SEQ ID NO:4) Another exemplary gly/ser polypeptide linker comprises the amino acid sequence $\text{S}(\text{Gly}_4 \text{Ser})_n$.

[0080] In one embodiment, $n=1$. In one embodiment, $n=2$. In another embodiment, $n=3$, i.e., $(\text{Gly}_4 \text{ Ser})_3$. In another embodiment, $n=4$, i.e., $(\text{Gly}_4 \text{ Ser})_4$ (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 $\text{Ser}(\text{Gly}_4\text{Ser})_n$ (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, $n=6$.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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).

[0089] 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.

[0090] 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).

[0091] 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.

[0092] As used herein the term "moiety" refers to a component part or constituent of a chimeric polypeptide.

[0093] 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.

[0094] 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.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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).

[0099] 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.

[0100] 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).

[0101] 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), CV1 (monkey kidney line), COS (a derivative of CV1 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.

[0102] 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.

[0103] 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.

[0104] 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.

[0105] 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.

[0106] In constructs that include more than one processing or cleavage site, it will be understood that such sites may be the same or different.

[0107] *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).

[0108] 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.

[0109] 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.

[0110] 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.

[0111] 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.

[0112] 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.

[0113] 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.

[0114] The chimeric molecules of the invention can be used prophylactically. As used herein the term "prophylactic treatment" refers to the administration of a molecule prior to a bleeding episode. In one embodiment, the subject in need of a general hemostatic agent is undergoing, or is about to undergo, surgery. The chimeric protein of the invention can be administered prior to or after surgery as a prophylactic. The chimeric protein of the invention can be administered during or after surgery to control an acute bleeding episode. The surgery can include, but is not limited to, liver transplantation, liver resection, or stem cell transplantation

[0115] 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 iliopsoas sheath. The subject may be in need of surgical prophylaxis, peri-operative

management, or treatment for surgery. Such surgeries include, e.g., minor surgery, major surgery, tooth extraction, tonsillectomy, inguinal herniotomy, synovectomy, total knee replacement, craniotomy, osteosynthesis, trauma surgery, intracranial surgery, intra-abdominal surgery, intrathoracic surgery, or joint replacement surgery.

[0116] As used herein the term "acute bleeding " refers to a bleeding episode regardless of the underlying cause. For example, a subject may have trauma, uremia, a hereditary bleeding disorder (e.g., factor VII deficiency) a platelet disorder, or resistance owing to the development of antibodies to clotting factors.

[0117] 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

[0118] The invention pertains to improved versions of factors VII.

[0119] 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.

[0120] 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.

[0121] 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.

[0122] 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.

[0123] 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.

[0124] 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.

[0125] 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).

[0126] Exemplary constructs are shown in the accompanying figures.

[0127] 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).

[0128] 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.

[0129] 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.

[0130] 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. 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.

[0131] The clotting factor of the invention is a mature form of Factor VII. 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.

[0132] Exemplary FVII variants include those with increased specific activity, e.g., mutations that increase the activity of FVII by increasing its enzymatic activity (K_{cat} or K_m). 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. 276:49027. Disclosed is a variant form of FVII that includes the mutations. Exemplary mutations include V158D-E296V-M298Q. It is disclosed that a variant form of FVII includes a replacement of amino acids 608-619 (LQQRKVGDSPN, 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. For 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 et al. 2003. J. Biol. Chem. 278:4121; and Sturzebecher et al. 1997. FEBS Lett 412:295.

[0133] Full activation, which occurs upon conformational change from a zymogen-like form, occurs upon binding to its 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.

[0134] It will be understood that in addition to wild type (WT) versions of 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.

[0135] 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.

[0136] As used herein, the term "Gla domain" refers to the conserved membrane binding motif which is present in vitamin K-dependent proteins, such as prothrombin, coagulation factors VII, IX and X, proteins C, S, and Z. These proteins require vitamin K for the posttranslational synthesis of γ -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 Ca^{2+} 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.

[0137] 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.

[0138] 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.

[0139] 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.

[0140] 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

[0141] 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.

[0142] 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

[0143] The 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. The targeted molecules are not expressed on cells or tissues other than platelets, i.e., the targeting moieties specifically bind to platelets.

[0144] 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.

[0145] 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.

[0146] The 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 include 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 GPIIb/IIIa. 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 mutant V86A/E277A/R338A) with augmented clotting activities has been described by Lin et al. 2010. Journal of Thrombosis and Haemostasis 8: 1773).

[0147] 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.

[0148] 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.

[0149] 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, or 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.

[0150] 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).

[0151] 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

[0152] In certain embodiments, a polypeptide of the invention comprises at least one antigen binding portion (e.g., binding site) of an antibody. The antigen binding portion targets the polypeptide to platelets

[0153] 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.

[0154] 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)).

[0155] 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 V_L and/or V_H 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).

[0156] 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.

[0157] 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 (Gly₄Ser)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.

[0158] Polypeptides of the invention may comprise a variable region or portion thereof (e.g. a V_L and/or V_H 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. 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.

[0159] 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".

[0160] 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.

[0161] 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 in 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.

[0162] DNA encoding the desired monoclonal antibody or 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.

[0163] 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 5,589,369e). 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. It will be appreciated that the

genetic material associated with these human antibodies may also be isolated and manipulated as described herein.

[0164] 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.

[0165] 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).

[0166] 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 V_H or V_L 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 (V_H) 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. Exemplary single domain antibodies comprising VHH domains include Nanobodies® (Ablynx NV, Ghent, Belgium).

[0167] 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 include 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 GPIIb/IIIa. 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

[0168] 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.

[0169] 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 GPIIb 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

[0170] 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.

[0171] 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. ALRPRVGGGA)).

[0172] In one embodiment, the cleavable linker may be flanked on one or more sides (upstream, downstream or both) by a spacer moiety.

[0173] 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.

[0174] Exemplary activatable constructs are shown in the accompanying Figures and following Examples.

V. Scaffold Moieties

[0175] The chimeric clotting factor of the invention comprise a scaffold moiety, which is a dimeric Fc moiety.

A . scFc Regions

[0176] 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.

[0177] 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).

[0178] A variety of polypeptides of alternative designs are within the scope of the invention. For example, in one embodiment, a polypeptide comprises the moieties:



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.

[0179] 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.

[0180] In one embodiment, F1 and F2 each comprise a CH2 and CH3 moiety.

[0181] 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.

[0182] 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

[0183] 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.

[0184] 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.

[0185] 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.

[0186] 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.

[0187] 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).

[0188] 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 CH₂, and a CH₃ 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.

[0189] Disclosed is a polypeptide that 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 K_A 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.

[0190] 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.

[0191] 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.

[0192] 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 γ 1) can be substituted without significant loss of Fc binding affinity for FcRn: P238A, S239A, K246A, K248A, D249A, M252A, T256A, E258A, T260A, D265A, S267A, H268A, E269A, D270A, E272A, L274A, N276A, Y278A, D280A, V282A, E283A, H285A, N286A, T289A, K290A, R292A, E293A, E294A, Q295A, Y296F, N297A, S298A, Y300F, R301A, V303A, V305A, T307A, L309A, Q311A, D312A, N315A, K317A, E318A, K320A, K322A, S324A, K326A, A327Q, P329A, A330Q, P331A, E333A, K334A, T335A, S337A, K338A, K340A, Q342A, R344A, E345A, Q347A, R355A, E356A, M358A, T359A, K360A, N361A, Q362A, Y373A, S375A, D376A, A378Q, E380A, E382A, S383A, N384A, Q386A, E388A, N389A, N390A, Y391F, K392A, L398A, S400A, D401A, D413A, K414A, R416A, Q418A, Q419A, N421A, V422A, S424A, E430A, N434A, T437A, Q438A, K439A, S440A, S444A, and K447A, where for example P238A represents 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).

[0193] 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).

[0194] 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.

[0195] 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.

[0196] 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.

[0197] 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.

[0198] 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 (Δ CH2 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 IgG₁ 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 IgG1 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.

[0199] 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.

[0200] The polypeptides of the invention 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.

[0201] 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.

[0202] 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.

[0203] 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.

[0204] 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.

[0205] 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.

[0206] 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

[0207] 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. 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.

[0208] 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.

[0209] 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.

[0210] 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).

[0211] Disclosed is a polypeptide that 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)).

[0212] 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

[0213] 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 contrast, 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.

[0214] 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.

[0215] 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 Xla, Xa, or thrombin cleavage site) and which may include additional spacer linkers on either the N terminal or 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.

[0216] 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.

[0217] 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 polypeptide 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.

[0218] 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.

[0219] 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.

[0220] In another embodiment, a polypeptide linker comprises or consists of a glyser 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 (Gly₄Ser)_n (SEQ ID NO: 4), wherein *n* is a positive integer (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10). A preferred gly/ser linker is (Gly₄Ser)₂ (SEQ ID NO:29), (Gly₄Ser)₄ (SEQ ID NO:6), or (Gly₄Ser)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 (Gly₄Ser)_n (SEQ ID NO:4)).

[0221] 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.

[0222] 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

[0223] 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 linker 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).

[0224] For example, in one embodiment, a nucleic acid molecule of the invention specifies a polypeptide represented by the formula:



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.

[0225] 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.

[0226] 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

[0227] 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.

[0228] 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.

[0229] 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.

[0230] 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.

[0231] 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).

[0232] 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.

[0233] 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.

[0234] 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.

[0235] 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. 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.

[0236] 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. 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.

[0237] 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 fluorescence-activated cell sorter analysis (FACS), immunohistochemistry and the like.

[0238] 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.

[0239] 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.

[0240] 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), 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.

[0241] 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 polypeptide. 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.

[0242] 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).

[0243] 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.

[0244] 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.

[0245] 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.

[0246] 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.

[0247] 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.

[0248] 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.

[0249] 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. 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.

[0250] 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.

[0251] *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.

[0252] 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.

[0253] 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 comprising a clotting factor comprising a FVII polypeptide, a targeting moiety which binds to platelets, and a scaffold moiety comprising an FcRn binding moiety, wherein the clotting factor is linked to the scaffold moiety directly or by a first linker, and wherein the targeting moiety is linked to the scaffold moiety directly or by a second linker and wherein the scaffold moiety is a dimeric Fc region comprising a first Fc moiety, F1, and a second Fc moiety, F2. 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.

[0254] 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

[0255] 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 clotting factor of the invention.

[0256] 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.

[0257] In one embodiment, an enhanced clotting 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 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.

[0258] 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.

[0259] 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.

[0260] 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.

[0261] For buccal and sublingual administration the composition may take the form of tablets, lozenges or fast dissolving films according to conventional protocols.

[0262] 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.

[0263] 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.

[0264] 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.

[0265] 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.

[0266] 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.

[0267] 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.

[0268] 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.

[0269] 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.

[0270] 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.

[0271] 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).

[0272] 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.

[0273] 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.

[0274] 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.

[0275] 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).

[0276] 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.

[0277] 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.

[0278] 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.

[0279] 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.

[0280] 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.

[0281] 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.

[0282] 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.

[0283] 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.

[0284] 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.

[0285] 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.

[0286] 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.

[0287] 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.

[0288] 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.

[0289] 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).

[0290] 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.

[0291] 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.

[0292] This invention is further illustrated by the following examples which should not be construed as limiting..

EXAMPLES

[0293] Throughout the examples, the following materials and methods were used unless otherwise stated.

General Materials and Methods

[0294] 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., C.S.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

[0295] 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.

[0296] 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.

[0297] As a first step a series of intermediate plasmid were generated using the following primers:

HindIII-Sall-BpsEI-Fc-F

AGTCAAGCTTGTCTGACTCCGGAACCTCCTGGGCGGACC

BamHI-linker-Fc-R

CATCGGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCCGCCACCTTTACCC
GGAGACAGGGAGAGG

BclI-Fc-F

CAGTCTTGATCAGACAAAACCTCACACATGCCCACC

scFc-EcoRI-R

ACTGACGAATTCTCATTTACCCGGAGACAGGGAG

HindIII-Kozak-FVII-F:

CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG

FVII-HC-BspEI-R:

AGGAGTTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTCTGGATCCCCCGCCACCGGAACCTCCACC
GCCTGATCCACCCCCACCTGATCCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCGGGAAATG
GGGCTCGCAGGAGG

A 50 ul PCR reaction was carried out with 25 pmol of HindIII-Sall-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-Sall-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 Sall 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 Bell 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.

[0298] Next, 2 DNA fragments were synthesized: Genescript-FVII-027-1 and Genescript-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. Genescript-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 EcoRI 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.

[0299] Genescript-FVII-027-1 was cloned into the SapI and EcoRI sites of pSYN-FVII-011 to generate pSYN-FVII-036. Next, Genescript-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

[0300] 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 GPIIb/IIIa is attached to the c-terminus of the second Fc moiety.

[0301] Synthesis of DNA fragment Genescript-FVII-037 was outsourced (Genescript) 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 Genescript-FVII-037 was subcloned into the SapI/EcoRI of pSYN-FVII-011 (refer to P0830) to generate an 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.

[0302] 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 Sall site (residues 412-413 EU numbering, GTG GAC to GTC GAC) in the second Fc.

Cloning of pSYN-FVII-044-, -045 and -046.

[0303] 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, and 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.

[0304] Synthesis of Genscript-FVII-044, -045 and -046 was outsourced (Genscript). These DNA fragments were cleaved with Sall/EcoRI and subcloned into the Sall/EcoRI sites of pSYN-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.

[0305] 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 Sall site (residues 412-413 EU numbering, GTG GAC to GTC GAC) in the first Fc. A HindIII/EcoRI fragment of pSYN-FVII-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.

[0306] 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 the N-terminus of the second Fc moiety.

[0307] 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

[0308] 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

[0309] 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.

[0310] 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

[0311] 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.

[0312] 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

[0313] 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

CGACTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTCGGGAAATGGGGCTCGCAGG

[0314] 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 µl 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:

GGGCATGTGTGAGTTTTGTCTGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCC
 GCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCGGGAAATGGGGCTCGCAGGAGG

Fc-linker-F:

GACAAAACTCACACATGCCCACC

Fc-linker-R:

GCAGAATTCTCATTACCCGGAG

[0315] 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

[0316] 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-Sall-BpEI-Fc-F

AGTCAAGCTTGTCTGACTCCGGAACCTCCTGGGCGGACC

BamHI-linker(PACE1)-Fc-R

CATCGGATCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCCGCCACCGCTCCGG
CGGCGCCGTTTACCCGGAGACAGGGAGAGG

HindIII-Kozak-FVII-F

CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG

BspEI-Fc-linker-FVIIIC-R

GAGTTCCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTCTGATCCCCCGCCACCGGAACCTCCACCGC
CTGATCCACCCCCACCTGATCCGCCGCCACCGGACCCACCTCCGCCGGAGCCACCGCCACCTCGGCCTTGG
GGTTTGCTGG

BamHI-2xlink-pace-HC-F

CAGTCTGGATCCGGCGGTGGAGGTTCCGGTGGGGGTGGATCAAGGAAGAGGAGGAAGAGGATTGTGGGGGG
CAAGGTGTGCC

Fc-EcoRI-R

ATGTCTGAATTCTCATTTACCCGGAGACAGGGAGAGG

[0317] To generate the first intermediate plasmid, a PCR reaction was performed with 25 pmol of primers HindIII-Sall-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-Sall-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-Sall-BpEI-Fc-F introduces a HindIII and Sall 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-FVIIIC-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-FVIIIC-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-FVIIIC-R adds a BspEI site at the 3' end of the molecule.

[0318] 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

[0319] A silent mutation was introduced in the first Fc moiety of FVII-024 by PCR-based site-directed mutagenesis methods, resulting in the generation of a Sall site at DNA region encoding amino acids in position 412 and 413 (EU numbering). This generated the intermediate construct FVII-073

Cloning of pSYN-FVII-057

[0320] The synthesis of the DNA sequence comprising nucleotides from the Sall to BsiWI sites of pSYN-FVII-057 was outsourced. This DNA was subcloned into the Sall/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

[0321] These constructs were cloned as described for pSYN-FVII-057 (outsourced synthesis of DNA from Sall to BsiWI and subcloned into pSYN-FVII-073)

Cloning of pSYN-FVII-066

[0322] The synthesis of the DNA sequence comprising nucleotides from the Sall to RsrII sites of pSYN-FVII-066 was outsourced. This DNA was subcloned into the Sall/RsrII sites of pSYN-FVII-043 to generate pSYN-FVII-066

Cloning of pSYN-FVII-067

[0323] The synthesis of the DNA sequence comprising nucleotides from the Sall to EcoRI sites of pSYN-FVII-067 was outsourced. This DNA was subcloned into the Sall/EcoRI sites of pSYN-FVII-041 to generate pSYN-FVII-067

Cloning of pSYN-FVII-090

[0324] 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

[0325] 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

[0326] 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

[0327] 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

[0328] 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

[0329] 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

[0330] 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

[0331] 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

[0332] 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

[0333] 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

[0334] 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:

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AQVQLQESGGGLVQPGGSLRLSCAASGFMFSRYAMSWVRQAPGKGPEWVSGISGSGGSTYYADSVKGRFTVS  
RDNSKNTLYLQMNSLRAEDTAVYYCARGATYTSRSDVPDQTSFDYWGQGTLVTVSSGSASAPKLEEGEFSEA  
RVSELTQDPAVSVALGQTVRITCQGDSLRNIFYASWYQQKPGQAPTLVIYGLSKRPSGIPDRFSASSSGNTAS  
LTITGAQAEDEADYYCLLYYGGGQQGVFGGCTKLTVLRQPKAAPSVTLFPPSSAA
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Cloning of pSYN-FVII-094

[0335] A DNA fragment comprising a sequence encoding a 6x(GGGGGS) 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

[0336] The synthesis of the DNA sequence comprising nucleotides from the Sall to RsrII sites of pSYN-FVII-088 was outsourced. This DNA was subcloned into the Sall/RsrII sites of pSYN-FVII-066 to generate pSYN-FVII-088

Cloning of pSYN-FVII-125

[0337] 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

[0338] 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).

[0339] 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 FVIII-Fc DNA sequence was cloned into the mammalian dual expression vector pBUDCE4.1 (Invitrogen) under control of the CMV promoter.

[0340] A second identical Fc sequence including the mouse Igk signal sequence was obtained by RT-PCR and cloned downstream of the second promoter, EF1 α , in the expression vector pBUDCE4.1. This final construct was designated pSYN-FVIII-013.

[0341] A second plasmid was created from similar constructs using PCR and standard molecular biology techniques, in order to express rFVIII-BDD-Fc-Fc in which the rFVIII-BDD-Fc coding sequence was fused to the second Fc sequence with a (GGGGS)₄ linker, allowing for production of only the rFVIII-BDD-Fc monomer-dimer hybrid in transient transfection. This construct was designated pSYN-FVIII-041.

Cloning of pSYN-FVIII-049

[0342] Generated intermediate pSYN-FVIII-048 by cloning NheI/XhoI fragment from pBUDCE4.1 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

[0343] 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

[0344] 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 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

[0345] 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.

[0346] 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.

[0347] 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

[0348]

FVII-HC-BspEI-R

AGGAGTTCGGAGCTGGGCACGGTGGGCATGTGTGAGTTTTGTTCGGATCCCC
CGCCACCGGAACCTCCACCGCCTGATCCACCCCCACCTGATCCGCCGCCACC
GGACCCACCTCCGCCGGAGCCACCGCCACCGGGAAATGGGGCTCGCAGGAG
G

FVII-HC-Hind3-IggKss-f

ACTGACAAGCTTGCCGCCACCATGGAGACAGACACACTCCTGCTATGGGTAC
TGCTGCTCTGGGTTCAGGTTCCACTGGTATTGTGGGGGGCAAGGTGTGC

FVII-LC-NotI-F

ACTGACGCGGCCGCGCCGCCACCATGGTCTCCCAGG

FVII-LC-XhoI-R

ACTGACCTCGAGTTATCGGCCTTGGGGTTTGCTGG

Cloning of pSYN-FVII-013

[0349] 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.

[0350] 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 and subclone in pSYN-FVII-012 to generate pSYN-FVII-013

Primers

[0351]

FVII-LC-6xlinker-BamHI-

RACTGACGGATCCCCCGCCACCGGAACCTCCACCGCCTGATCCACCCCCACC
TGATCCGCGCCACCGGACCCACCTCCGCGGAGCCACCGCCACCTCGGCCT
TGGGGTTTGCTGGC

HindIII-Kozak-FVII-F

CGACAAGCTTGCCGCCACCATGGTCTCCCAGGCCCTCAGG

FVII-HC-NotI-F

ACTGACGCGGCCGCGCCGCCACCATGGAGACAGAC

FVII-HC-XhoI-R

ACTGACCTCGAGTTAGGGAAATGGGGCTCGCAGGAG

Cloning of pSYN-FVII-018

[0352] For the FVII-018 construct, the heavy chain of FVII was expressed as an Fc fusion protein and the light chain of FVII was separately expressed as a separate Fc fusion protein.

[0353] 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**[0354]**

scFc-EcoRI-R

ACTGACGAATTCTCATTTACCCGGAGACAGGGAG

Fc-XhoI-R

AGCTCTCGAGTCATTTACCCGGAGACAGGG

Example 8. Attempts at Expression of Activatable Constructs**Cloning of FVII-039, -040**

[0355] 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.

[0356] 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

[0357] 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

[0358] 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% CO₂. Cells we subcultured every three to four days by seeding at cell density of 5x10⁵ cells/ml.

[0359] Twenty-four hours prior to transfection cells were seeded at a density of 7x10⁵ 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 FVIIFc Molecules (except FVII-028 and FVII-053) and FIXFc molecules

[0360] FVIIFc molecules were purified from conditioned media using the following columns: 1) Anion exchange chromatography with pseudo-affinity elution (e.g. Q sepharose 4FF (GE Healthcare) followed by elution with varying levels of CaCl₂ 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

[0361] 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

[0362] 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 FVIII_h proteins

[0363] FVIII_h 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

[0364] Fractions eluted from the FcRn column containing FVII_h were pooled, and total protein was concentrated to 4 mg/ml. The CaCl₂ concentration was raised to 5 mM and the sample was incubated at 4°C for 24 to 48 hours until at least 80% of FVII_h was activated. The extent of activation was assessed by SDS PAGE (Figure 10)

Example 15. FVIIa activity assays, soluble tissue factor method

[0365] 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 variant	IU/nM
FVII-011	991
FVII-024	929
FVII-027	790
FVII-037	1131
FVII-044	1300
FVII-045	906

FVIIaFc variant	IU/nM
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

[0366] In this example, the following reagents and methods were used:

Reagents

[0367]

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

[0368]

- Count platelets
- Add 20 ul of ~2-4x10⁸ 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 CaCl₂/1.5% 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

[0369] In this example, the following reagents and methods were used:

Reagents

[0370]

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

[0371]

- Set up software according to manufacturer's recommendations
- Prewarm water and Fluca buffer
- 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
- 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
- Make calibrator solution (1 ml of warm water in calibrator vial)
- Add 20 ul of buffer or calibrator to the wells
- Add 25 ul of clotting factor mix to the wells (or 25 ul of buffer to calibrator wells)
- Add 20 ul of FVIIaFc to the wells (or buffer to calibrator wells)
- Add 35 ul of platelets (previously add ADP and SFLLRN). Add platelets to calibrator well
- 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

[0372] 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).

[0373] 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

[0374] 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 μ M 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

[0375] 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.

[0376] 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

[0377] The constructs depicted in Figure 33 were (FVII-090, FVII-089 and FVII-062) were cloned, expressed and purified 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.

[0378] Thrombin generation assays were used to measure activity of activatable variant FVII-090. A thrombin generation assay 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 FVII-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

[0379] 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.

[0380] 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

[0381] 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.

[0382] 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.

[0383] 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.

[0384] 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

[0385] 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).

[0386] 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).

[0387] 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.

[0388] 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 μ M 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.

[0389] 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, FVIIIFc (100 nM) was activated with thrombin (100 nM) for 20 minutes at 37C. Hirudin was added to inhibit the thrombin. FX (10 μ M) 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

[0390] 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.

[0391] 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 FVIIIFc band with increasing thrombin concentration is similar for the FVII-118, FVII-119, and FVII-090 constructs.

[0392] 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

[0393] In this example the constructs illustrated in Figure 44A were cloned, transiently expressed, purified and activated as previously described. FVII-066 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 constructs employed the targeting moiety SCE5, a scFv against the active conformation of GPIIb/IIIa. 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 affinity from platelets. Since the SCE5 targeting moiety has been shown to interact with the mouse GPIIb/IIIa receptor, FVII-066 was tested in thrombin generation assays using mouse FVIII-deficient platelet rich plasma, 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 GPIIb/IIIa

[0394] 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 GPIIb/IIIa. 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 described (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 GPIIb/IIIa, demonstrating the effects are mediated by the interaction of MB9 with the activated form of GPIIb/IIIa. 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. Thrombin generation assays in a FVIII-deficient reconstituted system with platelets revealed 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

[0395] 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 GPIIb/IIIa. 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 GPIIb/IIIa 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.

[0396] 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 FVIIIFc to a final concentration of 100, 30 or 10 nM. The NATEM reaction was initiated by the addition of CaCl₂. 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

[0397] 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 GPIIb-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 FVIII Fc variant targeted to the active form of GPIIb/IIIa

[0398] The constructs illustrated in Figure 49A were made as previously described. FVIII-041 is wild type FVIII Fc, 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 FVIII Fc. 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

[0399] In this example a version of FVIIaFc illustrated in Figure 21 was generated. This protein has the MB9 scFv at the N-terminus and a deletion that removes the Gla domain. FVII-053 contains an RKRRKR sequence inserted between the light and heavy chain for intracellular activation. FVII-053 was transiently expressed (cotransfected 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 GPIIb/IIIa 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

[0400] 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.

[0401] 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

[0402] In this example, the FIX-089 construct illustrated in Figure 53A was cloned, transiently expressed and purified as previously described. The molecule comprises the OS 1 peptide, which binds to GPIIb-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.

[0403] 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 GPIIb increases the activity of FIXF

DRAFT SEQUENCE LISTING

[0404]

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

<u>MV</u> <u>SOALRLLC</u>	<u>LLLGLQGCLA</u>	<u>AVFVTQKEAH</u>	<u>GVLHRRRRAN</u>	<u>AFLEELRPGS</u>	<u>LERECKEEQC</u>
SFEAREIFK	DAERTKLEWI	SYSGDQCAS	SPCQNGGSCK	DQLQSYICFC	LEAFEGENCE
THKDDQLICV	NENGGCEQYC	SDHTGTKRSC	RCEGYSLLA	DGVSCTPTVE	YPCGKIPILE
KRNASKPQGR	IVGGKVCCKG	ECPWQVLLLV	NGAQLCGGTL	INTIWVVSAA	HCFDKIKNWR
NLLAVLGEHD	LSEHDGDEQS	RRVAQVLIIS	TYVPGFTNHD	IALLRLHQPV	VLTDHVVPLC
LPERTFSERT	LAFVRFSLVS	GWGQLLDRGA	TALELMVLNV	PRLMTQDCLQ	QSRKVGDSFN
ITEYMFCAGY	SDGSKDSCCK	DSGGPHATHY	RGTWYLTGIV	SWGQGCATVG	HFGVYTRVSQ
YIEWLQKLMR	SEPRPGVLLR	APFFGGGGSG	GGGSGGGGSG	GGGSGGGGSG	GGGSDKTHTC
PPCPAPELLG	GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN
AKTKFREEQY	NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	ALPAPIEKTI	SKAKGQPREP
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SGGGSGGGG	SGGGSRKRR	KRAEVQLVQS	GAEVNKEGAS	VKVSCKASGY	TFTGYIMHWV
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RGRALYNRND	RSPNWFDPWG	QGTLVTVSSG	SASAPFLKLE	EGEFSEARVQ	AVLTQFPPSVS
VAPGQFARLT	CGGNIGSKS	VQWYQQKPGQ	APVLLVYDDS	DRPSGIPERF	SGSNSGNMAF
LTISRVEAGD	EADYYCQVWD	SSSDHVVFGG	GTKLTVLGQP	KAAPSVTLFP	PSAAAGGGGS
GGGGSGGGGS	GGGGSGGGGS	GGGGSDKTHF	CPPCPAPELL	GGPSVFLFPP	KPKDTLMISR
TPEVTCVVVD	VSHEDPEVKF	NWYVDGVEVH	NAKTKFREEQ	YNSTYRVVSV	LTVLHQDWLN
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DIAVEWESNG	QPENNYKTFP	FVLDSDGSEF	LYSKLTVDKS	RWQQGNVFSC	SVMHEALHNH
YTQKSLSLSP	GK				

DNA sequence of FVII-027

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Genscript-FVII-027-1 DNA sequence

gaagagcctc tccctgtctc cgggtaaaac ggcgcgcgg agcgggtggc gccgatcagg
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atccgcccc acccttaagg ttgaagaagg tgaattc

Genscript--FVII-026-2 DNA sequence

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cagcagggga acgttttttc atgtctcgtg atgcatgagg ctctgcacaa ccactacacg
cagaagagcc tctcctgtc tccgggtaaa tgagaattc

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

MYSQALRLLC LLLGLOGCLA AVFVTQEEAH GVLHRRRRAN APTEELRPGS LERECKEEQC
SFEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
HKDDQLICV NENGGCEQYC SDHTGTRKSC RCHEGYSLA DGVSCPTPTVE YPCGKIFILE
KRNASKPQGR IVGGKVCPRG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLI AVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNID IALLRLHQPV VLTDHVVPLC
LPERTFSERT IAFVRFSLVS GWGQLLDGSA TALELMVLNV PRIMTQDCLQ QSRKVGDSFN
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YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDFEVKFN WYVDGVEVIN
AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTTP VLDSDGSFFL
YSKLTVDKSR WQQGNVFSQS VMHEALHNHY TQKSLSLSPG **KGSGSGSGSG** **SGSGSGSGSG**
SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
DGVEVINAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSRDELK KNQVSLTCLV KGFYPSDIIV EWESNGQPEN NYKTTTPVLD
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGKGG GGSGGGSGSG
GGSGGGSGSG GGSGGGSGAE VQLVQSGAEV NKPGASVKVS CKASGYTFTG YIMHWVRQAP
GQGLEWMGWI NPNSGCTNYA QKFGQWVTMT RDTISITAYM ELSRLSDDT AVYYCARGA
LYNRNDRSPN WFDPWGQGTI TVVSSGSASA PTLKLEGEF SEARVQAVLT QPFSVSVAPG
QTARITCGGN NIGSKSVQWY QOKPGDAPVL VVYDDSDRPS GIPERFSGSN SGNMATLTIS
RVEAGDEADY YCQVWDSSSD HVVFGGGTKL TVLGQPKAAP SVTLFPPPSAA A

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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MYSQALRLIC LLLGLQGCLA AEVQLVQSGA EVNKPASVK VSCKASGYTF TGYMHWVRQ
APGQGLEWMG WINFNSGGTN YAQKIQGWVT MTRDTSISTA YMELSRLRSD DTAVYYCARG
RALYNRNDRS PNWDPWGQG TLVTVSSGSA SAPTLKLEEG EFSEARVQAV LTQPPSVSVA
PGQTARITCG GNNIGSKSVQ WYQQKPGQAP VLVVYDDSDR PSGIPERFSG SNSGNMATLT
ISRVEAGDEA DYYCQVWDSS SDHVVEGGGT KLTVLGQPKA APSVTLEPPS AAARTKLEWI
SYSDGDOCAS SPCQNGGSCK DQLQSYLCFC LPAFEGRNCE THKDDQLICV NENGGCEQYC
SDHTGPKRSC RCHEGYSLLA DGVSCPTPTVE YPCGKIPILE KRNASKPQGR RKRRKRIVGG
KVCPKGECFW QVLLLVNGAQ LCGGTLLINTI WVSAAHCFD KIKNWRNLIA VLGEHDLSEH
DGDEQSRRVA QVIIPSTYVP GTTNHDIALL RLHQPVVLT D HVVPLCLPER TFSERTLAFV
RFSLVSGWGQ LLDRGATALE LMVLNVPRLM TQDCLOQSRK VGDSEFNITEY MFCAGYSDGS
KDSCKGDSGG PHATHYRGTW YLTGIVSWGQ GCATVGHFGV YTRVSQYIEW LQKLMRSEPR
FGVLLRAPFP GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKHTCPCPCP APELLGGPSV
FLFPPKPKDT LMISRPPEVT CVVVDVSHED PEVKFNWYVD GVEVIINAKTK FREEQYNSTY
RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTSKAK GQPREPQVYT LPISRDELTK
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NVFSCSVMIH ALHNHYTQKS LSLSPGKGGG GSGGGSGGGG GSGGGGSDKT ITCPCPCAPE
LGGPSVFLF PPKPKDTIMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE
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SRDELTRNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TFPVLDSDGS FFLYSKLTVD
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FVII-053 DNA sequence

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aaccactaca cgcagaagag cctctccctg tctcgggta 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

MVSOALRLLC ILLGLQGCLA AVFVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
SFEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LEAFEGRNCE
THKDDQLICV NENGCEQYC SDRTGTRKSC RCHGYSLLA DGVSCPTVE YPCGKIPLE
KRNASKPQGR IVGGRVCFKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDRHVPLC
LPERTFSERT LAFVRFSLVS GWGQLLDLGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
YIEWLQKLMR SEPRPGVLLR APFPGGGSG GGGSGGGSG GGGSGGGSG GGGSDKTHTC
PPCPARELLG GPSVLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREMQY NSTYRVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIENTI SKAKGQPREP
QVYTLPPSRD ELTKNQVSLF CLVKGFYPSD IAVEWESNGQ PENNYKTPFP VLDSGGSFEL
YSKLTVDKSR WQQGNVFSQS VMIEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGGSGGGG
SDKTHFCPPC PARELLGGPS VFLFPPKPKD TLMISRTPEV TCVVDVSHE DPEVKFNWYV
DGVEVHNAK KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTPFPVLD
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GGSGGGGSAC TERWALHNLC GG

FVII-044 DNA sequence

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aagaaccagg tcagcctgac ctgcctgggt aaaggcttct atccagoga catgcctgtg
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ggcgggtga

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

MYSOALRLIC LIIGLOGCLA AVFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
SFEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
THKDDQLICV NENGGEQYC SDHTGTRKSC RCEGYSLLA DGVSCPTVE YPCGKIPLE
KRNASKPQGR IVGGKVCPRG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDRHVPLC
LPERTFISRT LAFVRFSLVS GWGQLLDLGA TALELMVLNV PRMTQDCLQ QSRKVGDSFN
ITEYMFACGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
YIEWLQKLMR SEPRPGVILR APFFGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
PFCPAPELLG GPSVFLFPPK FKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY NSTYRVSVL TVLHQDWLNG KEYCKVSNK ALFAPIEKTI SKAKGQPREP
QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD LAVWEISNGQ PENNYKTTTP VLDSDGSFTL
YSKLTVDKSR WQQGNVFCSS VMHEALHNYH TQKSLSLSPG KGGGGSGGGG SGGGGSGGGG
SDKTHFCRPS PAVLLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHD DPEVKFNWYV
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY CKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSRDEL TKNQVSLTCLV KGFYPSDIIV EWESNGQPEN NYKTTTPVLD
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GGSGGGGSAC TERMALHNLC GG

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

MVSOALRLLC ILLGLOGCLA AVEVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
SFEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICEF LPAFEGRNCE
THKDDQLICV NENGGEQYC SDHTGTRKSC RHEGYSLA DGVSCPTPVE YPCGKIPILE
KRNASKPQGR IVGCKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLI AVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPSTTNHD IALLRLHQPV VLTDHVVPLC
LPERTFSERT IAFVRFSLVS GWQQLIDRGA TALELMVLNV PRIMTQDCLQ QSRKVCDSFN
LPEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGLV SWQGGCATVG HFGVYTRVSQ
YLEWLQKLMR SEPRPGVLLR APFPGGGGSG GGSGGGGSG GGSGGGGSG GGSGDKTHTC
PPCPAPELLG GPSVFLFPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY NSTYRVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPPP VLDSDSGFFL
YSKLTVDKSR WQQGNVFSQS VMREALHNHY FQKSLSLSPG **KGGGSGGGG** **SGGGSGGGG**
SDKTHFCPPC PAPELLGGPS VFLEPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
DGVEVHNAKT' KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSRDEL' KNQVSLTCLV KGFYPSDIIV EWESNGQPEN NYKTPPPVLD
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GGSGGGGSAC TERDALHNLC GG

FVII-046 DNA sequence

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ggcgggtga

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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 AVFVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEOC
SFEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
THKDDQLICV NENGCEQYC SDHTGTRKSC RCHEGYSLA DGVSCPTVE YFCGKIFILE
KRNASKPQGR IVGGKVCPKG ECPWQVLLLV NGAQLCGGTL INTIWWVSAA HCFDKIKNWR
NLIAVLGEHD LSEHDGDEQS RRVAQVILPS TYVPGTTNHD IALLRLHQPV VETDHRVPLC
LPERTFSERT LAFVRFSLVS GWQQLIDRGA TALELMVLNV PRMTQDCLQ QSRKVGDSFN
LTYMFACAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
YLEWLQKLMR SEPRKPGVLLR APFPGGGGSG GGGSGGGGSG GCGSDKTHTC
PCPAPELLG GPSVFLFPPK PKDTLMISRT EVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY NSTYRVSVL TVLHQDWLNG KEYKCRVSNK ALPAPIEKTI SKAKGQPREP

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QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP VLDSGGSFFL
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SGGGSGGGG SACTERWALH NLGGGGGSGG GSGGGGSGG GSGGGGSGG GGSCKTHTCP
PCPAPELLG PSVFLFPPK KDTLMISRT EVTCVVVDV SHEDPEVKFN WYVDGVEVHN
KTKPREEQYN STYRVSVLT VLHQDWLNGK EYKCRVSNK LPAPIEKTIS KAKGQPREPQ
VYTLPPSRDE LTKNQVSLT CLVKGFYPSD IAVEWESNGQ ENNYKTTPPV LDSGGSFFLY
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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 OS 1 to Fc regions is underlined, and OS1 peptide is italicized

MYSQALRLRL LLLGLOGCLA AVEVTVQEEAH GVLHRRRRAN APLEELRPGS LERECKEEQC
SFEAREIEFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
THKDDQLICV NENGCEQYC SDHTGTRKSC RCHEGYSLA DGVSCPTVE YPCGKIPILE
KRNASKPQGR IVGGKVCFKG ECPWQVLLLV NGAQLCGGTL INTIWWVSAA HCFDKIKNWR
NLI AVLGEHD LSEHDGDEQS RRVAVQVIIPS TYVPSTTNHD IALLRLHQPV VLT DNVVPLC
LPERTFSERT IAFVRFSLVS CWGQLLD RGA TALELMVLNV PRIMTQDCLQ QSRKVGDSFN
LTEMFCAGY SDGSKDSCKG DSGGPRATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
PCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
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YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KGGGSGGGGG SGGGSGGGGG
SGGGSGGGGG SACTERMALH NLCGGGGSGG GGSGGGSGG GGSGGGSGG GGSDKTHTCP
PCPAPELLGG ESVFLFPPKP KDTLMISRTPE EVTCTVVVDVS HEDPEVKFNW YVLDGVEVINA
KTKPREEQYN STYRVVSVLT VLNHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
VYFLPPSRDE LTKNQVSLTCLVKGFYPSDI AVEWESNGQP ENNYKTPPVV LDS DGSFFLY
SKLTVDKSRW QQGNVFSCSV MHEALEHNHYT QKSLSLSPGK

DNA sequence of FVII-048

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tga

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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

<u>MYSQALRLILC</u>	<u>ILLGLQGCLA</u>	<u>AVEVTQEEAH</u>	<u>GVLHRRRRAN</u>	APLEELRPGS	LERECKEEQC
SFEARELEFK	DAERTKLEWI	SYSDGDQCAS	SPCQNGGSCK	DQLQSYICFC	LPATFGRNCE
THKDDQLICV	NENGGCEQYC	SDHTGTKRSC	RCHEGYSLLA	DGVSCPTPVE	YPCGKIPILB
KRNASKPQGR	IVGGRVCPKG	ECPWQVLLLV	NGAQLCGGTL	INTIWVVSAA	HCFDKIKNWR
NLIAVLGEHD	LSEHDGDEQS	RRVAQVLIIPS	TYVPSTTNHD	IALLRLHQPV	VLTDHVVPIC
LPERTFSERT	LAFVRFSLVS	GWGQLLDRCG	TALELMVLNV	PRLMTQDCLQ	QSRKVQDSPN
ITEYMFCAGY	SDGSKDSCKG	DSGGPHATHY	RGTWYLTGIV	SWGQGCATVG	HFGVYTRVSQ
YIEWLQKLMR	SEPRPGVLLR	APFPGGGSGG	<u>GGGSGGGSGG</u>	<u>GGGSGGGSGG</u>	<u>GGGSDKTHTC</u>
PPCPAPELLG	GFSVFLFPPK	PKDTLMISRT	PEVTCVVVDV	SHEDPEVKFN	WYVDGVEVHN
AKTKFREEQY	NSTYRVVSVL	TVLHQDWLNG	KEYKCKVSNK	ALPAPIEKT	SKAKGQPREP
QVYTLPPSRD	ELTKNQVSLT	CLVKGFYPSD	IAVEWESNGQ	PENNYKTTPP	VLDSDGSFFL
YSKLTVDKSR	WQGNVFSCSV	VMHEALHNHY	TQKSLSLSPG	<u>KGSGSGSGSG</u>	<u>SGSGSGSGSG</u>
<u>SGSGSGSGSG</u>	<u>SACTERDALH</u>	<u>NLCGGGSGSG</u>	<u>GGSGGGSGSG</u>	<u>GGSGGGSGSG</u>	<u>GGSDKTHTCP</u>
PCPAPELLGG	FSVFLFPPKP	KDTLMISRTF	EVTCVVVDVS	HEDPEVKFNW	YVDGVEVHNA
RTKPREEQYN	STYRVVSVLT	VLHQDWLNGK	EYKCKVSNKA	LPAPIEKTIS	KAKGQPREPQ
VYTLPPSRDE	ITKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY
SKLTVDKSRW	QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK		

DNA sequence of FVII-049

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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

MYSOALRLIC ILLGLOGCIA AVFVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
SFEARELIFK DAERTKLEWL SYSDGDQCAS SPCQNGGSCK DQLQSYLCFC LPATFGRNCE
THKDDQLICV NENGCEQYC SDHTGTRSC RCEGYSLA DGVSCTPTVE YPCGKIPILE
KRNASKPQGR IVGGKVC PKG ECPWQVLLLV NGAQLCGGTL INTIWVVSAA HCFDKIKNWR
NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDFHVVPIC
LPERTFSERT LAFVRESLVS GWGQLLD RGA TALELMVLNV PRMTQDCLQ QSRKVGDSFN
ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
YIEWLQKLMP SEPRPGVILR APFFGGGGSG GGGSGGGSGG GGGSGGGSGG GGGSDKTHTC
PPCPAPELLG GPSVFLPPPK PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY NSTYRVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP
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SDKTHTCPPC PAPELLGGPS VFLPPPKPKD FLMISRTPEV TCVVVDVSHE DPEVKFNWYV
DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
KGQPREPQVY TLPPSRDEL T KNQVSLTCLV KGFYPSDI AV EWESNGQPEN NYKTPPVLD
SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK

FVII-011 DNA sequence

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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	<u>MQIELSTCEFF</u>	<u>LCLLRFCFSA</u>	TRRYYLGAWE	LSWDYMQSDL	GELPVDARFP
51	PRVPKSEFPN	TSVVYKKTLE	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY
101	DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFPG
151	GSHTYVWQVL	KENGPMASDP	<u>LCLTYSYLSH</u>	VDLVKDLNSG	LIGALLVCRE
201	GSLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM
251	HTVNGYVNR	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH
301	RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSQPE
351	EPQLRMKNNE	EAEDYDDDLT	DSEMDVVRFD	DDNSPSFIQT	RSVAKKHPKT
401	WVHYIAAHEE	WDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
451	TDETFKTREA	IQHESGILGP	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
501	DVRFLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
551	YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
601	NRSWYLTENI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDQLQLSVCL
651	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFMS
701	MENPGLWILG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYYED	SYEDISAYLL
751	SKNNAIEPRS	<u>ESQNPVLR</u>	<u>HQREITRTTL</u>	QSDQEEIDYD	DTISVEMKKE
801	DFDIYDEDEN	QSPRSFQKKT	RHYFLAAVER	LWDYGMSSSP	HVLRNRASQSG
851	SVFQFKKVVF	QBFTDGSFTQ	PLYRGELNEH	LCLLGPYIRA	EVEDNIMVTF
901	RNQASRPYSF	YSSLISYEED	QRQGAEPKRN	FVKPNETKTY	FWKVQHMAP
951	TKDEFDCKAW	AYFSDVDLEK	DVHSGLIGPL	LVCHTNLNP	AHGRQVTVQE
1001	FALFFTIFDE	TKSWYFTENM	ERNCRAPCNI	QMEDPTFKEN	YRPHAINGYI
1051	MDTLPLGLVMA	QDQIRWYLL	SMGSNENIHS	IHFSGHVPTV	RKKEEYKMAL
1101	YNLYPGVFET	VEMLPSKAGI	WRVECLIGEH	LHAGMSTLEL	VYSNKCQTPL
1151	GMASGHIRDF	QITASGQYCG	WAPKLARLHY	SGSINAWSTK	EPFSWIKVDL
1201	LAPMIHGIK	TQGARQKFSS	LYISQFILMY	SLDGKKWQTY	RGNSTGTLMV
1251	FFGNVDSSGI	KHNIFNPPII	ARYIRLHPTH	YSIRSTLRME	LMGCDLNSCS
1301	MPLGMESKAI	SDAQITASSY	FTNMPATWSP	SKARLHLQGR	SNAWRPQVNN
1351	PKEWLQVDFQ	KIMKVTGVTI	QGVKSLLTSM	YVKEFLISSS	QDGHQWTLFF
1401	QNGKVKVFQG	NQDSFTPVVN	SLDPELLTRY	LRIHPQSWVR	QIALRMEVLG
1451	CEAQDLY				

Full length FVIII amino acid sequence: Signal peptide underlined

1	<u>MQIELSTCEFF</u>	<u>LCLLRFCFSA</u>	TRRYYLGAWE	LSWDYMQSDL	GELPVDARFP
51	PRVPKSEFPN	TSVVYKKTLE	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY
101	DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFPG
151	GSHTYVWQVL	KENGPMASDP	<u>LCLTYSYLSH</u>	VDLVKDLNSG	LIGALLVCRE
201	GSLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM
251	HTVNGYVNR	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH
301	RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSQPE

301	NGASLEBISFI	ITETAGILEM	DLGQFHEFCH	ISSAQHAGME	ALVRYDSCEB
351	EPQLRMKNNE	EAEDYDDDLT	DSEMDVVREF	DDNSPSFIQI	RSVAKKHPKT
401	WVHYIAAEEE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
451	TDETFKTREA	IQHESGILGF	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
501	DVRPLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
551	YVSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVEDE
601	NRSWYL TENI	QRFLNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
651	HEVAYWYLLS	ICAQTFILSV	FFSGYTFKHK	MVYEDTITLF	PFSGETVFMS
701	MENPGLWILG	CHNSDFRNRC	MTALLKVSSC	DKNTGDYYED	SYEDISAYLL
751	SKNNAIEPRS	FSQNSRHPST	RQKQFNATTI	PENDIEKTDI	WFAHRTMPMK
801	IQNVSSSDLL	MLLRQSPTPH	GLSLSDLQEA	KYETFSDDPS	PGAIDSNNSL
851	SEMTHFRPQL	HHSGDMVFTF	ESGLQLRLNE	KLGTTAATEL	KKLDFKVSST
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951	SGGFLSLSEE	NNDSKLLESG	LMNSQESSWG	KNVSSTESGR	LFKGKRAHGP
1001	ALLTKDNALF	KVSISLLKTN	KTENN SATNR	KTHIDGPSLL	IENSPSVWQN
1051	ILESDTIEFKK	VTPLIHDRML	MDKNATALRL	NHMSNKTTSS	KNMEMVQQKK
1101	EGPIPPDAQN	PDMSEFKMLF	LPESARWIQR	THGKNSLNSG	QGPSFKQLVS
1151	LGPEKSVVEGQ	NPLSEKNKVV	VKGGEFTKDV	GLKEMVFPSS	RNLPLTNLDN
1201	LHENNTHNQE	KKIQEETIEKK	ETLIQENVVL	PQIHTVTGTR	NFMKNLFLLS
1251	TRQNVESYD	GAYAPVLQDF	RSLNDSTNRT	KKHTAHFSKK	GEEENLEGLG
1301	NQTKQIVEKY	ACTTRISPNT	SQQNFVTQRS	KRALKQFRLP	LEETELEKRI
1351	IVDDTSTQWS	KNMKHLTPST	LTQIDYNEKE	KCAITQSPLS	DCLTRSHSIP
1401	QANRSPLPIA	KVSSFP SIRP	IYLFVFLFQD	NSSHLPAASY	RKKDSGVQES
1451	SHFLQGAKKN	NLSLAILTLE	MTGDQREVGS	LGTSATNSVT	YKKVENTVLP
1501	KEDLPKTS GK	VELLPKVHIY	QKDLFFTETS	NGSPGHLDLV	EGSLLQGTEG
1551	AIKWNEANRP	GKVPFLRVAT	ESSAKTPSKL	LDPLAWDNHY	GTQIPKEEWK
1601	SQEKSP E KTA	FKK KDTILSL	NACESNHATA	AINEGQNKPE	IEVTWAKQGR
1651	TERLCSQNPP	VLKRHQREIT	RTTLQSDQEE	IDYDDTISVE	MKKEDFDIYD
1701	EDENQSPRSF	QKKTRHYFIA	AVERLWDYGM	ESSPHVLRNR	AQSGSVPQFK
1751	KVVFQEFTDG	SFTQPLYRGE	LNEHLGLLGP	YIRAEVEDNI	MVTFRNQASR
1801	PYSFYSSLIS	YEEDQRQGAE	PRKNFVKPNE	TKTYFWKVQH	HMAPTKDEFD
1851	CKAWAYFSDV	DLEKDVHSGL	IGPLLVCHTN	TLNPAHGRQV	TVQEEALFFT
1901	IFDETKSWYF	TENMERN CRA	PONIOMEDPT	EKENYRFHAI	NGYIMDTLPG
1951	LVMAQDQRIE	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	VDFQKIMKVT	CVTTQGVKSL	LTSMYVKEFL	ISSSQDGHQW	TLFPQNGKVK
2301	VEQGNQDSFT	PVVNSLDPPL	LTRYLRHPQ	SWVHQIALRM	EVLGCEAQDL
2351	Y				

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

MORVNMIMAE SPGLITICLL GYLLSAECTV FLDHENANKI LNRPKRYNSG KLEEFVQGNL
ERECEEEKCS FEEAREVFEN TERTTEFWKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP
FGFEGKNCEL DVTCKIKNGR CEQFCCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPFPFCGR
VSVSQTSKLT RAETVFPDVD YVNSTEAEI LDNITQSTQS FNDFTRVVGG EDAKPGQFPW

QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETEHT EQKRN VIRII
PHHYNAAIN KYNHDIALLE LDEPLVLNSY VTPICIADKE YTNIFLKFGS GYVSGWGRVF
HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
GTSFLTGIIS WGEECAMKGK YGIYTKVSRY VNWIEKTKL T

FIX DNA sequence

atgcagcgcg tgaacatgat catggcagaa tcaccagggc tcatcaccat ctgcctttta
ggatatctac toagtgetga atgtacagtt tttcttgatc atgaaaaagc acaaaatt
ctgaatcggc caaagaggta taattcaggt aaattggaag agtttggtca agggaatcta
gagagagaat gtatggaaga aaagtgtagt ttggaagaag cagcagaagt tttgaaaaac
actgaaagaa caactgaatt ttggaagcag tatgttgatg gagatcagtg tgagtccaat
ccatgtttaa atggcggcag ttgcaaggat gacattaatt cctatgaatg ttggtgtccc
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tgcgagcagt tttgtaaaaa tagtgctgat aacaagggtg tttgctcctg tactgaggga
tatcgacttg cagaaaacca gaagtccgtg gaaccagcag tgcatttcc atgtgggaga
gtttctgttt cacaacttcc taagctcacc cgtgctgaga ctgtttttcc tgatgtggac
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caggttgttt tgaatggtaa agttgatgca ttctgtggag gctctatcgt taatgaaaaa
tggattgtaa ctgctgcccc ctgtgttgaa actggtgtta aaattacagt tgtcgcaggt
gaacataata ttgaggagac agaacataca gagcaaaaagc gaaatgtgat tgaattatt
cctcaccaca actacaatgc agctattaat aagtacaacc atgacattgc ccttctggaa
ctggacgaac ccttaqtgct aaacagctac gtacaccta tttgcattgc tgacaaggaa
tacacgaada tcttctcaca atttggatct ggctatgtta gtggctgggg aagagtcctc
cacaaggga gatcagcttt agttcttcag taccttagag ttccacttgc tgaccagacc
acatgtcttc gatctacaaa gttcaccatc tataacaaca tgttctgtgc tggcttccat
gaaggaggta gagattcatg tcaaggagat agtgggggac ccatgttac tgaagtggaa
gggaccagtt tcttaactgg aattatttagc tgggggtgaag agtgtgcaat gaaaggcaaa
tatggaatat ataccaaggt atcccggtat gtcaactgga ttaaggaaaa aacaaggctc
acttga

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

MGRPLHLVLL SASLAGLLLL GESLFIRREQ ANNILARVTR ANSFLEEMKK GHLERECMEE
TCSYEEAREV FEDSDKTNEF WNKYKDGQOC ETSPCQNGGK CKDGLGEYTC TCLEGFEGKN
CELI¹TRKLCS LDNGDCDQFC HEEQNSVVCS CARGYTLADN GKACI¹PTGPFY PCGKQTLERR
KRSVAQATSS SGEAPDSL¹TW KFYDAADLDF TENPFDELDF NQTQPERGDN NLTRIVGGQE
CKDGECPWQA LLINEENESF CGGTILSEFY ILTAAHCLYQ AKRFKVRVGD RNTEQEEGGE
AVHEVEVVIK HNRFTKET¹YD FDI¹AVLRLKT PITFRMNVAP ACLPERDWAE STLMTQKTGI
VSGFGRTHEK GRQSTRLKML EVPYVDRNSC KLSSSFII¹PQ NMFCAGYDTK QEDACQGD¹SG
GPHVTRFKDT YFVTGIVSWG EGCARKGRYG IYTKVTAFLK WIDRSMKTRG LPKAKSHAPE
VITSSPLK

FX DNA sequence

atggggcgcc caactgaact cgtcctgctc agtgccctcc tggctgqccn cctgctgctc
ggggaaagtc tgttcatccg cagggagcag gccaaacaac tcctggcgag ggtcacgagg
gccaatctct ttcttgaaga gatgaagaaa ggacaccccg aaagagagtg catggaagag
acctgctcat acgaagaggc ccgcgaggtc tttagaggac gcgacaagac gaatgaattc
tggaaataaat acaaaqatgg cgaccagtgt gagaccagtc cttgccagaa ccaggggcaa
tgtaaagacg gctcggggga atacacctgc acctgtttag aaggattcga aggcaaaaac
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cacgaggaac agaactctgt ggtgtgctcc tgcgcccgcg ggtacacctn ggctgacaac
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aagaggtcag tggcccaggc caccagcagc agcggggagg ccctgacag catcacatgg
aagccatatg atgcagccga cctggacccc accgagaacc ccttcgacct gcttgacttc
aacagagcgc agcctgagag gggcgacaac aaactcaaca ggatcgtggg aggcacaggaa
tgcaaggacg gggagtgctc ctggcaggcc ctgctcatca atgaggaaaa cgagggtctc
tgtggtggaa ccattctgag cgagttctac acctaacgg cagcccactg tctctaccaa

gccaaagagat tcaaaggtgag ggtagggggac cggaaacacg agcaggaqga gggcggtgag
ggggtgcacg aggtggaggt ggtcatcaag cacaaccggt tcacaaagga gacctatgac
ttcgacatcg ccgtgctccg gctcaagacc cccatcaact tccgcatgaa cgtggcgccct
gcttgctcc ccgagcgtga ctgggcggag tcacacgtga tgacgcagaa gacggggatt
gtgagcggct tgggggcac ccacgagaag ggccggcagt ccaccaggct caagatgctg
gaggtgcctt acgtggaccg caacagctgc aagctgtcca gcagcttcac catcacccag
aacatgttct gtgcggcta cgacaccaag caggaggatg cctgccaggg ggacagcggg
ggcccgcacg tcaccgcctt caaggacacc tacttcgtga caggcatcgt cagctgggga
gagggctgtg ccctgaaggg gaagtacggg atctacacca aggtcaaccg cttcctcaag
tggatcgaca ggtccatgaa aaccaggggc tggcccaagg ccaagagcca tggcccggag
gtcataacgt cctctccatt aaagtga

DNA sequence of FVII--066

1 ATGGTCTCCC AGGCCCTCAG GTCCTCTGC CTTCTGCTTG GGCTTCAGGG
CTGCC¹GGCT¹
61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GGCCTCCTGC ACCGGCGCCG GCGCCCAAC
121 GCCTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TC¹TACAGTG ATGEGGACCA GTGTGCTTCA AGTCCA¹GCC AGAA¹TGGGG CTTC¹TGCAAG
301 GACCAGCTCC AGTCTATAT CTGCTCTGC CTCCCTGCC CTGAGGGCCG GAAC¹TGTGAC
361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACC GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCAACCA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCA¹TGTG GAAAAATACC TAT¹CTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGG GCAAGGTGTG CCCC¹AAAGGG
601 GAGTGTCCAT GGCAGGTCTT GTTGTGGTG AATGGAGCTC AGTTGTGTGG GGGG¹ACCC¹TG
661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAA¹AATCAA GAAC¹TGGAGG
721 AACCTGATCG CGGTGCTGGG CAGGCACGAC CTCAGCAGC ACGACGGGA TGAGCAGAGC
781 CCGCGGGTGG CCGAGGTGAT CATCCCGAGC ACGTACGTCC CCGGCACCC CAAC¹CACGAC
841 ATCGCGCTGC TCCGCTGCA CCAGCCCGTG GTCTCACTG ACCATGTGGT GCCC¹CTCTGC
901 CTGCCCGAAC GGACGTTCTC TGAGAGGACG CTGCCCTTCG TCGCTTCTC ATTG¹CTCAGC
961 GGCTGGGGCC AGCTGCTGGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CTT¹CAACGTG
1021 CCCC¹GGCTGA TGACCCAGGA CTGCCCTGAG CAGTCAAGGA AGGTGGGAGA CTCCC¹AAAT
1081 ATCAGGGAGT ACATGTTCTG TCCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAG¹GGAG GCCACAT¹GC CACCCAT¹AC CCGGGCAAG¹ GGTA¹CC¹TGAC GGGCA¹TGG¹C
1201 AGCTGGGGCC AGGCTCGGC AACCCTGGGG CACTTGGGG TGTACACCAG GGTCTCCAG
1261 TACATCGAGT GGCTGCAAAA GCTCATGGGC TCAGAGCCAC GCCAGGAGT CCT¹CTGCGA
1321 GCCCCATTTT CCGGTGGCGG TGGCTCCGGC GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
1381 GGGGG¹GGAT¹ CAGGCGGTGG AGG¹TTCGG¹ GGGGGGGGA¹ CCGACAAAAC TCACACA¹TGC
1441 CCACCGTGCC CAGCTCCGGA ACTCCGGGGA GGACCGTCAG TCTTCTCTT CCCCC¹AAAA
1501 CCCAAGGACA CCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG
1561 AGCCACGAAG ACCCTGAGGT CAAGTECAAC TGGTACGTGG ACGGCGTGGG GGTGCATAAT
1621 GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACG¹ ACCGTGTGG¹ CAGCGTCTTC
1681 ACCGTCTCTC ACCAGGACTG GCTGAATGGC AAGCAGTACA AGTGCAAGGT CTCCAACAAA
1741 GCCCTCCAG CCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAA¹CA
1801 CAGGTGTACA CCTGCCCC ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGGAC
1861 TGCCTGCTCA AAGGCTTCTA TCCAGCGAC ATGCCCTGG AGTGGGAGAG CAAT¹GGGACG
1921 CCGGAGAACA ACTACAAGAC CACGCTCC CTGTGGACT CCGACGGCTC CTTCTTCTC
1981 TACAGCAAGC TCACCGTCA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
2041 GTGATGCATG AGGCTCTGCA CAACACTAC ACGCAGAAGA GCTCTCCCT GTCT¹CGGGT

2101 AAACGGGCGCC GCCGGAGCGG TGGGCGCGGA TCAGGTGGGG GTGGATCAGG CGGTGGAGGT
2161 TCCGGTGGCG GGGGATCCGG CGGTGGAGGT TCCGGTGGGG GTGGATCAAG GAAAGGGAGE
2221 AAGAGGCGCG AGGTGCAGCT GCAGGAGCTT CCGGAGGCTT TGGTACAGCC TGGGGGCTCC
2281 CTGAGACTCT CCTCTGCAGC CTCTGGATTC ATGTTTAGCA GGTATGCCAT GAGCTGGGTC
2341 CGCCAGGCTC CAGGGAAGGG GCCAGAGTGG GTCTCAGGTA TTAGTGGTAG TGGTGGTAGT
2401 ACAACACACG CAGACTCCGT GAAGGGCCGG TCCACCGTCG CCAGAGACAA TCCCAAGAAC
2461 ACCCTGTATC TGCAAATGAA CAGCCTGAGA CCGCAGGACA CGGCTGTATA TTAAGCGGCC
2521 CGGGGCGCCA CCTACACCCG CCGGAGCGAC GTGCCCCACC AGACCAGCTT CGACTACTGG
2581 GGCCAGGGAA CCTGGGTCAC CGTCTCCTCA GGGAGTGCAT CCGCCCCAAA GCTTGAAGAA
2641 GGCGAATTTT CAGAAGCAGG CGTATCTGAA CTGACTCAGG ACCCTGCCTG GTCTGTGGCC
2701 TTGGGACAGA CAGTCAGGAT CACATGCCAA CGAGACAGCC TCAGAAACTT TTAGGCAAGC
2761 TGGTACCAGC AGAAGCCAGG ACAGGCCCTT ACTCTTGTCA TCTATGGTTT AAGTAAAAGG
2821 CCTCAGGGA TCCAGAGCCG ATTCTCTGCC TCCAGCTCAG GAAACACAGC TTCTTGACC
2881 ATCACGGGG CTTCAGGCGA AGATGAGGCT GACTATTTAC GCTTGGCTGA CTACGGGCGC
2941 GGCCAGCAGG CGGTGTTCGG CCGCGGCACC AAGCTGACCG TCCTACGCTA GCGCAAGGCT
3001 GCGGCTCGG TCACTCTGTT CCGGCGCTCT TCTCGGCGG GTGGGGGTGG CTCCGGCGGA
3061 GGGGGTCCG GTGGGGCGG ATCAGGTGGG GGTGGATCAG GCGGTGGAGG TTCCGGTGGC

3121 GGGGGAACAG AAAAAACCA CACAAGCCCA CCGGCGCAG CACCGGAACG CCGGGGCGA
3181 CCGTCAGTCT TCCTCTTCCC CCAAAAACCC AAGGACACCC TCATGATCTC CCGGAACCCCT
3241 GAGGTACAGT GCGTGGTGGT GGACGTGAGC CAGCAAGACC CTGAGGTCAA GTTCAACTGG
3301 TACGTGGAGC GCGTGGAGGT GCATAATGCC AAGACAAAGC CCGGGGAGGA GCAGTACAAAC
3361 AGCAGCTACC GTGTGGTCAG CGTCTCACC GTCTGCACC AGGACTGGCT GAAAGGCAAG
3421 GAGTACAGT GCAAGGTCTC CAACAAAGCC CTCCAGGCC CCATCGAGAA AACCATCTCC
3481 AAAGCCAAAG GGCAGCCCCG AGAACACAG GTGTACACCC TGCCCCATC CCGGGATGAG
3541 CTGACCAAGA ACCAGGTCAE CCTGACCTEC CTGCTCAAAG GCTTCTATCC CAGCGACATC
3601 GCGCTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAAC ACTAAGACCAC GCTCCCGGTG
3661 TTGGACTCCG ACGGCTCCTT CTCTCTCTAC AGCAAGCTCA CCGTGGACAA GAGCAGGTGG
3721 CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG ATGCATGAG CTCTGCACAA CCACTACAGC
3781 CAGAAGAGCC TCTCCCTGTC TCCGGGTAAG TGA

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

1 MVSQALRLC LLLGLQGCLA AVFVTQEEAH GVHRRRRAN AFLEELRPGS LERECKEEQC
51 SFDEAREILK DARETKLFWI SYSDGDQCAE SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NNNGGCEQYC SDHTGTKRSC RCHHYSILA DGVSCPTVH YPGCKIPLEH
181 KRNASKPQGR IVCGKVCPKG ECPWQVLLLV NGAQLCGSTL INTIWWVSAE HCFDKLENWR
241 NLIAVLGEHD LSEHDGDEQS RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTCHVVPIC
301 LPHKTFSRRT IAPVRPFLVS GWGQLIDRGA TALHIMVLNV PRIMTQDCIQ QSRKVG DSPN
361 ITEYMFCAGY SDGSKDECKG DSGGPHATHY RSTWYLTGIV SWGQGCATVG HPGVYTRVSQ
421 YLEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGGSG GGGSGGGGSG GGGSDKTHTC
481 PPGPAPELIG GPSVKLFPPK PKDILMISRT PVVTCVVVDV SHFDPPVKPN WYV DGVFVHN
541 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPLEKTI SKARGQPREP
601 QVYFLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPPP VLDSGGSFFL
661 YSKLTVDKSR WQGNVVFCS VMHEALHNHY TQKSLSLSPG **KRRRS****GGGG** **SGGGSGGGG**
721 **SGGGSGGGG** **SGGGSGRRR** **KRAQVQLQES** GGGVLQPGGS LRLSCAASEF MFSRYAMSWV
781 RQAPGKGFEW VSGISGSGGS TYYADSVKGR ETVSRDNSKN TLYLQMNSLR AEDTAVYYCA
841 RGATYTSRSD VEDQTSFDYW GCGTLVTVSS GSASAPKLEE GEFSEARVSE LTQDPAVSVA
901 LGQTVRIITCQ GDSTKNFYAS WYQQKPGQAP TLVITYGISKR PSGLPDRFSA SSSGNTASLT
961 ITGAQAEDEA DYYCLLYYGG GCGGVFGGGT KLTVLRLPKA APSVTLFPFS SAAGGGGSGG
1021 **GGSGGGGSGG** **GGSGGGGSGG** **GGSDKTHTCP** PCPAPPELLG PSVFLFPPKP KDTLMISRTF
1081 EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
1141 EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTCLVKGFYPSDI
1201 AVEWESNGQF ENNYKTPPV LDSGGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
1261 QKSLSLSPGK *

DNA sequence for FVII-057

1 ATGCTCTCCC AGGCCCTCAG GCTCCTCTGC CTCTGCTTG GGTTCAGGG CTGCCTGGCT
61 GCAGTCTTCG TAACCCAGGA GGAAGCCCAC GCGCTCCTGC ACCGGGCGCG CCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCCTTCGAG AGCCCCGGGA GATCTTCAAG CACGCGGAGA GGACGAAGCT GTTCTGCATT
241 TCTTACAGTG ATGGGAGCCA GTGTGCCVCA AGTCCATGCC AGAATGGGG CTCTGCAAG
301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCCTGCC CTGAGGGCCG GAACTGTGAG
361 ACGCACAAGC ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGAACCAA CGCTCCTGT CCGTGCCACG AGGCTACTTC TCTCTCGCA
481 GACGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATCTAGAA
541 AAAAGAAATG CCAGCAAAAC CCAAGGCCGA GGTGGGGTG GCTCCGGCGG AGGTGGGTCC
601 GGTGGCGGCG GATCAGGTGG GGTGGATCA GCGGGTGGAG GTTCCGGTGG CCGGGGATCC
661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCTGGGAGG ACCGTCAGTC
721 TTCTCTCTCC CCCCCAAACC CAAGGACACC CTCATGATCT CCGGACCCC TGAGGTACAC
781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CTTGAGGTCA AGTTCAACTG GTACGTGGAC
841 GCGGTGGAGG TGCAATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACCTAC
901 CGTGTGGTCA GCGTCTCAC CGTCTTGCAC CAGGACTGGC TGAATGGCAA GGAETACAAG
961 TGCAAGGTCT CCAACAAAGC CCTCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTTGACACC CTGCCCCCA CCGGGGATCA GCTGACCAAG
1081 AACCAGGTCA GCTGACCTG CTTGGTCAA GGTCTCTATC CCAGCGACAT CGCGGTGGAG
1141 TGGGAGAGCA ATGGGAGCCA GGAGAACAA TACAAGACCA CGCTCCCTGT GTTGGACTCC
1201 GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCCTCGACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTCTT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA

1331 GGTTCGGGTG GCGGGGGATC CGGCGGTGGA GGTTCGGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGCG TGAGCCAGAC CAGCAAGCTG ACCCGGATTC TGGGGGGCAA GGTGTGCCCC
1501 AAAGGGGAGT GTCCATGSCA GGTCCTGTTC TTGGTGAATG GAGCTCAGTT GTGTGGGGGG
1561 ACCCTGATCA ACACCATCTG GGLGGTCTTC GCGGGCCACG GTTTCGACAA AATCAAGAAC

1621 TGGAGGAACC TGATCGCGGT GCTGGGCGAG CACGACCTCA GCGAGCACGA CGGCGA TGAG
1681 CAGAGCCGGC GGGTGGCGCA GGTCAATCAT CCCAGCACGT ACGTCCCCGG CACCACCAAC
1741 CACGACATCG CGCTGCTCCG CCTGCACCAG CCCGTGGTCC TCACTGACCA TGTGGTGCCC
1801 CTCTGCCTGC CCGAACGGAC GTTCTCTGAG AGGACGCTGG CCTTCGTGGC CTTCTCATTC
1861 GTCAGCGGCT GGGGCCAGCT GCTGGACCGT GCGGCCACGG CCCTGGAGCT CATGGTCCTC
1921 AACGTGCCCC GGCTGATGAC CCAGGACTGC CTGCAGCAGT CACGGAAGGT GGGAGACTCC
1981 CCAAAATATCA CGGAGTACAT GTTCTGTGGC GCGTAC TGGCAGCAA GGACTCTTGC
2041 AAGGGGGACA GTGCAGGCCC ACATGCCACC GACTACCGGG GCACGTGCTA CCTCAAGGGC
2101 ATCGTCAGCT GGGGCCAGGG CTGCGCAACC GTGGGCCACT TTGGGGTGTA CACCAGGGTC
2161 TCCAGTACA TCGAGTGGCT GCAAAAGCTC ATGGCGTCA GGCACGGCC AGGAGTCTTC
2221 CTGCGAGCCC CATTTCCCGG TGGCGGTGGC TCCGCGGGAG GTGGGTCCGG TGGCGCGGA
2281 TCAGGTGGGG GTGGATCAGG CGGTGGAGGT TCCGGTGGCG GGGGATCAGA CAAAACTCAC
2341 ACATGCCCAC CGTCCCCAGC ACCTGAACCT CTGGGAGGAC CGTCACTCTT CCTCTTCCCC
2401 CCAAAACCCA AGGACACCCCT CATGATCTCC CGGACCCCTG AGGTCACTG CGTGGTGGTG
2461 GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
2521 CATAAAGCCA AGACAAAGCC GCGGGAGGAG CAGTACACA GCACGTACCG TGTGGTACGC
2581 GTCTTCACCG TCCTGCACCA GCACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC
2641 AACAAAGCCC TCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
2701 GAACCCACAG GTTACACCCCT GCGGCCATCC CGGGATGAGC TGACCAAGAA CCAGGTACGC
2761 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
2821 GGGCAGCCGG AGAACAACCTA CAAGACCAGG CCTCCCGTGT TGGACTCCGA CGGCTCTTTC
2881 TTTCTCTACA GCAAGCTCAC GTTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA
2941 TGCTCCCTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
3001 CCGGGTAAAT GA

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 Xla cleavage site is shown in bold, and the Factor Xla cleavage site is shown in dashed underline

1 MVSQALRLLC LLGLGGLCA AVFVTOEEMH GVLIRRRRAN AFLDELRLPGS LERECKEEQC
61 SFEEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYTCFC LPATFGRNCE
121 THKDDQLTCV NNNGCEQYVC SDHTGTKRSC RCHRGYSLLA DGVSCPTVVR YPGKIPILR
181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKTHTCPPCP APELLGGPSV
241 FLFPFKPRDT LMISRTPEVT CVVVDVSHED FEVKFNWYVD GVEVHMATK FREEQYNSTY
301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPFSRDELTK
361 NQVSDTCLVK GFTPSDIAVE WESNGQPENN YKTPPPVLDL DGSFELYSLK TVDRSRWQGG
421 NVTSCTSMHE ALHNHYTQKS LSLSPGKGGG **GGGGGGSGGG** **GGGGGGSGGG** **GGGGGGSGGG**
481 **GSSVSQESKL** TRIVGGKVCF KGECFQVLL LVNGAQLCGG TLINTIWWVS AAHCFDKIKN
541 WRNLIIVLGE HDLSEHDEDE QSRVAQVLI PSTYVPETTN HDIALRLRHQ PVVLTQHVVP
601 LCLPERTFSE RLIAFVRFSL VSGWGLIDR CATALELMVL NVPRLMQDC LQDSRKVGDS
661 PNIEYMFCA GYSDGSKDSC KGDGGPHAT HYRGTWILT IVSWGQGCAT VGHPGVYTRV
721 SQYTFWLQKL MRSEPRPGVI LRAPTPGSGG SGGGSGGGG SGGGSGGGG SGGGSGDKTH
781 TCPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
841 HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQFR
901 EPQVYTLPPS RDELTKNQVS LTCLVKGFPY SDIAVEWESN GQPENNYKTT PPVLDSGGSF
961 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTKQSLSLG PGK*

DNA sequence for FVII-058

1 ATGGTCTCCC AGGCCCTCAG SCTCCTCTGC CTTCTGCCTG GGTTCAGGG CTGCCTGGCT
61 GCAGTCTTCG TAACCCAGGA GGAAGCCAC GGCCTCCTGC ACCGGCCCGG GCGCGCCAAC
121 GCGTTCCTTG AGGAGCTGGG GCCTGGCTCC CTGGAGAGGG AGTGCAGGA GGAGCAGTGC
181 TCCTTCGAGG AGCCCCGGGA GATCTTCAAG CACCCGAGCA GGACGAAGCT CTTCTGCAAT
241 TCTTACAGTG ATGGGGACCA GTGTGCTTCA AGTCCATGCC AGAATGGGG CTCTTGCAAG
301 GACGAGCTTC AGTCTTATAT CTGCTTCCTG CTCCCTGCC TCGAGGGCCG GAACGTG GAG
361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAAGC GCGGCTGTGA CCAGTACTGC
421 AGTGACCACA CCGGCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTCTTGGCA
481 GACGGGTTCG CTTGCACACC CACAGTTGAA TATCCATGTC GAAAAATACC TATCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGCCCA GGTGGCGGTG CTTCCGGCGG AGGTGGGTCC
601 GGTGGCGGGG GATCAGGTGG GGGTGGATCA GCGGTGGAG GTTCGGGTGG CCGGGGATCC
661 GACAAAATTC ACACATGCCC ACGTGGCCA GCTCCGGAAC TCTTGGGAGG ACGGTCACTC
721 TTCTCTCTTC CCCCAAAACC CAAGGACACC CTCATGATCT CCGGACCCC TGAGGTACAC
781 TGGGTGGTGG TGGACGTGAG CCACGAAGAG CCTGAGGTCA AGTTCAACTG CTACGTGGAC
841 GCGGTGGAGG TGCATAATGC CAAGCAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
901 CGTGTGGTCA CCGTCTTCAC CGTCTTGCAC CAGGACTGGC TGAATGGCAA CGAGTACAGG
961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGGAGCCCC GAGAACACCA GGTGTACACC CTGCCCCCAT CCGGGGATGA GCTGACCAAG
1081 AACCAGTCA GCTTACCTG CCGGTCAAAA GGCCTCTATC CCAGCGACAT CGCGGTGGAG

1141 TGGGAGAGCA ATGGGCAACC GCAGAACAAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGACAC AGACAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAACAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GCAACAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTTCGGGTG GCGGGGGATC CCGCGGTGGA GGTTCGGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGACT TCTTGGCCGA GGGCGCGCGC GTGCGGATTG TGGGGGGCAA GGTGTGCCCC
1501 GGTTCAGACT TCTTGGCCGA GGGCGCGCGC GTGCGGATTG TGGGGGGCAA GGTGTGCCCC

1501 AAAGGGGAGT GTCCATGGCA GGTCTGTGTTG TTGGTGAATG GAGCTLAGTT GTGTGGGGGG
1561 ACCCTGATCA ACACCATCTG GGTGGTCTCC GCGGGCCACT GTTTCGACAA AATCAAGAAC
1621 TGGAGGAACC TGATCGCGGT GCTGGGCGAG CACGACCTCA GCGAGCACGA CGGGGATGAG
1681 CAGAGCCGGC GGGTTCGCGA GGTCAATCAT CCCAGCACGT ACSTCCCGGG CACCACCAAC
1741 CACGACATCG CCGTCTCTCC GCTGGACCCAG CCGGTGGTCC TCACTGACCA TGTGGTGCCC
1801 CTCTGCCTGC CCGAACGGAC GTTCTCTGAG AGGACGCTGG CCTTCGTCGG CTTCTCATTTG
1861 CTCAGCGGCCT GGGGCCAGCT GCTGGACCGT GCGGCCAAGG CCCTGGAGCT CATGGTCCTC
1921 AACGTGCCCC GCGTGATGAC CCAGGACTGC CTG-CAGCAGT CACGGAAGGT GGGGAGACTCC
1981 CCAAAATATCA CCGAGTACAT GTTCTGTGCC GCGTACTCGG ATGGCAGCAA GGACTGCTGC
2041 AAGGGGGACA GTGGAGGCCC ACATGCCACC CACTACCGGG GCACGTGGTA CCTGACGGGC
2101 ATCGTCAAGT GGGGCCAGGG CTGCGCAACC GTGGGCCACT TTGGGGTGTA CACCAGGGTC
2161 TCCCASTACA TCGAGTGGCT GCAAAAGCTC ATGCGCTCAG AGCCACGCCC AGGAGTCTTC
2221 CTGCGAGGCC CATTTCCCGG TGGCGGTGGT TCCGGTGGCG GTGGGTCCGG TGGCGCGGGA
2281 TCAGGTGGGG TCGGATCAGG CCGTGGAGGT TCCGGTGGCG GGGGATCAGA CAAAATCAC
2341 ACATGCCCAC CGTGCCCAGC ACCTGAACCT CTGGGAGGAC CGTCAGTCTTC CTTCTTCCCC
2401 CAAAAACCCA AGGACACCCCT CATGATCTCC CCGACCCCTG AGGTACATG CGTGGTGCTG
2461 GACGTGAGCC ACGAAGACCC TGAGGTCAAG TCCAACTGGT ACGTGGACGG CGTGGAGGTG
2521 CATAATGCCA AGACAAAGCC GCGCGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
2581 GTCTTCACCG TCCTGCACCA GGAATGGCTG AATGGCAAGG AGTACAACTG CAAGGTCTCC
2641 AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
2701 GAACCACAGG TGTACACCCCT GCGCCCATCC CCGGATGAGC TGACCAAGAA CCAGGTACAG
2761 CTGACCTTGC TTGTCAAAGC CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
2821 GGGCAGCCGG AGAACAACCTA CAAGACACCG CTTCCCTGT TGGACTCCGA GGGCTCCTTC
2881 TTCTCTTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA
2941 TGCTCGGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTTCCCTGTC
3001 CCGGGTAAAT GA

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 LLLGQQGCLA AMFVTQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61 SFEEAREIFK DAERTKLEWI SYSDGDDQAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGEQYC SDHTGTRKSC RCHEGYSLLA DGVSCPTPVE YPCGKIPILE
181 KRNASKPQGR GGGGGGGGGG GGGGGGGGGG GGGGGGGGGG DKHTTCPPCP APELLGPPSV
241 FLTPPKPKDT LMISRTPEVT CVVVDVSHFD PEVKFNWYVD GVEVHNAKTK PREEQYNSTY
301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTIKAK GQPREPQVYT LPFSRDELTK
361 NQVSTFCLVK GYFPSDIAVE WESNGQPENN YKTEPPVLDL DGSFFLYSKL TVDKSRWQQG
421 NVFSCSVMHF ALHNHYTQKS LSLSPGKGGG GSGGGGGGGG GSGGGGGGGG GSGGGGGGGG
481 GSDFLAEGGG VRIVGGKVPF KCECFWQVLL LVNGAQICGG TLINTIIVVS AANCFDKIKN
541 WRNLIAVLGE HDLSEHDGDE QSRRAQVII PSTYVPCTTN HDIALRLHQ PVVITDHVVP
601 LCLPERTFSE RFLAFVRFSL VSGWGQLDR GATALELMVL NVPRLMTPDC LQSRKVGDS
661 PNITEYMFCA GYSDGSKDSC KCDSGGPHAT HYRGTWYLTG TVSWGQGCAT VGHEFGVYTRV
721 SQVTEWLQKL MRSEPRPGVL LRAFPFGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSDKTH
781 TCPPCPA*FI LGGPSVELEF PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
841 HNAKTKPREE QYNSTYRVVS VLTVELHQDW NGKEYKCKVS NKALPAPTEK TISKAKGQPR
901 EFQVYILPPS RDELTKNQVS LITCLVKGFP SDIAVEHWSN GQFENNYKTT PFVLDSDGSE
961 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLG PKG*

DNA sequence for FVII-059

1 ATGGTCTCCC AGGCCCTCAG GTCCTCTCC CTTCTGCTTG GGTTCGAGG CTGCTTGGCT
61 GCAGTCTTTC TAAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGCGCCG GCGCGCCAAC
121 GCGTCTCCTG AGGAGCTGCG GCGCGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCACTGC
181 TCCCTCGAGG AGGCTCGGGA GATCTTCAAG GACGCGGAGA GCACCAACCT GTTCTGGATT
241 TCTTACAGTG ATGGGAGCCA GTGTGCTCA AGTCCATGCC AGAATGGGGG CTCTGCAAG
301 GACCAGCTCC AGTCCATATAT CTGCTTCTGC CTCCCTGCC TCGAGSGCCG GAACTGTGAG
361 ACGCACAAAG ALGACCACT GATCTGTGT AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CCGGCACCAA CCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGTGT CTGACACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GTTGGCGGTG GCTCCGGCGG AGGTGGGTCC
601 GGTGGCGGGG GATCAGGTGG GGGTGGATCA GCGGCTGGAG GTTCCGGTGG CCGGGGATCC
661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCTGGGAGG ACCGTCTAGT
721 TTCCTCTTCC CCCCCAAACC CAAGGACACC CTCATGATCT CCGGGAACCC TGAGGTCAAC

781 TGGGTGGTGG TGGAGGTGAG CCAAGAAAGAC CCTGAGGTCA AGTTCGAAC TGACGTGGAC
841 GCGGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGAGG AGCAGTACAA CAGCACGTAC
901 CGGTGTGTCA GCGTCTCAC CGTCCCTGC CAGGACTGGC TGAATGGCAA GGAGTACAA
961 TGCAAGGTCT CCAACAAAGC CTTCCCAAGC CCAATCGAGA AAACCACTTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GETGTACACC CTGCCCCCA CCGGGGATGA GCTCAACAA
1081 AACCAGGTCA GCGTGACCTG CTTGGTCAAA GCGTCTCTAT CCAGCGACAT CCGCGTGGAG
1141 TGGGAGAGCA ATGGCGAGCC GGAGAACAAC TACAAGACCA CCGCTCCCGT GTTGGAGTCC
1201 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCCGCTAA AGGTGGCGGC GATCAGGTG GCGGTGGATC AGGCGGCGGA
1381 GGTTCGGGTG CCGGGGATC CCGCGGTGGA GGTTCGGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCACCAA CCAAGATCAA CCGCGGATC GTGGGGGGA AGGTGTGCC CAAAGGGGAG
1501 TGLCCATGGC AGGTCTTGT GTTGGTGAAT GAGCTCAGT TGTGTGGGGG GACCTGATC
1561 AACACCATCT GGGTGGTCT CCGCGCCAC TGTTCGACA AAATCAAGAA CTGGAGGAAC
1621 CTGATCGCGG TGGTGGCGA GCACGACCTC AGCGAGCAG ACGGGGATGA GCAGAGCCGG
1681 CCGGTGGCGG AGGTATCAT CCGCAGCAG TACGTCCCG GCACCACCA CCACGACATC

1741. GCGCTGCTCC GCCTGCACCA GCCCGEGGEC CTCACTEACC ATGTGGTGCC CCTCTGCCTG
1801. CCGGAACGGA CGTTCTCTGA GAGGACGGTG GCCCTCGTGC GCTTCTCATT GGTGAGCGGC
1861. TGGGGCCAGC TGCTGGACCG TGGCGCCACG GCCCTGGAGC TCATGGTCCT CAACGTGCCC
1921. CGGCTGATCA CCCAGGACTC CCTGCAGCAG TCACCGAAGC TGGGAGACTC CCAAAATATC
1981. ACGGAGTACA TGTCTGTGTC CGGCTACTCG GATGGCAGCA AGGACTCCTG CAAGGGGGAC
2041. AGGGGAGGCC CACATGCCAC CCACTACCGG GCGAAGGGG ACCTGACGGG CATCGTCAGC
2101. TGGGGCCAGG GCTCCGCAAC CGTGGGCCCAC TTGGGGTGT ACACCAGGGT CTCCCAGTAC
2161. ATCGAGTGGC TGCAAAAGCT CATGCGCTCA GAGCCACGCC CAGGAGTCCT CCTGCGAGCC
2221. CCATTTCCCG GTGGCGGTGG CTCGGGCGGA GGTGGGTCCG GTGGCGGCGG ATCAGGTGGG
2281. GGGGATCAG GCGGTGGAGC TTCCGGTGGC GGGGATCAG ACAAACCTCA CACATGCCCA
2341. CCGTGCCAG CACCTGAACCT CCTGGGAGGA CCGTCAGTCT TCCTCTTCCC CCAAAACCC
2401. AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTACACG GCGTGGTGGT GGACGTGAGC
2461. CACCAAGACC CTGAGGTCAA GTTCAACTGG TACCTGGACC GCGTGGAGGT GCATAATGCC
2521. AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCAGGTACC GTGTGGTCAG CGTCCTCACC
2581. GTCCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAG GCAAGGTCTC CAACAAAGCC
2641. CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG
2701. GTGTACACCC TGCCCCCACC CCGGGATGAG CTGACCAAGA ACCAGGTGAG CCGTGAACCTG
2761. CTGGTCAAAG GCTTCTATCC CAGCGACATC GCGGTGGAGT GGGAGAGCAA TGGGACAGCC
2821. GAGAACAAC TACAAGACAC GCCTCCCGTG TTGGACTCCG ACGGCTCCTT CTTCCTCTAC
2881. AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACCTCTCTCT ATGCTCCGTG
2941. ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAG
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 MVSQAIKILC LLLGLQGC LA AVLVYTORHAK GYLHRRRRKAN AFHEKLRPGS LKKECKEEMQC
61 SFEEAREIFK DAERTKLEFI SYSDDGDCAS SPCQNGESCK DQLQSYICFC LEAFEGERNCE
121 THKDDQLICV NENGGEQYC SDHTGTRKRC RCHEGYSLLA DGVSCPTVE YPCGKIPILE
181 KRNASKPQGR GGGSGGGGS GGGSGGGGS GGGSGGGGS DKHTTQPPCP APELLGGPSV
241 FLTPPKPRDI LMSKTPFVT CVVVDVSHED PEVKFNWYVD GVEVHNAIK PREEQINSTY
301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTIKAK GQPREPQVYT LPFSRDELTK
361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN FKTEPPVLDL DGSFFLYSKL TVDKSRWQGG
421 NVFSCSVME ALHNHYTQKS LSLSFGKGGG **SGGGSGGGG** **SGGGSGGGG** **SGGGSGGGG**
481 GSFKIKPR VGGKVCPRGR CPWQVLIIVN GAGLCGGTLL NTIIVVSAAH CTDKIKNWRN
541 LIAVLGEHDL SEHDEDEQSR RVAQVLIIPST YVPGTTHNDI ALLRLHQPVV LTHIVVPLCL
601 PERTFSERTL AFVRFSLVSG WQQLIDRGT ALELMVLNVP RLMTQDCLQQ SRKVGDSFNI
661 TRVMEFCAGYS DGSKDSCKG SGGPHATHYR GTWYLTGIVS WQQGCAIVGH PGVYTRVSQY
721 LEWLQKLMRS EPRPGVLLRA PFPGGGSGG GSGGGGSGG GSGGGGSGG GSGDKTHTCP
781 PCPAPPELLG PSVTLFPFKK KDTLMISRTF EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
841 KTKPREKQYN SYRQVSV N VIHQDWLNGK FYKCKVSNKA LPAPIEKVIS KAKGQPRMFPQ
901 VYTLPPSRDE LTKNQVSLT LVKCFYPSDI AVEWESNGQP ENNYKTTPPV LDDSGSFPLY
961 SKLTVDKSRW QQGNVFSQSV MHEALHNHYT QKSLSLSPGK *

DNA sequence for FVII-060

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCCTGGCT
61 GCAGTCTTCC TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGCGCCG GCGGCGCAC
121 GCGTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GCAGCAGTGC
181 TCCCTTCGAG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTC ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGG CTCTGCAAG
301 GACCAGCTCC AGTCCTATAT CTGCTCTGCG CTCCTGCTT TCGAGGGCCG GAACTGTGAG
361 ACGCACAAAG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGCCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGCTGT CCTGCACACC CACAGETGAA TATCCAGTGC GAAAAATACC TATCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCCG AGGTGGGTCC
601 GGTGGCGGCG GATCAGGTGG GGTGGATCA GCGGTGGAG GTTCCGGTGG CGGGGATCC
661 GACAAAATC ACACATCCCC ACCGTGCCCA CCTCCGGAAC TCCTGGGAGG ACCCTCAGTC
721 TTCTCTTCC CCAAAAACC CAAGGACACC CTCATGATCT CCGGACCCC TGAGGTACA
781 TGGGTGGTGG TGGAGGTGAG CCACGAAGAC CTTGAGGTCA AGTTCAACTG GTACGTGGAC
841 GCGGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGAGG AGCAGTACAA CAGCACGTAC
901 CGGTGTGTCA GCGTCTCAC CGTCTGCGC CAGGACTGGC TGAATGGCAA GGAGTACAAG
961 TGGCAAGGTCT CCAACAAAGC CTTCCAGGC CCAATCGAGA AAACCAATCT CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCGGGATGA GCTCAACAAG
1081 AACCAGGTCA GCTGACCTG CCTGTCAAA GGTCTCTATC CCAGCGACAT CGCGGTGGAG
1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGGCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTCCACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCTG GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGCTAA AGGTGGCGGC GGAACAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTCCCGGTG GCGGGGGATC CCGCGGTGGA GGTCCCGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGCCC TGGCGCCCCG GGTGGTGGGC GCGCGCGTGG TGGGGGGCAA GGTGTGCCCC
1501 AAAGGGGAGT GTCCATGGCA GGTCTGTGG TTGGTGAATG GAGCTCAGTT GTTGGGGG
1561 ACCGTGATCA ACACCATCTG GGTGGTCTCC GCGGCCCCAT GTTTCGACAA AATCAAGAAC
1621 TGGAGGAACC TGATGCGCGT GCTGGGCGAG CAGGACCTCA GCGAGCACGA CCGGCATGAG
1681 CAGAGCCGCG GGGTGGCGCA GTTCAATCA CCGAGCAAGT ACGTCCCGGG CACCAACCAAC
1741 CACGACATCG CGCTGCTCCG CCTGCAGCAG CCGGTGGTCC TCACTGACCA TGTGGTGCCC
1801 CTCTGCCCTG CCGAACGGAC GTTCTCTGAG AGGACGCTGG CCTTCGTGGG CTTCTCATTT
1861 GTTACGGGCT TGGGCGAGCT GTTGGACGTT GCGGCCAGCG CCGTGGAGCT CATGGTCTCT
1921 AACGTGCCCC GCTGATGAC CCAGGACTGC CTGCAGCAGT CAGGGAAGCT GCGAGACTCC

1981 CCAAAATATCA CGGAGTACAT GTTCTGTGCC GCGTACGCGG ATGGCAGCAA GGACTGCTGC
2041 AAGGGGGACA GTGGAGGCCC ACATGCCACC CACTACCGGG GCACGTGGTA CCTGACGGGC
2101 ATCGTCAGCT GGGGCCAGGG CTGGCGAACC GTGGGCCACT TTGGGGTGTA CACCAGGGTC
2161 TCCCAGTACA TCGASTGGCT GCAAAAGCTC ATGCCCTCAG AGCCACGGCC AGGACTCCTC
2221 CTGCGAGCCC CATTTCCCGG TGGCGGTGGC TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA
2281 TCAGGTGGGG GTGGATCAGG CGGTGSAGGT TCCGCTGGCG GGGGATCAGA CAAAACTCAC
2341 ACATGCCCAC CGTGCCACAG ACCTGAACTC CTGGGAGGAC CGTCAGTCCT CCTCTTCCCC
2401 CAAAACCCCA AGGACACCCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG
2461 GACGTGAGCC ACGAAGACCC TGAGGTCAGG TTCAACGGG ACGTGGAGCG CGTGGAGGTG
2521 CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC
2581 GTCTTCACCG TCTGCAACA GGAATGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC
2641 AACAAAGCCC TCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
2701 GAACCACAGG TGTACACCCCT GCGCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTGAGC
2761 CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CGTGGAGTG GGAGAGCAAT
2821 GGGCAGCCCG AGAACAAGTA CAAGACGACG CCTCCCGTG TGGACTCCGA CGGCTGCTTC
2881 TTCTCTTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA
2941 TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT
3001 CCGGGTAAA GA

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 LLLGLQGLA AVEVTQEEAH GVLHRRRRAN APLEELRPFGS LEKECKEEQC
61 SFEEAREIFK DAERTKLEWI SYSDGDQCAS SFCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 EHKDDQLICV NENGGCEQYC SDHTGTRKSC RCHEGYSLA DGVSCPTVE YPCGKIPILE
181 ERNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKHTCPFP APELLGGPSV
241 ELFPKPKDL LMSKTPFVT CVVVDVSHED PEVKFNWYVD GVEVHNATK PREEQYNSTY
301 RVSVLTVLH QEWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTFPVLDS DGSFELYSKL TVDKSRWQQG
421 NVESCSVMHF ALHNHYTQKS LSLSFGGGGG **GGGGGGSGGG** **GGGGGGSGGG** **GGGGGGSGGG**
481 GSALRPRVVG GAVVGGKVP KGNCPWQVLL LYNQAQLCGG TLENTIHWVS AAHCIDKIKN
541 WRNLIATLGE HDLSHHDGDE QSRFVAQVLI PSTYVPETTN HDIALRLHQ FVVLTDHVP
601 LCLPERCFSE ETLAFVRESL VSGWGQLDR GATALELMVL NVPRIMTQDC LQQSRKVGDS
661 PNITRYMFCA GYSDESKDSC KGDSGGPHAT HYRGTYWLTG TVSWGQGCAT VGHFGVYTRV
721 SQYLEWLQKL MRSEPRPQVL LRAPTPGGGG SGGGSGGGG SGGGSGGGG SGGGSGDKTH
781 TCFPCFAPFL LGGPSVTLFF PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
841 HNARTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCRVS NEALPAPTEK TISKAKGQPR
901 EPQVYTLPPS RDELTKNQVS LITCLVKGFPY SDIAVEWESN GQPENNYKTT PPVLDSGSGF
961 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLG PKK*

DNA sequence for FVII-061

1 ATGGTCTCCC AGGCCCTCAG GCTGCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCCTTCG TAACCCAGGA GGAAGCCAC GCGGTGCTGC ACCGGGCGCG GCGCGGCAAC
121 GCGTTCTCTG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACCGGAGA GGACGAAGCT GTTCTGGATT
241 TCCTACAGTG ATGGGGACCA GTGTGCTCA AGTCCATGCC AGAATGGGG CTCTGCAAG
301 GACCAAGCTTC AGTCCATATAT CTGCTCTGTC CTCCCTGCC TCGAGGGCGCG GAAGTGTGAG
361 AGCCACAAGG ATGACCAGCT GATCTGTGCG AACCAGAACC GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTE GAAAATACC TATCTAGAA
541 AAAAGAAATG CCAGCAAAACC CCAAGGCCGA GGTGCGGGTG GCTCGGCGCG AGGTGGGTCC
601 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GCGGCTGGAG GTTCCGGTGG CCGGGGATCC
661 GACAAAATC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGAGG ACCGTGAGTC
721 TTCTCTTTC CCCAAAACC CAAGGACAGC CTCATGATCT CCGGACCCC TGAGGTGACA
781 TGGCTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGTCA AGTTCAACTG GTACGTGGAC
841 GCGGTGGAGG TGCATAATGC CAAGACAAG CCGCGGAGG AGCAGTACAA CAGCACGTAC
901 CGGTGTGGTCA CGCTCTCAC CGTCTGAC CAGGACTGGC TGAATGGCAA GGAGTACAA
961 TGCAAGGTC CCAACAAGC CCTCCAGGC CCAATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCA CCGGGGATGA GCTEACCAA
1081 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GCGTTCTATC CCAGCGACAT CGCCGTGGAG
1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGACCA AGAGCAGGTG GCAGCAGGGG
1261 AACCTCTTCT CATGCTCCGT GATGCATGAG CTTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTE GGGGTGGATC AGGCGGTGGA
1381 GGTTCGGGTG GCGGGGATC CCGGGTGGG GGTTCGGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGCCC TCGGCCCCG GTTGGTGGG GCGGCAATTG TGGGGGGCAA GGTGTGCCCC
1501 AAAGGGGAGT GTCCATGGCA GTTCTGTGTT TTGGTGAATG GAGCTCAGTT GTGGGGGGG
1561 ACCCTGATCA ACACCATCTG GGTGGTCTCC GCGGCCCAC GTTTCGACAA AATCAAGAAC
1621 TGGAGGAACC TGATGCGGGT GCTGGGCGAG CACGACCTCA GCGAGGACGA GGGGGAAGAG
1681 CAGAGCCGCG GGTGGCGCA GGTGATCACT CCCAGCACGT AGTCCCGGG CACCAACAA
1741 CAGGACATCG CGCTGCTCCG CCTGCACCAG CCGGTGGTCC TCACTGACCA TGTGGTGCCC
1801 CTCTGCCTGC CCGAAGCGAC GTTCTCTGAG AGGACGCTGG CTTCTGTCGG CTTCTCATTE
1861 GTCAGGGGCT GGGGCCAGCT GCTGGACCTT GCGGCCACGG CCGTGGAGCT CATGGTCTTC
1921 AACGTGCCCC GGTGATGAG CCAGGACTGC CTGCAGCAG CACGGAAGGT GGGAGACTCC
1981 CCAAAATATCA CGGAGTACAT GTTCTGTGCC GCGTACTCGG ATGGCAGCAA GGAATCTTGC
2041 AAGGGGGACA GTGGAGGCC ACATGCCACC CACTACCGGG GCACGTGGTA CCTGACGGG
2101 ATCGTCAGCT GGGGCCAGGG CTGGCGAAGC GTGGGCCACT TTGGGGTGTA CACCAGGGTC
2161 TCCCAGTACA TCGASTGGCT GCAAAAGCTC ATGCCCTCAG AGCCACGGCC AGGAGTCTCT
2221 CTGCGAGCCC CATTTCCCGG TGGCGGTGGC TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA
2281 TCAGGTGGGG GTGGATCAGG CGGTGSAGGT TCCGCTGGCG GGGGATCAGA CAAAACTCAC

2341. ACATGCCCCAG CGTGCCCCAGC ACCTGAACTC CTGGGAGGAC CGTCAGTCCTT COTCTTCCCC
2401. CCAAAACCCCA AGGACACCCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG
2461. GACGTGAGCC ACGAAGACCC TGAGGTCAAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG
2521. CATAAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCAGGTACCG TGTGGTCAGC
2581. GTCCTCACCAG TCCTGCACCA GGA CTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC
2641. AACAAAGCCC TCCAGCCCC CATCGAGAAA ACCATCTCCA AAGCCAAAGG GCAGCCCCGA
2701. GAACCACAGG TGTACACCTT GCCCCATCC CGGATGAGC TGACCAAGAA CCAGGTGAGC
2761. CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT
2821. GGGCAGCCCG AGAACAAC TA AAGACCAAG CTTCCCGTGT TGGACTCCGA CGGCTCCTTC
2881. TTCTCTTACA GCAAGCTCAC GGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA
2941. TGCTCCTGA 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 ILLGLQCCLA AVEVTQEEAH CVLHRRRRAN AFLEELRFGS LERECKEEQC
61. SFEETAREITK DAERTKLEWI SYSDGDQCAS SFCQNGGSK DQLQSYICFC LPATFGRNCE
121. THKDDQLICV NENGCCQYC SDHTGTKRSC RCHFGYSILA DGVSCTPCVF YPCGKIPTLE
181. KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKHTQPPCP APELLGGPSV
241. FLFFPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAETK FREEQYNSTY
301. RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
361. NQVSLFCLVR GFYPSDIAVE WESNGQPENN YKTEPPLVDS DGSFFLYSKL TVDKSRWQGG
421. NVFSCSVMHF ALHNHYTQKS LSLSFGKGGG GGGGGGGGGG GGGGGGGGGG GGGGGGGGGG
481. GSALRPRVVG GATVGGKVCP KGECPWQVIL LVNGAQICGG TLINTTWVVS AAHCPTDKIKN
541. WRNLIAVLGE HDLSEHDSDE QSRRAQVLI PSTYVPQTIN HDIALERLHQ PVVLTDHVVV
601. LCLPERTFSE RTLAFVRFSL VSGWGQLIDR GATALELMVL NVPRIMTQDC LQQSRKVGDS

661. PNITEYMFCA GYSDESKDSC KGDSGGPHAT HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV
721. SQYIEWLQKL MRSEFRPGVL LRAPFPGGGG SGGGSGGGGG SGGGSGGGGG SGGGSGDKTH
781. TCPPCPAPEL GGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVFV
841. HNARTKPREL QYNSTYRVVS VLTCLVQDWL NGKEYKCRVS NKALPAPIEK TISKAKGQFR
901. EPQVYTLPPS RDELTKNQVS LTCLVKGTFP SDIAVEWESN GQPENNYRFT PPVLDSGDSF
961. FLYSKLTVDK SRWQGGNVFS CSVMHFATHN HYTQKSLSLG PKK*

DNA sequence for FVII-062

1. ATGGTCTCTCC AGGCCCTCAG GCTCCTCTGCT CTCTCTGCTTG GGCTTCAGGG CTGCCTGGCT
61. GCAGTCCTTCG TAACCCAGGA GCAAGCCAC GCGCTCCTGC ACCGGCGCCG GCGCGGCAAC
121. GCGTTCTCTCG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GCAGCAGTGC
181. TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241. TCTTACAGTG ATGGGGACCA GTGTGCTCA AGTCCATGCC AGAATGGGG CTCTTGCAAG
301. GACCAGCTCC AGTCTATAT CTGCTCTGCT CTCCCTCCCT TCGAGGGCCG GAACTGTGAG
361. ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACE GCGGCTGTGA GCAGTACTGC
421. AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
481. GACGGGGTCTT CTTGCACACC CACAGTTGAA TATCCAATGT GAAAAATACC TATCTAGAA
541. AAAAGAAATG CCACCAAACC CCAAGCCCA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
601. GGTGGCGGCG GATCAGGTGG GGTGGATCA GCGGTTGGAG GTTCCGGTGG CGGGGGATCA
661. GACAAAACTC ACACATGCCC ACCGTGCGCA CTTCCGGAAC TCTGGGCGG ACCGTGAGTC
721. TTCTCTCTTC CCCCCAAACC CAAGGACACC CTCATGATCT CCGGACCCC TGAGGTGACA
781. TGGCTGGTGG TGGACGTGAG CCACGAAGAC CTTGAGGTCA AGTTCAACTG GTACGTGGAC
841. GCGGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
901. CGTGTGTCTA GCGTCTTACC CTTCTTGCAC CAGGACGGC TGAATGGCAA GGAETACAAE
961. TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACATCTC CAAAGCCAAA
1021. GGGCAGCCCC GAGAACACCA GGTGTACACC CTGCCCCCA CCGGGGATGA GCTGACCAAG
1081. AACCAGGTCA GCTGACCTG CTTGGLCAA GGLTCTTATC CCAGCGACAT CGCGGTGGAG
1141. TGGGAGAGCA ATGGGAGCC GAGAAACAAC TACAAGACCA CGCTCCCGT GTTGGACTCC
1201. GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTGACA AGAGCAGGTG GCAGCAGGGG
1261. AACGTCTTCT CATGCTCCGT GATGCAATGAG GCTCTTGCACA ACCACTACAC GCAGCAAGAGC
1321. CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GATCAGGTG GGGGTGGATC AGGCGGTGGA
1381. GGTTCGGGTG GCGGGGATC CCGCGGTGGA GGTTCGGGTG GGGGTGGATC AGGAGGAGGT
1441. GGTTCAGGTG GTGGAGGATC CATTGTGGGG GCAAGGTGT GCCCAAAGG GGAGTGTCCA
1501. TGCCAGGTCC TGTGTGTGGT GAATGGAGGT CAGTGTGTGT GGGGGACCCCT GATCAACACC
1561. ATCTGGGTGG TCTCCGCGGC CCACTGTCTT GACAAAATCA AGAACTGGAG GAACCTGATC
1621. GCGGTGCTGG GCGAGCACGA CCTCAGCGAG CACGACGGGG ATGAGCAGAG CCGCGGGGTG
1681. GCGCAGGTCA TCAATCCCCA CACGTACGTC CCGGGCAACA CCAACCACGA CATCGGCTTG
1741. CTCCGCTCTC ACCAGCCCTG GTCTCTCACT GACCATGTGG TGCCCTCTCT CCTGCCGAA
1801. CGGACGTTCT CTGAGAGGAC GCTGGCCTTC GTGGCCTCT CATTGGTTCAG CGGCTGGGGC
1861. CAGCTGCTTG ACCGTGGGCG CACGACCTTG GAGCTCATGG TCTTCAACGT GCCCGGGCTG
1921. ATGACCCAGG ACTGCTGCA GCAGTCACGG AAGGTGGGAG ACTCCCCAAA TATCAAGGAG
1981. TACATGTTCT GTGCCGCTA CTGGGATGGC AGCAAGGACT CTTGCAAGGG GGACAGTGGG
2041. GGCCACATG CCACCCACTA CCGGGGCAAG TGGTACCTGA CCGGCATCGT CAGCTGGGGC
2101. CAGGGCTGCG CAACCGTGGC CCACTTTGGG GTGTACACCA GGGTCTCCCA GTACATCGAG
2161. TGGCTGCAAA AGCTCATGCG CTCAGAGCCA CGCCAGGAG TCTCTCTGCG AGCCCCATTT
2221. CCGGTGGGCG GTGGCTCCGG CCGAGGTGGG TCGGTGGGCG GCGGATCAGG TGGGGGTGGA
2281. TCAGGCGGTT GAGTTTCGG TCGCGGGGGA TCAGACAAAA CTCACACAAT GCCACGTTGC
2341. CCAGCACCTG AACTCTTGG AGGACCTTCA CTCCTCTCTT TCCCCCAAAC ACCCAAGGAC
2401. ACCCTCATGA TCTCCCGGAC CCTTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA
2461. GACCCGAGG TCAAGTTCAA CTGGTACGTC GACGGCTTGG AGGTGCAATA TGCCAGACA
2521. AAGCGCGGG AGGAGCAGTA CAACAGCACC TACCTCTTGG TCAGCGTCTT CACCTCTCTG

2581. CACCAGGACT GGCTGAATGG CAAGGAGTAC AAGTCCAAGG TCTCCAACAA AGCCCTCCCA
2641. GCCCCCATCG AGAAAAACCAT CTCCAAAGCC AAAGGGCAGC CCGGAGAACC ACAGGTGTAC
2701. ACCCTGCCCC CATCCCGGGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC
2761. AAAGGC"TC" A"CCGAGCGA CATCGCCG"CG GAG"GGGAGA GCAATGGGCA GCGCGAGAAC
2821. AACTACAAGA CCACGCTCC CGTGTEGGAC 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 ILLGLQCCLA AVFVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61 SFEEARELTK DAERIKLEWI SYSDGDDQAS SPCQNGGSCK DQLQSYLCFC LPATEGRNCE
121 FHKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCTEPVE YPCGKIPILE
181 KRNASKPQGR GGGSGGGGS GGGSGGGGS GGGSGGGGS DKTHTCPPCP APELLGGPSV
241 FLTPPKPKDT LMISKTP EVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK FREEQYNSTY
301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPFSRDELTK

361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTFPVLDS DGSTFLYSKL TVDKSRWQQG
421 NVFSCSVMHF ALHNHYTQKS LSLSPEK**GGG GSGGGSGGGG GSGGGSGGGG GSGGGSGGGG**
481 **GSGGGGS**LVG GKVCPKGECP WQVLLLVNSA QLQSGTLINT IWVVSAAHCF DK.KNWRNLI
541 AVLGEHDLSE HEGDEQSRRV AQVIIPSTYV PGTINHDIAL IRLHQPVVLT DHVVPLCLFE
601 RTFSERTLAF VRFSLVSGWG QLLDRGATAL ELMVLNVERL MQDCLEQSR KVGDSFNITE
661 YMFCAGYSDE SKDSCKGDSG GHATHYRGT WYLTGIVSWG QGCATVGHFG VYTRVSQYIE
721 WLQKLMSREF RFGVLLRAPF PGGGSGGGG SGGGSGGGG SGGGSGGGG SDKTHTCPPC
781 PAPFTIGGPS VFLPFPKPKD TIMISRTPEV TCVVVDVSHF DPEVKFNWYV DGVEVHNAKT
841 KPREEQYNST YRVVSVLTVL HQDWLNGKEY CKVSNKALP APIEKTISKA KGQPREPQVY
901 LTPFSRDELK KQVSLTCLV KGFYPSDIAV EWESNGQPENN YKTTFPVLD SDGSTFLYSK
961 LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPK*

DNA sequence for FVII-090

1 ATGGTCTCCC AGGCCCTCAG GCTCCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCTTCG TAAACCAGGA GGAAGCCCAC GGCGTCTGC ACCGGCGCCG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGGG GCGGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGCCCCGGGA GATCTTCAAG CACCCGAGAG GCACCAAGCT GTTCTGCATT
241 TCCTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCTGCAAG
301 GACCAGCTCC AGTCCATATAT CTGCTTCTGC CTCCCTGCCCT TCGAGGGCCG GAACTGTGAG
361 ACCCACAAGG ATGACCAGCT GATCTGTGTG AACCAGAACG GCGGCTGTGA CCACTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAAATG CCAGCAAAAC CCAAGGCCGA GGTGGCGGTG GCTCCGCGCG AGGLGGGTCC
601 GGTGGCGCGG GATCAGGTGG GGGTGGATCA GCGGTGGAG GTTCCGGTGG CGGGGGATCA
661 GACAAAACTC ACACATGCCC ACCGTGCCCA GCTCCGGAAC TCCTGGGCGG ACCGTCAGTC
721 TTTCTCTTCC CCCCCAAAAC CAAGGACACC CTCA"TGATC" CCGGACCCC "TGAGGT"CA
781 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTCAGGTCA AGTTCAACTG GTACGTGGAC
841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
901 CGGTGTGTCA GCGTCTCAC CGTCCGTGC CAGGACTGGC TGAATGGCAA GGAGTACAA
961 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCCAGA AAACCACTC CAAAGCCAA
1021 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCGGGGATGA GCTGAOCAA
1081 AACCAGGTCA GCGTGACCTG CTTGGTCAAA GCGTCTTATC CCAGCGACAT CGCGGTGGAG
1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCTTCCCGT GTTGGACTCC
1201 GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTCCACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1321 CTCTCCC"TG" CTCCGGCTAA AGGTGGCGGC GGA" CAGGTG GGGGTGGATC AGGCGG"GG
1381 GGTTCGGGTG GCGGGGGATC CCGCGGTGGA GGTTCGGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGCCC TGGCGCCCCG GATTGGGGGG GCGAAGGTGT GCGCCAAAGG GGAGTGTCCA
1501 TGGCAGGTCC TGTGTTGGT GAATGGAGCT CAGTTGTGTG GGGGGACCCCT GATCAACACC
1561 ATCTGGGTGG TCTCCGCGGC CCACTGTTEC CACAAAATCA AGAACTGGAG GAACCTGATC
1621 GCGGTGCTGG GCGAGCAGCA CCTCAGCGAG CAGGACGGG ATGAGCAGAG CCGCGGGGTG
1681 GCGCAGGTCA TCATCCCCAG CACGTACGTC CCGGGCACCA CCAACCACGA CATCGCGCTG
1741 CTCCGCTTGC ACCAGCCCGT GGTCC"CACT" GACCA"GTGG TGGCCCTCTG CTTCGGGAA
1801 CGGAGGTTCT CTGAGAGGAC GCTGGCCTEC GTGCGCTTCT CATTGGTTCAG CGGCTGGGGC
1861 CAGCTGCTGG ACCGTGGCGC CACGGCCCTG GAGCTCATGG TCCTCAACGT GCGCCGGCTG
1921 ATGACCCAGG ACTCCCTGCA GCAGTCACGG AAGGTGGGAG ACTCCCAAA TATCACGGAG
1981 TACATGTTCT GTGCGGGCTA CTCGGATGGC AGCAAGGACT CCTGCAAGGG GGACAGTGG
2041 GGCCACATG CCACCCACTA CCGGGGCACG TGGTACCTGA CCGGCATCGT CAGCTGGGGC
2101 CAGGGCTGCG CAACCGTGGG CCACTTGTGG GTGTACACCA GGGTCTCCCA GTACATCGAG
2161 TGGCTGCAAA AGCTCATGCG CTCAGAGCCA CCGCCAGGAG TCCTCTTGG AGCCCCATTT
2221 CCGGTTGGCG GTGGCTCCGG CCGAGTGGG TCCGTTGGCG GCGGATCAGG TGGGGGTGG
2281 TCAGGCGGTG GAGGTTCGG TGGCGGGGGA TCAGACAAA CTCACACATG CCAACCGTGC
2341 CCAGCACCTG AAC"CC"GGG AGGACCGTCA GTCT"CCCTC" TCCCCCAAA ACCCAAGGAC
2401 ACCCTCATGA TCTCCCGGAC CCTTGAGGTC ACATGGCTGG TGGTGGACGT GAGCCACGAA
2461 GACCTTCAGG TCAAGTTCAA CTGGTACGTC GACGGCGTGG AGGTGCATAA TGCCAAGACA
2521 AAGCGCGCGG AGGAGCAGTA CAACAGCAGG TACCGTGTGG TCAGCGTCTT CACCGTCTCT
2581 CACCAGGACT GGCTGAATGG CAAGGAGTAC AAGTCCAAGG TCTCCAACAA AGCCCTCCCA
2641 GCCCCCATCG AGAAAAACCAT CTCCAAAGCC AAAGGGCAGC CCGGAGAACC ACAGGTGTAC
2701 ACCCTGCCCC CATCCCGGGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC
2761 AAAGGCTTCT ATCCCAACGA CATCGCCGTG CAGTCCGAGA GCAATGGGCA GCGCGAGAAC
2821 AACTACAAGA CCACGCTCC CGTGTEGGAC 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 MVSQALRLLLGLQGCLA AVFVTOEEFAH GVLHRRRRAN AFLEELRPGS LERECKEEQC

61 SFEEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGCEQYIC SDHTGTEKRSK RCHEGYSLLA DGVSCPTPVE YPOGKIPILE
181 ERNASKPQGR GGGSGGGGS GGGSGGGGS GGGSGGGGS DKTHTCPPCP AFELLGGPSV
241 FLPPPKPKDT IMTSRTPFVT CVVVDVSHED PEVKFNWYVD GVEVHNARKT PREEQYNSTY
301 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK QPREPQVYT LPPSRDELTK
361 NOVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG
421 NVEKCSVMHE ALHNHYTQKS LSLSPGK**GGG** **GGGGGGGGG** **GGGGGGGGG** **GGGGGGGGG**
481 **GS**ALRPRIVG GKVCPRKGECE WQVLLLVNGA QLCGGTLINT IWWVSAAHCT DKTKNWRNLI
541 AVLGEHDLSE HDGDEQSRRV AQVIIPSTYV PGTENHDIAL LRLHQPVVLT DHVVPLCLFE
601 RTFSERTLAF VRFSLSVSWG QLLDRGATAL ELMVLNVPRL MQDCLQQSR KVGDSFNITE
661 YMTFCAGYSDG SKDSCKGDSG GFHATHYRGT WYLTGIVSWG QGCATVGHFG VYTRVSYIE
721 WLQKLMRSEF RFGVLLRAPF PGGGSGGGG SGGGSGGGG SGGGSGGGG SDKTHTCPPC
781 PAPF LGGPS VFLPPPKPKD IMTSRTPFVT CVVVDVSHED PEVKFNWYVD GVEVHNARKT
841 KPREEQYNST YRVVSVLTVL HQDWLNGKEY CKVSNKALP APIEKTISKA KQPREPQVY
901 LPPSRDELTK KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK
961 LTVDKSRWQQ GNYTSCSVMH EALHNHYTQK SLSLSPGK*

DNA sequence for FVII-100

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCTTGG TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACCGGGCGCG GCGCGCCAA
121 GCCTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGACCACTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG CACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCCTACAGTG ATGGGGAACA GTGTGCTTCA AGTCCATGCC AGAATGGGGG CTCTGCAAG
301 GACCAGCTCC AGTCTTATAT CTGCTTCTGC CTCCCTGCTC TCGAGGGCCG GAACCTGLGAG
361 ACGCACAAAG ATGACCAGCT GATCTGTGTG AACGAGAACC GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CGGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCGGCAACCC CACAGTGAAG TATCCATGTG GAAAAATACC TATCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
601 GGTGGCGCGG GATCAGGTGG GGGTGGATCA GCGGCTGGAG GTTCCGGTGG CCGGGGATCC
661 GACAAAAGTC ACACATGCCC ACCGTGCCCA CTTCCGGAAC TCGTGGGAGG ACCGTGAGTC
721 TTCTCTCTTC CCCCCAAACC CAAGGACACC CTCATGATCT CCGGACCCCT TGAGGTGACA
781 TGGCTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
841 GGGCTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
901 CGGTGTGTCG CCGTCTCTAC CTTCTCTCAC CAGGACTGGC TGAATGGCAA GGAGTACAA
961 TGCAAGGTCT CCAACAAAGC CCTCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGGCAGCCCC GAGAAACCACA GGTGTACACC CTGCCCCCAT CCGGGGATGA GCTGACCAAG
1081 AACCAGGTCA GCGTACCTG CTTGTGCAAA GGCCTGATAT CCAGGACATG CGCGGTGGAG
1141 TGGGAGAGCA ATGGGAGGCC GGAGAACAAC TACAAGACCA CGCTCCCGT GTTEGACTCC
1201 GACGGCTCCT TCTTCTCTTA CAGCAAGCTC ACCGTGACCA AGAGCAGGTG GCAGCAGGGG
1261 AAGCTCTTCT CATCTTCTGT GATGCAATGAG CTTCTGCAAA ACCATACAC GCAGAAAGAC
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTCCGGGTG GCGGGGATC CCGCGGTGGA GGTCCGGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTTCAGCCC TGGGGCCCCG GATTTTGGGG GGCAAGGTG GCGCCAAAGG GGAGTGTCCA
1501 TGCCAGGTCC TGTGTGTTGT GAATGGAGCT CAGTGTGTGT GGGGGACCCCT GATCAACACC
1561 ATCTGGGTGG TCTCCGCGGC CCACTGTCTT GACAAAATCA AGAACTGGAG GAACCTGATC
1621 GCGGTGCTTG GCGAGCACGA CTTGAGCGAG CACGACGGGG ATGAGGAGAG CCGGCGGGTG
1681 GCGCAGGTCA TCATCCCCAG CACGTACGTC CCGGGCACCA CCAACCACGA CATCGCGCTG
1741 CTCCGCTTGC ACCAGCCCGT GGTCTTCACT GACCATGTGG TGGCCCTCTG CTTGCCCGAA
1801 CGGAGGTCTT CTGAGAGGAC GCTGGCTTCT GTGGCTTCTT CATTTGGTCAG CCGCTGGGGC
1861 CAGCTGCTGG ACCGTGCGGC CACGGCCCTG GAGCTCATTG TCCCTCAACCT GCGCCGGCTG
1921 ATGACCCAGG ACTGCGAGGC CAGCTACCCC GGCAAGATCA CGGAGTACAT GTTCTGTGCC
1981 GGCTACTCGG ATGGCAGCAA GCACTCTTGC AAGGGGGACA GTGGAGGCCC ACATGCCACC
2041 CACTACCGGG GCACGTGTTA CTTGACGGGC ATTCTCAGCT GGGGCCAGGG CTTCGCAACC
2101 GTGGGCCACT TTGGGGTGTG CACCAGGCTC TCCCAGTACA TCGAGTGGCT GCAAAAAGCTC
2161 ATGCGCTCAG AGCCACGCCC AGGAGTCCCT CTGCGAGCCC CATTTCCCGG TGGCGGGGGC
2221 TCCGGCGGAG GTGGGTCCGG TCGCGGCGGA TCAGGTGGGG GTGGATCAGG CCGTGGAGGT
2281 TCCGGTGGCG GGGCATCAGA CAAAACCTAC ACATCCCCAC CGTGCCGAGC ACCTGAACTC
2341 CTGGGAGGAC CGTCAGTCTT CTTCTTCCCC CCAAAACCCA AGGACACCTT CATGATCTCC
2401 CGGACCCCTG AGGTACATG CTTGGTGGTG GACCTGAGCC ACGAAGACCC TGAGGTCAAG
2461 TTCAACTGGT ACGTGGACGG CTTGGAGGTG CATAATCCCA AGACAAAGCC GCGGGAGGAG
2521 CAGTACAACA GCACGTACCG TGTGGTCAGC GTCTTCACCG TCTTGACCCA GCACTGGCTG
2581 AATGGCAAGG AGTACAAGTG CAAGTCTCC AACAAAGCCC TCCAGGCCCT CATCGAGAAA
2641 ACCATCTCCA AAGCCAAAGC GCAGCCCCGA GAACCACAGG TGTACACCTT GCGCCCATCC
2701 CGGGATGAGC TGACCAAGAA CCAGGTGAGC CTGACCTGCC TGGTCAAGAG CTTCTATCCC
2761 AGCGACATCG CCGTGGAGTG GGAGAGCAAT GGGCAGCCCG AGAACAACCTA CAAGACCACG
2821 CCGCCCGTGT TGGACTCCGA CCGCTCCTTC TTCTCTTACA GCAAGCTCAC CGTGGACAA
2881 AGCAGGTGGC AGCAGGGGAA CTTCTTCTCA TGCTCCGTGA TGCATGAGGC TCTGCACAA
2941 CACTACACGC AGAAGAGCCT CTCCCTGTCT CCGGCTAAAT 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  MVSQAIRRLC LIHLGIQGC LA AVFVYTOEHAAH GVLRHRRRRAN APLEH IRPGS IRRECKKKEQC
61  SFEEAREIFE DAERTKLEWI SYSDGDQCAS SFCQNGESCK DQLQSYICPC LEAFEGRNCE
121 CHKDDQLICV NENGGCEQYC SDHTGTRKSC RCHEGYSLLA DGVSCTEFVE YPCGKIPILE
181 KRNASKPQGR GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS DKHTTCPPCP APELLGGESV
241 ELPPPKPKD IMISRT PKVT CVVVDVSHED PEVKFNWYVD GVRVHNAKTK PRKMQYNSLY
301 RVVSVLEVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELEK
361 NQVSDTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDG DGSPTFLYSL TVDKSRWQQG
421 NVFSCSVMHE ALHNHYTQKS LSLSPGKGGG SGGGSGGGG SGGGSGGGG SGGGSGGGG
481 GSALRPRIVE GKVCFKGECF WQVLLLVNCA QLCGGTLINT IWVVSAAHCF DKIKNWRNLI
541 AVLCEHDLSE HDQDEQSRRV AQVILPSTEV PGTTHNDIAL LRLHQPVVLT DHVVPLCLPE
601 RTPSERELAF VRFSLVSGWG QLLDRGATAL ELMVLNVREL MQDCEASYP GKITEYMTCA
661 GYSDGSKDSC KGDGGGPHAT HYRGTYWYLG TVSWGQGCAT VGHFGVYTPV SQYIENLQKL
721 MRSEPRQVL LRAFFEGGGG SGGGSGGGG SGGGSGGGG SGGGSGGGG SGGGSGGGG SKTH TCPFPAPPEL
781 LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDEPKV FNWYVDGVEV HNAKTKPREE
841 QYNSTYRVVS VLTVLHQDWL NGKEYECKVS NKALPAPIEK TISKAKGQPR EPQVYTLRPS
901 RDELITRYQVS ITCLVKGFP SDIAVEWESN GQPEMN YKTT PPVLDG DSF PIYSKILTVDR
961 SRWQQGNVFS CSYMHEALHN HYTQKSLSL SL PGK
```

DNA sequence for FVII-115

```

1      ATGGGTCTCC AGCCCCCAG GCTCCTCTGC CTCTCTCTTG GGCTTCAGG
      CTGCCTGGCT
61  GCAGTCTTCC TAACCCAGGA GGAAGCCAC GCGTCTCTC ACCGGCGCGG CCGCGCCAAC
121 GCCTTCCTCG AGCAGCTGCG GCCGGCTCC CTGCAGAGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGCCCGCGGA GATCTTCAAG GACCCGAGG GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATGGGGACCA GTGTGCTCA AGTCCATGCC AGAATGGGG CTCTGCAAG
301 GACCAGCTCC AGTCCTATAT CTGCTCTCTC CTCCCTCCCT TCGAGGGCGG GAAGTGTGAG
361 ACQCACAAGG ATGACAGCT GATCTCTG GACGAGAAG CGGGCTGTGA CCAGTACTGC
421 AGTGACCACA CGGCACCAA GCGTCTCTC CCGTGCCAG AGGGGTAGTC TCTCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAATACC TATCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCCG GGTGGCGGTG GCTCGGGCG AGGTGGGTCC
601 GGTGGCGGGG GATCAGGTGG GGGTGGATCA GCGCGTGGAG GTTCGGGTGG CCGGGGATCA
661 GACAAAATTC ACACATGCCC ACCGTGCCA GCTTCGGGAG TCTGGGGCG ACCGTGAGTC
721 TTCTCTCTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCGGACCCC TGAGGTGACA
781 TGCGTGGTGG TGAAGGTGAG CCACGAAGC CTTGAGGTCA AGTTCAACTG GTACGTGGAC
841 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCAAGTAC
901 CGTGTGGTCA GCCTCTCAC CGTCTCTCAC CAGCACTGCC TGAATGGCAA CGAGTACAA
961 TGCAAGGTCT CCAACAAAGC CCTCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1021 GGCCAGCCCC GAGAAACACA GGTGTACACC CTGCCCCCA CCGGGATGGA GCTACCCAA
1081 AACGAGGTCA GCCTGACCTG CCTGGTCAAA GGTTCCTATC CCAGCGACAT CGCCGTGGAG
1141 TGGGAGAGCA ATGGGCAGCC GGAGAACAA TACAAGACCA CGCTCCCGT CTTEGACTCC
1201 GACGGCTCTT TCTTCTCTA CAGCAAGCTC ACCGTGACA AGAGCAGGTG GCAGCAGGGG
1261 AACGTCTTCT CATCTCTCGT GATGCATGAG CTTCTGCACA ACCACTACAC GCAGAGAGAG
1321 CTCTCCCTGT CTCCGGGTAA AGGTGGCGGC GGATCAGGTG GGGGTGGATC AGGCGGTGGA
1381 GGTTCGGGTG CCGGGGATC CCGCGGTGGA GGTTCGGGTG GGGGTGGATC AGGAGGAGGT
1441 GGTCAGCCC TGGGCCCCG GATTTGGGG GGCAGGAC TCCCCAAAGG GGAATTTCCA
1501 TGCCAGGTCC TGTGTTGGT GAATGGAGCT CAGTTGTGTG GGGGGACCCG GATCAACACC
1561 ATCTGGGTGG TCTCCGGGCG CCACTGTCTC GACAAAATCA AGAAGTGGAG GAACCTGATC
1621 GCGGTGCTTG GCGAGCAGCA CTTACCGGAG CACGACGGG ATGAGCAGAG CCGCGGGGTG
1681 GCGCAGGTCA TCATCCCCAG CACGTACGTC CCGGGCACCA CCAACCAGGA CATCGCGCTG
1741 CTCCGCTCTC ACCAGCCCGT GGTCTCTCAT GACCATGTGG TGCCCCCTCT CCTGCCCGAA
1801 CGGAGCTTCT CTGAGAGGAC GCTGGCTTTC GTGGCTTCT CATTTGGTTCAG CCGCTGGGGC
1861 CAGCTGCTCG ACCGTGGGGC CACGGCCCTG EFACTCCAAG TCCTCAACGT CCCCCGCTG
1921 ATGACCCAGG ACTGCCTGCA GCAGTCAAGG AAGGTGGGAG ACTCCCCAAA TATCACGGAG
1981 TACATGTTCT GTGCCGCTA CTGGGATGCG AGCAAGGACT CCTGCAAGGG GGACAGTGGG
2041 GGCCACATG CCACCCACTA CCGGGGCACG TGGTACCTGA CGGGCATCGT CAGCTGGGGC
2101 CAGGGCTGCG CAACCGTGGG CCACTTTGGG GTGTACACCA GGGTCTCCCA GTACATCGAG
2161 TTGCTGCAAA AGCTCATGCG CTGAGAGGCA CGCCAGGAG TCTTCTTGG AGCCCAATTT
2221 CCGGTGGGCG GTGGCTCCCG CCGAGCTGGC TCCCGTGGCG CCGCATCAGG TGGCGGTGGA
2281 TCAGGCGGTG GAGGTTCGGG TGGCGGGGGA TCAGACAAAA CTCACACATG CCCACCGTGC
2341 CCAGCACCTG AACTCCTGGG AGGACCGTCA GTCTTCTCT TCCCCCAAA ACCCAAGGAC
2401 ACCCTCATGA TCTCCCGGAC CCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA
2461 GACCTGAGG TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA

2521 AAGCCGCGGG AGGAGCAGTA CAACAGCAG TACCGTGTGG TCAGCGTCTT CACCGTCTCT
2581 CACCAGGACT GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
2641 GCGCCCATCG AGAAAAACAT CTCCAAAGCC AAAGGGCAGC CCGGAGAACG ACAGGTGTAC
2701 ACCCTGCCCG CATCTCGGGA TGAGCTGACC AAGAACGAGG TCAGCTGAC CTGCTGGTC
2761 AAAGGCTTCT ATCCAGCGA CATCGCCGCG GAGTGGGAGA GCAATGGGCA GCGCGAGAAC
2821 AACTACAAGA CCACGCTCTC CGTGTGGGAC TCCGACGGCT CCTTCTCTCT CTACAGCAAG
2881 CTCACCGTGG ACAAGAGCAG GTGGCAGCAG GGGAACGCTT TCTCATGCTC CGTGATGCAT
2941 GAGGCTCTGC ACAACCACTA CAGGCAGAG AGCCCTTCCC 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 LLLGLOGCLA AVFVTQEEAH CVLHRRRRAN AFLEDELRPQS LERECKEEQC
61  SFEEAREIFK DAEETKLEFWI SYSDGDQCAS SPQNGGSCK DQLQSYICFC LPAFEGRNCE
121 PHKDDQLICV NENGGCEQYC SDHTGCKRSC RCHEGYSLLA DGVSCTEPVE YPCGKIPILE
181 KRNASKPQGR GGGSGGGGGS GGGSGGGGGS GGGSGGGGGS DKTHTCPPCF APELLGGFSV
241 FLFPFKPKDT LMISRTPEVT CVVVDVSHRD PEVKFNWYVD GVEVHNAKTK FREEQYNSTY
301 KVVSVLEVLI QDWLNGKEYK CKVSNKALPA PIEKTISKAK QGPREPQVYT LPPSRDELTK
361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDG DGSEFLYSKL TVDKSRWQCG
421 NVFSCSVMHE ALHNHYTQKS LSLSFGKGGG GSGGGSGGGG GSGGGSGGGG GSGGGSGGGG
481 GSALRPRIVG GKDCFKGECF WCVELLLVNGA QLCGGTHINT FWVSAAHCF DKIKNWRNLI
541 AVLGEHDLSE HDGDEQSERV AQVIIPSTTV PGTNRDIAL LRLHQPVVLT DHVVPCLLPE
601 RTESRTTAE VRTSLVSGWG QLLDRGATAL VLDVNLVPER MTQDCLQCSR KVGDSFNITE
661 YMFCAQYSDG SKDSCKGDSG GPHATHYRCF WYITGIVSWG QGCATVGHFG VYTRVSQYLE
721 WLQKLMRSEP RPGVLLRAPE PCGGSGGGGG SGGGSGGGGG SGGGSGGGGG SGGGSGGGGG
781 PAPETLGGPS VFLFPFKPKD TLMISRTPEV TCVVVDVSHD DPEVKFNWYV DQVEVHNAKT
841 KPREEQYNST YRVVSVITVI HQDWLNGKEY KCKVSNKALP APIEKTISKA KGPREPQVY
901 TLFPSRDELK KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD DGSEFLYSKL
961 LTVDKSRWQG GNVFSCSVMH EALHNHYTQK SLLSLSPGK*
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DNA sequence for FVII-118

```

1  ATGGCTCTCC AGGCCCTCAG GCTCCTCTGC CTCTGTCTTG GGCTTCAGGG CTGCCCTGGCT
61  GCAGTCTTCC TAACCCAGGA GGAAGCCAC GCGGTCTCTG ACCGGCGCCG GCGCGCCAAC
121 GCGTCTCTGG AGGAGCTGCG GCCGGCTCC CTGGAGAGGG AGTCCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATCGGCACCA GTGTGCCCTCA AGTCCATGGC AGAATGGGGG CTCTGCAAG
301 GACCAGCTCC AGTCCATATC CTGCTCTCTG CTCCCTGCCT TCGAGGGCCG GAACCTGTCAG
361 ACGCACAAAG ATGACCAGCT GATCTCTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCTGTG CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CTTGCACACC CACAGTGGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAATG CCAAGCAAAAC CCAAGGCGCC CTGCGGCGCC GGATCTGTGG GGGCAAGGTG
601 TGCCCCAAAG GGGAGCTCTC ATGCGCAGGC CTGCTGTGTG TGAATGGAGC TCAGTGTGT
661 GGGGGGACCC TGATCAACAC CATCTGGGTG GTCTCCGCGG CCCACTGTTC CGACAAAATC
721 AAGAATCGGA GGAACCTGAT CGCGGTGCTG GCGGAGCACG ACCTCAGCGA GCACGACGG
781 GATGAGCAGA CCGGCGGGGT GGCGCAGGTC ATCATCCCCA GCACGTACCT CCGGGGCACC
841 ACCAACCAGC ACATCGCGCT GCTCCGCGTG CACCAGCCCG TGCTCTCAC TGAACATGTG
901 GTGCCCTCTC GCTGCCCCGA ACGGACGCTT TCTGAGAGGA CGCTGGCCTT CGTGCCTTC
961 TCATTGCTCA CCGGCTGGGG CCAGCTGGTG GAAGGTGGCG CCACGCGCCT EGAGCTCATG
1021 GTCTCAACG TCCCCGGCT GATGACCCAG GACTGCTTGC AGCAGTCAAG GAAGGTGGGA
1081 GACTCCCCAA ATATCAGCGA GTACATGTTC TGTGCGGGCT ACTCGGATGG CAGCAAGGAC
1141 TCCTGCAAGG CGGAGAGTGG AGGCCAGCA ATCAACCCAT ACCGGGCGAC GTGGTACCTG
1201 ACGGGCATCG TCAGCTGGGG CCAGGCTGTC GCAACCGTGG GCCACTTGG GGTGTACACC
1261 AGGGTCTTCC AGTACATCGA GTGGCTGGCA AAGCTCATGC GCTCAGAGCC ACGCCAGGA
1321 GTCTCTCTGC GAGCCCCATT TCCCGGTGGC GGTGGCTCCG GCGGAGGTGG GTCCGGTGGC
1381 GGCGGATCAG GTGGGGGTGG ATCAGGCGGT GGAGGTTCGG GTGGCGGGGG ATCCGACAAA
1441 ACTCACACAT GCGCACCGTG CCGAGCTCCG GAACCTCTGG GCGGACCGTC AGTCTTCTC
1501 TTCCCCCAA AAGCCAGGA CACCTCATG ATCCTCGGGA CCCCTGAGGT CACATGCGTG
1561 GTGGTGGACG TGAGCCACGA AGACCTGAG CTCAAAGTTC ACTGGTACGT GGAAGGCGTG
1621 GAGGTGCATA ATGCCAAGAC AAGCGCGGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG
1681 GTCAGCGTCC TCACCGTCTT GCACAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG
1741 GTCTCCAACA AAGCCCTCCC AGCCCGCATC GAGAAAACCA TCTCCAAGC CAAAGGGCAG
1801 CCGCGAGAAC CACAGGTGTA CACCTGCGCC CCATCCCGGG ATGAGCTGAC CAAGAACCAG
1861 GTCAACCTGA CCTGCTGGT CAAAGGCTTC ATCCCGAGC ACATCGCGCT GGAGTGGGAG
1921 AGCAATGGGC AGCCGAGAA CAACTACAAG ACCACGCTTC CCTGTGTGGA CTCCGACGGC
1981 TCTTCTTCTC TCTACAGCAA GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGCAACCTC
2041 TTCTCATGCT CCGTGAAGCA TGAGGCTCTG CACAACCACT ACACGAGAA GAGCCTCTCC

2101 CTGTCTCCGG CTAAAGGTGG CCGCGGATCA GGTGGGGGTG GATCAGGCGG TGGAGGTTC
2161 GGTGGCGGGG GATCAGACAA AACTCACACA TCCCCACCGT GCCCAGCACC TGAACCTCTG
2221 GGAGGACCGT CAGTCTTCTT CTTCGCCCA AAACCCAGG ACACCTCAAT GAATCTCCGG
2281 ACCCTCGAGG TCACATCGGT GGTGGTGGAC GTGAGCCACG AAGACCTTGA GGTCAAGTTC
2341 AACTGGTACG TGGAGGCGGT GGAGGTGCAT AATGCCAAGA CAAAGCGCGG GGAGGAGCAG
2401 TACAACAGCA CGTACCGTGT GGTACCGTTC CTACCGTCC TCAACAGGA CTGGCTGAAT
2461 GGCAAGGAGT ACAAGTCAAA GTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC
2521 ATCTCCAAAG CCAAGGGGCA GCCCGAGAA CCACAGGTGT ACACCTGCC CCATTCGGC
2581 GATGAGCTGA CAAAGAAACA GTTACCGTTC ACCTGCTTGG TCAAGGCTT CTATCCAGC
2641 GACATCGCGG TGGAGTGGGA GAGCAATGGG CAGCCGGAGA CAACTACAA GACCAAGCCT
2701 CCGGTGTTGG ACTCGACGG CTCTCTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC
2761 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC
2821 TACACGCAGA AGAGCCTCTC CTTGTCTCCG 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 LLLGLOGCLA AVFVTQEEAH CVLHRRRRAN AFLEDELRPQS LERECKEEQC
61  SFEEAREIFK DAEETKLEFWI SYSDGDQCAS SPQNGGSCK DQLQSYICFC LPAFEGRNCE
121 PHKDDQLICV NENGGCEQYC SDHTGCKRSC RCHEGYSLLA DGVSCTEPVE YPCGKIPILE
181 KRNASKPQGR GGGSGGGGGS GGGSGGGGGS GGGSGGGGGS DKTHTCPPCF APELLGGFSV
241 FLFPFKPKDT LMISRTPEVT CVVVDVSHRD PEVKFNWYVD GVEVHNAKTK FREEQYNSTY
301 KVVSVLEVLI QDWLNGKEYK CKVSNKALPA PIEKTISKAK QGPREPQVYT LPPSRDELTK
361 NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDG DGSEFLYSKL TVDKSRWQCG
421 NVFSCSVMHE ALHNHYTQKS LSLSFGKGGG GSGGGSGGGG GSGGGSGGGG GSGGGSGGGG
481 GSALRPRIVG GKDCFKGECF WCVELLLVNGA QLCGGTHINT FWVSAAHCF DKIKNWRNLI
541 AVLGEHDLSE HDGDEQSERV AQVIIPSTTV PGTNRDIAL LRLHQPVVLT DHVVPCLLPE
601 RTESRTTAE VRTSLVSGWG QLLDRGATAL VLDVNLVPER MTQDCLQCSR KVGDSFNITE
661 YMFCAQYSDG SKDSCKGDSG GPHATHYRCF WYITGIVSWG QGCATVGHFG VYTRVSQYLE
721 WLQKLMRSEP RPGVLLRAPE PCGGSGGGGG SGGGSGGGGG SGGGSGGGGG SGGGSGGGGG
781 PAPETLGGPS VFLFPFKPKD TLMISRTPEV TCVVVDVSHD DPEVKFNWYV DQVEVHNAKT
841 KPREEQYNST YRVVSVITVI HQDWLNGKEY KCKVSNKALP APIEKTISKA KGPREPQVY
901 TLFPSRDELK KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD DGSEFLYSKL
961 LTVDKSRWQG GNVFSCSVMH EALHNHYTQK SLLSLSPGK*
```

181 KRNASKPQGA LRPRIVGGKV CRRGECRWQV LLLVNGAQLC GGTLINTLWV VSAAHCFDKI
241 KNWRMLLAVL GEHDLSEHDG DEQSRRAQV LIPSTYVFGT TNHDIALLR HQPVVLTQHV
301 VPLCLPERTE SERTLAFVRF SLVSGWCQLL DRGATALELM VLNVPRLMTQ DCLQQSRKVG
361 DSPNITEYMF CAGYSDGSKD SCKGDSGGPH ATHYRGTWYL TGIVSWGQGC ATVGHFGVYT
421 KVSQYIEWLQ KLMRSEPRPG VILRAPFFCG GGSGGGGSGG GGSGGGGSGG GGSGGGGSDR
481 ZHTCPFCFAP ELLGGPSVFL FFPKPFDTL LSRTEVTCV VVDVSHEDPE VKENWYVDGV
541 KVNNAKTKPR HHQYNSTYRV VSVITVLHQD WINGKHYKCK VSNKA LPAP KKTL SKAKGQ
601 PREPQVYTLR PSRDELTKNQ VSLTCLVKCF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
661 SFTLYSKLTV DKSRWQQENV FSCSVMHEAL HNHYTQKSL ISPGRKGGGS GGGGSGGGGS
721 GGGSDKTHT CFPCFAPELL GGPSVFLFPP KFKDTLMISR TPEVTCVVVD VSHEDFEVKF
781 NWYVDGVEVH NAKTKPREEQ YNSTYRVSV LTVLHQDWLN GKEYCKVSN KALPAPIEKT
841 SKAKGQPRP PQVYTLRPSR DELTKNQVSL CLVKGYPSD AVIEWESNG QENNYKTTP
901 PVLDSDGSEF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK*

DNA sequence for FVII-119

1 ATGGTCTGCC AGCCCTCTAG GCTCCTCTTC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
51 GCAGTCTTTC TAACCCAGGA GGAAGCCAC GCGCTCTTGC ACCGGGCGCG GCGCGCCAA
121 GCGTTCCTTC AGCAGCTGCG GCCGGCTTCC CTGCGAGAGG AGTCGAAGGA GGACCACTGC
181 TCCCTCGAGG AGCCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGG CTCTTGCAAG
301 GACCAGCTTC AGTCTATAT CTGCTCTTC CTCCCTGCTT TCGAGGGCCG GAACTGTGAG
361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACC GCGGCTGTGA CCACTACTGC
421 AGTGACCAACA CCGGCACCAA CGCTCTCTGT CCGTGCCACG AGGGGTACLC TCTECTGGCA
481 GACGGGCTGT CCTGGACACC CACASTGAA TATCCATSTG GAAAAATACC TATCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGCGGGA GGAGGTGTT CAGCCCTGCG GCCCGGATT
601 GTGGGGGGCA AGGTGTGCC CAAAGGGGAG TGTCCATGGC AGGTCTGTT GTTGGTGAAT
661 GGAGCTCAGT TGTGTGGGG GACCTGATC AACACATCT GGTGTGCTC CGCGGCCAC
721 TGTTCGACA AAATCAAGAA CTGGAGGAAC CTGATCGCG TGCTGGGCGA GCACGACCTC
781 AGCGAGCAGC ACGGGGATGA GCAGAGCCCG CCGGTGGCGC AGGTCACTAT CCCCAGCAG
841 TACGTCCCGG CCACCAACAA CCAGACATC CCGCTGCTCC GCGTGCACCA GCGGTGGTC
901 CTCACGTACC ATGTGGTGCC CTTCTGCTTG CCGGAACGGA CGTCTCTGA GAGGACGCTG
961 GCGTTCGTTC GCTTCTCATT GGTGAGCGG TGGGGCCAGC TGCTGGACCG TGGCGCCACG
1021 GCGGTGGAGG TCAATGGTCT CAACGTGCC CCGCTGAGA CCCAGGACCG CTTGACGAG
1081 TCACGGAAGG TGGGAGACTC CCCAAATATC ACGGAGTACA TGTCTGTGTC CCGCTACTCG
1141 GATGGCAGCA AGGACTCCTG CAAGGGGGAC AGTGGAGGCC CACATGCCAC CCACTACCGG
1201 GGCACGTGGT ACGTGACGGG CATCTGTCAG TGGGGCCAGG GCTGCGCAAC CGTGGGCCAC
1261 TTTGGGCTGT ACACCAAGGT CTCCAGTAC ATCCAGTGGC TGCAAAAGCT CATCCGCTCA
1321 GAGCCACGCG CAGGAGTCTT CCGCGAGGCC CCATTTCCTG GTGGCGGTGG CTCCGCGGGA
1381 GGTGGGTCCG GTGGCGCGCG ATCAGGTGGG GGTGGATCAG GCGGTGGAGG TTCCGGTGGC
1441 GGGGGAATCG ACAAAACTCA CACATGCCCA CCGTGGCCAG CTCCGGAAC CTGGGGGGA
1501 CCGTCAGTCT TCTCTTCTCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT
1561 GAGGTCAAT GCGTGGTGGT GGACGTGAG CACGAAGACC CTGAGGTCAA GTTCAATGG
1621 TACGTGGAGG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA GCACTACAAC
1681 AGCACGTACC GTGTGGTCAG CTTCTTCACC CTCCTGCACC AGGACTGGCT GAATGGCAAG
1741 GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCAGGCC CCATCGAGAA AACCATCTCC
1801 AAAGCCAAAG GGCAGCCCGG AGAACCACAG GTGTACACCC TGCCCCCATC CCGGGATGAG
1861 CTGACCAAGA ACCAGGTGAG CTTGACCTGC CTGCTCAAA GCTTCTATCC CAGGACATC

1921 GCGGTGGAGT GGGAGAGCAA TGGGCAGCCG GAGAACAAC ACAAAGACC GCGTCCCGTG
1981 TTGGACTCCG ACGGTCTCTT CTCTCTCTAC AGCAAGCTCA CCGTGGACAA GAGCAGGTGG
2041 CAGCAGGGGA ACGTCTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACAGG
2101 CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA GGTGGCGGCG GATCAATGTC GCGTGGATCA
2161 GCGGGTGGAG GTTCCGGTGG CCGGGGATCA GACAAAATC ACACATGCC ACCGTGCCCA
2221 GCACCTGAAC TCTGGGAGG ACCGTCAATG TCTCTCTTCC CCGAAAAC CAAGGACACC
2281 CTCATGATCT CCGGACCCCT TGAGGTCACT TCGGTGCTGG TGGACGTGAG CCAAGAGAGC
2341 CCGGAGTCA AGTCAAACTG GTACGTGGAC GCGGTGGAGG TGCTAATGC CAAGACAAAG
2401 CCGCGGGAGG AGCAGTACAA CAGCAGGTAC CGTGTGCTCA GCGTCTCAC CTTCTGCAC
2461 CAGGACTGGC TGAATGCCAA GGAGTACAAG TGCAAGGTCT CCAACAAAGC CTTCCAGCC
2521 CCGATCGAGA AAACCATCTC CAAAGCCAAA GGCAGCCCC GAGAACCA GGTGTACAC
2581 CTGCCCCAT CCGCGATGA GCTGACCAAG AACAGETCA GCGTGAACG CTTGGTCAAA
2641 GCGTCTATC CCAGCGACAT CCGCGTGGAG TGGGAGAGCA ATGGGCAGCC GGAGAACAA
2701 TACAAGACCA CGCTCCCGT GTTGAACCTC GACGGCTCT TCTCTCTCTA CAGCAAGCTC
2761 ACGTGGACA AGAGCAGGTG GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGATGAG
2821 GCTCTGCACA ACCACTACAC GAGAGAGAG CTCTCCCTGT CTCCGGGTAA ATGA

FVII-119 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underline, the thrombin cleavage site with GGGS linker 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 LLLGLOGCLA AVFVTQEEAH GVLHRRRRAN AFVLEELRFGS LERECKEEQC
61 SFEEAREIFK DAERTKLFWI SYSDGDQCA SPOQNGGSCR DQLQSYICFC LPAFMGRNCF
121 THKDDQLICV NENGGCEQYC SDHTGTRKSC RCHEGYSLLA DGVSCPTVE YPCGKIPILE
181 KRNASKPQGE GGGSALRPRI VGGKVCPKGE CPWQVLLLVN GAQLCGGTLI NTIWWVSAAH
241 CFDKIKNWRN LAVLGEHDL SEHDGQWQSR RVAQVLIPST YVPGTTNHDI ALRLHQPVV
301 LTDHVVPCLCL PERTFSERTL AFVRFSLVSG WGQLLDRGAT ALEMVINVP RMTQDCLQQ
361 SRKVGDSFNI TEYMFCAGYS DGSKDSCKGD SGGPHATHYR GTWYLTGIVS WQCCCATVGH
421 FGVTYTRVSQY TEWLQKLMRS EFPRGVLLRA PFPGGGGSGG GGSGGGGSGG GGSGGGGSGG
481 GGSDKTHTCP PCFAPRLYGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDFEVRFNW
541 YVDSVEVHNA KTKPREEQYN STYRVSVILI VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
601 KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LYKGYPSDL AVIEWESNGQP ENNYKTTPFV
661 EDGGGCGTTHH CHTEDGEDG CGGHHGCGG AGGSTHHHHT CGCTCGCGG GGGGGGGGGG

661 LDSDGSEFLY SKLVVDKSRW QQGNVFSLSV MHEALHNHYT QKSLSLSPGK GGGSGGGGS
721 **GGGSGGGGS** DKHTTCPPCP APPELLGGFSV FLFPPKPKDT I M I SRTPEVT CVVVDVSHED
781 PEVKFNWYVD GVEVHNATK PREEQYNSTY RVVSVITVLH QDWLNGKEYK CKVSNKALPA
841 PIKKT SKAK GQPREPQVYT LPFSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN
901 YKTTPEVLDS DGSEFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*

DNA sequence for FVII-127

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTCC CTCTTGCTTG GGCTTCAGGG CTGCCCTGGGT
61 GCAGTCTTCG TAACCCAGGA GGAAGCCAC GGCCTCCTCC ACCGCGCCCG GCGCGCCAAC
121 CCGTTCCTGG AGGAGCTGCG GC CGGGCTCC CTGGAGAGGG AGTCAAGGA GGAGCACTGC
181 TCCTTCGAGG AGCCCCGGCA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCCTTACAGT ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGG CTTCCTGCAAG
301 GACCAAGCTCC ATCCCTATAT CTGCTTCTCC CTCCCTGCC TCGAGGGCCG GAACTGTGAG
361 ACCCACAAGG ATGACCAAGT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CTTGCACACC CACAGTGAAG TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAATC CCAGCAAAAC CCAAGGCGCC CTGCGGCCCC GGATTGTGGG GGGCAAGGTG
601 TGCCCCAAAG GGGAGTGTCC ATGCGAGGTC CTGTTGTGTG TGAATGGAGC TCAGTGTGT
661 GGGGGGACCC TGATCAACAC CATCTGGGTG GTCTCCCGCG CCCACTGTTT GCACAAAATC
721 AAGAACTGGA GGAACCTGAT CGCGGTGCTG GGGGAGCACG ACCTCAGCGA GCACGACGGG
781 GATGAGCAGA CCGGCGCGGT GCGGCAGGTC ATCATCCCA GCACGTACCT CCGGGGCACC
841 ACCAACCAGC ACATCGCGCT GCTCCGCTG CACCAGCCCG TGGTCCCTCA TGAACATGTG
901 GTGCCCCCTC GCCTGCCCCA ACGGACGTTT TCTGAGAGGA CGCTGGCCCT CGTGCGCTTC
961 TCATTGCTCA CCGGCTGGGG CCAGCTGCTG GACCTGGCG CCACGGCCCT GGAGCTCATG
1021 GTCTTCAAGC TSCCCCGGCT GATGACCCAG CACTGCGAGG CCAGCTACCC GGGCAAGATC
1081 ACGGAGTACA TGTCTGTGTC CCGCTACTCG GATGGCAGCA AGGACTCCTG CAACGGGGAC
1141 AGTGGAGGCC CACAAGCCAC CCACTACCG GGCACGTGGT ACCTGACGGG CATCTCAGC
1201 TGGGGCCAGG GCTCCGCAAC CTTGGGCTAC TTTTGGGTGT ACACCAGGGT CTCCCAGTAC
1261 ATCGAGTGGC TGCAAAAGCT CATGCGCTCA GAGCCACGCC CAGGAGTCC TCTGCGAGCC
1321 CCATTTCCTG GTGGCGGTGG CTCCGGCGGA GGTGGGTCCG GTGGCGGCGG ATCAGGTTGG
1381 GGTGGATCAG GCGGTGGAGG TTCCGGTGGC GGGGGATCAG ACAAACTCA CACATGCCCA
1441 CCGTGCCCA GCTCCGGAAC CTTGGGCGCA CCGTCACTCT TCCCTCTCCC CCAAAAACCC
1501 AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTACAT GCGTGGTGGT GGACGTGAGC
1561 CAAGAAAGAC CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATCCC
1621 AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCAGGTACC GTGTGGTCAG CGTCTCACC
1681 GTCTTGACCC AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC
1741 CTCCCAGCCC CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG

1801 GTGTACACCC TGCCCCCATC CCGGGAATGAG CTGACCAAGA ACCAGTTCAG CCTGACCTGC
1861 CTGCTCAAAG CCTTCTATCC CAGCGACATC GCGCTGGAGT GGGAGAGCAA TGGGCAGCCG
1921 GAGAACAAC TACAAGACC ACCTCCCGTG TTGGACTCCG ACGGCTCCTT CTTCCTCTAC
1981 AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG
2041 ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA
2101 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GCGGCTGGAG GTTCGGGTGG CGGGGATCA
2161 GACAAAAC TCACATGCCC ACCGTGCCCC GCACCTGAAC TCCTGGGAGG ACCCTCAGTC
2221 TTCCTCTTCT CCCCCAAAACC CAAGGACACC CTCATGATCT CCGGACCCC TGAGGTGACA
2281 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTCAACTG GTACGTGGAC
2341 GCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
2401 CGTGTGGTCA GCGTCTCTAC CTTCTCTCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
2461 TGCAAGGTCT CCAACAAAGC CCTCCGAGCC CCGATCGAGA AAACCATCTC CAAAGCCAAA
2521 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCGCGGATGA GCTGACCAAG
2581 AACCAGGTCA GCCTGACCTG CTTGGTCAA GCGTCTCTAT CCAGCGACAT CGCCGTGGAG
2641 TGGGAGAGCA ATGGGACAGC GGAGAACAAC TACAAGACCA CGCTCCCGT GTTGGACTCC
2701 GACGGCTCCT TCTTCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGCTG 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 AVFVTOEEAH CVLHRRRRAN AFLEELRPGS LERECKEEQC
61 SFEEAREIFK DAEKTLFWI SYSDGDQCAS SFCQMGESC DQLQSYICFC LPAPEGRNCE
121 THKDDQLTCV WNNGGCEQYC SDHTGCKRSC RCHRGYSLLA DGVSCPTVIR YPCGKIPIIR
181 KRNASKPQGA LRPRIVGCKV CPKGECPWQV LLLVNGAQLC GSTLINTLWV VSAAHCFDKI
241 KNWRNLIAVL GEHDLSEHDG DLQSRVAQV LIPSTYVFGT TNHDIALRL HQPVVLTQHV
301 VPLCLPPERTF SERTLATVRF SIVSGWGQLL DRGATALELM VLNVPRLMTQ DCEASYPGKI
361 HYMFCAGYS DGSKDSCKG SGGPHATHYK GTWYLTGIVS WGQGCATVGH PGVYTRVSQY
421 LEWLQKLMRS EPRPGVLERA PFPGGGGSGG GGSGGGGSGG GGSGGGGSGG GGSDKHTCP
481 PCPAPELLGG PSVTLFPPKF KDTLMISRCP EVTCVVVDVS HEDPEVKFNW YVDGVLVINA
541 KTKFREEQYN STYRVSVLT VLHQDWLNGK EYCKVSNKA LPAPIEKTIS KAKGQPREPQ
601 VYTLPPSRDE LKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTPPV LDSDGSFFLY
661 SKLVVDKSRW QQGNVFSLSV MHEALHNHYT QKSLSLSPGK **GGGSGGGGS** **GGGSGGGGS**
721 DKHTTCPPCP APPELLGGFSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
781 GVEVHNATK PREEQYNSTY RVVSVITVLH QDWLNGKEYK CKVSNKALPA PIKKTISKAK
841 GQPREPQVYT LPFSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPEVLDS
901 DGSEFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*

DNA sequence for FVII-125

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTCC CTCTTGCTTG GGCTTCAGGG CTGCCCTGGGT

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1  ATGGTCTCCG AGGCCCTCAG GCTCCTCTGC CTTCCTGCTG GGCTTCAGGG CTGCCTGGCT
61  GCAGTCTTTC TAACCCAGGA GGAAGCCAC GCGTCTCTGC ACCGGCCCG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCG GCGCGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATGGGGACCA GTGTGCTCA AGTCCATGCC AGAATGGGG CTCTGCAAG
301 GACCAGCTTC AGTCTTATAT CTGCTTCTGC CTCCCTGCC CTGAGGGCCG GAACGTGAG
361 ACCCACAAGG ATGACCAGCT GATCTCTGTC AACGAGAACC GCGCTCTGTA CCACTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATCTTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTCTGGGG GCAAGGTGTG CCCCAAAGGG
601 GAGTGTCCAT GGCAGGTCTT GTTGTGGTG AATGGAGCTC AGTTGTGTGG GGGGACCCTG
661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAAATCAA GAACGTGGAG
721 AACCTGATCG CCGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGT TGAGCAGAGC
781 CCGCGGGTGG CGCAGGTCAT CATCCCCAGC ACGTACGTCC CCGGCACCAC CAACCACGAC
841 ATCGCGCTGC TCCGCCTGCA CCAGCCCGTG GTCTCACTG ACCATGTGGT GCCCTCTGC
901 CTGCCCCAAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TGCGCTTCTC ATTGGTCAGC
961 GGCTGGGGCC AGCTGCTGGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
1021 CCCCAGCTGA TGACCCAGGA CTGCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
1081 ATCAGGAGT ACATGTTCTG TCCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAGTGGAG GCCCACATGC CACCCACTAC CGGGGCACGT GGTACCTGAC GGCATCGTC
1201 AGCTGGGGCC AGGGCTGCGC AACCGTGGGC CACTTTGGGG TGTACACCAG GGTCTCCAG
1261 TACATCGAGT GCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCAGGAGT CCTCCTGCGA
1321 GCCCCATTTC CCGGTGGCGG TGGCTCCGGC GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
1381 GGGGTTGGAT CAGGCGGTGG AGGTTCCGGT GCGGGGGGAT CCGACATCGT GATGACCCAG
1441 GCGGCCCCCA CCGTGCCCGT GACCCCGGCG GAGAGCGTGA GCATCAGCTG CCGGAGCAGC
1501 CGGAGCCTGC TGCACAGCAA CGGCAACACC TACCTGTGCT GGTTCCTGCA GCGGCCCGGC
1561 CAGAGCCCCC AGCTGCTGAT CTACCGGATG AGCAACCTGG CCAGCGGCGT GCCCGACCGC
1621 TTCAGCGGCA CGGCGAGCGG CACCGCCTTC ACCCTGGGGA TCAGCCGGT GGAGGCCGAG
1681 GAGTGGGGG TGTACTACTG CATGCAGCAC CTGGAGTACC CCTTCACCTT CCGCAGCGGC

1741 ACCAAGCTGG AGATCAAGCG GGGCGGCGGC GGCAGCGGCG GCGGCGGCAG CCGCGCGGC
1801 GGCAGCCAGG TGCAGCTGCA GCAGAGCGGC GCGGAGCTGG TCGGGCCCGG CACCAGCGTG
1861 AAGATCAGCT GCAAGGCCAG CCGCTACACC TTCACCAACT ACTGGCTGG CTGGGTGAAG
1921 CAGCGGCCCG GCCACGGCCT GGAGTGGATC GCGGACATCT ACCCGCGCGG CGCTTACAAC
1981 AAGTACAACG AGAACTTCAA GGGCAAGGCC ACCCTGACCG CCGACACCAG CAGCAGCACC
2041 GCCTACATGC CCTGAGCAG CCTGACCAGC GAGGACAGCG CCGTGTACTT CTGCGCCCG
2101 GAGTACGGCA ACTACGACTA CGCCATGGAC AGCTGGGGCC AGGGCACCGG 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  MYSQALRLIC LHLGLQGCLA AVEVTEQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61  SFEEAREIFK DAERTKLFWI SYSDGDOCAS SPCQNGGSECK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGCEQYC SDHTGTRKRC RCHEGYSLLA DGVSECTPTVE YFCGKIPILE
181 KRNASKPQGR IVGKVCPEKG ECPWQVLLL NVGAQLCGGTL INTIWWVSAA HCFDKIKNWR
241 NLIAVLGEHD LSEHDGDEQS RRVAAVLIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPIC
301 LPERTFSERT LAFVRFSLVS GWGQLLDIGA TALELMVLNV PRLMTQDCLQ QSRKVGDSFN
361 ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
421 YIEWLQKLNR SEPRPGVLLR ABFPGGGGGG GGGSGGGGGG GGGSGGGGGG GGGSDIVMTQ
481 AAPSVVPTPG ESVSISCRSS RSLHNSNGNT YLCWFLQRPQ QSPQLLIYRM SNLASGVFDR
541 FSGSGSGTAF TLRISRVEAE DVGVIYCMQH LYBPPTFGSG TKLEIKRGGE GSGGGSGSGG
601 GSQVQLQSG AEIVRPGTSV KISCKASGYT FTNYWLGWYK QRPGHGLEWI GDIYPGGGYN
661 KYNENFRGKA TLTADTSBST AYMQLSSLTS EDSAVYFCAR EYGNVDYAMD SWGQGTSTVT
721 SS*
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DNA sequence for FVII-067

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1  ATGGTCTCCG AGGCCCTCAG GCTCCTCTGC CTTCCTGCTG GGCTTCAGGG CTGCCTGGCT
61  GCAGTCTTTC TAACCCAGGA GGAAGCCAC GCGTCTCTGC ACCGGCCCG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCG GCGCGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATGGGGACCA GTGTGCTCA AGTCCATGCC AGAATGGGG CTCTGCAAG
301 GACCAGCTTC AGTCTTATAT CTGCTTCTGC CTCCCTGCC CTGAGGGCCG GAACGTGAG
361 ACCCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACC GCGCTCTGTA CCACTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATCTTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGG GCAAGGTGTG CCCCAAAGGG
601 GAGTGTCCAT GGCAGGTCTT GTTGTGGTG AATGGAGCTC AGTTGTGTGG GGGGACCCTG
661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAAATCAA GAACGTGGAG
721 AACCTGATCG CCGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGT TGAGCAGAGC
781 CCGCGGGTGG CGCAGGTCAT CATCCCCAGC ACGTACGTCC CCGGCACCAC CAACCACGAC
841 ATCGCGCTGC TCCGCCTGCA CCAGCCCGTG GTCTCACTG ACCATGTGGT GCCCTCTGC
901 CTGCCCCAAC GGACGTTCTC TGAGAGGACG CTGGCCTTCG TGCGCTTCTC ATTGGTCAGC
961 GGCTGGGGCC AGCTGCTGGA CCGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
1021 CCCCAGCTGA TGACCCAGGA CTGCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
1081 ATCAGGAGT ACATGTTCTG TCCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAGTGGAG GCCCACATGC CACCCACTAC CGGGGCACGT GGTACCTGAC GGCATCGTC
1201 AGCTGGGGCC AGGGCTGCGC AACCGTGGGC CACTTTGGGG TGTACACCAG GGTCTCCAG
1261 TACATCGAGT GCTGCAAAA GCTCATGCGC TCAGAGCCAC GCCAGGAGT CCTCCTGCGA
1321 GCCCCATTTC CCGGTGGCGG TGGCTCCGGC GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
1381 GGGGTTGGAT CAGGCGGTGG AGGTTCCGGT GCGGGGGGAT CCGACAAAC TCACACATGC
1441 CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG TCTTCCTCTT CCCCCAAAA
1501 CCAAGGACA CCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG
1561 AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
1621 GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCTC
1681 ACCCTCTCTG ACCAGACTC GCTGAATGCG AAGGAGTACA ACTGCAAGGT CTCCAACAAA
1741 GCCCTCCAG CCCCACGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
1801 CAGGTGTACA CCTGCCCCC ATCCCGGGAT GAGCTGACCA AGAACCAGGT CAGCCTGACC
1861 TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCGA
1921 CCGGAGACAA ACTACAAGAC CACGCTTCCC GTGTTGGAAT CCGACGGCTC CTTCCTCTC
1981 TACAGCAAGC TCACCGTGA CAAGCGTAGG TGGCAGCAGG GGAACGTCTT CTGATGCTCC
2041 GTGATGCATG AGGCTCTGCA CAACCCTAC ACCCAGAGA GCCTCTCCCT GTCTCCGGT
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2161 AAAGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGCGGTG GAGTTTCGG TGGCGGGGGA
2161 TCAGACAAAA CTCACACATG CCCACCGTGC CCAGCACCTG AACTCCTGGG AGGACCGTCA
2221 GTCTTCCTCT TCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTG
2281 ACATGCGGTG TGGTGGACGT GAGCCACGAA GACCTTGAGG TCAAGFTCAA CTGGTACGTG
2341 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCGCGGGG AGGAGCAGTA CAACAGCACG
2401 TACCGTGTGG TCAGCGTCTC CAACGTCCTG CAACAGGACT GGCFTGAATGG CAAGGAGTAC
2461 AAGTGCAAGG TCTCCAAACA AGCCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAGCC
2521 AAAGGGCAGC CCGGAGAACC ACAGGTGTAC ACCCTGCCCC CATCCCGCGA TGAGCTGACC
2581 AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCAGCGCA CATCGCCGTG
2641 GACTGGGAGA GCAATGGGCA GCCCGAGAAC AACTACAAGA CCACGCTTCC CGTCTTGGAC
2701 TCCGACGGCT CTTCTCTCTC CTACAGCAAG CTCACCGTCC ACAAGAGCAG GTGGCAGCAG
2761 GGGAAACGTC TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAAG
2821 AGCCTCTCCC TGTCTCCGGG TAAAGGTGGC GGTGGCTCCG GCGGAGGTGG GTCCGGTGGC
2881 GCGCGATCAG GTGGGGGTGG ATCAGGCGGT GGAGGTTCCG GTGGCGGGGG ATCAGGCGAG

2941 GTGCAGCTGC AGGAGTCTGG GGGAGGCTTG GTACAGCCTG GGGSGTCCCT GAGACTCTCC
3001 TGTGCAGCCT CTGGATTCAT GTTTAGCAGG TATGCCATGA GCTGGGTCCG CCAGGCTOCA
3061 GGGAAAGGGG CAGAGTGGGT CTCAGGTATT AGTGGTAGTG GTGTAATGAC ATACTACGCA
3121 GACTCCGTGA AGGGCGGGTT CACCGTCTCC AGAGACAATT CCAAGAACAC GCTGTATCTG
3181 CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTATATT ACTGCGCCCG GGGCGCCACC
3241 TACCCAGGCC GGAGCGACGT GCCCGACCA ACCAGCTTCC ACTACTGGGG CCAGGGAACC
3301 CTGGTCAACG TCTCCTCAGG GAGTGCATCC GCCCCAAAGC TTGAAGAAGG TGAATTTTCA
3361 GAAGCACGGG TATCTGAAC TACTCAGGAC CCTGCTGTGT CTGTGGCCTT GGGACAGACA
3421 GTCAGGATCA CATGCCAAGG AGACAGCCTC AGAAACTTTT ATGCAAGCTG GTACCAGCAG
3481 AAGCCAGGAC AGGCCCTTAC TCTTGTCTAT TATGGTTTAA GTAAAAGGCC CTCAGGGATC
3541 CCAGACCGAT TCTCTGCCTC CAGCTCAGGA AACACAGCTT CCTTGACCAT CACTGGGGCT
3601 CAGGCGGAAG ATGAGGCTGA CTATTACTGC CTGCTGTACT ACGGCGGGCG CCAGCAGGGC
3661 GTGTTCCGGG GCGGCACCAA GCTGACCGTC CTACGTCAGC CCAAGGCTGC CCCCCTGGTC
3721 ACTCTGTCTC CGCCCTCTTC TGGGGCTTGA

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 SCES is in bold

1 MVSQALRLIC MLGLGGCLA AVEVTQEEAH GVLRRRRRAN AFLEELRPGS LERECKEEQC
61 SFEEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSECK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGCEQYC SDHTGTRKSC RCHGGYSLLA DGVSCTPTVE YPCGKIPILE
181 ERNASKPOGR LVGGKVCPRK ECPWQVLLLV NGAOLCGGTL INTIWWVSAA HCFDKIKNWR
241 NLIAVLGEHD LSEHDGDEQS RRVAQVLIIPS TYVPGTINHD IALLRLHQPV VLTDEHVVPLC
301 LPERTFSERT LAFVRFSLVS GWGQLLDLGA TALELMVLNV PRLMTQDCLQ QSRKVGDSPN
361 ITEYMFACGY SDGSKDSCKG DSGGPHATHY RGTWYLIGIV SWGQGCATVG HPGVYTRVSQ
421 YIEWLQKLMR SEPRPGVLLR AFFFGGGGSG GGGSGGGGGG GGGSGGGGGG GGGSDKHTTC
481 PPCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVDV SHEDPEVKFN WYVDQVEVHN
541 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYRCKVSNK ALPAPIEKTI SKARGQPREP
601 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKITPP VLDSGGSFFL
661 YSKLTVDKSR WQGNVVFSCS VMHEALHNHY TQKSLSLSPG KGGGGGGGGG GGGGGGGGGG
721 SDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVDVSHE DPEVKFNWYV
781 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
841 KGQPREPQVY TLPFSRDEL TKNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD
901 SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGRGG GGGGGGGGGG
961 GSGGGGGSGG GSGGGGGGSAQ VQLQESGGGL VQPGSLRLS CAASGMFMR YAMSWVRQAP
1021 GKGPFWVSGI SSGGGSTYYA DSVKGRFTVS RDNSENTLYL QMNSLRADT AVYYCARGAT
1081 YTSRSDVFDQ TSFDYWGQT LVTSSGGAS APKLEEGERFS EARVSELTQD PAVSVALGQT
1141 VRITCQGDSL RNFYASWYQQ KPGQAPTLVI YGLSKRPSGI PDRFSASSSG NTASLTITGA
1201 QAEDADYYC LLYYGGGQQG VFGGGTKLTV LRQPKAAPSV TLFPSSAA*

DNA sequence for FVII-094

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCTTCC TAACCCAGGA GGAAGCCAC GGCCTCCTGC ACCGCGCGCG GCGCGCCAAC
121 GCCTTCCTGG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTSCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACCGCGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGSGGG CTCTGCAAG
301 GACCAGCTCC AGTCTATAT CTGCTTCTGC CTCCTGCCT TCGAGSGCCG GAACTGTGAG
361 ACCCAGACAG ATGACCAAGT GATCTGTGTG AACGAGAACC GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCACCAA GCGCTCCTGT CCGTGCCACG AGSGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCCCAGAGG
601 GAGTGTCCAT GTCAGTCTCT GTTGTGTGTG AATGGAGCTC AGTTGTGTGG GGGGACCTG
661 ATCAACACCA TCTGGGTGGT CTCCGCGGGC CACTGTTTCC ACAAATCAA GAACTGGAG
721 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
781 CGGCGGGTGG CGCAGGTGAT CATCCCCAGC ACGTACGTCC CGGGCACCA CAAACACGAC
841 ATCGCGCTGC TCCGCTGCA CCAGCCCGTG GTCTCACTG ACCATGTGGT GCCCTCTGTC
901 CTGCCCCGAC GACGTTCTC TGAGAGGAGC CTGGCCTTCG TCGCCTTCTC ATTGGTCAGC
961 GGCTGSGGGC AGCTGCTGGA CCGTGCGGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTC
1021 CCCCAGCTGA TGACCCAGGA CTGCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAT
1081 ATCAGCGAGT ACATGTTCTG TGCCGCGTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAGTGGAG GCCACATGTC CACCCACTAC CGGGGCACGT GGTACCTGAC GGGCATCGTC
1201 AGCTGGGGGC AGGCTGCGC AACCTGGGC CACTTGGGG TGTACACCAG GGTCTCCAG
1261 TACATCGAGT GCCTGCAAAA GCTCATGGCC TCAGAGCCAC GCCAGAGT CCTCCTGCGA
1321 GCCCATTTTC CCGATATCGG TGGCGGTGGC TCCGGCGGAG GTGGGTCCGG TGGCGGCGGA
1381 TCAGGTGGGG GTGGATCAGG CGGTGGAGGT TCCGTTGGCG GGGGATCAGC GCAGGTGCAG
1441 CTGCAGGAGT CTGGGGGAGC CTTGCTACAG CCTGGGGGGT CCTGAGACT CTCCTGTGCA
1501 GCTCTCTGAT TCATGTTTAC CAGGTATGCC ATGAGCTGGG TCCGCCAGGC TCCAGGGAAG
1561 GGGCCAGAGT GGGTCTCAGG TATTAGTGGT AGTGGTGGTA GTACATACTA CGCAGACTCC
1621 GTGAAGGGCC GGTTCACCGT CTCCAGAGAC AATTCGAAGA ACACGCTGTA TCTGCAATG
1681 AACAGCCTGA GAGCCGAGGA CACGGCTGTA TATTACTGCG CCGGGGGCGC CACCTACACC
1741 AGCCGAGCGC ACGTCCCCGA CCAGACCTGA TTGCACTACT GGGGCCAGGG AACCTTGGTC
1801 ACCGTCTCCT CAGGGAGTGC ATCCGCCCCA AAGCTTGAAG AAGGTGAATT TTCAGAAGCA
1861 CGCGTATCTC AACTGACTCA GCACCTGCT GTGTCTGTGG CCTTGGGACA GACAGTCAGG
1921 ATCACATGCC AAGGAGACAG CCTCAGAAAC TTTTATGCAA GCTGGTACCA GCAGAAGCCA
1981 GGACAGGCCC TACTCTTGT CATCTATGGT TTAAGTAAAA GGCCCTCAGG GATCCCAGAC
2041 CCACTCTCTG CTTCTCCTGC AGCAACACCA GCTTCTCTGA CCACTCCTGC GCTCTCCTGC

2101. GAAGATGAGG CTGACTATTG CTGCCTGCTG TACTACGGCG GCGGCCAGCA GGCCTGTTC
2161. GCGGGCGGCA CCAAGCTGAC CGTCTACGT CAGCCCAAGG CTGCCCTC GGTCACTCTG
2221. TCCCCCCTT CTTCTGGGG 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 AEVYTOEEAH GVLIHRRRRAN AFLEELRPGS LERECKEEQC
61. SFEEAREIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121. THKDDQLICV NENGGCEQYC SDHTGTRKRC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
181. KRNASKPQGR IVGKVCVCPKG ECPWQVLLLV NGAQLCGGTL INTIIVVSAA HCFDKIKNWR
241. NLI AVLGEHD LSEHDGDEQS RRVAQVIIPG TYVPGTTNHD IALLRLHQPV VLTDHVVPLC
301. LPERTFSEST LAFVRFSLVS GWGQLLDRA TALELMVLNV PRIMTQDCLQ QSRKVCDSFN
361. ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HPGVYTRVSQ
421. YIEWLQKLMR SBRPQVLLR APFPDIGGGG SGGGGGGGGG SGGGGGGGGG SGGGGSAQVQ
481. LQESGGGLVQ PGSLRLSCA ASGFMFERYA MSWVRQAPGK GPEWVSGTSG SGGSTYYADS
541. VKGRFTVSRD NSKNTLYLQM NSLRAEDTAV YYCARGATYT SRSDVPDQTS FDYWGQGTIV
601. TVSSGSASAP KLEEGEFSEA RVSELTQDPA VSVALGQTVR ITCQGDLSLN FYASWYQQKP
661. GQAPTLVIYQ LSKRPSGIPD RFSASSSNT ASLTITGAQA EDEADYYCLL YYGGGQQGVF
721. GGGTKLTVLR QPKAAPSVTL FPPSSAA*

DNA sequence for FVII-028

1. ATGCTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCACGG CTGCCTGGCT
61. GCGGAAGTGC AGCTGGTGCA GTCTGGAGCT CAGGTGAATA AGCCTGGGGC CTCAGTGAAG
121. GTCTCCTGCA AGCCTTCTGC ATACACCTTC ACCGCTACT ATATGCACTG GGTGCGACAG
181. GCGCCTGGAC AAGGGCTTGA GTGGATGGGA TGGATCAACC CTAACAGTGG TGGCACAAAC
241. TATGCACAGA AGTTTCAGGG CTGGGTCAAC ATGACCAGGG ACACGTCCAT CAGCACCGCC
301. TACATGGAGC TGAGCAGGCT GAGATCTGAC GACACGGCCG TGTATTACTG TCGGAGAGGC
361. CGTGCCTTGT ATAACCGGAA CGACCGGTCC CCCAACTGGT TCGACCCCTG GGGCCAGGGA
421. ACCCTGGTCA CGCTCTCTC AGGGAGTGCA TCCGCCCCAA CCCTTAAACT TGAAGAAGGT
481. GAATTTTCAG AAGCACGCGT ACAGGCTGTG CTGACTCAGC CGCCCTCGGT GTCAGTGGCC
541. CCAGGACAGA CGGCCAGGAT TACCTGTGGG CGAAACAACA TTGGAAGTAA AAGTGTGCAG
601. TGGTACCAGC AGAAGCCAGG CCAGGCCCTT GTGCTGGTCC TCTATGATGA TAGCGACCGG
661. CCCTCAGGGA TCCCTGAGCG ATTCTCTGGC TCCAACTCTG GGAACATGGC CACCCTGACC
721. ATCAGCAGGG TCGAAGCCGG GGATGAGGCC GACTATTACT GTCAGGTGTG GGATAGTAGT
781. AGTGATCATG TGGTATTCGG CGSAGGGACC AAGCTGACCG TCCTAGGTCA GCCCAAGGCT
841. GCGCCCTCGG TCACTCTGTT CCGCGCGTCC GCGGCGGCTA GGACGAAGCT GTTCTGGATT
901. TCTTACAGTG ATGGGGACCA GTGTGCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
961. GACCAGCTCC AGTCTATAT CTGCTTCTGC CTCCTGCTT TCGAGGGCCG GAACTGTGAG
1021. ACGCACAAGG ATGACCAGCT GATCTGTGTG AAGCAGAACG GCGGCTGTGA GCAGTACTGC
1081. AGTGACCACA CGGGCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
1141. GACGGGGTGT CCGTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
1201. AAAAGAAATG CCAGCAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCCCAGGGG
1261. GAGTGTCCAT GCGTGTGGT GTTGTGGTG AATGGAGCTC AGTTGTGTGG GGGGACCTG
1321. ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTTTCG ACAAATCAA GAAGTGGAGG
1381. AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
1441. CGCGGGGTGG CGCAGGTGAT CATCCCCAGC ACGTACGTCC CGGSCACCAC CAACACGAC
1501. ATCGCGCTGC TCCGCCTGCA CCAGCCCGTG GTCCTCACTG ACCATGTGGT GCCCCTCTGC
1561. CTGCCCCGAA CGAGCTTCTC TGAGAGTACG CTGCGCTTCC TGCGCTTCTC ATTGGTCAGC
1621. GGCTGGGGCC AGCTGCTGGA CGGTGGCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
1681. CCGCGGCTGA TGACCCAGGA CTGCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
1741. ATCACGGAGT ACATGTTCTG TCGCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1801. GACAGTGGAG GCCACATGC CACCCACTAC CGGGGCACGT GGTACCTGAC GGGCATCGTC
1861. AGCTGGGGCC AGGCTGGGC AAGCGTGGC CACTTTGGGG TGTACACCAG GGTCTCCAG
1921. TACATCGAGT GGCTGCAAAA GCTCATGGCC TCAGAGCCAC GCGCAGGAGT CCTCCTGCGA
1981. GCGCCATTTC CCGGTGGCGG TGGCTCCGCC CGAGGTGGGT CCGGTGGCGG CGGATCAGGT
2041. GGGGGTGGAT CAGGCGGTGG AGGTTCCGGT GCGGGGGGAT CCGACAAAAC TCACACATGC
2101. CCACCGTGCC CAGCTCCGGA ACTCTGSGC GGAACGTCAG TCTTCTCTCT CCCCCAAAA
2161. CCCAAGGACA CCTCATGAT CTCCCGGACC CTTGAGGTCA CATGCGTGGT GGTGGACGTG
2221. AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT
2281. GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCTCT
2341. ACCGTCTTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTSCAAGGT CTCCAACAAA
2401. CCGCTCCAG CCGCTCCAG GAAAACCA AAGGGCAGCC CCGAGAACCA CCGAGAACCA
2461. CAGGTGTACA CCTGCGGCC ATCCCGGGAT GAGCTGACCA AGAACAGGT CAGCCTGACC
2521. TGCCTGCTCA AAGCCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG
2581. CCGGAGAAC ACTACAAGAC CACGCTCTCC GTGTTGGACT CCGACGGCTC CTCTCTCTCT
2641. TACAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
2701. GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT
2761. AAAGGTGGTG GCGCATCAGG TGGGGGTGGA TCAGGCGGTG GAGGTTCCGG TGCGGGGGGA
2821. TCGACAAAA CTCACACATG CCCACCGTGC CCAGCACCTG AACTCCTGGG AGGACCGTCA
2881. GTCTTCTCTT TCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
2941. ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCTTGAGG TCAAGTTCAA CTGGTACGTG
3001. GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA CAACAGCACG
3061. TACCGTGTGG TCAGCGTCTT CACCGTCTCT CACCAGGACT GGCTGAATGG CAAGGAGTAC
3121. AAGTGCAAGG TCTCCAACAA AGCCTCTCCA GCGCCCATCG AGAAAACCAT CTCCAAGGCC
3181. AAAGGGCAGC CCGGAGAAC ACAGGTGTAC ACCCTGCCCC CATCCCGCGA TGAGCTGACC
3241. AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCAGCGA CATCGCGGTG

3301. GAGTGGGAGA GCAATGGGCA GCGGAGAAC AACTACAAGA CCACGCTCTC CGTGTGGAG
3361. TCGACGGCTT CCTTCTTCTT CTACAGCAAG CTCACCGTGG ACAAGAGCAG GTGGCAGCAG
3421. GGGAACTGCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA CAGCAGAAAG
3481. AGCCTCTCTC TGTCTCCGGG TAAATGA

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

1. MVSQALRLLC LLLGLQGCLA AEVQLVQSGA EVNKPQASVK VSCKASGYTF TGYNHNVNQ

61 APGQGLEWMG WINENSGGTN YAQKFQGNVT MTRDTSISTA YMELSRRLRSD DTAVYYCARG
121 RALYNRNDRS PNWFDPWQQC TLVTVSSGSA SAPTLKLEEG EFSEARVQAV LTQPPSVSVA
181 PGQTARITCG GNNIGSKSVQ WYQKPGQAP VLVVYDDSDR PSGIPERFSG SNSGNMATLT
241 ISRVEAGDEA DYFCOVWDS SDHVVFGGGT KLTVLGQPKA APSVTLFPFS AAARTKLEWI
301 SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE THKDDQLICV NENGGCEQYC
361 SDHTGTRKSC RCHRGYSLA DGVSCPTVE YPCGKIPILE KRNASKPQGR IVGGKVCCKG
421 ECPWQVLLLV NGAQLCGGTL INTIWWVSAA HCFDKIKNWR NLIAVLGEHD LSEHDGDEQS
481 RRVAQVIIPS TYVPGTTNHD IALLRLHQPV VLTDHVVPLC LPERTFSERT LAFVRFLSVS
541 GWGQLLDRGA TALEBLMVLNV PRLMTQDCLO QSRKVGDSNP ITEYMFPCAGY SDGSKDCKG
601 DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ YIEWLQKLMR SEPRPGVLLR
661 APFPGGGGSG GGSGGGGGSG GGSGGGGGSG GGSGDKHTC PPCPAPELLG GPSVFLFPPK
721 PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL
781 TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTRNQVSLT
841 CLVKGFYPSD IATVEWESNGQ PENNYKTTTP VLDSDGSEFL YSKLTVDKSR WQGNVPSCS
901 VMHEALHNHY TQKSLSLSPG KGGGGSGGGG SGGGSGGGG SDKTHTCPPC PAPELLGGPS
961 VFLFPPKPKD TLNISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
1021 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
1081 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD SDGSFFLYSK LTVDKSRWQQ
1141 GNVFSCSVMH EALHNHYTQK SLSLSPGR*

DNA sequence FVII-039

1 ATGCTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCTGCTTG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCTTCC TAACCCAGGA GGAAGCCAC GCGCTCCTGC ACGGGGCGCG GCGCGCCAAC
121 GCGTTCTCTG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGCCCGGGA GATCTTCAAG CACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCCTTACAGT ATGGGGACCA GTGTGCTTCA AGTCCATGCC AGAATGSGGG CTCTGCAAG
301 GACCAGCTCC AGTCTATAT CTGCTTCTGC CTCCCTGCCT TCGAGGCGCG GAAGTGTGAG
361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAAGC GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGGGCAACCA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCCGA GCGGAGGAG ACTTCACTCG GGTGTGGGG
601 GGCAAGGTGT GCGCCAAAGC GGAGTGTCCA TGGCAGGTCC TGTGTGTGGT GAATGGAGCT
661 CAGTTGTGTG GGGGACCTT GATCAACACC ATCTGGGTGG TCTCCGCGGC CCACTGTTTC
721 GACAAAATCA AGAAGTGGAG GAACCTGATC GCGGTGTCTG GCGAGTACGA CCTCAGCAG
781 CACGACGGGG ATGAGCAGAG CCGGCGGGTG CCGCAGGTCA TCATCCCCAG CACGTACGTC
841 CCGGGCACCA CCAACCACGA CATCGCGCTG CTCCGCTGCG ACCAGCCCGT GGTCTCACT
901 GACCATGTGG TGCCCTCTCT CCTGCGCGAA CGGACGTTCT CTGAGAGGAC GCTGGGCTTC
961 GTGCGCTTCT CATTGGTCAG CCGCTGGGGC CAGCTGCTGG ACCGTGCGC CACGGCCCTG
1021 GAGCTCATGG TCCTCAACGT GCGCCGGCTG ATGACCCAGG ACTGCTGCA GCAGTCAAG
1081 AAGGTGGGAG ACTCCCCAAA TATCACGGAG TACATGTTCT GTGCCGCTA CTGGATGGC
1141 AGCAAGGACT CCTGCAAGGG GGACAGTGGG GCGCCACATG CCACCCACTA CCGGGGCACG
1201 TGGTACCTGA CCGGCATCGT CAGCTGGGGC CAGGGCTGCG CAACCGTGGG CCACTTTGGG
1261 GTGTACACCA GGGTCTCCCA GTACATCGAG TGGCTGCAAA AGCTCATGCG CTCAGAGCCA
1321 CGCCCGAGGAG TCCTCTGTGG AGCCCATTTT CCGGCTGCGG GTGGCTCCCG CCGAGGTGGG
1381 TCCGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGCGGTG GAGGTTCCCG TGGCGGGGGA
1441 TCCGACAAA CTCACACATG CCCACCGTGC CCAGCTCCGG AACTCCTGGG CCGACCGTCA
1501 GTCTTCCTCT TCCCCCAAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCTGAGGTC
1561 ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCTGAGG TCAAGTTCAA CTGGTACGTG
1621 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA CAACAGCAG
1681 TACCGTGTGG TCAGCGTCTT CACCGTCTCT CACCAGGACT GGCTGAATGG CAAGGAGTAC
1741 AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCGCCCATCG AGAAAACCAT CTCCAAGCC
1801 AAAGGGCAGC CCGGAGAAAC ACAGGTGTAC ACCCTGCCCC CATCCCGGGA TGAGCTGACC
1861 AAGAACCAGG TCAGCCTGAC CTGCTGGTGC AAAGGCTTCT ATCCACGCGA CATCGCGTGC
1921 GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCTTCC CGTGTGGAC
1981 TCCGACGGGT CCTTCTTCTT CTACAGCAAG CTCACCGTGG ACAAGAGCAG GTGGCAGCAG
2041 GGGAACGTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC ACAACCACTA CACGCAGAA
2101 AGCCTCTCCC TGCTCTCGGG TAAAGGTGGC GCGGATCAG GTGGGGGTGG ATCAGGCGGT
2161 GGAGGTTCCG GTGGCGGGGG ATCAGACAAA ACTCACACAT GCCACCGTG CCCAGCACCT
2221 GAACCTCTGG GAGGACCGTC AGTCTTCTCT TCCCCCAAA AACCAAGGA CACCTCATG
2281 ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCTGAG
2341 GTCAAGTTCA ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG
2401 GAGGAGCAGT ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCTT GCACAGGAC
2461 TGGCTGAATG GCAAGGAGTA CAAGTGCAGG GTCTCCAACA AAGCCCTCCC AGCCCCATC
2521 GAGAAAACCA TCTCCAAGC CAAAGGGCAG CCGCGAGAAC CACAGGTGTA CACCTGCCC
2581 CCATCCCGCG ATGAGCTGAC CAAGAACCAG GTCAGCCTGA CCTGCTGCTT CAAAGGCTTC
2641 TATCCAGCGC ACATCGCCGT GGAGTGGGAG AGCAATGCGC AGCCGGAGAA CAACTACAAG
2701 ACCACGCTC CCGTGTGGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA GCTCACCGTG
2761 GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG

2821 CACAACCACT ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAATGA

FVII-039 amino acid sequence. Signal sequence is shown in dotted underline, propeptide is double underline, 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 MVSOALRLLC LLLGLOGCLA AVFVTOEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61 SFEAREEIFK DAERTKLEWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGCEQYC SDHTGTRKSC RCHRGYSLA DGVSCPTVE YPCGKIPILE
181 KRNASKPQGR GGGDFTRVVG GKVCCKGECF WQVLLLVNGA QLOGGTLINT IWVVSAAHCF
241 DKIKNWRNLI AVLGEHDLSE HDGDEQSRV AQVIIPSTYV PGTTNHDIAL LRLHQFVVLIT
301 DHVVPICLPE RTFSERTLAF VRFLSVSGWG QLLDRGATAL ELMVLNVPLR MTQDCLOQSR
361 KVGDSNPITE YMFPCAGYSD SKDSCKGDSG GPHATHYRGT WYLTGIVSWG QGCATVGHFG
421 VYTRVSQYIE WLQKLMRSEP RPGVLLRAPF PGGGGSGGGG SGGGGSGGGG SGGGGSGGGG
481 SDKTHTCPFC PAPELLGGPS VFLFPPKPKD TLNISRTPEV TCVVVDVSHE DPEVKFNWYV
541 DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA
601 KGQPREPQVY TLPPSRDELT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTTPVLD
661 SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGRGG GGSGGGSGGG
721 GGSGGGSGG SDKTHTCPPC PAPELLGGPS ELGGGPSVFL FPKPKDITLM ISRTPEVTCV VVDVSHEDPE

781 VKENWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNKEYKCK VSNKALPAPI
841 EKTISKAKGQ PREPQVYTLR PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQENNYK
901 TTPFVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLSL LSPGK*

DNA sequence for FVII-040

1 ATGGTCTCTCC AGCCCTCTCAG GCTCCTCTTGC CTTCTGCTTG GGCTTCAGGG CTGCTTGGCT
61 GCAGTCTCTCG TAACCCAGGA GGAAGCCAC GGCGTCCTGC ACCGGCGCCG GCGCGCCAAC
121 GCGTTCCTTG AGGAGCTGCG GCCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACCGCGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATGGGGACCA GTGTGCCCTCA AGTCCATGCC AGAATGGGG CTCCTGCAAG
301 GACCAGCTCC ACTCCTATAT CTGCTTCTTGC CTCCCTGCTT TCGAGGSCCG GAACTGTGAG
361 ACGCACAAGG ATGACCAGCT GATCTGTGTG AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCACA CGCGCACCAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAATG CCAGCAAACC CCAAGGCGGC GGAGGAGACT TCACTCGGGT TGTGGGGGGC
601 AAGGTGTGCC CCAAAGGGGA GTGTCCATGG CAGGTCTGTG TGTGGTGA TGGAGCTCAG
661 TTGTGTGGGG GACCCCTGAT CAACACCATC TGGGTGGTCT CCGCGGCCCA CTGTTTCGAC
721 AAAATCAAGA ACTGGAGGAA CTTGATCGCG GTGCTGGGCG AGCAGGACCT CAGCGAGCAC
781 GACGGGGATG AGCAGAGCCG GCGGGTGGCG CAGGTCTATC TCCCAGCAC GTACGTCCCG
841 GGCACCACCA ACCACGACAT CCGCTGCTC CGCTGCACC AGCCCGTGGT CCTCACTGAC
901 CATGTGGTGC CCTCTGCTT GCCCGAACCG ACGTTCTCTG AGAGCAGCTT GGCCTTCTGT
961 CGCTTCTCAT TGGTCAGCGG CTGGGGCCAG CTGCTGGACC GTGGCGCCAC GGCCTTGGAG
1021 CTCATGGTCC TCAACGTGCC CCGGCTGATG ACCCAGGACT GCGTGCAGCA GTCACGGAAG
1081 GTGGGAGACT CCCCAAATAT CACGAGTAC ATGTTCTGTG CCGGCTACTC GGATGGCAGC
1141 AAGGACTCCT GCAAGGGGGA CAGTGGAGGC CCACATGCCA CCACTACCG GGCACGCTG
1201 TACCTGACGG GCATCGTCAG CTGGGGCCAG GGCTGCGCAA CCGTGGSCCA CTTTGGGGTG
1261 TACACCAGCG TCTCCAGTA CATCGAGTGG CTGCAAAAGC TCATGCGCTC AGAGCCACCG
1321 CCAGGAGTCC TCTGCGAGC CCCATTTCCG GGTGGCGGTG GCTCCGCGG AGGTGGGTCC
1381 GGTGGCGGCG GATCAGGTGG GGGTGGATCA GCGGTGGAG GTTCCGGTGG CCGGGGATCC
1441 GACAAAACTC ACACATGCCC ACGTGCCTCA GCTCCGGAAC TCCTGGGCGG ACGTCAAGTC
1501 TTCTCTTTCC CCCCAAACC CAAGGACACC CTCATGATCT CCCGAGCCC TGAGGTCAAC
1561 TCGTGGTGG TGGACGTGAG CCACGAAGAC CTTGAGGTCA AGTTCAACTG GTACGTGGAC
1621 GCGGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCAGTAC
1681 CGTGTGGTCA GCTCTCTCAC CGTCTCTCAC CAGGACTGGC TGAATGGCAA GGAGTACAA
1741 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1801 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCGGGATGA GCTGACCAAG
1861 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1921 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCTCCCGT GTTGGACTCC
1981 GACGGCTCTT TCTTCTCTTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
2041 AACGTCTTCT CATGCTCCTT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
2101 CTCTCCCTGT CTCGGGGTAA AGGTGGCGGC GCATCAGGTG GGGGTGGATC AGCCGTGGGA
2161 GGTTCCGGTG GCGGGGGATC AGACAAAAC CACACATGCC CACCGTGCCC AGCACCTGAA
2221 CTCCTGGGAG GACCGTCAGT CTTCTCTTTC CCCCCAAAC CCAAGGACAC CCTCATGATC
2281 TCCCGGACCC CTGAGGTAC ATGGTGGTG GTGGACGTGA GCCACGAAGA CCTGAGGTTC
2341 AAGTTCAACT GGTACGTGGA CCGCGTGGAG GTGCATAATG CCAAGACAAA GCGCGGGAG
2401 GAGCAGTACA ACAGCACGTA CCGTGTGGTC AGCGTCTTCA CCGTCCGTGA CCAGGACTGG
2461 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC CCCCATCGAG
2521 AAAACCATCT CCAAAGCCAA AGGGCAGCTC CGAGAACCAC AGGTGTACAC CCGCCCCCA
2581 TCCCGCGATG AGCTGACCAA GAACCAAGCT AGCTGGTCAA GCGGTGGTCA AGGCTTCTAT
2641 CCCAGCGATA TCGCCGTGGA GTGGGAGAGC AATGCGGAGC CGGAGACAA CTACAAGACC
2701 ACGGCTCCCG TGTGGGACTC CGAAGGCTCC TTCTTCTCTT ACAGCAAGCT CACCGTGGAC
2761 AAGAGCGGTT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC
2821 AACCACACAA 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 MLLGLOCCIA AVEVTOEEAH GVLHRRERAN AFLEELRPGS LERECKEEQC
61 SFEEAREIFK DAERTKLEFWI SYSDDGQCAS SPOQNGGSCK DQLQSYICFC LPAFEGRNCE
121 THKDDQLICV NENGGEQYVC SDHTGTRSC RCHEGYSLLA DGVSCTPTVE YPCGKIPILE
181 KRNASKPQGG GGDETRVVGG KVCPRGECFW OVLLLVNGAQ LCGTLINTI WVVSAAHCFD
241 KIKNWRNLIA VLGEHDLSEH DGDEQSRRVA QVLIPTSTYVF GTTNHDIALL RLHQFVVLTD
301 HVVPLCLPER TFSERTLAFV RFSLVSGWQG LLDGCATALE LMVLNVPRLM TQDCLQQRK
361 VGDEPNITEY MFCAGYSDGS KDSCKGDSGG PHATHYRGTW YLTGLVSWGQ GCATVGRFGV
421 YTRVSQYIEW LQKLMRSEPR PGVLLRAPFP GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS
481 DKHTTCPPCP APPELLGGPSV FLFPPPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
541 GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNKEYK CKVSNKALPA PIEKTISKAK
601 GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPEMN YKTTPEVLDS
661 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGRGGG GSGGGSGGGG
721 GSGGGGSDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV
781 KFNWYVDGVE VNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNKKEYKCKV SNKALEPAPIE
841 KTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT
901 TPFVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLSL SPGK*

DNA sequence for FIX-042

1 ATGACGCGCG TGAACATGAT ATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCCTTTA
61 GGATATCTAC TCAGTGTCTGA ATGTACAGGT TTGTTTCTTT TTTTAAATA CATTGAGTAT
121 GCTTGCCCTTT TAGATATAGA AATATCTGAT GCTGTCTTCT TCACTAAATT TTGATTACAT
181 GATTTGACAG CAATATTGAA GAGTCTAACA GCCAGCACGC AGGTGTGTAA GTACTGTGGG
241 AACATCACAG ATTTTGGCTC CATGCCCTAA AGAGAAATTG GCTTTCAGAT TATTTGGATT
301 AAAAACAAAG ACTTTCCTTA GAGATGTAAA ATTTTCATGA TGTTTTCTTT TTGCTAAAA
361 CTAAGAATTT ATTCTTTTAC ATTTCAAGTT TTCTTGATCA TGAAAACGOC AACAAAATTC
421 TGAATCGGCC AAAGAGGTAT AATTCAAGTA AATTGGAAGA GTTTGTTCAG GGAATCTAG
481 AGAGAGAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA
541 CTGAAAGAAC AACTGAATTT TGAAGCAGT ATGTTGATGG AGATCASTGT GAGTCCAATC
601 CATGTTTAAA TGGCGGCAGT TGCAAGGATG ACATTAATTC CTATGAATGT TGGTGTCCCT
661 TTGGATTTGA AGGAAAGAAC TGTGAATTAG ATGTAACATG TAACATTAAG AATGGCAGAT
721 GCGAGCAGTT TTGTAAAAAT AGTGCTGATA ACAAGGTGGT TTGCTCTCTG ACTGAGGGAT

781 ATCGACTTGC AGAAAACCAG AAGTCCTGTG AACCAGCAGT GCCATTTCCA TGTGGAAGAG
841 TTTCTGTTTC ACAAACCTCT 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 TGTGTGAAA CTGGTGTAA AATTACAGTT GTCCGAGGTG
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 CTTATGTAAG TGGCTGGGGA AGAGTCTTCC
1381 ACAAAGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTT GACCGAGCCA
1441 CATGCTCTCG ATCTACAAAG TTCACCATCT ATAACAACAT GTTCTGTGCT GGCTTCCATG
1501 AAGGAGGTAG AGATTCATGT CAAGGAGATA GTGGGGGACC CCATGTTACT GAAGTGGAAAG
1561 GGACCAGTTT CTTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCCAAAT
1621 ATGGAATATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAGGAAAAA ACAAAGCTCA
1681 CTGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG
1741 TCTTCCCTCT CCCCCCAAAA CCCAAGGACA CCTTCATGAT CTCCCGGACC CCTGAGGTCA
1801 CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
1861 ACGGCGTGGG GGTGCATAAT GCCAAGACAA AGCCCGGGGA GGAGCAGTAC AACAGCAOGT
1921 ACCGTGTGGT CAGCGTCCTC ACCGTCTGTC ACCAGGACTG GCTGAATGGC AAGGAGTACA
1981 AGTGCAAGGT CTCCAACAAA GCCCTCCGAC CCCCATCGA GAAAACCATC TCCAAAGCCA
2041 AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCTTGCCTCC ATCCCGGGAT GAGCTGAOCC
2101 AGAACCAGGT CAGCCTGACC TGCTGTGTC AAGGCTTCTA TCCAGCGAC ATCGCCGTGG
2161 AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGTTGGACT
2221 CCGACGGCTC CTTCCTCCTC TACAGCAAGC TCACCGTGGG CAAGAGCAGG TGGCAGCAGG
2281 GGAACGTCCT CTCATGCTCC GTGATGCAAG AGGCTCTGCA CAACCACTAC ACGCAGAAGA
2341 GCTCTCCCTT GTCTCCGGGT AAAGSTGGCG CCGGATCAGG TGGGGGTGGA TCAGGCGGTG
2401 GAGGTTCCGG TGGCGGGGGA TCAGACAAA CTCACACATG CCCACCTGTC CCAGCACCTG
2461 AACTCCTGGG AGGACCGTCA GTCTTCCTCT TCCCCCAAAA ACCCAAGGAC ACCCTCATGA
2521 TCTCCCGGAC CCTTGAGGTC ACATGCGTGG TGGTGGAGCT GAGCCACGAA GACCCTGAGG
2581 TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCGCGGGG
2641 AGGAGCAGTA CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
2701 GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCATCG
2761 AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCGGAGAACC ACAGGTGTAC ACCCTGCCCC
2821 CATCCCGCGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT
2881 ATCCCGCGGA CATCGCGGTG GAGTGGGAGA GCAATGGGCA GCCCGAGAAC AACTACAAGA
2941 CCACGCGCTC CGTGTGGGAC TCCGACGGCT CCTTCTTCCT CTACAGCAAG CTCACCGTGG
3001 ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT GAGGCTCTGC
3061 ACAACCACCT CACGCAGAAG AGCCTCTCCC TGTCTCCGGG TAAATGA

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

1 MQRVNMIMAE SEGLITICLL GYLLSAECTV FLDHENANKI LNRPKRYNSG KLEEFVQGNL
61 ERECMEEKCS FEEAREVFEN TERTTEPWKQ YVDGDQCESN PCLNGGSCKD DINSYECWCP
121 FGFEGKNCEL DVTGNIKNGR CEQFCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPFFPCGR

181 VSVSQTSKLT RAETVFPDVD YVNSTEAEFI LDNITQSTQS FNDFTRVVGG EDAKPGQFPW
241 QVVLNGKYDA PCCGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETHT EQKRNVIRLI
301 PHHNYNAAIN KYNHDIALLE LDEPLVNSY VTFICIADE YTNIFLKFGS GYVSGWGRVF
361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGEHVTEVE
421 GTSFLTGLIS WGEFCAMKKG YGIYTKVSRY VNWIKETKL TDKTHTCPPC PAPELLGGPS
481 VFLFPPKPKD TLMISRTPEV TCVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
541 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISRA KGQPREPQVY TLEPPSRDELT
601 KNQVSLTCLV KGFYPSDIAY EWESNGQPEN NYKITPPVLD SDGSFFLYSK LTVDKSRWQQ
661 GNVFSCSVMH EALHNHYTQK SLSLSPCKQG QSGSGGSGSG QSGSGGSGDK THTCPPCPAP
721 ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR
781 EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLF
841 PSRDELTKNQ VSLTCLVKGF YPSDIAVWEW SNGQPENNYK TTPFVLDSDG SFFLYSKLTV
901 DKSRWQQGNV FSCSVMHREAL HNHYTQKSLK LSPGK*

DNA sequence for FIX-068

1 ATGCAGCGCG TGAACATGAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCTTTTA
61 GGATATCTAC TCAGTGCTGA ATGTACAGGT TTGTTTCCTT TTTTAAAATA CATTGAGTAT
121 GCTTCCCTTT TAGATATAGA AATATCTGAT CCTGTCTTCT TCACTAAATT TTGATTACAT
181 GATTTGACAG CAATATTGAA GAGTCTAACA GCCAGCACGC AGGTTCGTAA GTACTGTGGG
241 AACATCACAG ATTTTGGCTC CATGCCCTAA AGAGAAATTG GCTTTCAGAT TATTTGEATT
301 AAAAAACAAAG ACTTCTTTAA GAGATGTAAA ATTTTCATGA TGTTTTCTTT TTTGCTAAAA
361 CTAAAGAATT ATTCTTTTAC ATTTTCAGTTT TTCTTGATCA TGAAAACGCC AACAAAATTC
421 TGAATCGGCC AAAGAGGTAT AATTCAGGTA AATTGGAAGA GTTTGTTCOA GCGAATCTAG
481 AGAGAGAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA
541 CTGAAAGAAC AACTGAATTT TGGAGCAGT ATGTTGATGG AGATCASTGT GAGTCCAATC
601 CATGTTTTAA TGGCGGCAGT TGCAAGGATG ACATTAATTC CTATGAATGT TGGTGTCCCT
661 TTGGATTTGA AGGAAAGAAC TGTGAATTAG ATGTAACATG TAACATTAAG AATGGCAGAT
721 GCGAGCAGTT TPGTAAAAAT AGTGCTGATA ACAAGGTGGT TTGCTCCTGT ACTGAGGGAT
781 ATCGACTTGC AGAAAACCAG AAGTCCGTGT AACCAGCAGT GCCATTTCCA TGTGGAAGAG
841 TTTCTGTTTC ACAAACCTCT 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 GTCCGAGGTG
1141 AACATAATAT TGAGGAGACA GAACATACAG AGCAAAAGCG AAATGTPGAT CGAATTATTC
1201 CTCACCACAA CTACAATGCA GCTATTAATA AGTACAACCA TGACATTGCC CTTCTGGAAC
1261 TGGACGAACC CTTAGTGCTA AACAGCTACG TTACACCTAT TTGCATTGCT GACAAGGAAT
1321 ACACGAACAT CTTCCTCAAA TTTGGATCTG CTTATGTAAG TGGCTGGGGA AGAGTCTTCC
1381 ACAAAGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTT GACCGAGCCA
1441 CATGCTCTTC ATCTACAAAG TTCACCATCT ATAACAACAT GTTCTGTGCT GGCTTCCATG
1501 AAGGAGGTAG AGATTCATGT CAAGGAGATA GTGGGGGACC CCATGTTACT GAAGTGGAAAG
1561 GGACCAGTTT CTTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCCAAAT
1621 ATGGAATATA TACCAAGGTG TCCCGGTATG TCAACTGGAT TAAGGAAAAA ACAAAGCTCA
1681 CTGACAAAAC TCACACATGC CCACCGTGCC CAGCTCCGGA ACTCCTGGGC GGACCGTCAG
1741 TCTTCTCTCT CCCCCCAAAA CCCAAGGACA CCTTCATGAT CTCCCGGACC CCTGAGGTCA
1801 CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
1861 TGGCGGGGGA TGGCGGGGGA TCAGACAAA CTCACACATG CCCACCTGTC CCAGCACCTG

1861 ACCGCTGTGGT GGTGCAIAAI GGCAGACAA AGCGCGGGA GCAATCAIAI AACAGACAI
1921 ACCGTGTGGT CAGCGTCTTC ACCGTCTCTC ACCAGGACTG GCTGAATGGC AAGGAGTACA
1981 AGTGCAAGGT CTCCAACAAA GCCCTCCAG CCCCATCGA GAAAACCATC TCCAAAGCCA
2041 AAGGGCAGGC CCGAGAACCA CAGGTGTACA CCTGCCCC ATCCCGGGAT GAGCTGACCA
2101 AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCITCTA TCCCAGCGAC ATCGCCGTGG
2161 AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCCTCC GTGTTGGACT
2221 CCGACGGCTC CTCTTCTCTC TACAGCAAGC TCACCGTCGA CAAGAGCAGG TGGCAGCAGG
2281 GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC ACCGAGAAGA
2341 GCCTCTCCCT GTCTCCGGGT AAACGGCGCC GCCCGAGCGG TGGCGGCGGA TCAGGTGGGG
2401 GTGGATCAGG CGGTGGAGGT TCCGCTGGCG GGGGATCCGG CGGTGGAGGT TCCCGTGGGG
2461 GTGGATCAAG GAAGAGGAGG AAGAGGGCGC AGGTGCAGCT GCAGGAGTCT GGCGGAGGCT
2521 TGGTACAGCC TGGGGGGTCC CTGAGACTCT CCTGTGAGC CTCTGGATTG ATGTTTAGCA
2581 GGTATGCCAT GAGCTGGGTC CGCCAGGCTC CAGGGAAGGG GCCAGAGTGG GTCTCAGGTA
2641 TTAGTGGTAG TGGTGGTAGT ACATACTACG CAGACTCCGT GAAGGGCCCG TTCAOCCGCT
2701 CCAGAGACAA TTCCAAGAAC ACGCTGTATC TGCAAAATGA CAGCCTGAGA GCGGAGACA
2761 CGGCTGTATA TTACTGCGCC CGGGCGGCA CCTACACAG CCGGAGCGAC GTGCGCGACC
2821 AGACCAGCTT CCACTACTGG GCGCAGGGA CCTGGTCA CGTCTCTTCA GCGAGTGCAT
2881 CGCCCCAAA GCTTGAAGAA GGTGAATTTT CAGAAGCACG CGTATCTGAA CTGACTCAGG
2941 ACCCTGCTGT GTCTGTGGCC TTGGGACAGA CAGTCAGGAT CACATGCCAA GGAGACAGCC
3001 TCAGAAACTT TTATGCAAGC TGGTACCAGC AGAAGCCAGG ACAGGCCCCT ACTCTTGTCA
3061 TCTATGGTTT AAGTAAAAGG CCTCAGGGA TCCCAGACCG ATTCTCTGCC TCCAGCTCAG
3121 GAAACACAGC TTCTTGAAC ATCACTGGGG CTCAGGCGGA AGATGAGGCT GACTATTACT
3181 GCCTGCTGTA CTACGGCGGC GCGCAGCAGG CGGTGTTCGG CGGCGGCACC AAGCTGACCG
3241 TCCTACSTCA GCCCAAGGCT GCGCCCTCGG TCACTCTGTT CCGCCCTCTT TCTGCGGCCG
3301 GTGGCGGTGG CTCCGGCGGA GGTGGGTCCG GTGGCGGCGG ATCAGGTGGG GGTGGATCAG
3361 GCGGTGGAGG TTCCGGTGGC GGGGGATCAG ACAAAACTCA CACATGCCCA CCGTGCCCAG
3421 CACCGGAACF CCGGGCGGA CCGTCAGTCT TCCCTCTCCC CCCAAAACCC AAGGACACCC
3481 TCATGATCTC CCGGACCCCT GAGGTACAT GCGTGGTGGT GGACGTGAGC CACGAGAGCC
3541 CTGAGGTCAA GTTCAACTGG TACGTGGAGC GCGTGGAGGT GCATAATGCC AAGACAAAGC
3601 CGCGGAGGA GCAGTACAAC AGCAGTACC GTGTGGTCAG CGTCTCACC GTCTGCACC
3661 AGGACTGGCT GAATGGCAAG GAGTACAAGT GCAAGGTCTC CAACAAAGCC CTCCCAGCCC

3721 CCATCGAGAA AACCATCTCC AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC
3781 TGCCCCCATC CCGCATGAG CTGACCAAGA ACCAGGTGAG CCTGACCTGC CTGGTCAAAG
3841 GCTTCTATCC CAGCGACATC GCGGTGGACT GGGAGAGCAA TGGGCAGCCG GAGAACAAC
3901 ACAAGACCAC GCCTCCCGTG TTGGACTCCG ACGGCTCCTT CTTCCTCTAC AGCAAGCTCA
3961 CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTCTC ATGCTCCGTG ATGCATGAGG
4021 CTCTGCACAA CCACTACAG 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 FLDHENANKL LNRPKRYNSG KLEEFVQGNL
61 ERECMEEKCS FREAREVPEN TERTTEFWKQ YVDGDQCESN PCLNGGSKD DINSYECWCP
121 PGFEGKNCGL DVTCNIKNGR CEQFCINSAD NKVVCSTEG YRLAENQKSC EPAPVPFCGR
181 VSVSQTSKLT RAETVFFDVD YVNSTEASTI LDNITQSTQS ENDFTRVVG EDAPGQFPW
241 QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIETEHT EQRNVIRII
301 PHHNYNAIN KYNHDIALLE LDEPLVLSY VTPICIADE YTNIFLKEFS GYVSGWGRVF
361 HKGRSALVLQ YLRVPLVDRA TCLRSTFTPI YNMFCAGFH EGGRDSCQGD SGGPHVTEVE
421 GTSFLTGIS WCEECAMKCK YGIYTRVSRV VNWIKETIKL TDKHTCPPC PAPELLOGPS
481 VFLFPPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV DGVEVHNAKT KPREEQYNST
541 YRVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDEL
601 KNQVSLTCLV KGFYPSDIIV EWESNGQPEN NYKTTPEVLD SDGSFFLYSK LTVDKSRWQQ
661 GNVFSCSVMH EALHNHYTQK LSLSPGKRR **RRSGGGSGSG** **GGSGGGSGSG** **GGSGGGSGSG**
721 **GGSRRRRRRA** QVQLQESGGG LVQPGGSLRL SCAASGMFPS RYAMSWVRQA PGKPEWVSG
781 ISGSGGSTYV ADSVKGRFTV SRDNSKNILY LQMNSLRAED TAVYYCARGA TYTSRSDVPD
841 QTSFDYWGQG TLVTVSSGSA SAPKLEEGEF SEARVSELTQ DPAVSVALLQ TVRITCQGDS
901 LRNFYASWYQ QKPGQAFITV IYGLSKRPSG IPDRFSASS GNTASLTITG AQAEDADYY
961 CLLYYGGGQQ GVFGGGTKLT VLRQPKAAPV VTLEPPSSAA GGGGSGGGSG GGGGSGGGSG
1021 GGGGSGGGSG DKHTCTCPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
1081 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
1141 PIEKTIKAK GQPREPQVYT LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPEN
1201 YKTTPEVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMH EALHNHYTQKS LSLSPGK*

DNA sequence for FIX-088

1 ATGCAGCGCG TGAACATGAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCTTTT
61 GGATATCTAC TCAGTGCTGA ATGTACAGGT TTGTTTCTCT TTTTAAAATA CATTGAGTAT
121 GCTTGCCTTT TAGATATAGA AATATCTGAT CCTGTCTTCT TCACTAAATT TTGATTACAT
181 GATTTGACAG CAATATTGAA GAGTCTAACA GCCAGCACCG AGGTTCGTAA GTACTGTGGG
241 AACATCACAG ATTTTGGCTC CATGCCCTAA AGAGAAATTG GCTTTCAGAT TATTTGGATT
301 AAAAACAAAG ACTTTCTTAA GAGATGTAAA ATTTTCATGA TGTTTTCTTT TTGCTAAAA
361 CTAAAGAATT ATCTTTTAC ATTTTCAGTTT TTCTTGATCA TGAAAACGCC AACAAAATTC
421 TGAATCGGUC AAAGAGCTAT AATTCAGGTA AATTGGAAGA CTTTGTTCAT GCCAATCTAG
481 AGAGAGAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTGAAAGACA
541 CTGAAAGAAC AACTGAATTT TGGAGCAAGT ATGTTGATGG AGATCAGTGT GAGTCCAATC
601 CATGTTTTAA TGGCGGCAGT TCCAAGGATG ACATTAATTC CTATGAATGT TGGTGTCCCT
661 TTGGATTGTA AGGAAAGAAC TGTGAATTAG ATGTAACATG TAACATTAAG AATGGCAGAT
721 GCGAGCAGTT TTGTAAAAAT AGTGTCTGATA ACAAGGTGGT TTGCTCCTGT ACTGAGGAT
781 ATCGACTTGC AGAAAACCAG AAGTCTCTGTG AACCAGCAGT GCCATTTCCA TGTGGAAGAG
841 TTTCTGTTTC ACAAACCTCT AAGCTCAGCC GTGCTGAGAC TGTTTTCTCT GATGTGGACT
901 ATGTAAATTC TACTGAAGCT GAAACCATTT TGGATAACAT CACTCAAAGC ACCCAATCAT
961 TTAATGACTT CACTCGGGTT GTTGGTGGAG AAGATGCCAA ACCAGGTCAA TTCCCTTGGC
1021 AGGTGTTTTT GAATGGTAAA GTTGAATGAT TCTGTGGAGG CTCTATCGTT AATGAAAAAT
1081 GGAATTGTAAC TGTGTTGAAA TGTGTTGAAA CTGGTGTATA AATTACAGTT GTCGCAAGTG
1141 AACATAATAT TGAGGAGACA GAACATACAG AGCAAAAGCG AAATGTGATT CGAATTATTC
1201 CTCACCACAA CTACAATGCA GCTATTAATA AGTACAACCA TGACATGACC CTTCTGGAAC
1261 TGGACGAACC CTAGTGTGTA AACAGCTACG TTACACCTAT TTGCAATGCT GACAAGGAAT
1321 ACACGAACAT CTTCTCTCAA TTTGGATCTG CCTATGTAAG TGGCTGGGGA AGAGTCTTCC
1381 ACAAAGGGAG ATCAGCTTTA GTTCTCTCAG ACCTTAGAGT TCCACTGTTT GACCGAGCCA
1441 CATGTCTTUG ATCTACAAAG TTCACCATCT ATAACAACAT GTTCTGTGCT GGCTTCCATG
1501 AAGGAGGTAG AGATTCATGT CAAGGAGATA GTGGGGGACC CCATGTTACT GAAGTGGAG

1561 GGACCAAGTTT CTAACTGGA ATTATTAGCT GGGGTGAAGA GTGTGCAATG AAAGGCAAAT
1621 ATGGAATATA TACCAAGGTC TCCCCGATG TCAACTGGAT TAAGCAAAA ACAAGGCTCA
1681 CTGACAAAAAC TCACACATGC CCACCGTGGC CAGCTCCGGA ACTCCCTGGC GGACCGTCAG
1741 TCTTCCCTCTT CCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGAAC CCTGAGSTCA
1801 CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
1861 ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT
1921 ACCGTGTGGT CAGCGTCCTC ACCGTCCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA
1981 AGTGCAAGGT CTCCAACAAA GCCCTCCAG CCCCCTCGA GAAAACCATC TCCAAAGCCA
2041 AAGGGCAGTC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAT GAGCTGACCA
2101 AGAACCAGGT CAGCCTGACC TCCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGNGG
2161 AGTGGGAGAG CAATGGGCAG CCGGAGAAC ACTACAAGAC CACGCTCTCC GTGTTGGACT
2221 CCGACGGCTC CTCTTCTCTC TACAGAGTCT TACCCGTGGA CAAGAGCAGG TGGCAGCAGG
2281 GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA
2341 GCCTCTCCCT GTCTCCGGGT AAAGGTGGCG GCGGATCAGG TGGGGGTGGA TCAGGCGGTG
2401 GAGGTTCCCG TGGCGGGGGA TCAGACAAA CTCACACATG CCCACCGTGC CCAGCACCTG
2461 AACTCCTGGG AGGACCGTCA GTCTTCTCTT TCCCCCAAA ACCCAAGGAC ACCCTCATGA
2521 TCTCCCGGAC CCCTGAGGTC ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG

2581 TCAAGTTCAA CTGGTACGTG GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCGCGGGG
2641 AGCAGCAGTA CAACAGCAGC TACCGTGTGG TCAGCGTCTT CACCGTCTCT CACCAGGACT
2701 GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG
2761 AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCGGAGAACC ACAGGTGTAC ACCCTGCCCC
2821 CATCCCGCGA TGAGCTGACC AAGAACCAGG TCAGCCTGAC CTGCCCTGTC AAAGGCTTCT
2881 ATCCCGCGGA CATCGCCGTG GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA
2941 CCACGCTCTC CGTGTGGGAC TCCGACGGCT CCTTCTTCTT CTACAGCAAG CTCACCGTCC
3001 ACAAGAGCAG GTGGCAGCAG GGAACGTCTT TCTCATGCTC CGTGATGCAT GAGGCTCTGC
3061 ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG TAAAGGTGGC GGTGGCTCCG
3121 GCGGAGGTGG GTCCGCTGGC GCGGATCAG GTGGGGGTGG ATCAGGCGGT GGAGGTTCGG
3181 GTGGCGGGGG ATCAGCGCAG GTGCAGCTGC AGGAGTCTGG GGGAGGCTTG GTACAGCCTG
3241 GGGGCTCCCT GAGACTCTCC TGTGCAGCCT CTGATTCTAT GTTTAGCAGG TATGCCATGA
3301 GCTGGGTCCG CCAGGCTCCA GGAAGGGGGC CAGAGTGGGT CTCAGGTAAT AGTGGTAGTG
3361 GTGGTAGTAC ATACTACGCA GACTCCGTGA AGGCGCGGTT CACCGTCTCC AGACACAATT
3421 CCAAGAACAC GCTGTATCTG CAAATGAACA GCCTGAGAGC CGAGGACAGG GCTGTATATT
3481 ACTGCGCCCG GGGCGCCACC TACACCAGCC GGAGCGACGT GCCCGACCAG ACCAGCTTCG
3541 ACTACTGGGG CCAGGCAACC CTGCTCACC TCTCCTCAGG GAGTGCATCC GCCCCAAGC
3601 TTGAAGAAGG TGAATTTTCA GAAGCAGCGG TATCTGAACG GACTCAGGAC CCGCTGTGT
3661 CTGTGGCCTT GGGACAGACA GTCAGGATCA CATGCCAAGG AGACAGCCTC AGAAACTTTT
3721 ATGCAAGCTG GTACCAGCAG AAGCCAGGAC AGGCCCCCTA TCTTGTCTAT TATGGTTTAA
3781 GTAAAAGGCC CTCAGGGATC CCAGACCGAT TCTCTGCCTC CAGCTCAGGA AACACAGCTT
3841 CCTTGACCAT CACTGGGGCT CAGGCGGAAG ATGAGGCTGA CTATTACTGC CTGCTGTACT
3901 ACGGCGGGCG GTGTTCCGGC GCGGCACCAA GCTGACCGTC CTACGTGAGC
3961 CCAAGGCTGC CCCCTCGGTC ACTCTGTCTC CGCCTCTCTT TCGGCGCTGA

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

1 MQRVNMIMAE SPGLITICLL GYLLSAECTV RLDHENANKI LNRPKRYNSG KLEEFVQGNL
61 ERECMEEKCS FEEAREVPEN TERTTEFWKQ YVDGDOCESN PCLNGGSCKD DINSYECWCP
121 PGFEGKNCGL DVTCNIKNGR CEQPCRN SAD NKVVCSCTEG YRLAENQKSC EPAVPFPCGR
181 VSVSQTSKLT RAETVFFDVD YVNSTEAETI LDNITQSTQS FNDFTRVVG EDAKPGQFPW
241 QVVLNGKVDA PCGGSIVNEK WIVTAAHCVE TGVKITVVAG EHNIEETEHT BQKENVIRII
301 PHHNYNAAIN KYNHDIALLE LDEPLVLNSY VTPICIADKE YTNIFLKFGS GYVSGWGRVP
361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SCGPHVTEVE
421 GTSFLTGLIS WGEECAMK GK YGIYTKVSRY VNWIKEKTKL TDKHTTCPPC PAPELLGGPS
481 VFLFPKPKDK TLMISRTPEV TCVVDVSH E DPEVKFNWYV DGVEVHNAKT KPREEQYNST
541 YRVVSVLTVL HQDWLNGKEY KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT
601 KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
661 GNVFSCSVMH EALHNHYTOR SLSLSPGRGG GGSGGGSGSG GGSGGGSGDK THTCPPCPAP
721 ELLGCFSVFL PPKPKKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDCV EVHNAKTKPR
781 EEQYNSTYRV VSVLTVLHQD WLNGKEYCK VSNKALPAPI EKTISKAKGQ PREPQVYTL P
841 PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV
901 DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPCKGGGGS GGGGSGGGGS GGGGSGGGGS
961 GGGGSAQVQL QESGGGLVQF GGSLRLSCAA SGFMPSTRYAM SWVRQAPGKG PEWVSGISGS
1021 GGSTYYADSV KGRFTVSRDN SKNTLYLQMN SLRAEDTAVY YCARGATYTS RSDVPDQTSF
1081 DYWGQSTLV VSSGSASAPK LEEFGSLEAR VSELTQDPAV SVALGQTVRI TCQGDLSLENF
1141 YASWYQKPKG QAPTLVLYGL SKRPSGIPDR PSASSSGNTA SLTITGAQAE DEADYYCLEY
1201 YGGGQQGVFG GGTKLTVLRQ PKAAPSVTLF PPSSAA*

DNA sequence for FIX-089

1 ATGCACGCGC TGAACATGAT CATGGCAGAA TCACCAGGCC TCATCACCAT CTGCCCTTTTA
61 GGATATCTAC TCAGTGCTGA ATGTACAGGT TTGTTTCTTT TTTTAAAAATA CATTGAGTAT
121 GCTTGCCCTT TAGATATAGA AATATCTGAT CCTGTCTTCT TCACTAAATT TTGATTACAT
181 GATTTGACAG CAATATTGAA GAGTCTAACA GCGAGCAGCG AGGTTGGTAA GTACTGTGGG
241 AACATCACAG ATTTTGGCTC CATGCCCTAA AGAGAAATTC GCTTTCAGAT TATTTGCAAT
301 AAAAACAAG ACTTTCTTAA GAGATGTAAA ATTTTCATGA TGTTTCTTT TTTGCTAAAA
361 CTAAAGAATT ATTCTTTTAC ATTTCACTTT TTCTTGATCA TGAACACGCC AACAAAATTC
421 TGAATCGGCC AAAGAGGTAT AATTGAGGTA AATTGGAAGA GTTTGTTCAT GGGAACTCTAG
481 AGAGAGAATG TATGGAAGAA AAGTGTAGTT TTGAAGAAGC ACGAGAAGTT TTTGAAAACA
541 CTGAAAGAAC AACTGAATTT TGAAGCAGT ATGTTGATGG AGATCAGTGT GAGTCCAATC
601 CATGTTTAAA TGGCGGCAGT TCAAGGATG ACATTAATTC CTATGAATGT TGGTGTCCCT
661 TTGGATTGTA AGCAAGAAGC TGTGAATTAG ATGTAACATG TAACATTAAG AATGGCAGAT
721 GCGAGCAGTT TTGTAAAAAT AGTGCTGATA ACAAGGTGGT TTGCTCCTGT ACTGAGGGAT
781 ATCGACTTGC AGAAAAACAG AAGTCCTGTG AACCAGCAGT GCCATTTCCA TGTGGAAGAG
841 TTTCTGTCTT ACAAACTTCT AAGCTCACCC GTGCTGAGAC TGTTTTCTCT GATGTGGACT
901 ATGTAAATTC TACTGAAGCT GAAACCATTT TGGATAACAT CACTCAAAGC ACCCAATCAT
961 TTAATGACTT CACTCGGGTT GTTGGTGGAG AAGATGCCAA ACCAGGTCAA TTCCCTTGGC
1021 AGSTTGTTTT CAATGCTAAA GTTGATGCAT TCTGTGGAGG CTCTATCGTT AATGAAAAAT
1081 GGATTGTAA C TGCTGCCAC TGTGTTGAAA CTGGTGTATA AATTACAGTT GTCCGAGGTG
1141 AACATAATAT TGAGGAGACA GAACATACAG AGCAAAAGCG AAATGTGATT CGAATTATTC
1201 CTCACCACAA CTACAATGCA GCTATTAATA AGTACAACCA TGACATFGCC CTTCTGGAAC
1261 TGGACGAACC CTTAGTGCTA AACAGCTACG TTACACCTAT TTGCATTGCT GACAAGGAAT

1321 ACACGAACAT CTTCCTCAAA TTTGGATCTG GCTATGTAAG TGGCTGGGGA AGAGTCTTCC
1381 ACAAAGGGAG ATCAGCTTTA GTTCTTCAGT ACCTTAGAGT TCCACTTGTG GACCGAGCCA
1441 CATGTCTTCG ATCTACAAAG TTCGCCATCT ATAACAACAT GTTCTGTGCT GGCTTCCATG
1501 AAGGAGGTAG AGATTCATGT CAAGGAGATA GTGGGGGACC CCATGTTACT GAAGTGGAAAG

1561	GGACCAGTTT	CTTAACTGGA	ATTATTAGCT	GGGGTGAAGA	GTGTGCAATG	AAAGGCCAAAT
1621	ATGGAATATA	TACCAAGGTG	TCCCGGTATG	TCAACTGGAT	TAAGGAAAAA	ACAAAGCTCA
1681	CTGACAAAAC	TCACACATGC	CCACCGTGCC	CAGCTCCGGA	ACTCCTGGGA	GGACCGTCAG
1741	TCTTCTCTTT	CCCCCAAAA	CCCAAGGACA	CCCTCATGAT	CTCCCGGACC	CTCGAGGTCA
1801	CATGCGTGGT	GGTGGACGTG	AGCCACGAAG	ACCCTGAGGT	CAAGTTCAAC	TGCTACGTGG
1861	ACGGCGTGGA	GGTGCAATAT	GCCAAGACAA	AGCCGCGGGA	GGAGCAATAC	AACAGCAAGT
1921	ACCGTGTGGT	CAGCGTCTCT	ACCGTCTCTC	ACCAGGACTG	GCTGAATTGG	AAGGAGTACA
1981	AGTGCACAGT	CTCCAAACAA	GCCCTCCAG	CCCCATCGA	GAAAACCATC	TCCAAAGCCA
2041	AAGGGCAGCA	CCGGAACCA	CAGGTGTACA	CCCTGCCCC	ATCCCGGAT	GAGCTGACCA
2101	AGAAACCAGT	CAGCCTGACC	TGCTTGGTCA	AAGGCTTCTA	TCCCAGCGAC	ATCGCCGTGG
2161	AGTGGGAGAG	CAATGGGCAG	CCGGAGAACA	ACTACAAGAC	CACGCCCTCC	GTGTTGGACT
2221	CCGACGGCTC	CTTCTTCTCT	TACAGCAAGC	TCAACCGTGA	CAAGAGCAGG	TGGCAGCAGG
2281	GGAACTCTTT	CTCATGCTCC	GTGATGCATG	AGGCTCTGCA	CAACCCATAC	ACGCAGAGA
2341	GCTCTCTCTT	GTCTCCGGT	AAAGCCGGTG	CGGGTTCAGG	TGAGGAGAGG	TACGGCGGT
2401	GTGGATCCGG	CGGGGGCGGA	TCCGGTGGCG	GAGGGTCAGG	CGGTGGCGGA	TCAGCCTGCA
2461	CCGAGCGGAT	GCCCTGAC	AACCTGTGCG	GTGGCGGTGG	CTCCGGCGGA	GGTGGGTCCG
2521	GTGGCGCGCG	ATCAGGTGGG	GGTGGATCAG	CGCGTGGAGG	TTCGGGTGGC	GGGGGATCCG
2581	ACAAAACCTCA	CACATGCCCA	CCGTGCCAC	CACCGGAAT	CCTGGGCGGA	CCGTGAGTCT
2641	TCTTCTCTCT	CCCAAAACCC	AAGGACACC	TCATGATCTC	CCGACCCCT	GAGTCAAT
2701	GCGTGGTGGT	GGACGTGAGC	CACGAAGACC	CTGAGGTCAA	GTTCAAATGG	TACGTGGAAG
2761	GCGTGGAGGT	GCATAATGCC	AAGACAAAGC	CGCGGGAGGA	GCAGTACAAC	AGCAAGTACC
2821	GTGTGGTCA	CGTCTCTACC	GTCTCTGACC	AGGACTGGCT	GAATGGCAAG	GAGTACAAGT
2881	GCAAGGTCTC	CAACAAAGCC	CTCCAGGCC	CCATCGAGAA	AACCATCTCC	AAAGCCAAAG
2941	GGCAGCCCCG	AGAACCACAG	GTGTACACCC	TGCCCCCATC	CCGGATGAG	CTGACCAAGA
3001	ACCAGETCAG	CCTGACCTGC	CTGGTCAAAG	GCTTCTATCC	CAGCGACATC	GCCGTGGAGT
3061	GGGAGAGCAA	TGGGCAGCCG	GAGAACAACT	ACAAGACCAC	GCCTCCCGTG	TTGGAATCCG
3121	ACGGTCTCCT	CTTCTCTTAC	AGCAAGCTCA	CCGTGGACAA	GAGCAGGTGG	CAGCAGGGGA
3181	AGCTCTTCTT	ATGCTCCGTG	ATGCATGAGG	CTCTGCACAA	CCACTACACG	CAGAAGAGCC
3241	TCTCCCTGTC	TCCGGGTAAA	TGA			

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

1	MORVMIMIAE	SPGLITICLL	GYLLSAECTV	FLDHENANKI	LNRPKRYNSG	KLBEFVQGNL
61	ERECEEEKCS	FEEAREVPEN	TERTTEFWKQ	YVDCGQCESN	PCLNGCSCKD	DINSYECWCP
121	PGFEGKNCCL	DVTCNIKNGR	CEQPCKNASD	NKVVCSCTEG	YRLAENQKSC	EPAPVPFPCGR
181	VGVSQTSKLT	RAETVFPPDV	YVNSTEAETI	LDNITQSTQS	FNDFTRVVG	EDAKPFGQFPW
241	QVVLGKQVDA	FCGGSIYNBK	WIVTAACHVE	TGVKITVVAG	EHNIETEETH	EQRKNVIRII
301	PHHNYNAAIN	KYNHDIALLE	LDEPLVLNSY	WPTICADKE	YTNI FLKFGS	GYVSGWGRVF
361	HKGRSALVLQ	YLRVPLVDRA	TCLRSTKPTI	YNNMFCACFH	EGGRDSCQGD	SGGPHVTEVE
421	GTSPLTGLIS	WGEECAMKCK	YGIYTKVSR	VNWIKBKIKL	TDKTHTCPPC	PAPELLCGPS
481	VPLFPFKPKD	TLMISRTPEV	TCVVVDVSH	DPEVTFNMYV	DGVEVHNATK	KPREEQYNST
541	KRVVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIETISKAK	KGQPREPQVY	TLTSPSRDEL
601	YKVVSLTCLV	KGFYPSPDIA	EWESNGQPEN	NYKTTTPVLD	SDGSFFLYSK	TLVDKSRWQQ
661	GNVFPSCVMH	EALHNHYTQK	LSLSLSPGKG	GGSGGGGGSG	GGSGGGGGSG	GGSGGGGGSG
721	TERMALHNLQ	GGGGGGGGSG	GGGGGGGGSG	GGGGGGGGSG	DKTHTCPCP	APELLGCPSP
781	FLFPPKPKDT	LMISRTPEVT	CVVVDVSHED	PEVYKFNWYD	GVEVHNATK	PREEQYNSTY
841	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALP	PIETISKAK	KGPREPQVY	LPPSRDELTK
901	NQVSLTCLVK	GFYPSPDIAE	WEESNGQPEN	YKTTTPVLD	DGSFFLYSKL	TVDKSRWQQG
961	GNVFPSCVMH	ALHNHYTQKS	LSLSLSPGK*			

DNA sequence for FIX-090

1	ATGCAGCGCG	TGAACATGAT	CATGGCAGAA	TCACCAGGCC	TCATCACCAT	CTGCCTTTTA
61	GGATATCTAC	TCAGTGCTGA	ATGTACAGGT	TTGTTTCCTT	TTTTAAAATA	CATTGAGTAT
121	GCTTGCCTTT	TAGATATAGA	AATATCTGAT	GCTGTCTTCT	TCACTAAATT	TTGATTACAT
181	GATTTGCACG	CAATATTGAA	GAGTCTAACA	CCCAGCACGC	AGGTTGGTAA	GTACTGTGGG
241	AACATCCACG	ATTTTGGCTC	CATGCCCTAA	AGAGAAATFG	GCTTTCAGAT	TATTTGGATT
301	AAAAACAAG	ACTTCTTAA	GAGATGTAAA	ATTTTTCATGA	TGTTTTCTTT	TTTGCTAAAA
361	CTAAAGAATT	ATTCTTTTAC	ATTTCAAGTT	TTCTTGATCA	TGAAAACGCC	AACAAAATTC
421	TGAATCGGCC	AAGAGGGTAT	AATTCAGGTA	AATTGGAAGA	GTTTGTTCAA	GGGAATCTAG
481	AGAGAGAATG	TATGGAAGAA	AAGTGTAGTT	TTGAAGAAGC	ACGAGAAGTT	TTTGAANAAC
541	CTGAAAGAAT	AACATGAATTT	TGGAAGCAGT	ATGTTGATGG	AGATCAGTGT	GATGCCAATC
601	CATGTTTAAA	TGCGCGCACT	TGCAAGCATC	ACATTAATTC	CTATCAATGT	TGGTCTCCTT
661	TTGGATTTGA	AGGAAAGAAC	TGTGAATTAG	ATGTAACATG	TAACATTAAG	AATGGCAGAT
721	GCGAGCAGTT	TTGTAAAAAT	AGTGCCTGATA	ACAAGGTGGT	TTGCTCTCTG	ACTGAGGGAT
781	ATCGAGTTCC	AGAAAACCCG	AAGTCCCTGT	AATCCAGCAGT	GCCATTTTCCA	TGTGGAAGAG
841	TTTCTGTTTC	ACAAACTTCT	AAGCTCACCC	GTGCTGAGAC	TGTTTTCTCT	GATGTGGACT
901	ATCTAAATTC	TACTGAAGCT	GAAACCATTT	TGGATAACAT	CACTCAAAGC	ACCCAATCAT
961	TTAATGACTT	CATCGGGTT	GTTGGTGGAG	AAGATGCCAA	ACCAGGTCAA	TTCCCTTGGC
1021	AGGTTGTCTT	GAATGGTAAA	GTTGATGCAT	TCTGTGGAGG	CTCTATCGTT	AATGAAAAAT
1081	GGAATGTAAAC	TGCTGCCCCA	TGTGTTGAAA	CTGGTGTATA	AAATTACAGT	GTCGCAGGTG
1141	AACATAATAT	TGAGGAGACA	GACATAACAG	AGCAAAAGCG	AAATGTGATT	CGAATTATTC
1201	CTCACCACAA	CTACAATGCA	GCTATTAAATA	AGTACAACCA	TGACATTGCC	CTTCTGGAAC
1261	TGGACGAACC	CTTAGTGCTA	AACAGCTACG	TTACACCTAT	TTGCATTGCT	GACAGGGAAT
1321	ACACAGACAT	CTTCTCTCAA	TTTGGATCTG	GCTATTGTAAG	TGGCTGGGGA	AGAGCTTCTC
1381	ACAAAGGGAG	ATGACCTTTA	TTCTCTCAGT	ACCTTAGAGT	TCCACTTGTG	GACCGGACCA
1441	CATGTCTTCG	ATCTACAAAG	TTCCACCATCT	ATAACAACAT	GTTCTGTGCT	GGCTTCCATG

1501	AAGGAGGTAG	AGATTCATGT	CAAGGAGATA	GTGGGGGACC	CCATGTTACT	GAAGTGGAAAG
1561	CGACCACTTT	CTTAACTGGA	ATTATTAGCT	CGGCTGAAGA	GTGTGCCAATG	AAAGGCCAAAT
1621	ATGGAATATA	TACCAAGGTG	TCOCGGTATG	TCAACTGGAT	TAAGGAAAAA	ACAAAGCTCA
1681	CTGGTGGCGG	TGCGTCCGGC	GGAGTTCGGT	CCGGTGGCGG	CGGATCAGGT	GGCGGTGGAT
1741	CAGGCGGTGG	AGGTTCCGGT	GGCGGGGGAT	CAGCGCGCGG	GCAGCTGCAG	GAGTCTGGGG
1801	GAGGCTTGGT	ACAGCCTGGG	GGGTCCCTGA	GACTCTCCTG	TGACAGCCTCT	GGATTCATGT

1861 TTAGCAGGTA TGCCATGAGC TGGGTCCGCC AGGCTCCAGG GAAGGGGCCA GAGTGGGTCT
1921 CAGGTATTAG TGGTAGTGST GGTAGTACAT ACTACGCAGA CTCCGTGAAG GGCCGGTTCA
1981 CCGTCTCCAG AGACAATTCC AAGAACACGC TGTATCTGCA AATGAACAGC CTGAGAGCCG
2041 AGGACACGGC TGTATATTAC TGCGCCCGGG GCGCCACCTA CACCAGCCGG AGCGACGTGC
2101 CCGACCAGAC CAGCTTCGAC TACTGGGGCC AGGGAACCCCT GGTCACCGTC TCCTCAGGGA
2161 GTGCATCCGC CCCAAAGCTT GAAGAAGGTG AATTTTCAGA AGCACGCGTA TCTGAACCTGA
2221 CTCAGGACCC TGCTGTGTCT GTGGCCTTGG GACAGACAGT CAGGATCACA TGCCAAGGAG
2281 ACAGCCTCAG AAACCTTTAT GCAAGCTGGT ACCAGCAGAA GCCAGGACAG GCCCCTACTC
2341 TTGTCATCTA TCGTTTAAGT AAAAGGCCCT CAGGGATCCC AGACCGATTG TCTGCCTCCA
2401 GCTCAGGAAA CACAGCTTCC TTGACCATCA CTGGGGGTCA GGCGGAAGAT GAGGCTGACT
2461 ATTACTGCCT GCTGTACTAC GGCGGCGGGC AGCAGGGCGT GTTCGGCGGC GGCACCAAGC
2521 TGACCGTCCG ACGTCAGCCC AAGGCTGCCC CCTCGGTACG TCTGTTCCTG CCTCTTCTG
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.

1 MQRVNMIMAE SPGLITICLL GYLLSABCTV PLDHEENANKL LNRPKRYNSG KLEEFVQGNL
61 ERECMEEKCS FEAREVPEN TERTTEFWKQ YVDGDQCESN PCLNGGCKD DINSYECWCP
121 FGFEGKNCEL DVTCKIKNGR CEQFCNSAD NKVVCSTEG YRLAENQKSC EPAVPFFQGR
181 VSVSQTSKLE RAETVFFDVD YVNSTAEETI LDNITQSTQS FNDFTRVVG EDAKPGQFPW
241 QVVLNGKVDA FCGGSIVNER WIVTAAHCVE TGVKITVVG EHNIEETHT EQRNVIRII
301 PHHNYNAAIN KYNHDIALLE LDEPLVNSY VTPICIAKRE YTNIFLRFGS GYVSGWGRVP
361 HKGRSALVLQ YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
421 GTSFLTGIIS WGEBCAMKCK YGIYTKVSRV VNWIKETKTL TGGGGSGGGG SGGGGGGGGG
481 SGGGGGGGGG SAQVQLQESG GGLVQPGGSL RLSCAASGFM FSRYAMSWVR QAPGKGPWV
541 SGISGSGGST YYADSVKGRF TVSRDNSKNT LYLQMNLSLA EDTAVVYCAR GATYTSRSDV
601 PDQTSFDYWG QGTLVTVSSG SASAPKLEEG EFSEARVSEL TQDPFVSVL GQTVRITCQG
661 DSLRNFYASW YQKPGQAPT LVIYGLSRKP SGIPDRFSAS SSGNTASLTI TGAQAEDAD
721 YYCLLYYGGG QQGVFGGGK LTVLRQPKAA PSVTLFPSS AA*

DNA sequence for FVII-088

1 ATGGTCTCCC AGGCCCTCAG GCTCCTCTGC CTTCCTGCTTG GGCTTCAGGG CTGCCTGGCT
61 GCAGTCTTCG TAACCCAGGA GGAAGCCAC GGCGTCTGC ACCGGCGCCG GCGCGCCAAC
121 GCGTTCCTGG AGGAGCTGCC GCGGGCTCC CTGGAGAGGG AGTGCAAGGA GGAGCAGTGC
181 TCCTTCGAGG AGGCCCGGGA GATCTTCAAG GACGCGGAGA GGACGAAGCT GTTCTGGATT
241 TCTTACAGTG ATGGGGAACA GTGTGCCCTCA AGTCCATGCC AGAATGGGGG CTCCTGCAAG
301 GACCAGCTCC AGTCCTATAT CTGCTTCTGC CTCCTGCCT TCGAGGSCCG GAACTGTGAG
361 ACGCACAAGG ATGACCAGCT GATCTGTGTC AACGAGAACG GCGGCTGTGA GCAGTACTGC
421 AGTGACCAAA CGGGCACAAA GCGCTCCTGT CCGTGCCACG AGGGGTACTC TCTGCTGGCA
481 GACGGGGTGT CCTGCACACC CACAGTTGAA TATCCATGTG GAAAAATACC TATTCTAGAA
541 AAAAGAAATG CCAGCAAAACC CCAAGGCCGA ATTGTGGGGG GCAAGGTGTG CCCCAGAGG
601 GAGTGTCCAT GGCAGGTCTT GTTGTGGTG AATGGAGCTC AGTTGTGTGG GGGGACCCCTG
661 ATCAACACCA TCTGGGTGGT CTCCGCGGCC CACTGTCTCG ACAAATCAA GAACTGGAGC
721 AACCTGATCG CGGTGCTGGG CGAGCACGAC CTCAGCGAGC ACGACGGGGA TGAGCAGAGC
781 CGGCGGGTGG CGCAGGTCTAT CATCCCAGC ACGTACGTCC CGGGCACCAC CAACCAAGAC
841 ATCGCGCTGC TCCGCTTGA CACAGCCGTG CTCTCACTG ACCATGTGGT GCGCCTCTGC
901 CTGCCCCAAC GGACGTTCTC TGAGAGGAGC CTGGCCTTCG TGCCTTCTC ATTGGTCAGC
961 GCGTGGGGCC AGCTGCTGGA CCGTGCCGCC ACGGCCCTGG AGCTCATGGT CCTCAACGTG
1021 CCGCGGCTGA TGACCCAGGA CTGCCCTGCAG CAGTCACGGA AGGTGGGAGA CTCCCCAAAT
1081 ATCACGGAGT ACATGTTCTG TCCCGGCTAC TCGGATGGCA GCAAGGACTC CTGCAAGGGG
1141 GACAGTGGAG GCCACATGTC CAOCCACTAC CGGGGCACGT GGTACCTGAC GGGCATCGTC
1201 AGCTGGGGCC AGGGCTGCGC AACCGTGGG CACTTTGGGG TGTACACCAG GGTCTCCCAG
1261 TACATCGAGT GGCTGCAAAA GCTCATGGC TCAGAGCCAC GCCCAGGAGT CCTCCTGCGA
1321 GCGCCATTTG CCGGTGGCGG TGGCTCCGGC GGAGGTGGGT CCGGTGGCGG CGGATCAGGT
1381 GGGGGTGGAT CAGGCGGTGG AGGTTCCGGT GCGGGGGGAT CCGACAAAAC TCACACATGC
1441 CCACGFTGCC CAGCTCCGGA ACTCCTGGGA GGAACGTCAG TCTTCTCTT CCCCCAAAA
1501 CCAAGGACA CCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG
1561 AGCCACGAAG ACCCTGAGGT CAAGTCAAC TGGTACGTGG ACGGCGTGGG GGTGCATAAT
1621 GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCTC
1681 ACCGTCTCTG ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA
1741 GCGCTCCCGC CCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
1801 CAGGTGTACA CCTGCCCC ATCCCGGAT GAGCTGACCA AGAACAGGT CAGCCTGACC
1861 TGCCCTGTC AAGGCTTCTA TCCCAGCGAC ATCCCGGTGG AGTGGGAGAG CAATGGGCAG
1921 CCGGAGAACT ACTACAAGAC CACGCTCCC GTGTTGGACT CCGACGCTC CTTCTTCTC
1981 TACAGCAAGC TCACCGTCCA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
2041 GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCTCTCCCT GTCTCCGGT
2101 AAACGGCGCC GCGGAGCGG TGGCGCGGA TCAGGTGGGG GTGGATCAGG CCGTGGAGGT
2161 TCCGTTGGGG GGGGATCGGG CCGTGGAGGT TCCGTTGGGG GTGGATCAAG GAAGAGGAGG
2221 AAGAGGGACA TCGTGTGAC CCAGGCCGCC CCCAGCGTGC CCGTGACCCC CCGCGAGAGC

2281 GTGAGCATCA GCTGCCGGAG CAGCCGGAGC CTGCTGCACA GCAACGGCAA CACCTACCTG
2341 TGCTGGTTCC TGCAGCGGCC CGGCCAGAGC CCCCAGCTGC TGATCTACCG GATGAGCAAC
2401 CTGGCCAGCG GCGTGCCCGA CCGGTTCAAG GGCAGCGGCA GCGGCACCGC CTTACCCCTG
2461 CGGATCAGCC GGGTGGAGGC CGAGGACGTG GCGGTGTACT ACTGCATGCA GCACCTGGAG
2521 TACCCCTTCA CCTTCGGCAG CGGCACCAAG CTGGAGATCA AGCGGGCGGG CGGCGGCAGC
2581 GCGGGCGGGC GCAGCGCGCG CCGCGGCAGC CAGGTGACAG TGCAGCAGAG CCGCGCCGAG
2641 CTGGTGCAGC CCGGCACCA CGTGAAGATC AGCTGCAAGG CCAGCGGCTA CACCTTCACC
2701 AACTACTGGC TGGGCTGGGT GAAGCAGCGG CCGCGCCAGG GCTTGGAGTG GATCGGCGAC
2761 ATCTACCCCG GCGGCGGCTA CAACAAGTAC AACGAGAACT TCAAGGGCAA GGCCACCCCTG
2821 ACCGCCGAG CACGAGCAG CACCGCTTAC ATGCAGTCA GCAGCTGAC CAGCGAGGAC
2881 AGCGCCGTGT ACTTCTGCGC CCGGGAGTAC GGCAACTACG ACTACGCCAT GCACAGCTGG
2941 GCGCAGGCA CCAGCGTGAC CGTGAGCAGC GGTGGCGGTG GCTCCGGCGG AGGTGGGTCC
3001 GGTGGCGGGG GATCAGGTGG GGGTGGATCA GCGGTTGGAG GTTCCGGTGG CCGGGGATCA
3061 GACAAAATC ACACATGCC ACCGTGCCCA GCACCGGAAC TCTTGGGCGG ACCGTCAAGT
3121 TTCTCTTTC CCCCCAAACC CAAGGACACC CTCATGATCT CCGGACCCC TGAGGTCAAC
3181 TGGCTGGTGG TGGACGTGAG CCACGAGAC CCGAGGTCA AGTTCAACTG GTACGTGGAC
3241 GCGGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
3301 CGTGTGGTCA GGTCTCTCAC CGTCCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
3361 TGCAAGGTCT CCAACAAAGC CCTCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
3421 GGCACGCCCT GAGAACCAAC GGTGTACACC CTGCCCCAT CCGGGATGA GCTGACCAAG
3481 AACCAGGTCA GCTGACCTG CCTGTCAAA GCTTCTATC CCAGCGACAT CCGCGTGGAG
3541 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GTTGGACTCC

3601 GACGGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGCTG GCAGCAGGGG
3661 AACGTCTCTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAAGAGC
3721 CTCTCCCTGT CTCCGGGTAA ATGA

FVII-038 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

1 MVEQALRLLC MLIGLGGCLA AVEVYQEEAH GVLHRRRRAN AFLEELRPGS LERECKEEQC
61 SFEEAREIFK DAERTKLFWI SYSDGDQCAS SPCQNGGSCK DQLQSYICFC LPAFEGFNCE
121 THKDDQLICV NENGGCEQYC SDHTGTKRSC RCHEGYSLLA DGVSCPTTVE YPCGKIPILE
181 KRNASKPQGR IVGCKVCPKG ECPWQVLLLV NGAQLCCGTL INTIWVVSAA HCFDKIKNKR
241 NLI AVLGEHD LSEHDGDEQS RRV AQV IIPS TYVP GTTNHD IALLRLHQPV VLTDHVVPLC
301 LPERTFSERT LAFVRFSLVS GWGQLLDRA TALELMVLNV PRLMTQDCLO QSRKVGDSFN
361 ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV SWGQGCATVG HFGVYTRVSQ
421 YIEWLQKLMR SEPRPGVLLR APFPGGGGSG GGGSGGGSGG GGGSGGGSGG GGGSDKTHTC
481 PFCPAPELLG GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVRFN WYVDGVEVHN
541 AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI SKAQGPREF
601 QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTTP VLDSDGSFFL
661 YSKLTVDKSR WQGNVFSQS VMHEALHNHY TQKSLSLSPG KRRRRS**GGGG** **SGGGSGGGG**
721 **SGGGSGGGG** **SGGGSGRRRR** KRDI VMTQAA PSVPVTPGHS *VSISCRSSRS* *LLHSNGNTYL*
781 *CWFLQRPQS* *PQLLIYRMSN* *LASGVDFRFS* *GSQSGTAFTL* *RISRVEADV* *GVYYCMQHL*
841 *YPFTFGSGTK* *LEIKRGGGGS* *GGGGSGGGGS* *QVQLQDSGAE* *LVRPGTSVKI* *SCKASGYTPT*
901 NYWLGVVKQR *PGHGLEWIGD* *IYPGGGYNKY* *NENFKGKATL* *TADTSSSTAY* *MQLSSLTSED*
961 SAVYFCAREY GNYDIAMDSW GQGTSEVTSS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS
1021 DKTHTCFPCP APELLGGPSV FLFPPKPKDT LMSPTPEVTV CVVVDVSHED PEVKFNWYVD
1081 GVEVHNATK PREEQYNSTY RVVSVLTVLV QDWLNGKEYK CKVSNKALPA PIEKTIKAK
1141 GQPREPQVYV LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDL
1201 DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS LSLSPGK*

DNA sequence for FVIII-041

1 ATGCAAATAG AGCTCTCCAC CTGCTCTCTT CTGTGCCTTT TGCGATTCTG CTTTAGTGCC
61 ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG ACTATATGCA AAGTGATCTC
121 GGTGAGCTGC CTGTGGACGC AAGATTTCCT CCTAGAGTGC CAAAATCTTT TCCATTCAAC
181 ACCTCAGTCC TGTACAAAAA GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC
241 GCTAAGCCAA GCGCACCCCT GATGGGTCTG CTAGGTCTTA CCATCCAGGC TGAGGTTTAT
301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT TCATGCTGTT
361 GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
421 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGCTCT GCAGGTCTCT
481 AAAGAGAATG GTCCAATGGC CTCGTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT
541 GTGACCTTGG TAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
661 TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAGAAGCT CCTTGATGCA GGATAGGGAT
721 GCTGCATCTG CTCGGGCCTG GCCTAAAGAT CACACAGTCA ATGGTTATGT AAACAGGTCT
781 CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TGGAAATGGG
841 ACCACTCCTG AAGTGCACCT AATATTCCTC GAAGGTACAA CATTTCCTTG GAGGAACCAT
901 CGCCAGGCGT CCTTGGAAT CTGCGCAATA ACTTTCCTTA CTGCTCAAAC ACTCTTGATG
961 GACCTTGGAC AGTTTCTACT GTTTTGTCAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
1021 GCTTATGTC AAGTAGACAG CTGTCCAGAG GAACCCCAAC TACGAATGAA AAATAATGAA
1081 GAAGCGGAAG ACTATGATGA TGATCTTACT CATTCTGAAA TGGATGTGGT CAGGTTTGAT
1141 GATGACAACT CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAGCT
1201 TGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT AGTCTCGCC
1261 CCCGATGACA GAAGTTATAA AAGTCAATAT TTGAACAATG GCCCTCAGCG GATTGGTAGG
1321 AAGTACAAAA AAGTCCGATT TATGGCATAC ACAGATGAAA CCTTTAAGAC TCGTGAAGCT

1381 ATTCAGCATG AATCAGGAAT CTTGGGACCT TTACTTTATG GGAAGTTGG AGACACACTG
1441 TTGATTATAT TTAAGAAATC AGCAAGCAGA CCAATAACA TCTACCCCTCA CGGAATCACT
1501 GATGTCGGTC CTTTGTATTC AAGGAGATTA CCAAAGGTG TAAAACATTT GAAGGATTTT
1561 CCAATTCTGC CAGGAGAAAT ATTCAATAT AAATGGACAG TGACTGTAGA AGATGGGCCA
1621 ACTAAATCAG ATCCTCGGTG CCTGACCCGC TATTACTCTA GTTTCGTTAA TATGGACAGA
1681 GATCTAGCTT CAGGACTCAT TGGCCCTCTC CTCATCTGCT ACAAGAATC TGTAGATCAA
1741 AGAGGAAACC AGATAATGTC AGACAAGAGG AATGTCATCC TGTTTCTGT ATTTGATGAG
1801 AACCGAAGCT GTTACCTCAC AGAGAATATA CAACGCTTTC TCCCCAATCC AGCTGGAGTG
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1921 TTTGATAGTT TGCAAGTTGTC AGTTTGTGTT CATGAGGTGG CATACTGGTA CAFTCTAAGC
1981 ATTGGAGCAC AGACTGACTT CCTTCTCTCT TTCTTCTCTG GATATACCTT CAAACACAAA
2041 ATGGTCTATG AAGACACACT CACCTATFTT CCATTCTCAG GAGAAACTGT CTTCATGTCT
2101 ATGGAAAACC CAGGTCTATG GATTCCTGGG TGCCACAACCT CAGACTTTCT GAACAGAGGC
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2221 AGTTATGAAG ATATTTTCA GATACTTGCT AGTAAAACA ATGCCATTGA ACCAAGAAGC
2281 TTCTCTCAAA ACCCACCAGT CTTGAAACGC CATCAACGGG AAATAACTCG TACTACTCTT
2341 CAGTCAGATC AAGAGGAAAT TGACTATGAT GATACCATAT CAGTTGAAAT GAAGAAGGAA
2401 GATTTTGACA TTTATGATGA GGATGAAAAT CAGAGCCCCC GCAGCTTTCA AAAGAAAACA
2461 CGACACTATT TTATGCTGTC AGTGGAGAGG CTCTGGGATT ATGGGATGAG TAGCTCCCCA
2521 CATGTTCTAA GAAACAGGGC TCAGAGTGGC AGTGTCCCTC AGTTCAAGAA AGTTGTTTTT
2581 CAGGAATTTA CTGATGGCTC CTTTACTCAG CCCTTATACC GTGGAGAACT AAATGAACAT
2641 TTGGGACTCC TGGGGCCATA TATAAGAGCA GAAGTTGAAG ATAATATCAT GGTAACTTTC
2701 AGAAATCAGC CCTCTCGTCC CTATTCCTTC TATTCAGCC TTATTTCTTA TGAGGAAGAT
2761 CAGAGGCAAG GAGCAGAAAC TAGAAAAAAC TTTGTCAAGC CTAATGAAAC CAAAACCTAC
2821 TTTTGGAAAG TGCAACATCA TATGGCAACC ACTAAAGATG AGTTTGACTG CAAAGCCTCG
2881 GCTTATTTCT CTGATGTTGA CTTGAAACAA GATGTGCACT CAGGCTGAT TGGACCCCTT
2941 CTGGTCTGCC AACTAACAC ACTGAACCCT GCTCATGGGA GACAAGTGAC AGTACAGGAA
3001 TTTGCTCTGT TTTTCAACAT CTTTGATGAG ACCAAAAGCT GGTACTTCAC TGAATATATG
3061 GAAAGAAACT GCAGGCTCTC CTGCAATATC CAGATGGAAG ATCCCACTTT TAAAGAGAT
3121 TATCGCTTCC ATGCAATCAA TGGCTACATA ATGGATACAC TACCTGGCTT AGTAATGGCT
3181 CAGGATCAAA GATTCGATG GATCTGCTC AGCATGGGCA GCAATGAAAA CATCCATTCT
3241 ATTCATTTCA GTGGACATGT GTTCACTGTA CGAAAAAAG AGGAGTATAA AATGGCACTG
3301 TACAATCTCT ATCCAGGTGT TTTTGAGACA GTGGAAATGT TACCATCCAA AGCTGGAATT
3361 TGGCGGGTGG AATGCCTTAT TGGCGAGCAT CTACATGCTG GGATGAGCAC ACTTTTTCTG
3421 GTGTACAGCA ATAAGTGTCA GACTCCCCCT GGAATGGCTT CTGGACACAT TAGAGATTTT
3481 CAGATTACAG CTTCAGACA ATATGGACAG TGGGCCCAA AGCTGGCCAG ACTTCATTAT
3541 TCCGATCAA TCAATGCCTG GAGCACCAAG GAGCCCTTTT CTTGGATCAA GGTGGATCTG

3601 TTGGCACCAA TGATTATFCA OGGCATCAAG ACCCAGGGTG CCCGTCAGAA GTTCTCCAGC
3661 CTCTACATCT CTCAGTTTAT CATCATGTAT AGTCTTGATG GGAAGAAGTG GCAGACTTAT
3721 CGAGGAAATT CCACTGGAAAC CTTAATGGTC TTCTTTGGCA ATGTGGATTG ATCTGGGATA
3781 AAACACAATA TTTTAAACCC TCCAATTATT GCTCGATACA TCCGTTTGCA CCCAACTCAT
3841 TATAGCATTC GCAGCACTCT TCCGATGGAG TTGATGGGCT CTGATTTAAA TAGTTGCAGC
3901 ATGCCATTGG GAATGGAGAG TAAAGCAATA TCAGATGCAC AGATTACTGC TTCATCCTAC
3961 TTTACCAATA TGTTTGCCAC CTGGTCTCCT TCAAAAGCTC GACTTCACCT CCAAGGCAGG
4021 AGTAATGCCT GGAGACCTCA GGTGAATAAT CCAAAAGAGT GGCTGCAAGT GGACTTCCAG
4081 AAGACAATGA AAGTCACAGG AGTAACTACT CAGGGAGTAA AATCTCTGCT TACCAGCATG
4141 TATGTGAAGG AGTTCTCAT CTCCAGCAGT CAAGATGGCC ATCAGTGGAC TCTCTTTTTT
4201 CAGAATGGCA AAGTAAAGGT TTTTCAGGGA AATCAAGACT CCTTCACACC TGTGGTGAAC
4261 TCTCTAGACC CACCGTTACT GACTCGCTAC CTTCGAATTC ACCCCCAGAG TTGGGTGCAC
4321 CAGATTGCCC TGAGGATGGA GGTTCCTGGG TCGGAGGCAC AGGACCTCTA CGACAAAACCT
4381 CACACATGCC CACCGTGCCC AGCTCCAGAA CTCTTGCGCC GACCGTCAGT CTTCCTCTTC
4441 CCCCCAAAAC CCAAGGACAC COTCATGATC TCCCAGACCC CTGAGGTGAC ATGCGTGGTG
4501 GTGGACGTGA GCCACGAAGA CCTGAGGTC AAGTTCAACT GGTACGTGGA CGGCGTGGAG
4561 GTGCATAATG CCAAGACAAA GCGGCGGGAG GAGCAGTACA ACAGCACGTA CCGTGTGGTC
4621 AGCGTCTCTA CCGTCTGCA CCAGGACTGG CTGAATGGCA AGGAGTACAA GTGCAAGGTC
4681 TCCAACAAAG CCTCTCCAGC CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC
4741 CGAGAACCAC AGGTGTACAC CCTGCCCCCA TCCCAGGATG AGCTGACCAA GAACCAGGTC
4801 AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCGGTGGA GTGGGAGAGC
4861 AATGGGCAGC CGSAGAACAA CTACAAGACG ACGCCTCCCG TGTGGACTC CGACGGCTCC
4921 TTCTTCTCT ACACCAAGCT CACCGTGGAC AAGAGCAGGT GGCAGCAGGG GAACGTCTTC
4981 TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCCTACA CGCAGAAGAG CCTCTCCCTG
5041 TCTCCGGGTA AAGGTGCGCG CCGATCAGGT GGGGTGGAT CAGGCCTGG AGGTTCCGCT
5101 GGCGGGGGAT CAGACAAAAC TCACACATGC CCACCGTGCC CAGCACCTGA ACTCCTGGGA
5161 GGACCGTCAG TCTTCTCTT CCCCCAAA CCAAGGACA CCTCATGAT CTCCCGGACC
5221 CCTGAGGTCA CATGCGTGGT GGTGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAC
5281 TGGTACGTGG ACGGCGTGG GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC
5341 AACAGCACGT ACCGTGTGGT CAGCGTCTCT ACCGTCTGCT ACCAGGACTG GCTGAATGGC
5401 AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCAG CCCCATCGA GAAAACCATC
5461 TCCAAGGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCC ATCCCGCGAT
5521 GAGCTGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC
5581 ATCGCCGTGG AGTGGGAGAG CAATGGGCAG CCGSAGAACA ACTACAAGAC CACGCCTCCC
5641 GTGTTGGA CTGACGGCTC CTCTCTCTCT TACAGCAAGC TCACCGTGA CAAGAGCAGG
5701 TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC
5761 ACGCAGAAGA GCCTCTCCT GTCTCCGGT AAATGA

FVIII-041. amino acid sequence. Signal sequence is shown in dotted underline, and linker region connecting the Fc regions is underlined

1 MQIELSTCFE LCLLRFCPSA TRRYVLGAVE LSWDYMQSDL GELPVDARFP PRVPKSFPPN
61 TSVVYKTLF VETDHLFNI AKPRPPWMGL LGPTIQAEVY DTVVITLKNM ASHPVSLHAV
121 GVEYWKASEG AEYDDQTSQR EKEDDKVFPG CSHTYVWQVL KENGFMASDP LCLTYSYLSH
181 VDLVKDLNSG LIGALLVCRE GSLAKEKTQT LHKFILLFAV FDEGKSNHSE TKNSLMQDRD
241 AASARAWPKM HTVNGYVNRSLPGLICCHRK SVYWHVIGMG TTPEVHSIFL ECHTFLVRNH
301 RQASLEISPI TPLTAQTLLM DLGQFLLPCH ISSHQHDMGE AYVKVDSQPE BPQLRMKNNE
361 EAEDYDDDLT DSEMDVVRFD DSNPSPIQI RSVAKKHPKT WVHYIAAEEB DWYAPLVLA
421 PDRSYKSQY LNNGPQRIGR KYKKVRFMAY TDETFTKREA IQHESGILGP LLYGEVGDTL
481 LIIFKNQASR PYNIPPHGIT DVRPLYSRRL PKGVKHLKDF PILPGEIPKY KWTVTVEDGP
541 TKSDPRCLTR YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
601 NRSWYLTENI QRFLNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL HEVAYWYILS
661 IGAQIDELSV PFSGYTFKHK MVEEDTLTLF PFSGETVFMS MENPGLNLLG CHNSDFRNRG
721 MTALLKVSSC DKNTGDYYED SYEDISAYLL SKNNALEPRS FSQNPVLKR HQREITRTTL
781 QSDQEEIDYD DTISVEMKKE DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP
841 HVLNRNRAQS SVPQFKKVVFP QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
901 RNQASRFYSP YSSLISYEED QRQGAEPKRN FVKENETKTY FWKVQHMAP TKDEFDCKAW
961 AYFSDVDLEK DVHSLIGPL LVCHTNFLNP AHGRQVTVQE FALFFTFIDE TKSIFYTENM
1021 ERNCRAPCNI QMEDPTFKEN YRPHAINGYI MDTLPGLVMA QDQRIRWYLL SMGSNENIHS
1081 IRFSGHVFTV RKKEEYKMAI YNLVPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL
1141 VYSNKCQTFE QMASGHIRDF QITASQYQYG WAPKLARLHY SGSINAWSTK EPPSWIKVDL
1201 LAPMIIHGIR TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV PFGNVDSGGI
1261 KHNIFNPPII ARYIRLHPFH YSIRSTLRME LMGCDLNSCS MFLGMESKAI SDAQITASSY
1321 FTMNFATWSP SKARLHLQGR SNAWRPQVNN PREWLQVDFQ KIMKVTGVTT QGVKSLLTSM
1381 YVKEFLISSQ QDGHQWTLFF QNGKVKVFOG NQDSFTPVVN SLDPPLLTRY LRHPQSWVH
1441 QIALRMEVLG CBAQDLYDKT HTCPFPAPE LLGCFPVFLF PPKPKDTLMI SRTPEVTCVV
1501 VDVSHEDFEV KFNWYVDGVE VHNARTKPRE BOYNSTYRVV SVLTVLHQDW LNKKEYKCKV
1561 SNKALPAPIE RTISKAKGQP REPQVYTLPP SRDELTKNQV SLTCLVRGFY PSDIAVEWES
1621 NGQPENNYKT TPPVLDSGDS FFLYSKLTVD KSRWQQGNVF SCSVMEEALH NHYTQKSLSL
1681 SPQKGGGGSG CCGGGGGGGSG GGGSDKTHTC PFCFAPELLG GPSVFLFPPK PKMTLMISRT
1741 PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG
1801 KEYKCKVSNK ALPAPIEKTI SKAKGQPREP QVYTLPPSRD ELTKNQVSLT CLVKGFYPSD
1861 IAVEWESNGQ PENNYKTTTP VLDSDGSFPL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
1921 TQKSLSLSPG K*

DNA sequence for FVIII-108

1 ATGCAAAATG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TCGATTCTG CTTTAGTGGC
61 ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTGATGGG ACTATATGCA AAGTGATCTC
121 GGTGAGCTGC CTGTGGACGC AAGATTTTCT CTTAGAGTGC CAAAATCTTT TCCATTCAAC
181 ACCTCAGTCC TGTACAAAAA GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAAACATC
241 GCTAAGCCAA GGCCACCCTG GATGGGTCTG CTAGGTCTTA CCATCCAGGC TGAGGTTTAT
301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTGAGTCT TCATGCTGT
361 GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
421 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCTCT
481 AAAGAGAATG GTCCAATGGC CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT
541 GTGACCTTGG TAAAAGACTT GAATTCAGCG CTCATTGGAG CCTTACTAGT ATGTAGAGAA
601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
661 TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAGAAGCT CCTTGATGCA GGATAGGGAT
721 GCTGCATCTG CTCGGGCCTG GCCTAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCT
781 CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TCGAATGGGC
841 ACCACTCTCT AAGTGCATCT AATATTCCTC GAAGGTCAAC CATTTCTTGT GAGGAACCAT
901 CGCCAGGCGT CTTGGGAAAT CTCGCCAATA ACTTTCTCTA CTGCTCAAAC ACTCTTGATG
961 GACCTTGGAC AGTTTCTACT GTTTTGTGAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
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1201 GCTAAGCCAA GGCCACCCTG GATGGGTCTG CTAGGTCTTA CCATCCAGGC TGAGGTTTAT
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1321 GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
1381 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCTCT
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1561 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
1621 TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAGAAGCT CCTTGATGCA GGATAGGGAT
1681 GCTGCATCTG CTCGGGCCTG GCCTAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCT
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1861 CGCCAGGCGT CTTGGGAAAT CTCGCCAATA ACTTTCTCTA CTGCTCAAAC ACTCTTGATG
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2101 ACCTCAGTCC TGTACAAAAA GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAAACATC
2161 GCTAAGCCAA GGCCACCCTG GATGGGTCTG CTAGGTCTTA CCATCCAGGC TGAGGTTTAT
2221 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTGAGTCT TCATGCTGT
2281 GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
2341 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCTCT
2401 AAAGAGAATG GTCCAATGGC CTCTGACCCA CTGTGCCTTA CCTACTCATA TCTTTCTCAT
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2521 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
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2761 ACCACTCTCT AAGTGCATCT AATATTCCTC GAAGGTCAAC CATTTCTTGT GAGGAACCAT
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3601 GCTGCATCTG CTCGGGCCTG GCCTAAAATG CACACAGTCA ATGGTTATGT AAACAGGTCT
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4081 GCTAAGCCAA GGCCACCCTG GATGGGTCTG CTAGGTCTTA CCATCCAGGC TGAGGTTTAT
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4261 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT GGAAGCCATA CATATGTCTG GCAGGTCTCT
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4441 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
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4621 CTGCCAGGTC TGATTGGATG CCACAGGAAA TCAGTCTATT GGCATGTGAT TCGAATGGGC
4681 ACCACTCTCT AAGTGCATCT AATATTCCTC GAAGGTCAAC CATTTCTTGT GAGGAACCAT
4741 CGCCAGGCGT CTTGGGAAAT CTCGCCAATA ACTTTCTCTA CTGCTCAAAC ACTCTTGATG
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5041 GCTAAGCCAA GGCCACCCTG GATGGGTCTG CTAGGTCTTA CCATCCAGGC TGAGGTTTAT
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6001 TCCGGCGGAG GTGGGTCCGG TGGCGCGGGA TCAGGTGGGG GTGGATCAGG CCGTGGAGGT
6061 TCCGGTGGCG GGGGATCAGA CAAAACCTAC ACATGCCAC CCGTCCAGC ACCTGGAACCT
6121 CTGGGCGGAC CGTCAGTCTT CCTCTTCCCT CCAAAACCCA AGGACACCTT CATGATCTTC
6181 CGGACCCCTG AGTCAACATG CGTGTGTTG GACGTGAGCT ACGAAGACCT TGAGGTCAAG
6241 TTCAACTGGT ACGTGGACGG CGTGGAGGTG CATAATGCCA AGACAAAGCC GCGGGAGGAG
6301 CAGTACAACA GCACGTACCG TGTGGTTCAG GTCTCTACCG TCCCTGCACCA GGAATGGCTG
6361 AATGGCAAGG AGTACAAGTG CAAGGTCTCC AACAAAGCCC TCCAGCCCC CATCGAGAAA
6421 ACCATCTCCA AAGCCAAAGG GCAGCCCCGA GAACCAAGG TGTACACCTT GCGCCCATCC
6481 CGGGATGAGC TGACCAAGAA CAGGCTCAGC CTGACCTGCC TGGTCAAAGG CTTCTATCTC
6541 AGCGACATCG CCGTGGAGTG GGAGAGCAAT GGGCAGCCGG AGAAACAATA CAAGACCAAG
6601 COTCCCGTGT TGGACTCCGA CCGCTCCTTC TTCTCTTACA GCAAGCTCAC CGTGGACAA
6661 AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA TGCTCCGTGA TGCAATGAGC TCTGCACAA
6721 CACTACACGC AGAAGAGCCT CTCCCTGTCT CCGGTAAAT GA

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  MQIELSTQFF LCLLRFCFSA FRRVYLGAVE LSWDYMQSDL GELPVDARFP PRVPKSEFPFN
61  TSVVYKKTLE VEETDHLFNI AKPRPPWMGL LGPTIQAEVY DTVVITLKNM ASHPVSLHAV
121 GVSYWKASEG AEYDDQTSQR EKEDDKVFPQ GSHTYVWQVL KENGPMASDP LCLTYSYLSH
181 VDLVKDLNSG LIGALLVCRE GSLAKEKTQT LHKFILLFAV FDEGKSNHSE TKNSLMQDRD
241 AASARAWPKM HTVNGYVNRS LPGLIGCHRK SVYWHVIGMC TTPEVHSIFL EGHTFLVRNH
301 RQASLEISPI TFLTAQTLLM DLGQFLLECH ISSHQHDGME AYVRVDSCEP EPQLSMENNE
361 EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT WVHYIAEEE DWDYAPLVLA
421 PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY TDETFKIREA IQHESGILGP LLYGEVGDTL
481 LIIFKNQASR FYNIFYPHGIT DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGF
541 TKSDPRCLTR YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
601 NRSWYLTENI QRPLENPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL HEVAYWYILS
661 IGAQTDFLSV PFSGYTFKKH MVEDTLTLEP PFSGETVFMS MENPGLWILG CHNSDFRNRC
721 MTALLKVSSC DKNTGDYYED SYEDISAYLL SKNNAIEPRS FSQNPFVLKR HQREITRTTL
781 QSDQEEIDYD DTISVEMKKE DEDIYDEDED QSPRSFQKKT RHYFTAAVER LDWYGMSSSP
841 HVLRNRRAQSG SVPOFKKVVF QEFTDGSFTQ ELYRGELNEH LGLLGPYIRA EVEDNIMVTF
901 RNQASRPYSF YSSLISYEED QRQGAEPKRN PVKPNETKTY FWKVQHMAP TKDEPDCKAW
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1081 IHFSGHVFTV RKKEEYKMAL YNLYPGVFET VEMLPKSKI WRVECLIGEH DHAGMSTLFL
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1381 YVKEFLISS QDGHQWTLFF QNGKVVFQF NQDSFTEVVN SLDPFLTRY LRINPQSVVH
1441 QIALRMEVLG CRAQDLYDKT HTCPPCPAPE LGGPSVFLF PKPKDILMI SRTPVTCVV
1501 VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHGDW LNKKEYKCKV
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1861 LEEGEFSEAR VSELTQDFAV SVALGQTVRI TCQGDSLRF YASWYQQKPG
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1921 SKRPSGIPDR FSASSSGNTA SLTITGAQAE DEADYYCLLY YGGGQQGVFG
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2041 LGGPSVFLFP PKPKDILMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV
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2101 QYNSTYRVVS VLVVLHGDWL NGKEYKCKVS NKALPAPIEK TISKAKQPR
      EPQVYTLPPS
2161 RDELTKNOVS LTCLVKGFYP SDIAVEWESN QQPENNYKTT PPVLDSGGSF
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2221 SRWQQGNVFS CSVMHEALHN HYTQKSLSL PGK*
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DNA sequence for pSYN-FVIII-049

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121 GGTGAGCTGC CTGTGACGCG AAGATTCCCT CCTAGAGTGC CAAAATCTTT TCCATTCAAC
181 ACCTCAGTGC TGTACAAAAA GACTCTGTCT GTAGAATTCA CGGATCACCT TTCAACATC
241 GCTAAGCCAA GGCCACCCTG GATGGGCTCT CTAGGTCTTA CCATCCAGGC TGAGGTTTAT
301 GATACAGTGC TCATTACACT TAAGAACATG CTTTCCCATC CTGTCACTCT TCATGCTGTT
361 GGTGTATCCT ACTGGAAGAG TTCTGAGGGA GCTGAATATG ATGATCAGAC CAGTCAAAGG
421 GAGAAAGAAG ATGATAAAGT CTTCCCTGGT CGAAGCCATA CATATGTCTG GCAGGTCTCT
481 AAAGAGAATG GTCCAATGGC CTCTGACCCA CTGTGCTTAA CCTACTGATA TCTTCTCAT
541 GTGGACCTGG TAAAAGACTT GAATTCAGGC CTCATTGGAG CCCTACTAGT ATGTAGAGAA
601 GGGAGTCTGG CCAAGGAAAA GACACAGACC TTGCACAAAT TTATACTACT TTTTGCTGTA
661 TTTGATGAAG GGAAAAGTTG GCACTCAGAA ACAAGAAGCT CCTTGATGCA GGATAGGGAT
721 GCTGCATCTG CTCGGGCTCT GCCTAAATG CACACAGTCA ATGTTTATGT AAACAGTCT
781 CTGCCAGGTC TGATTGGATC CCACAGGAAA TCACTCTATT GGCATGTGAT TGGAAATGGC
841 ACCACTCCTG AAGTGCACTC AATATTCCTC GAAGGTACA CATTCTTGT GAGGAACCAT
901 CGCCAGGCGT CCTTGSAAAT CTCGCCAATA ACTTTCCTTA CTGCTCAAAC ACTCTTGATC
961 GACCTTGGAC AGTTTCTACT GTTTGTCTAT ATCTCTTCCC ACCAACATGA TGGCATGGAA
1021 GCTTATGTCA AAGTAGACAG CTGTCCAGAG GAACCCCAAC TACGAATGAA AAATAATGAA
1081 GAAGCGGAAG ACTATGATGA TCACTTACT GATTCTGAAA TGGATGTGGT CAGGTTTGAT
1141 GATGACAACT CTCCTTCCTT TATCCAAATT CGCTCAGTTG CCAAGAAGCA TCCTAAAACT

1201 TGGGTACATT ACATTGCTGC TGAAGAGGAG GACTGGGACT ATGCTCCCTT AGTCCTCGCC
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1921 TTTGATAGTT TGCAGTTGTC ACTTTGTTTC CATGAGGTGC CATACTGCTA CATTCTAAGC
1981 ATTGGAGCAC AGACTGACTT CTTTTCTGTC TTCTTCTCTG GATATACCTT CAAACACAAA
2041 ATGCTGCTATG AAGAGTCTCT CAGCTGCTATG CCAATCTCTG CAGAAAGCTCT CTGCTGCTGCT
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2161 ATGACCGGCT TACTGAAGGT TTCTAGTTGT GACAAGAACA CTGGTGATTA TTACGAGGAC
2221 AGTTATGAAG ATATTTCAGC ATACTTGCTG AGTAAACA 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 AGTTCAGAA AGTTGTTTTT
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2641 TTGGGACTCC TGGGCCATA TATAAGAGCA GAAGTTGAAG ATAATATCAT GGTAACTTTC
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2761 CAGAGGCAAG GAGCAGAACG TAGAAAAAAC TTTGTCAAGC CTAATGAAAC CAAAACCTAC
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2881 GCTTATTTCT CTGATGTTGA CCTGSAAAA GATGTGCACT CAGGCCTGAT TGGACCCCTT
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3181 CAGGATCAAA GGATCGATG GTATCTGCTC AGCATGGGCA GCAATGAAAA CATCCATTCT
3241 ATTCAATTTCA GTGACATGT GTTCACTGTA CGAAAAAAG AGGAGTATAA AATGGCACTG
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4081 AAGACAATGA AAGTCACAG AGTAAGTACT CAGGGAGTAA AATCTCTGCT TACCAGCATG
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4261 TCTCTAGACC CACCGTTACT GACTCGCTAC CTTCGAATTC ACCCCAGAG TGGGTGAC
4321 CAGATTGCCC TGAGGATGGA GGTCTCTGGG TCGGAGGCAC AGGACCTCTA CGACAAAAC
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4501 GTGGACGTGA GCCACGAAGA CCTGAGGTC AAGTTCAACT GGTACGTGGA CGGCGTGGAG
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4681 TCCAACAAAG CCTCCAGC CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGGCC
4741 CGAGAACCA AGGTGTACAC CCTGCCCCCA TCCCGGATC AGCTGACCA GAACCAGGT
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5161 AAGAGGAGGA AGAGGGACAA AACTCACACA TGCCCACCGT GCCAGCTCC AGAATCTCTG
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5281 ACCCTGAGG TCATGCGT GGTGTGGAC GTGAGCTACG AAGACCTGA GGTCAAGTTC
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5521 ATCTCCAAG CCAAAGGCA CCCCCGAA CCACAGTGT ACACCTGCC CCCATCCCG
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5641 GACATCGCCG TGGAGTGGGA GAGCAATGG CAGCCGAGA ACAACTACAA GACCAGGCT
5701 CCGGTGTTGG ACTCGAAGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC
5761 AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC

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5821 TACACGCAGA AGAGCCTCTC CCGTCTCCG 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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1 MQIELSTCTF LCLLRFCFSA FRRYYLGAVE LSWDYMQSDI GELFVDARFF PRVPKSEFFN
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181 VDLVKDLNSG LIGALLVORE GSLAREKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD
241 AASARAWPRM HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHFTPLVRNH
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421 PDDRSYKSYQ LNNGPQRIGR KYKKVRFMAY TDETFTKREA IQHESGILGP LLYGEVGDTL
481 LIIFKNQASR PYNIPHGIT DVRPLYSRRL PKGVKHLKDF PILPGEIPKY KWTVTIVEDGP
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841 HVLNRNRAQSG SVFQFKKVVF QEFTDGSFTQ PLYRGELNER LGLLGPYIRA EVEDNIMVTF
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961 AYFSDVDLEK DVHSGLIGPL LVCHTNLNP AHGRQVTVOE FALFFTIEDE TKSWEYETENM
1021 ERNCRAPCNI QMEDPTFKEN YRFHAINGYI MDTLPLGLVMA QDQIRRWYLL SMGSNENIHS
1081 IHFSGHVFTV RKKEEYKMAI YNLYPGVFET VEMPLSKAGI WRVECLIGEH LHAGMSTLFL
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1201 LAPMIIHGIR TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV FFGNVDESGL
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1741 GGFSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
1801 YNSTYRVVSV LTVLHQDWLN GREYKCKVSN KALPAIEKT ISKAKGQPRE PQVYTLPPSR
1861 DELTKNOVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP FVLDSGSGFP LYSKLTVDKS
1921 RWQGNVFSB SVMHEALHNH YTQKSLSLSP GR*

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Patentkrav

1. Kimær koagulationsfaktor, som omfatter
 - i) en koagulationsfaktor, der omfatter et FVII-polypeptid,
 - 5 ii) en målretningsdel, der binder til trombocytter, og
 - iii) en skeletdel, der omfatter en FcRn-bindingsdel,hvor koagulationsfaktoren er koblet til skeletdelen direkte eller via en første linker, hvor målretningsdelen er koblet til skeletdelen direkte eller via en anden linker, og hvor
- 10 skeletdelen er en dimer Fc-region, der omfatter en første Fc-del, F1, og en anden Fc-del, F2.
2. Kimær koagulationsfaktor ifølge krav 1, hvor FVII-polypeptidet omfatter et heterologt enzymatisk spaltningssted,
- 15 der kan aktiveres af en bestanddel af koagulationskaskaden.
3. Kimær koagulationsfaktor ifølge krav 2, hvor koagulationsfaktoren er koblet til skeletdelen via den første linker.
- 20 4. Kimær koagulationsfaktor ifølge et hvilket som helst af kravene 1 til 3, som omfatter en spaltbar scFc (cscFc)-linker, der er skudt ind mellem de to Fc-dele, hvor cscFc-linkeren støder op til mindst ét enzymatisk spaltningssted, hvilket
- 25 resulterer i spaltning af cscFc-polypeptidlinkeren.
5. Kimær koagulationsfaktor ifølge krav 4, hvor det mindst ene enzymatiske spaltningssted er et intracellulært processeringssted.
- 30 6. Kimær koagulationsfaktor ifølge et hvilket som helst af kravene 1 til 5, hvor målretningsdelen binder til hvilende trombocytter eller aktiverede trombocytter.
- 35 7. Kimær koagulationsfaktor ifølge et hvilket som helst af kravene 1 til 6, hvor målretningsdelen er valgt fra gruppen, der består af: et antistofmolekyle, et antigenbindende fragment af et antistofmolekyle, et scFv-molekyle, en

receptorbindingsdel af en receptor og et peptid.

8. Kimær koagulationsfaktor ifølge krav 2, hvor det heterologe enzymatiske spaltningssted er valgt fra gruppen,
5 der består af: et faktor XIa-spaltningssted, et faktor Xa-spaltningssted og et thrombinspaltningssted.

9. Kimær koagulationsfaktor ifølge et hvilket som helst af kravene 1 til 8, hvor koagulationsfaktoren omfatter to
10 polypeptidkæder.

10. Nukleinsyremolekyle, der koder for en kimær koagulationsfaktor ifølge et hvilket som helst af kravene 1
til 9.

15 11. Ekspressionsvektor, der omfatter nukleinsyremolekylet ifølge krav 10.

20 12. Værtscelle, der omfatter ekspressionsvektoren ifølge krav 11.

25 13. Fremgangsmåde til fremstilling af en kimær koagulationsfaktor, som omfatter dyrkning af værtscellen ifølge krav 12 i dyrkningsmedium og indvinding af den kimære koagulationsfaktor fra mediet.

30 14. Farmaceutisk sammensætning, der omfatter den kimære koagulationsfaktor ifølge et hvilket som helst af kravene 1 til 9 og et farmaceutisk acceptabelt bæremateriale, til anvendelse til forbedring af hæmostase hos et individ.

DRAWINGS

Generic examples of Enhanced Clotting Factor Fc fusions:
Platelet targeting for enhanced efficacy

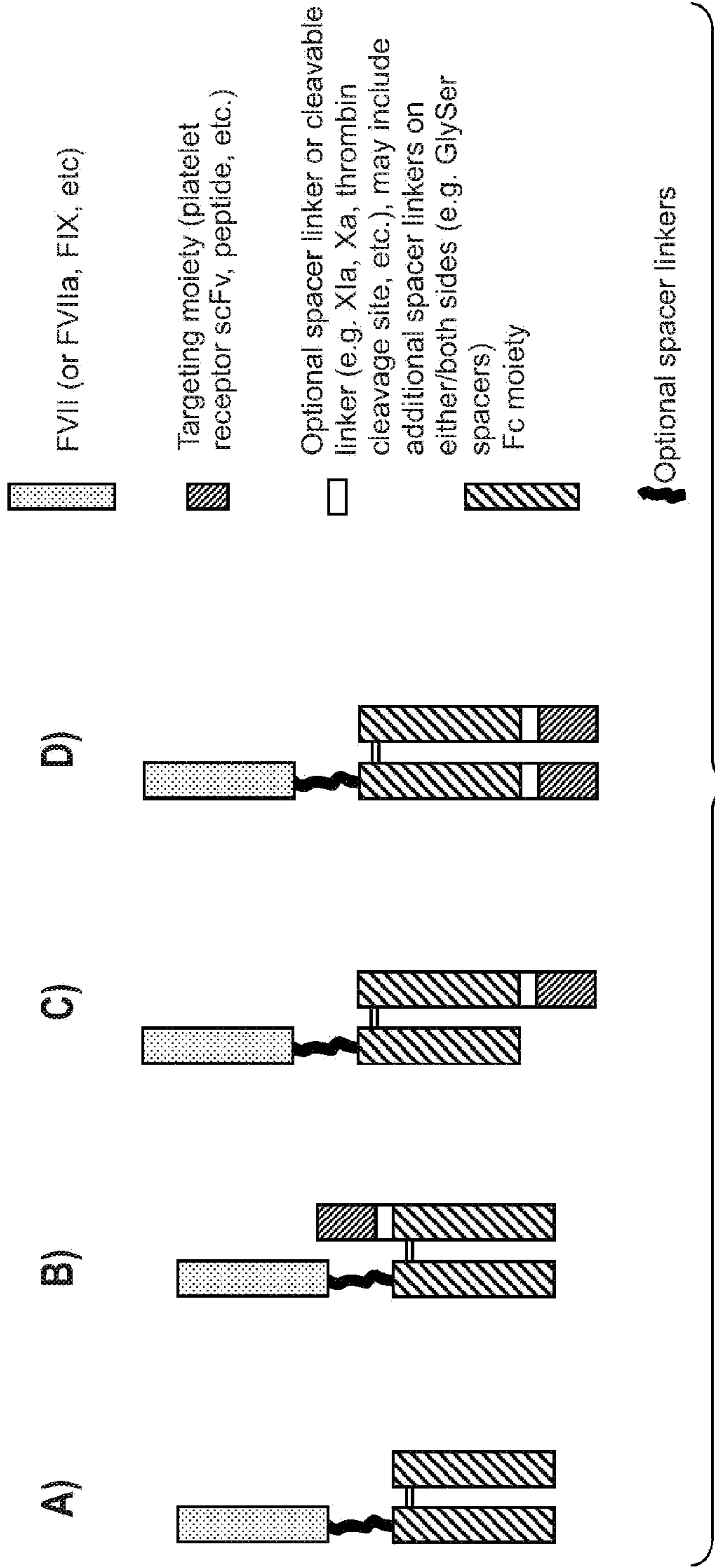


Fig. 1

Generic examples of Enhanced Clotting Factor Fc fusions
(with protease processing sites): Platelet targeting for enhanced efficacy

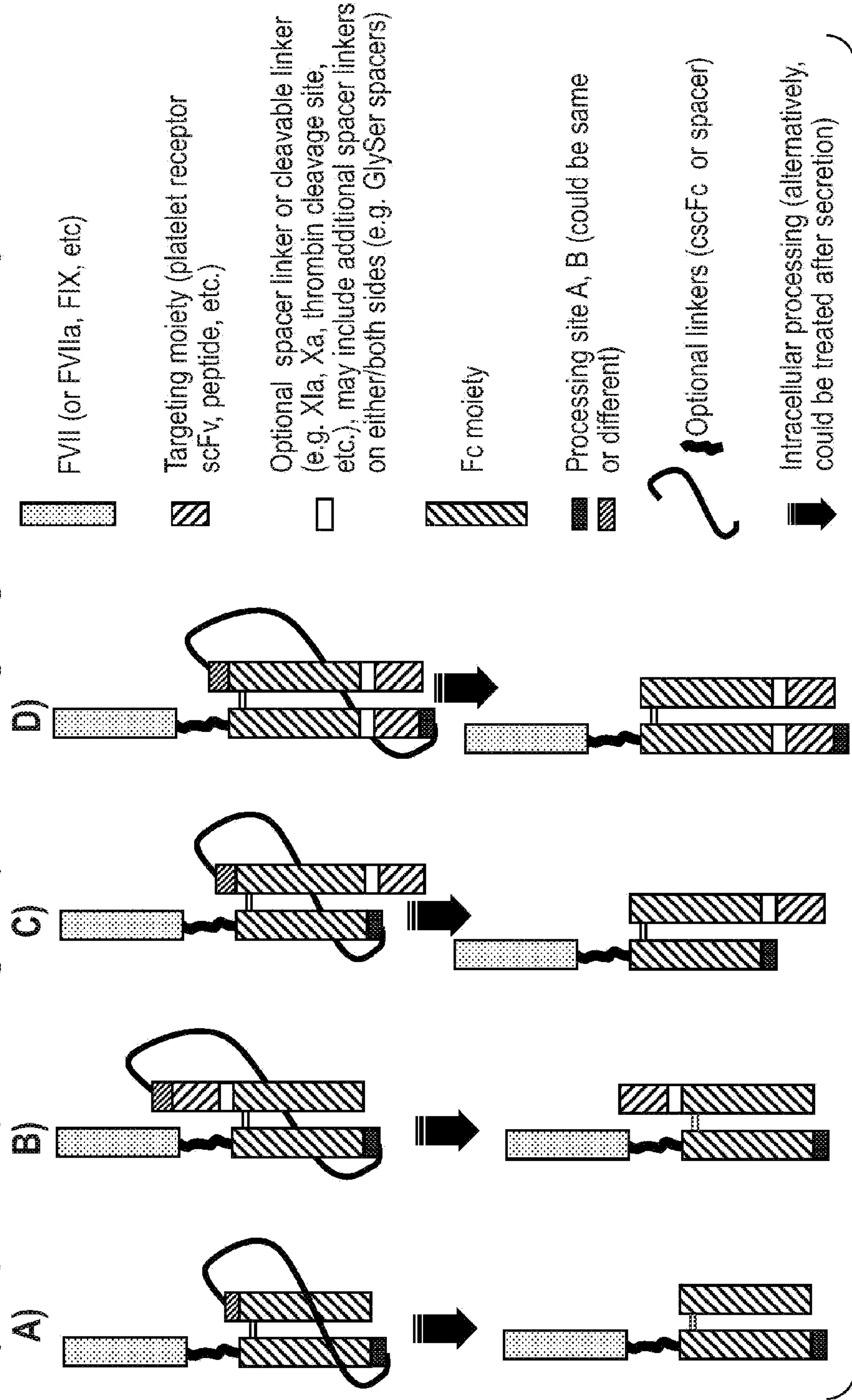


Fig. 2

Generic examples: Platelet targeting with Gla replace/deletion to decrease clearance

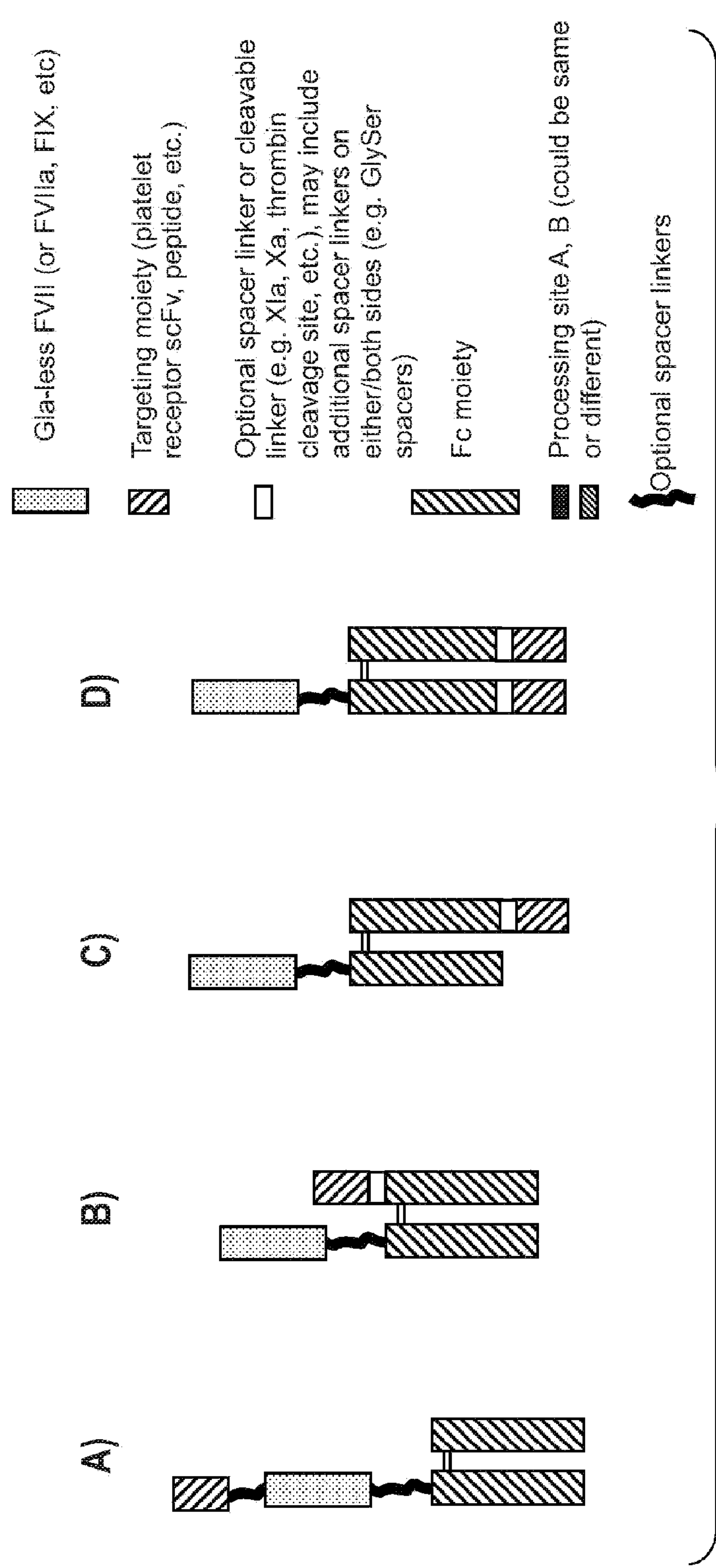


Fig. 3

Generic examples: Platelet targeting with Gla replace/deletion to decrease clearance, with single chain cleavable linkers

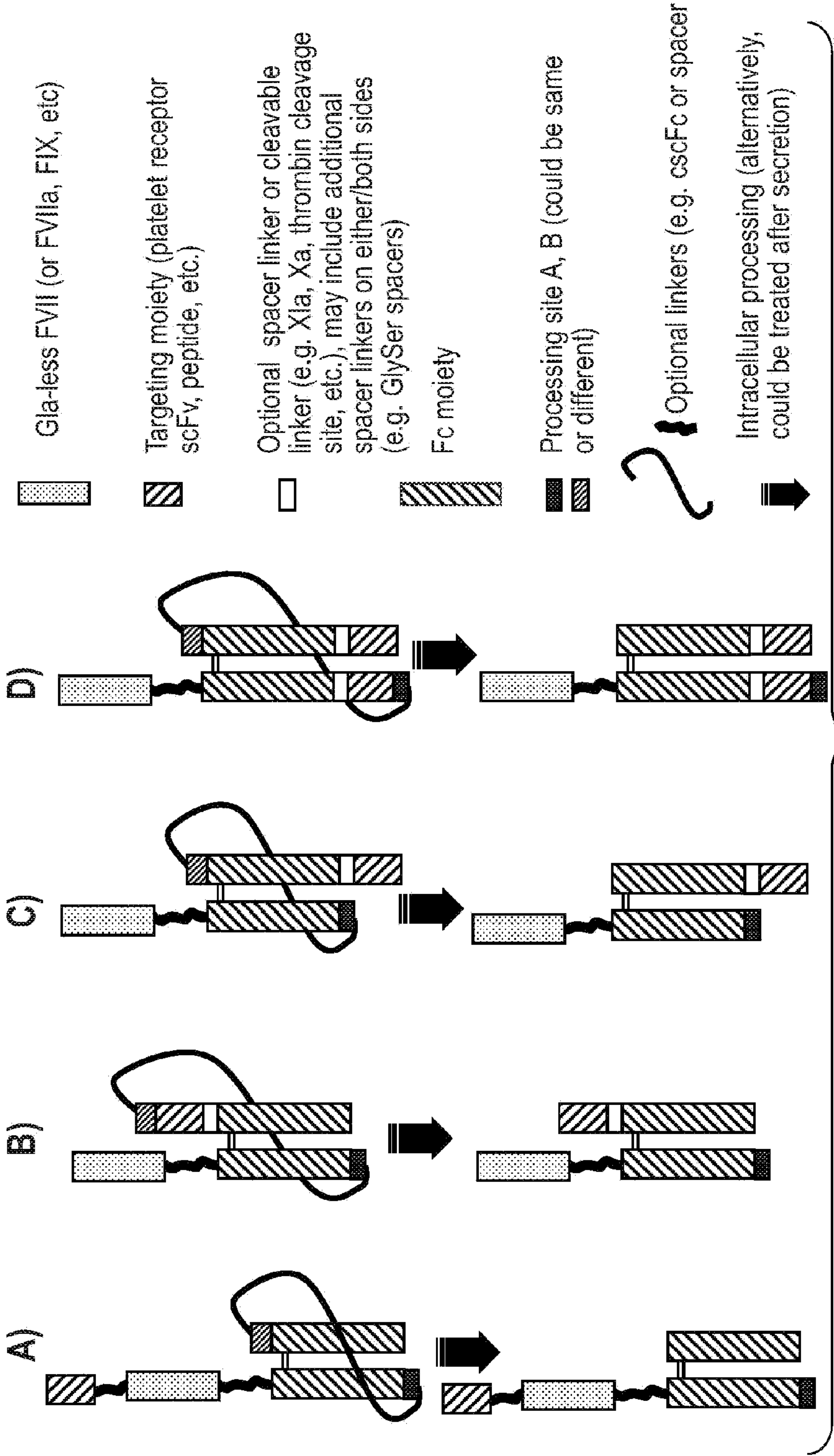


Fig. 4

Generic examples: Activated FVII constructs, activatable FVII constructs

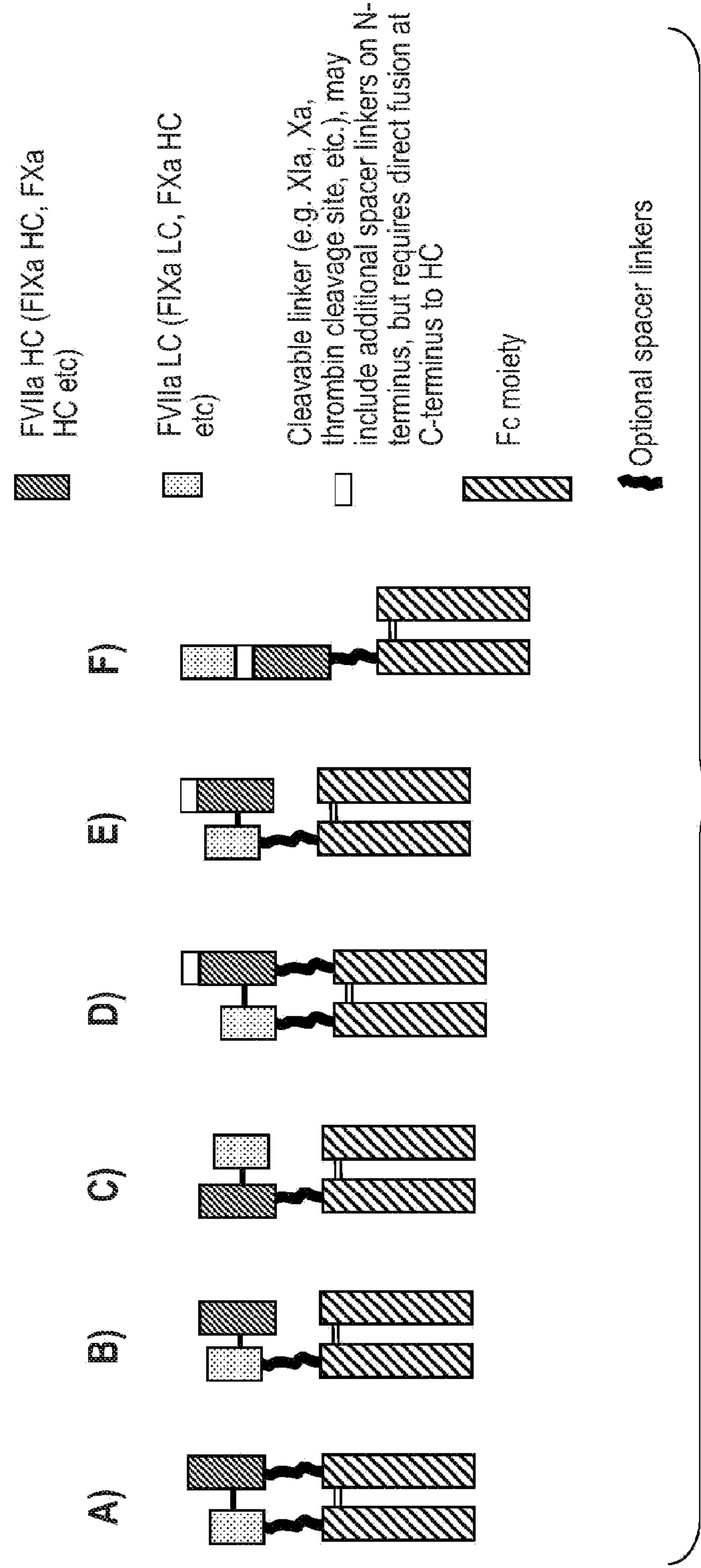


Fig. 5

Activated FVII constructs, activatable FVII constructs with single chain
cleavable linkers

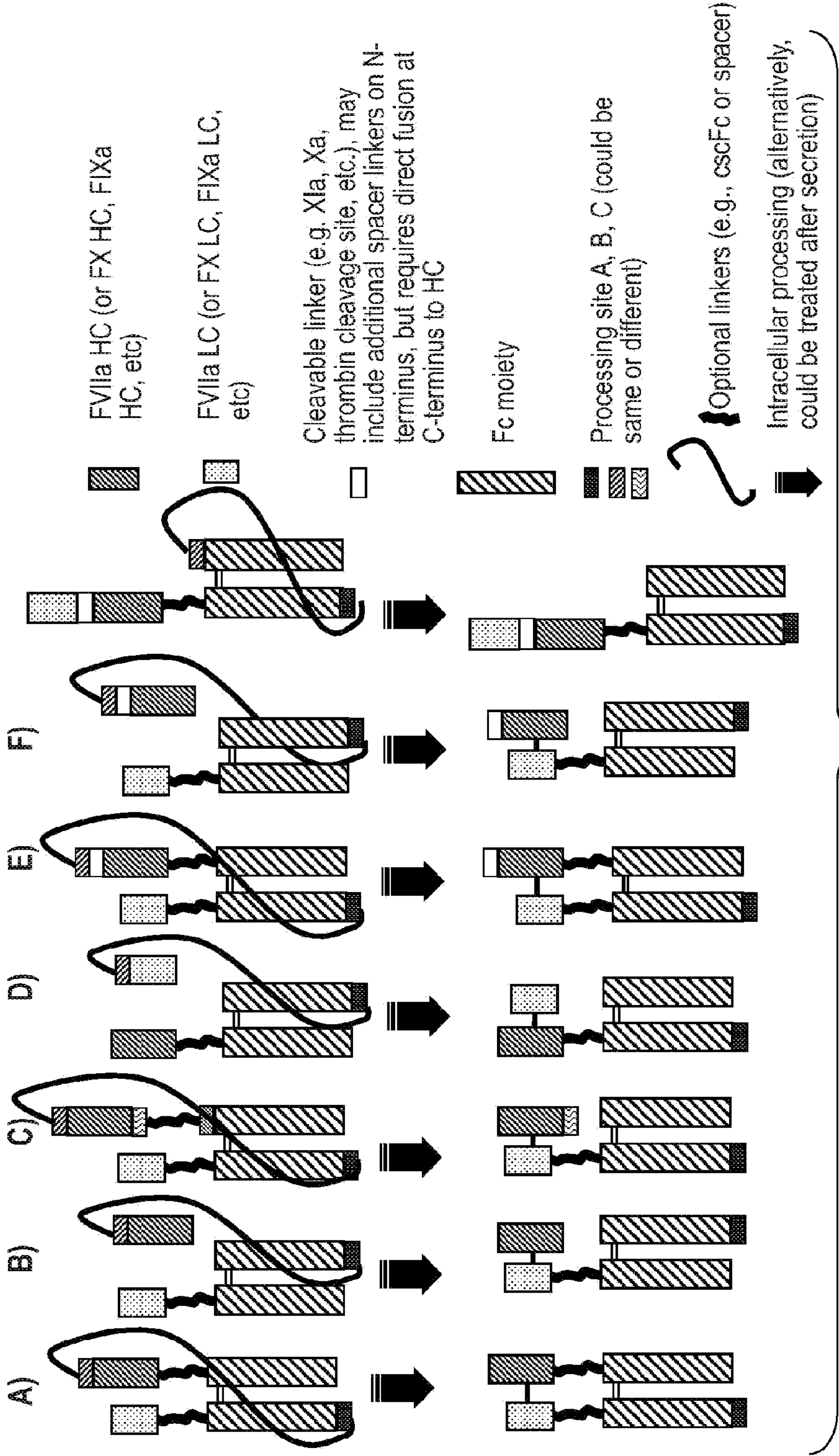


Fig. 6

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

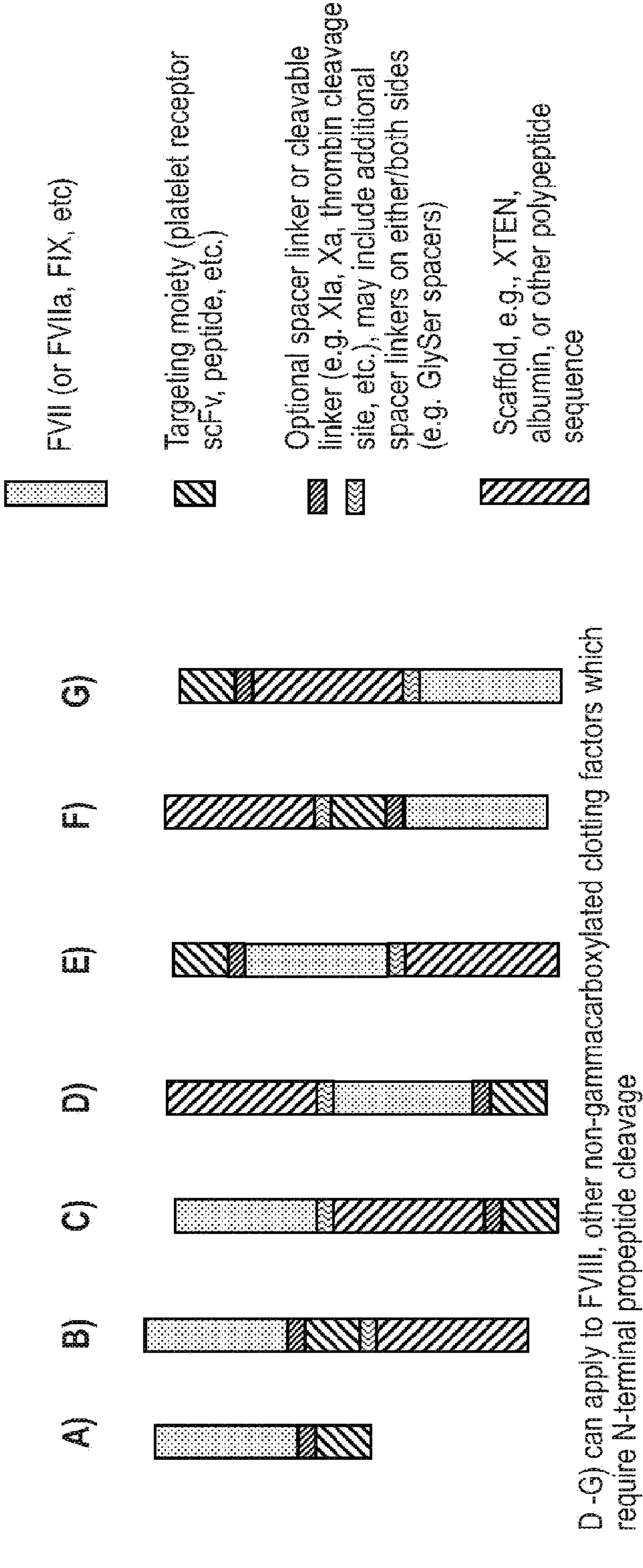
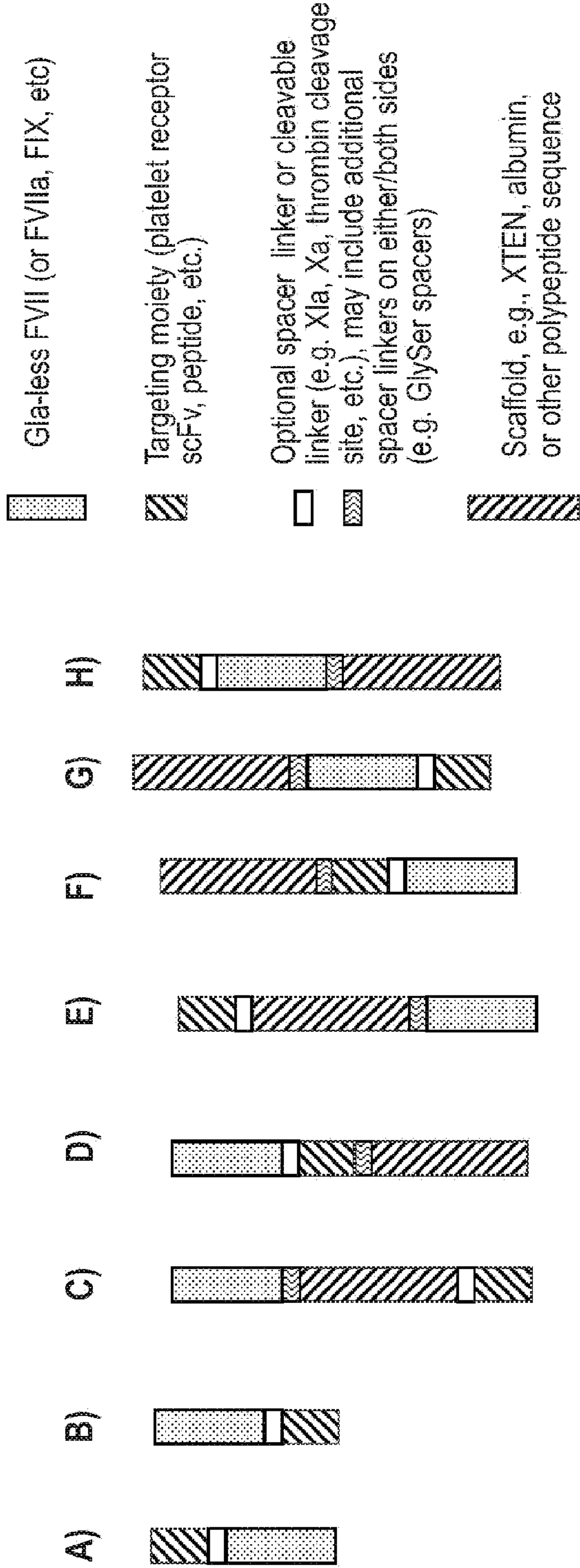


Fig. 7

Generic examples: Platelet targeting with Gla replace/deletion to decrease clearance, to FVII, FIX, FX
(non-Fc fusions)



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

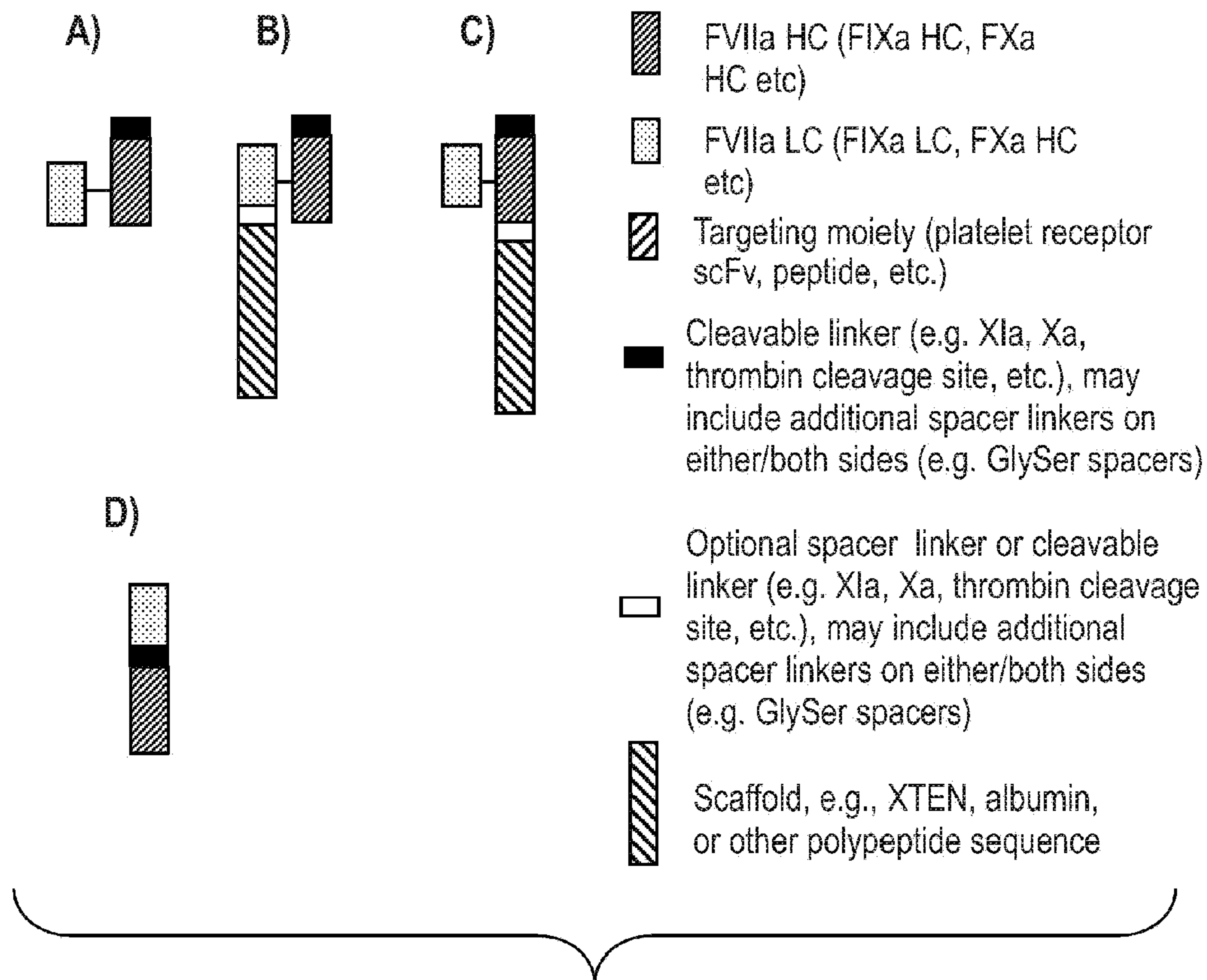


Fig. 9

SDS PAGE for purification and activation of FVII-011

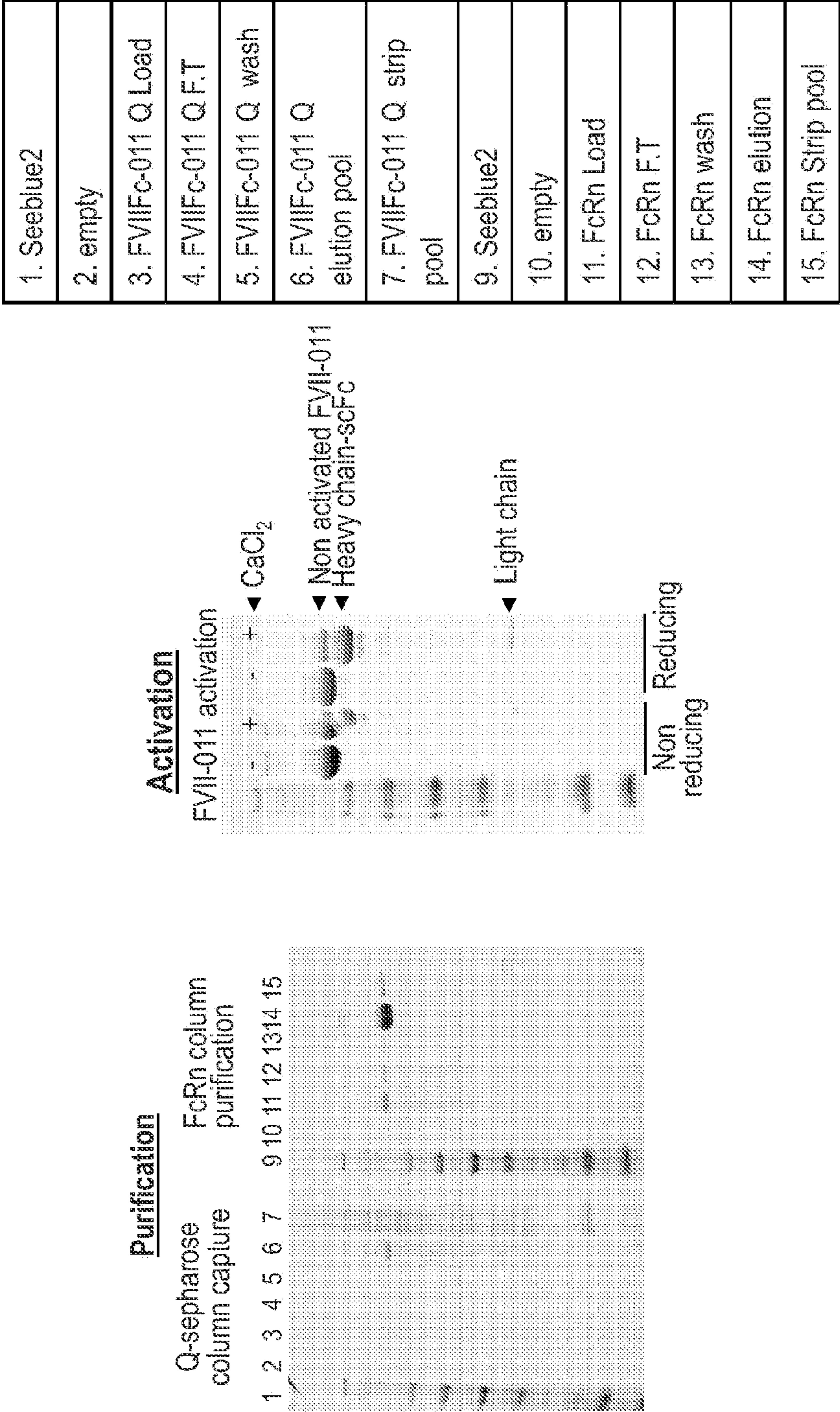


Fig. 10

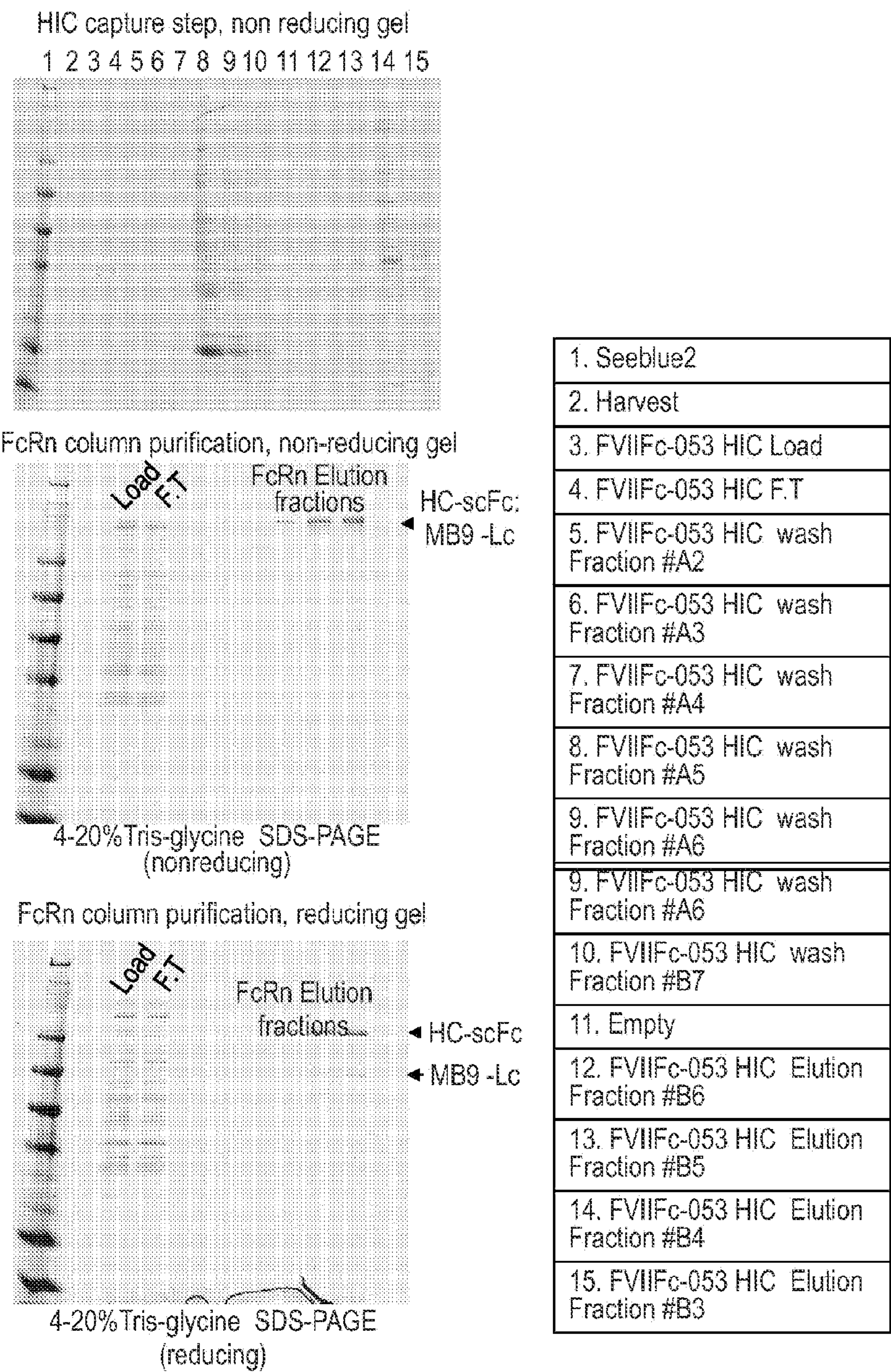


Fig. 11

Binding of FVII-011 and FVII-027 to activated
platelets determined by FACS

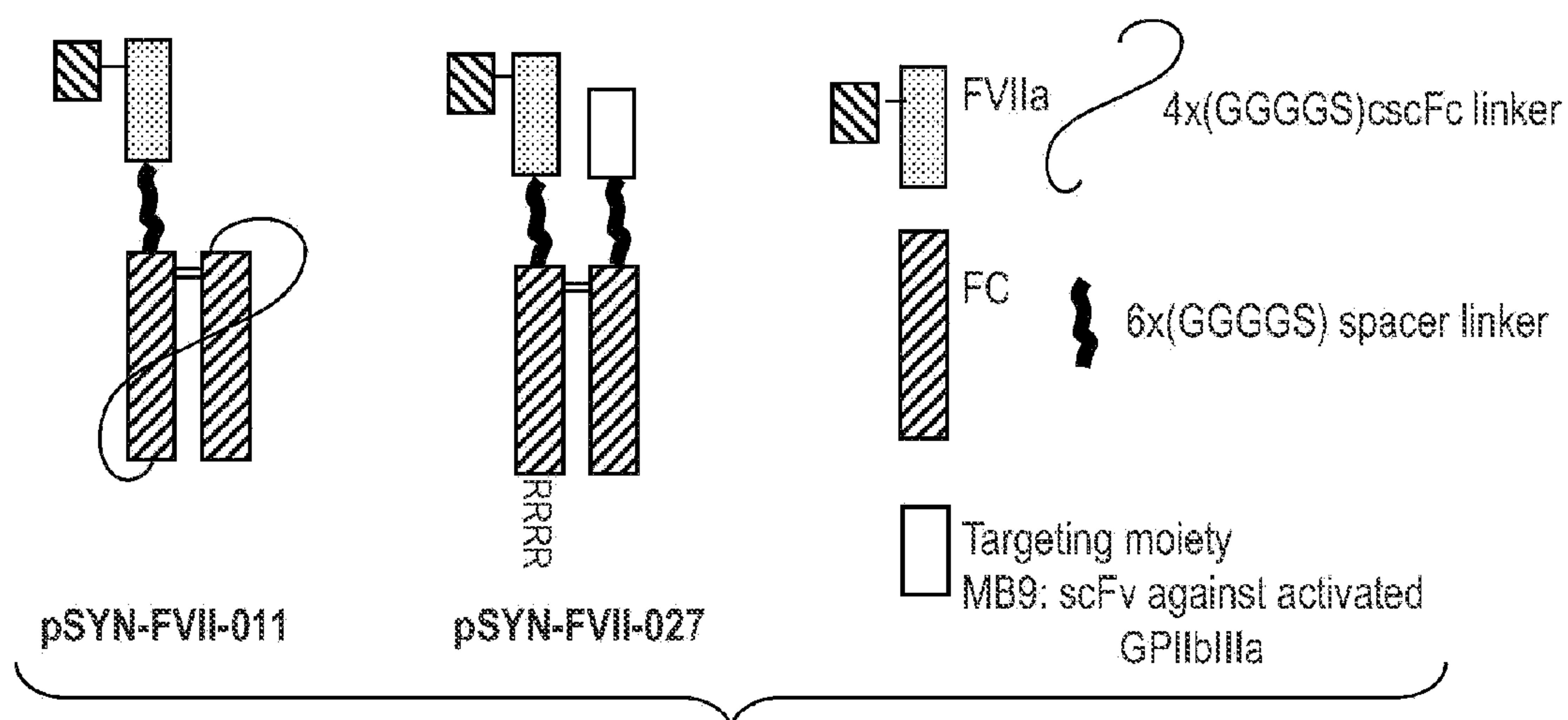


Fig. 12A

Binding of FVII-011 and FVII-027 to activated
platelets determined by FACS

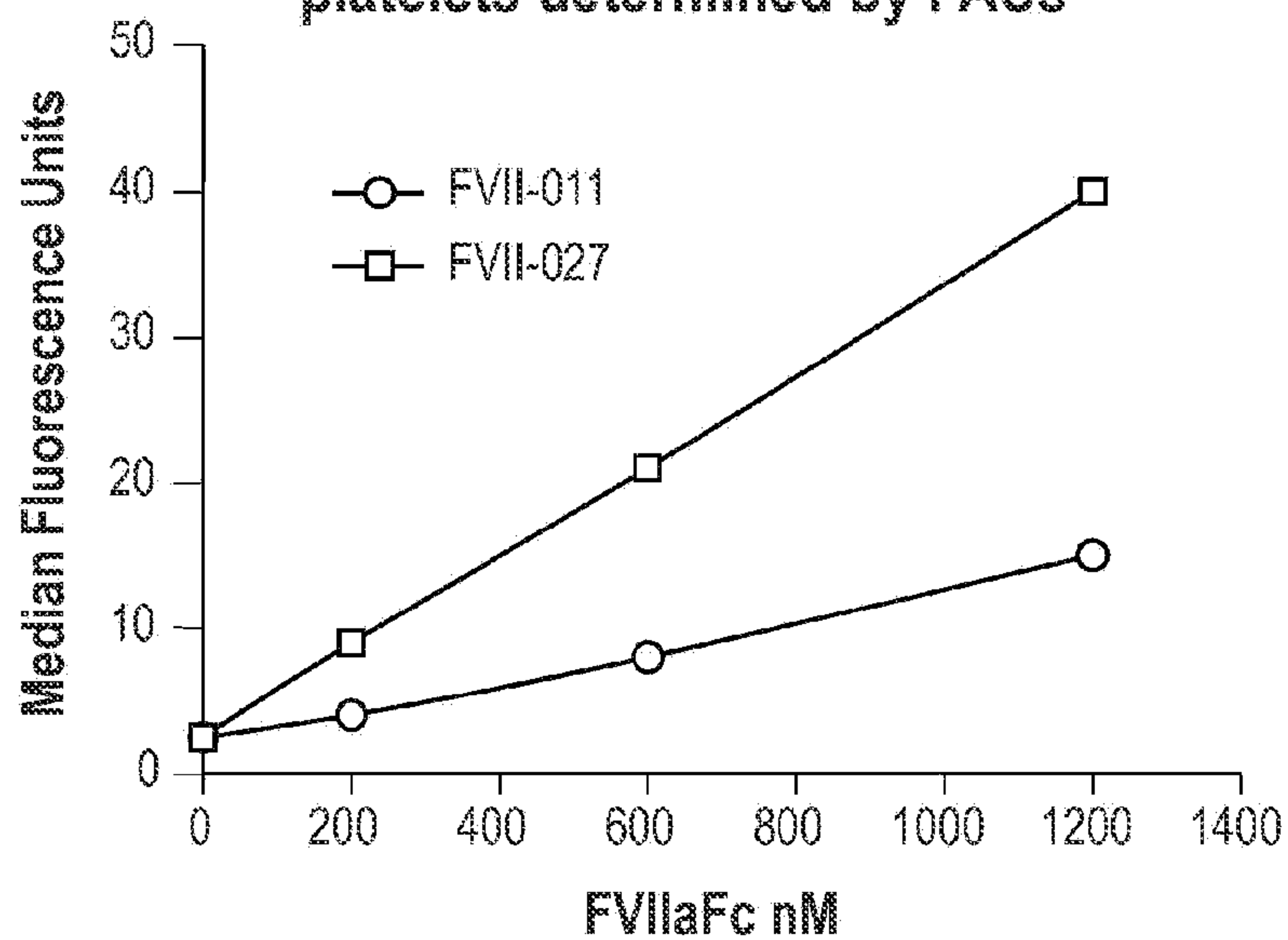


Fig. 12B

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

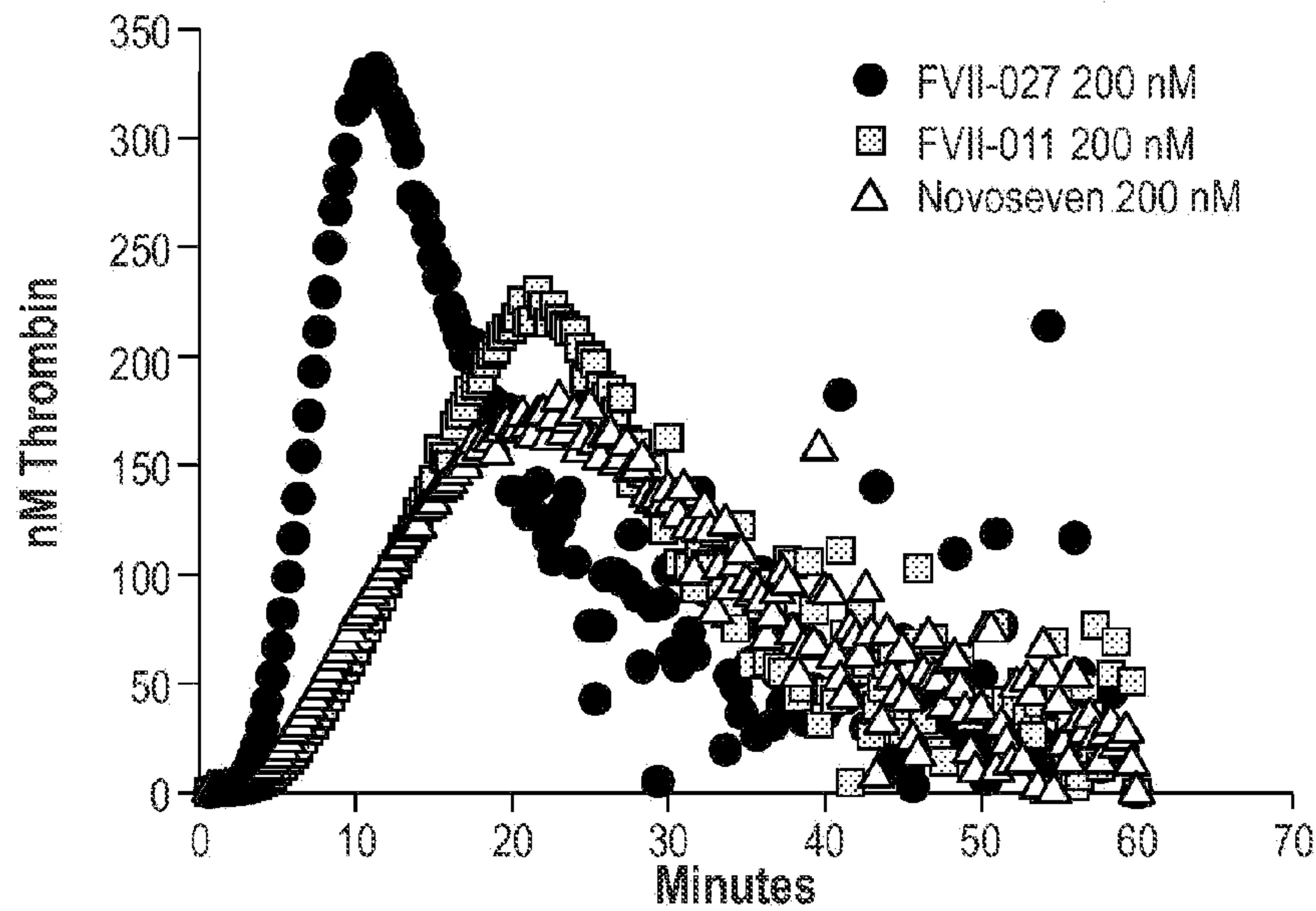


Fig. 13A

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

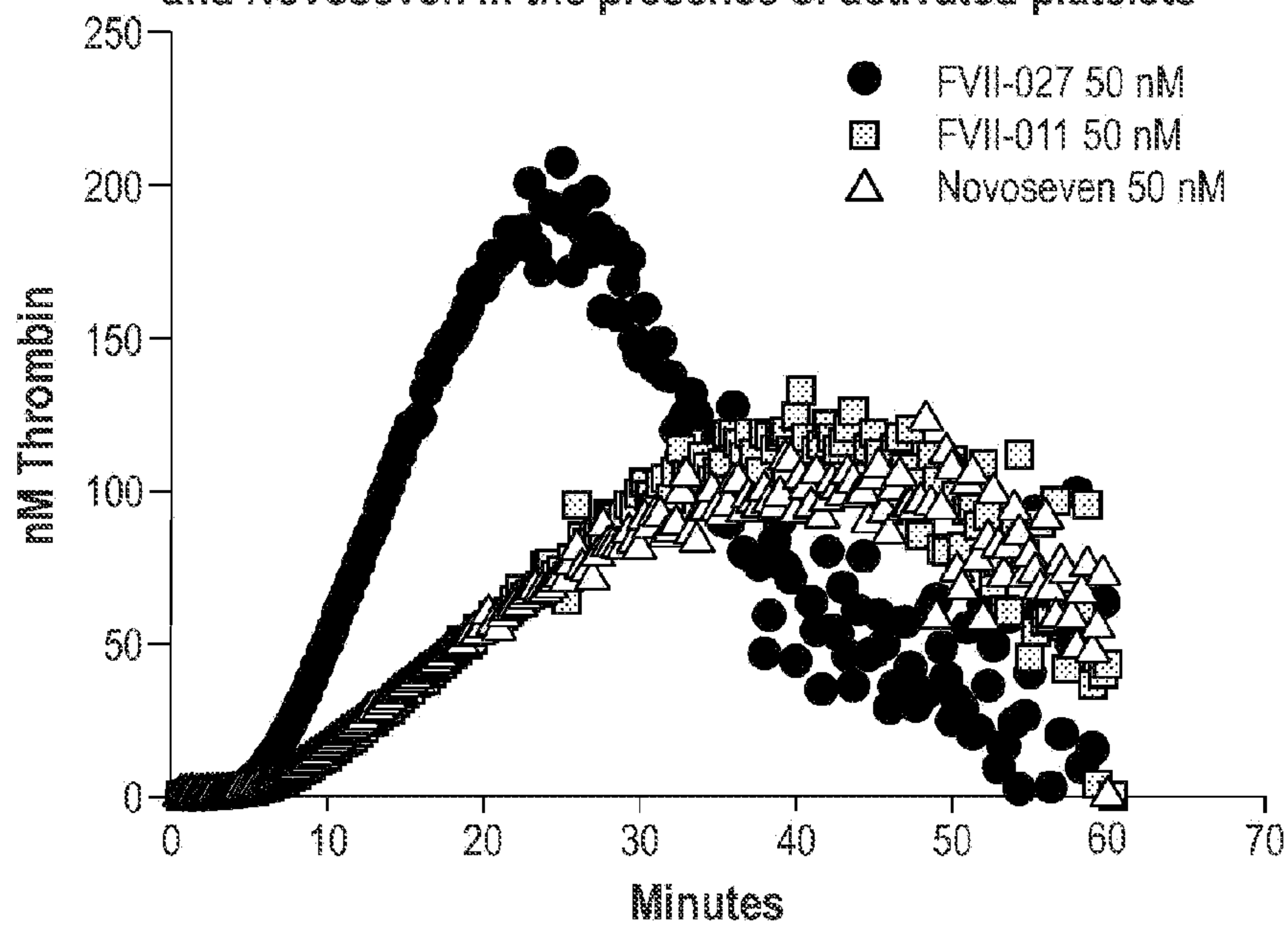


Fig. 13B

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

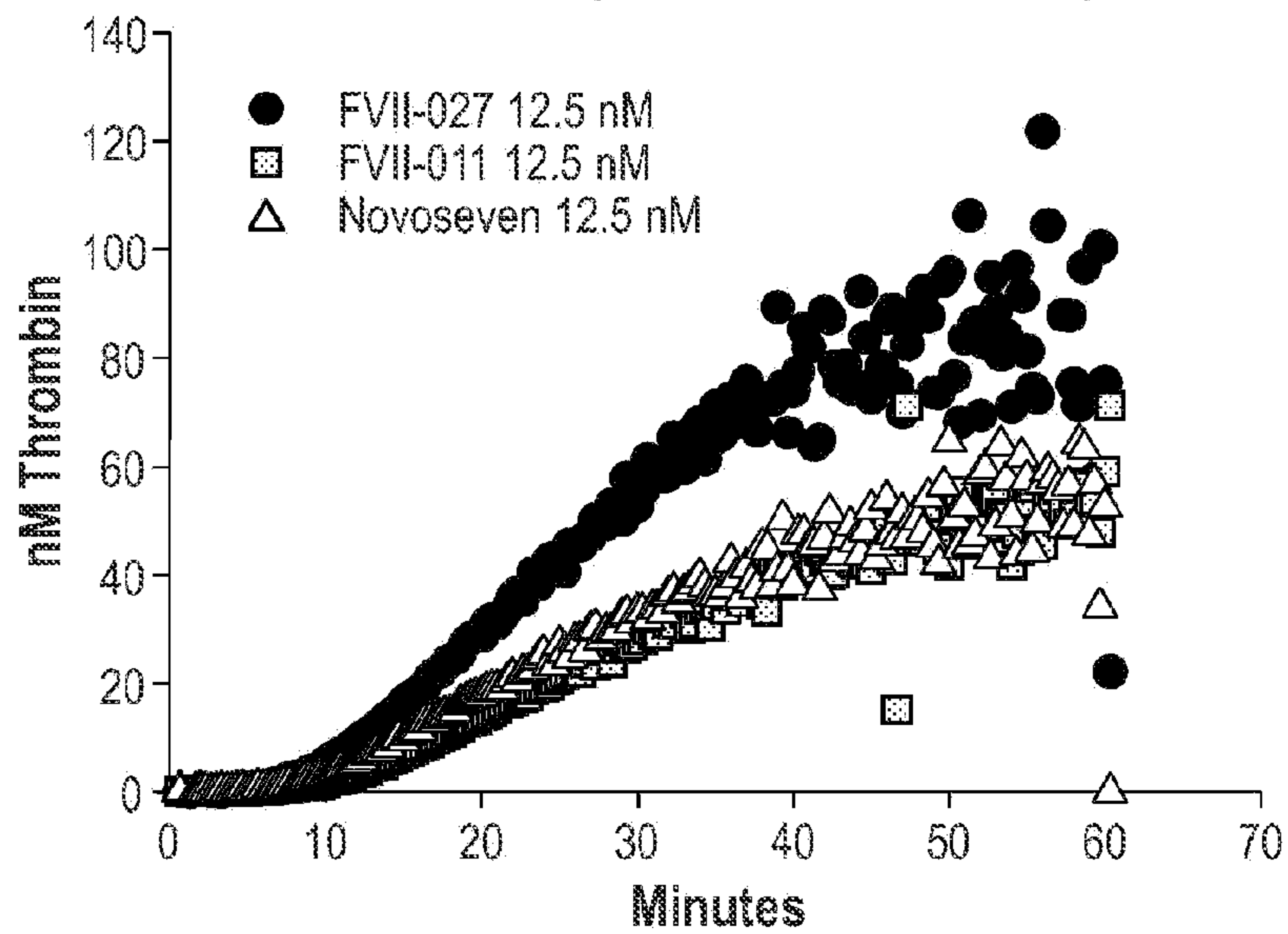


Fig. 13C

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

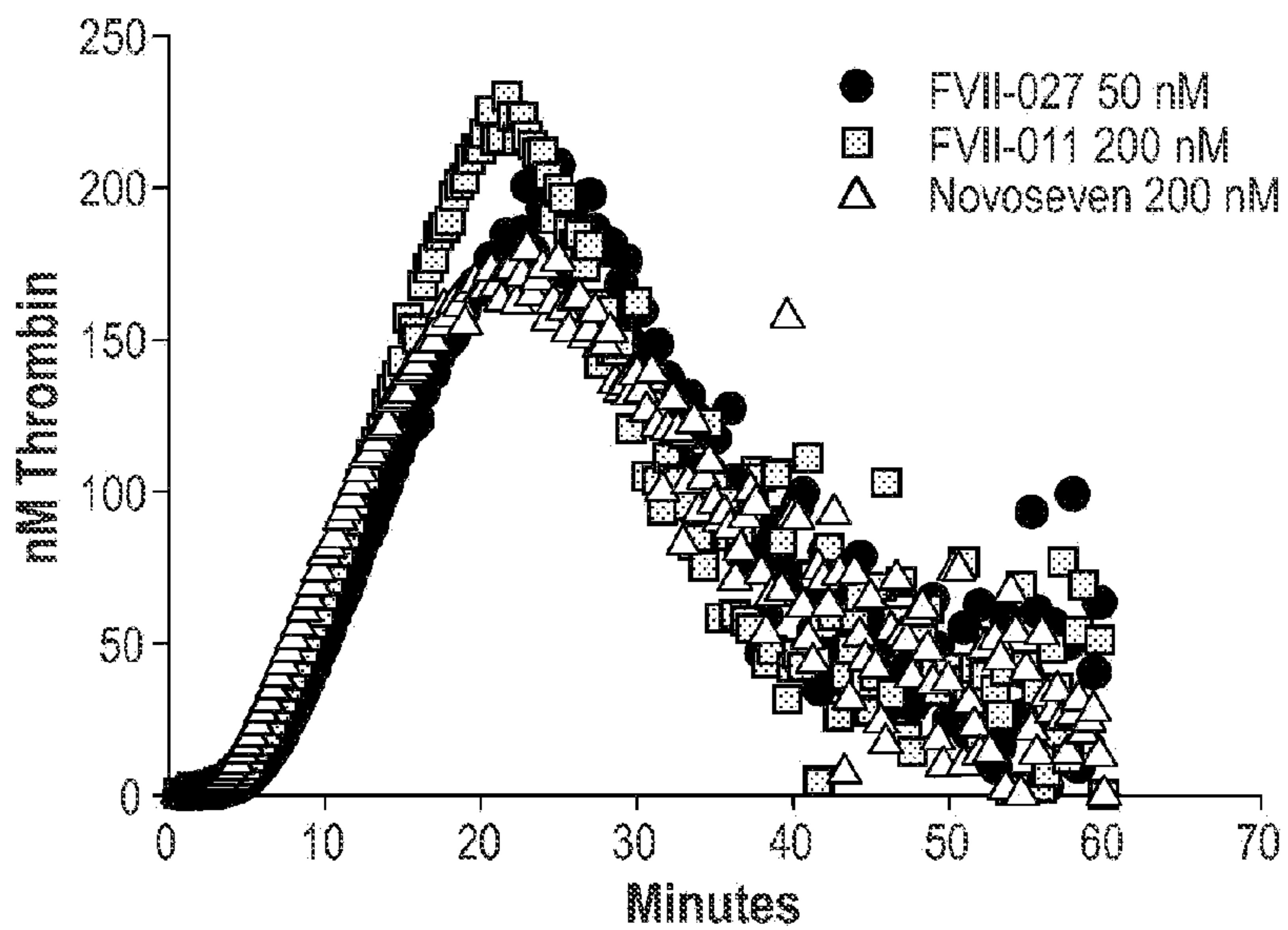


Fig. 13D

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

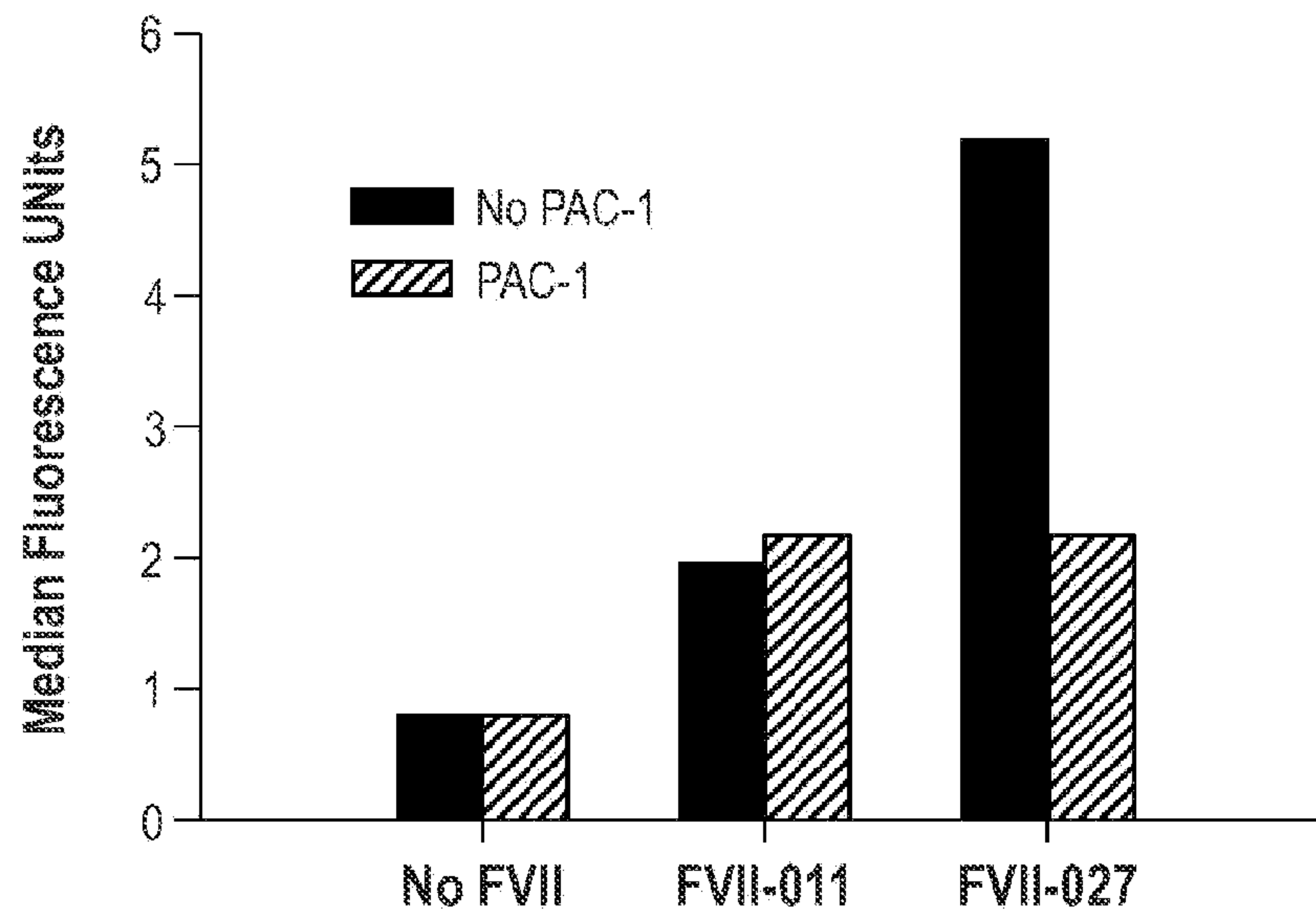


Fig. 14A

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

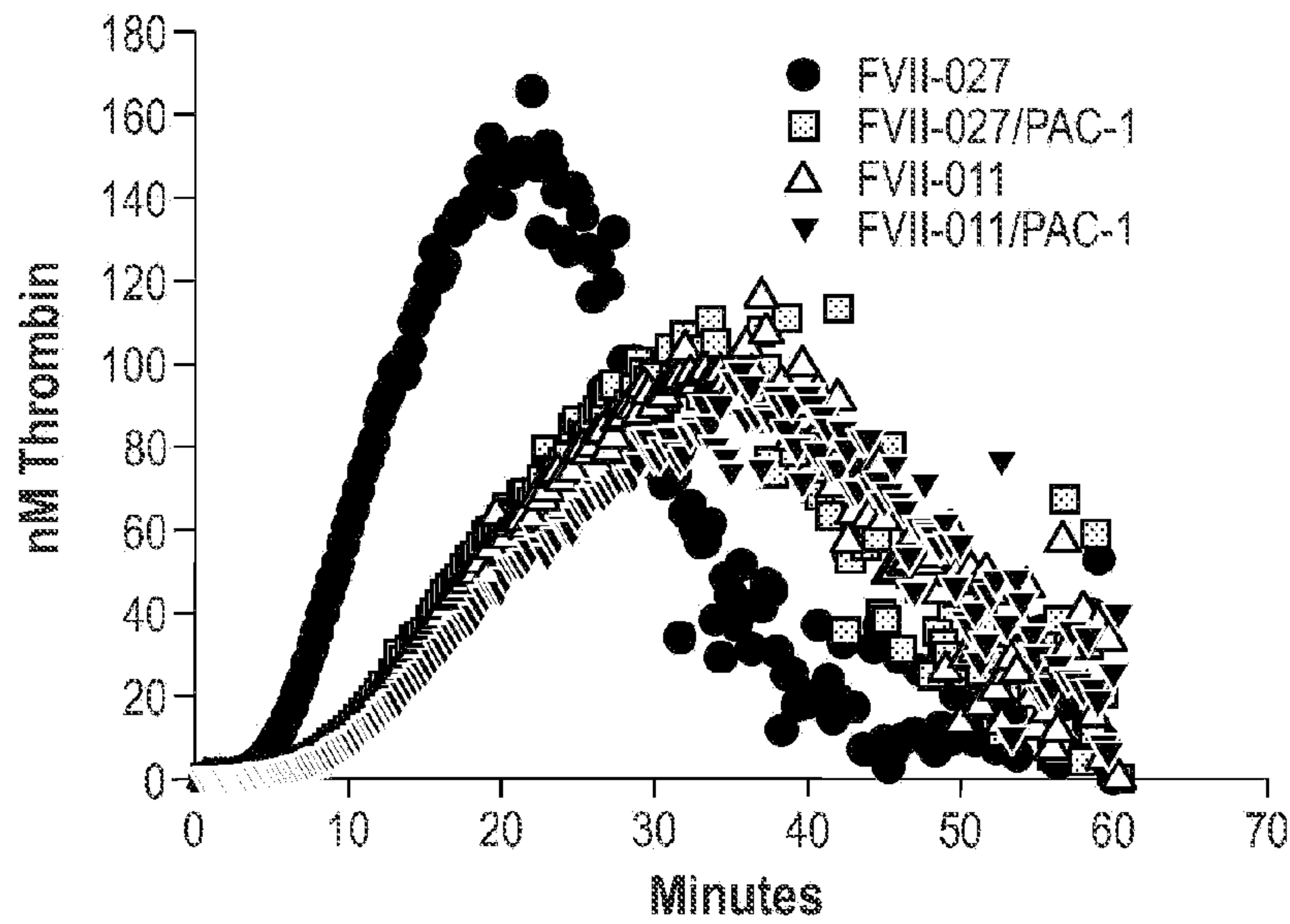


Fig. 14B

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

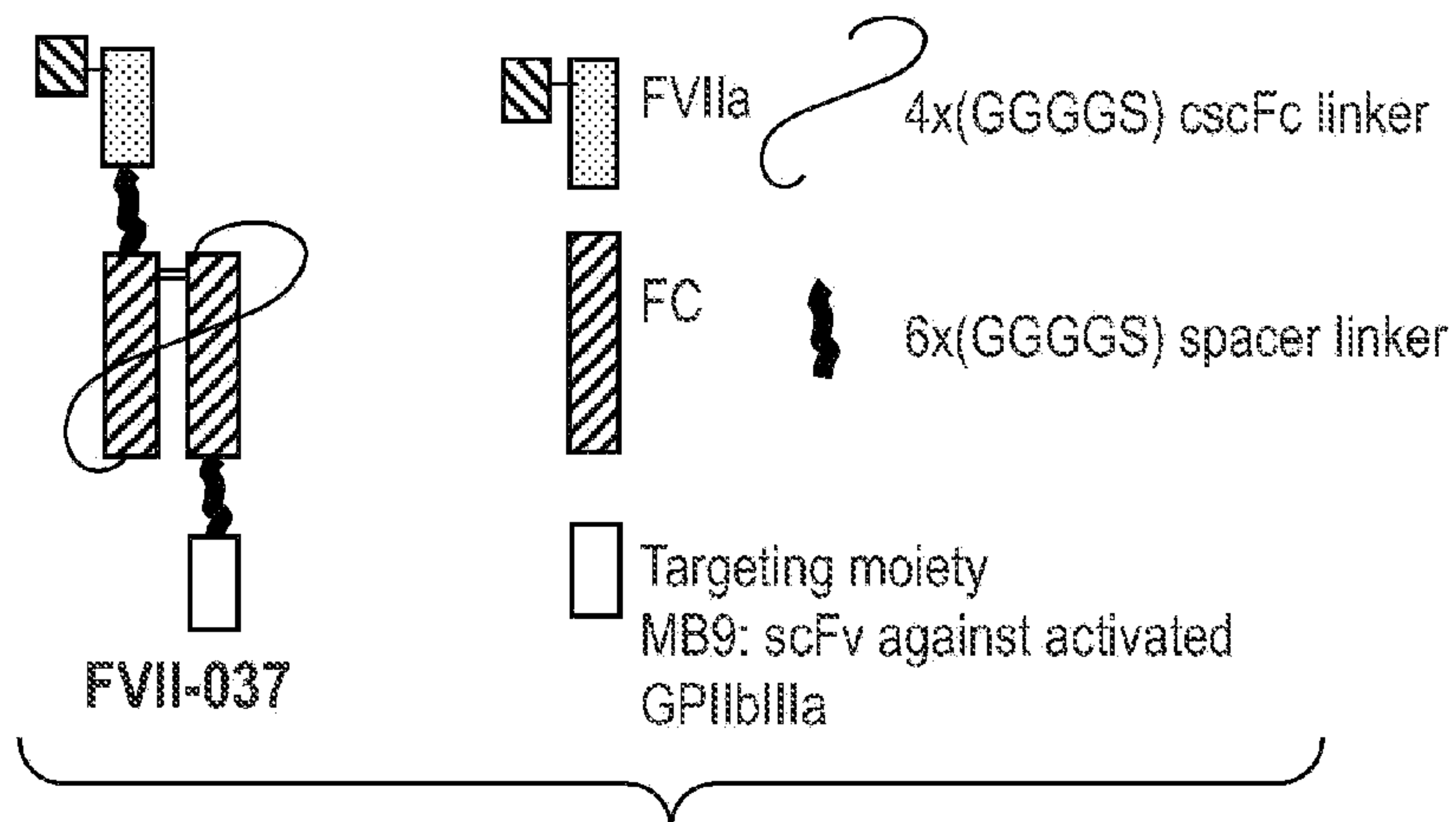


Fig. 15

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

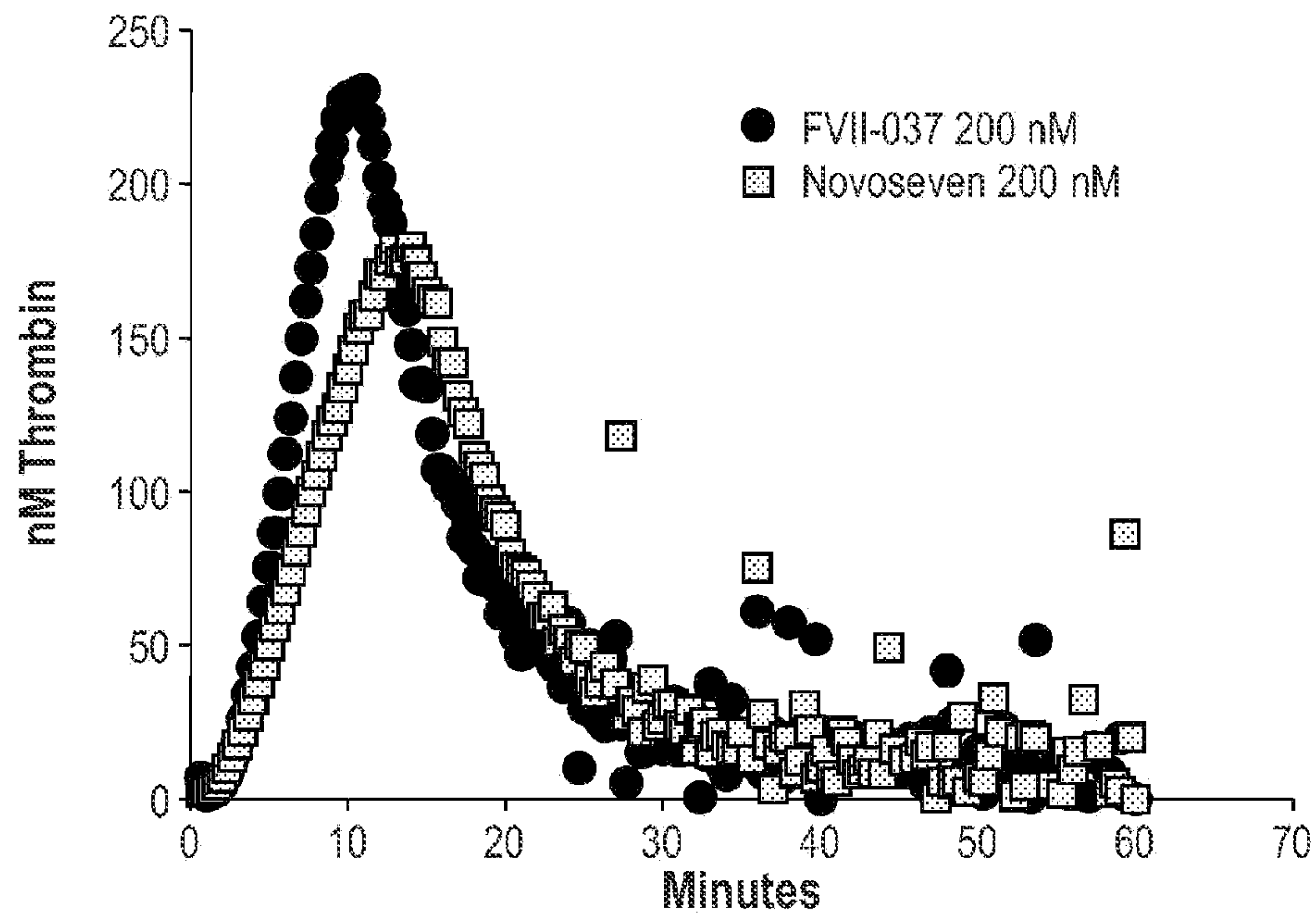


Fig. 16A

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

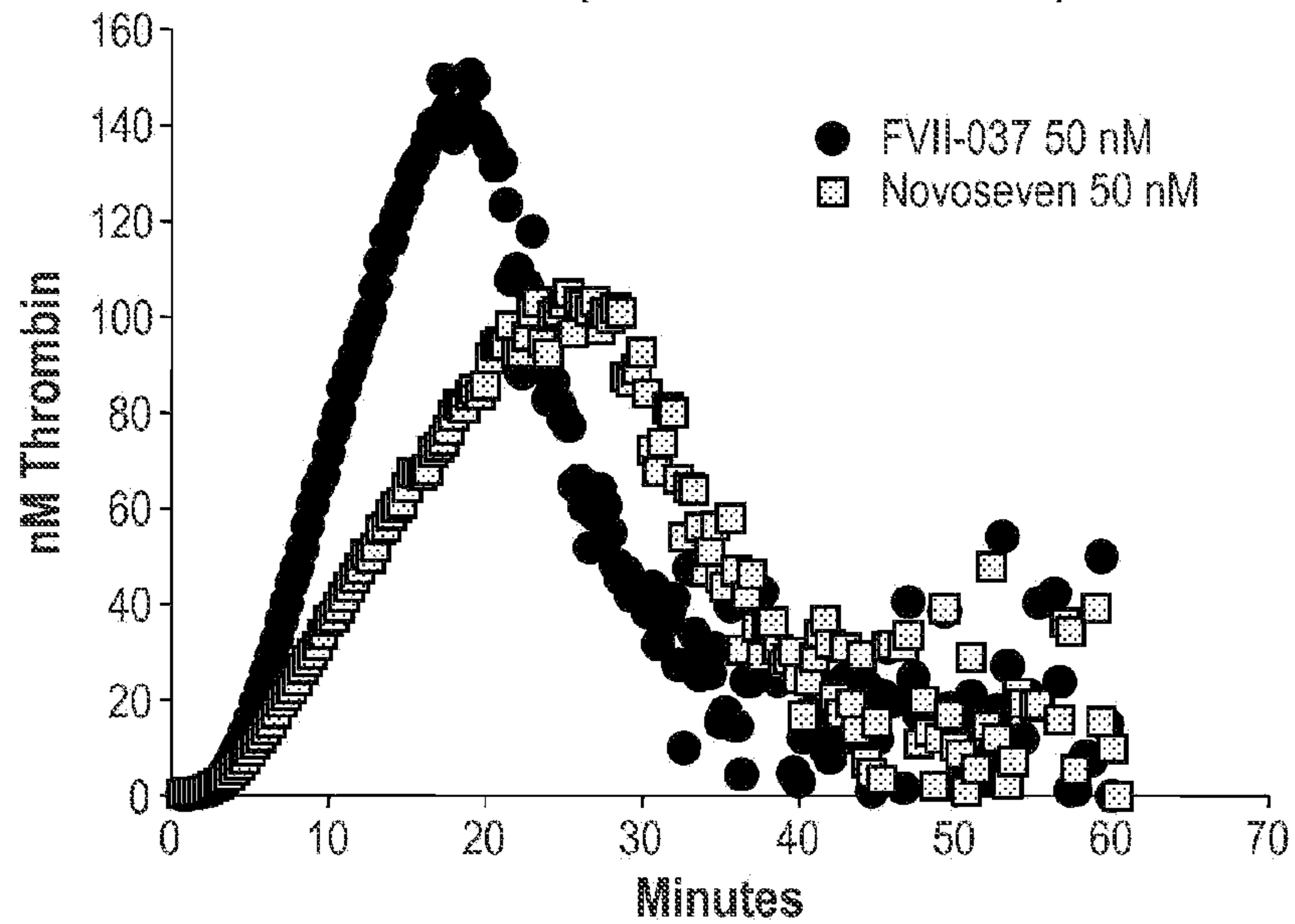


Fig. 16B

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

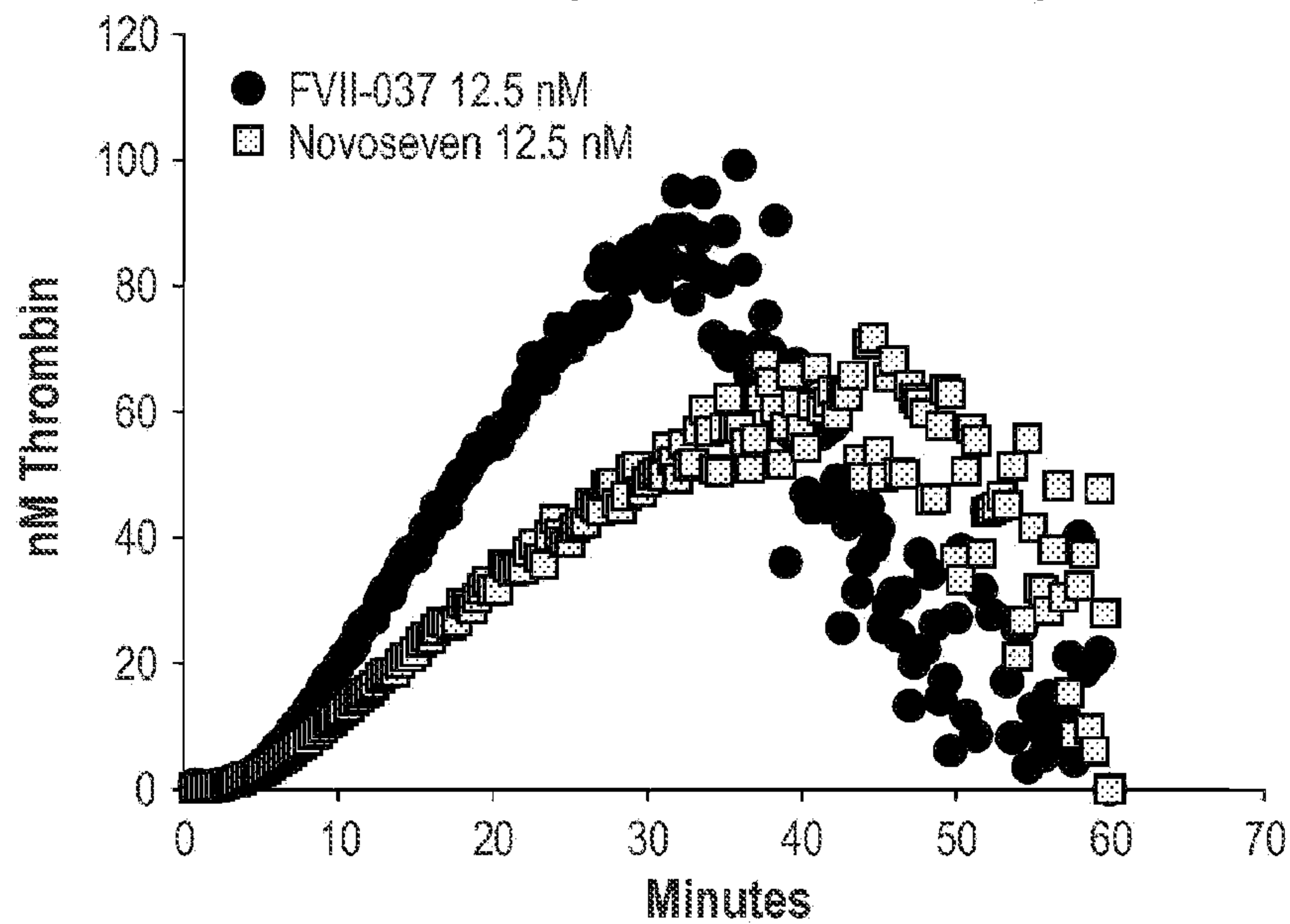
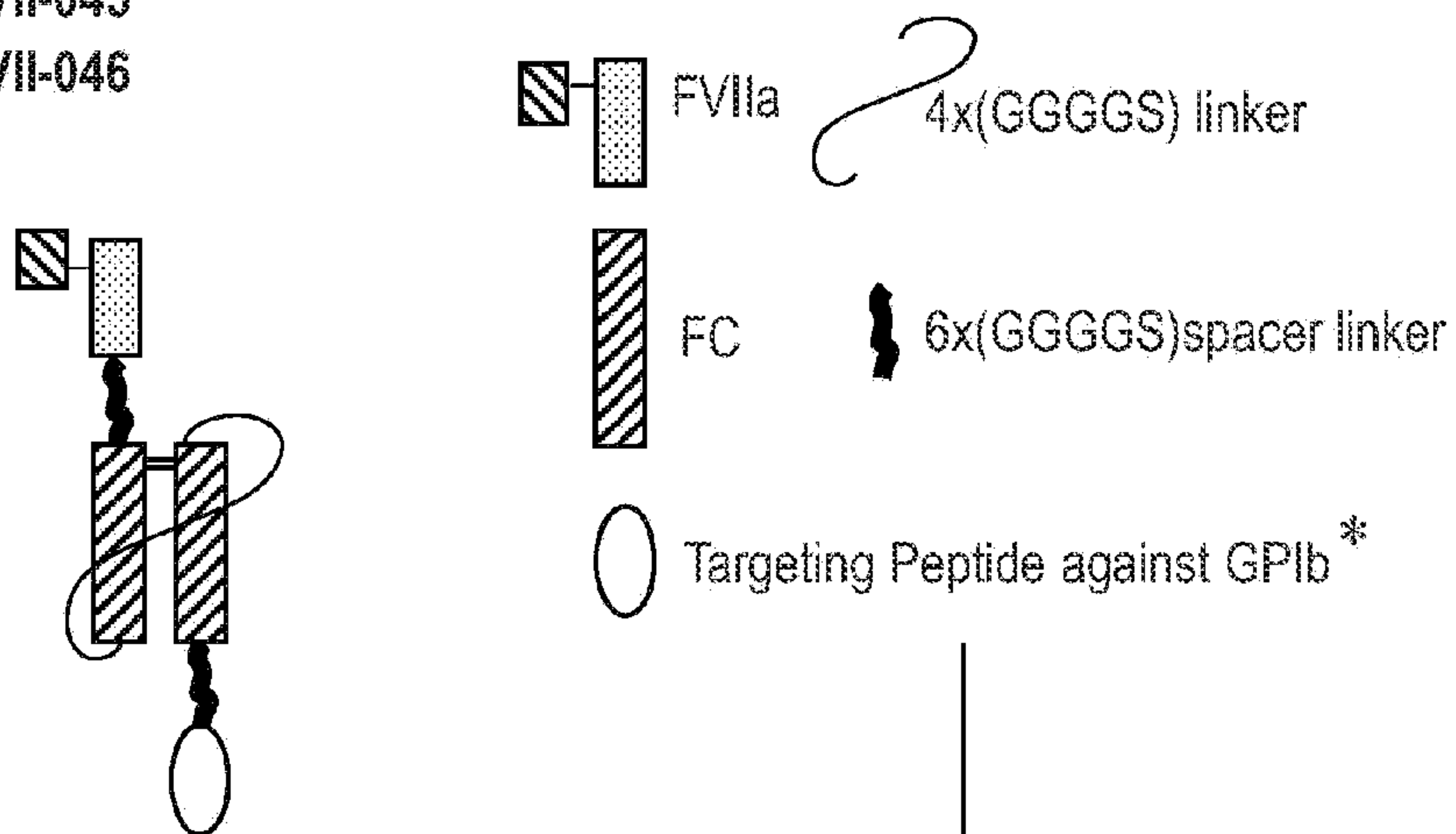


Fig. 16C

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

FVII-044
FVII-045
FVII-046



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

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Fig. 17

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

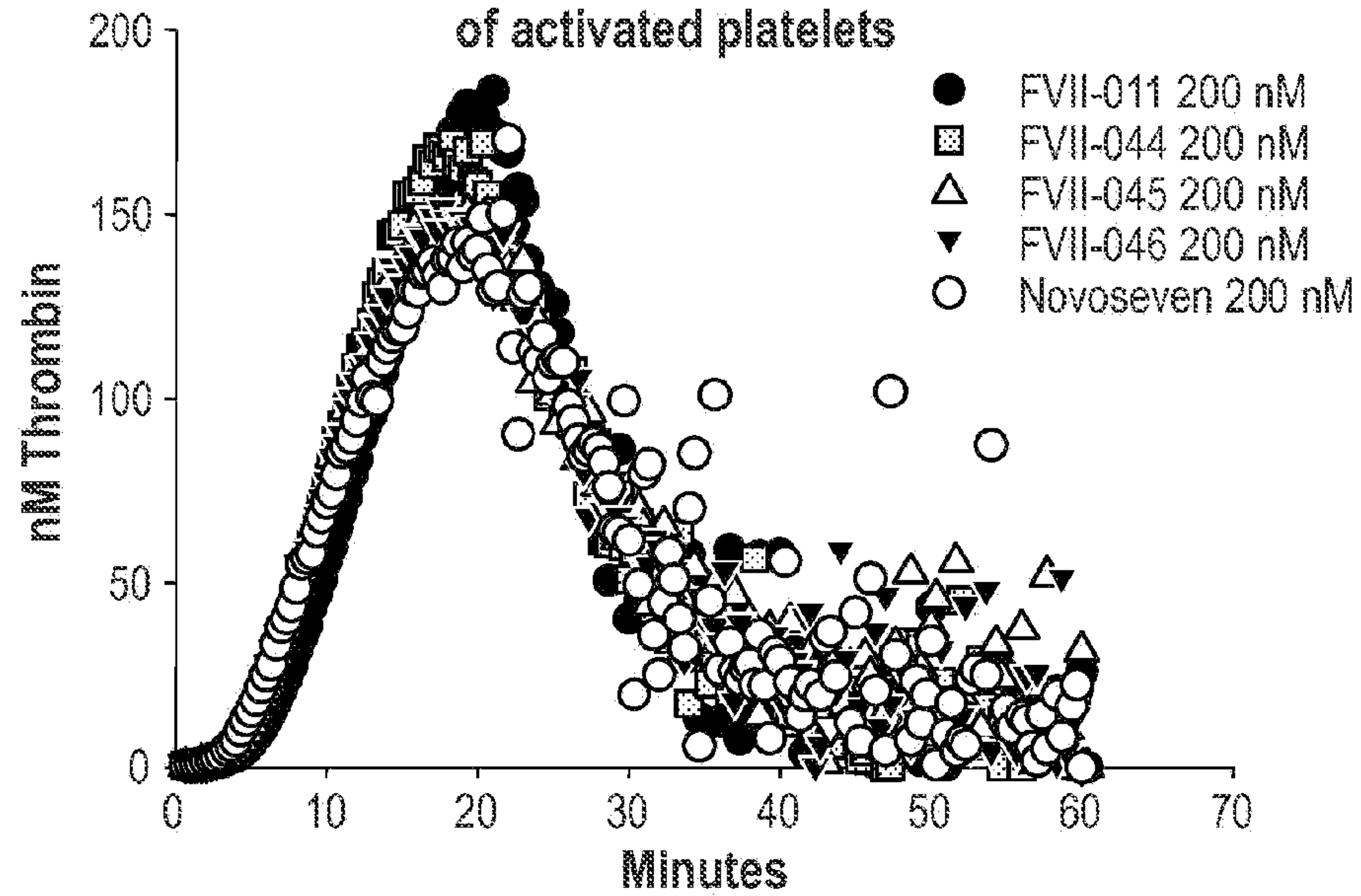


Fig. 18A

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

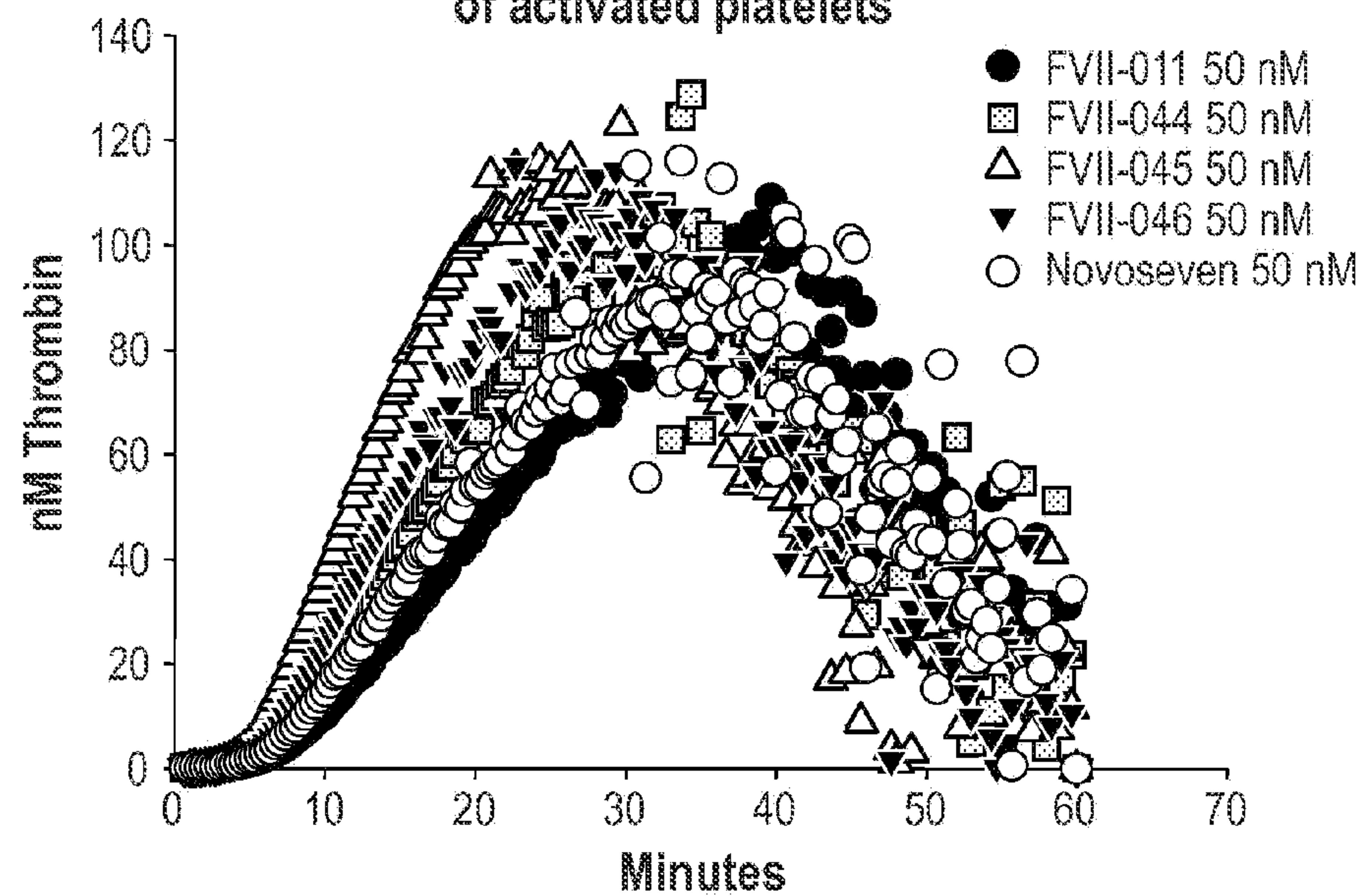


Fig. 18B

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

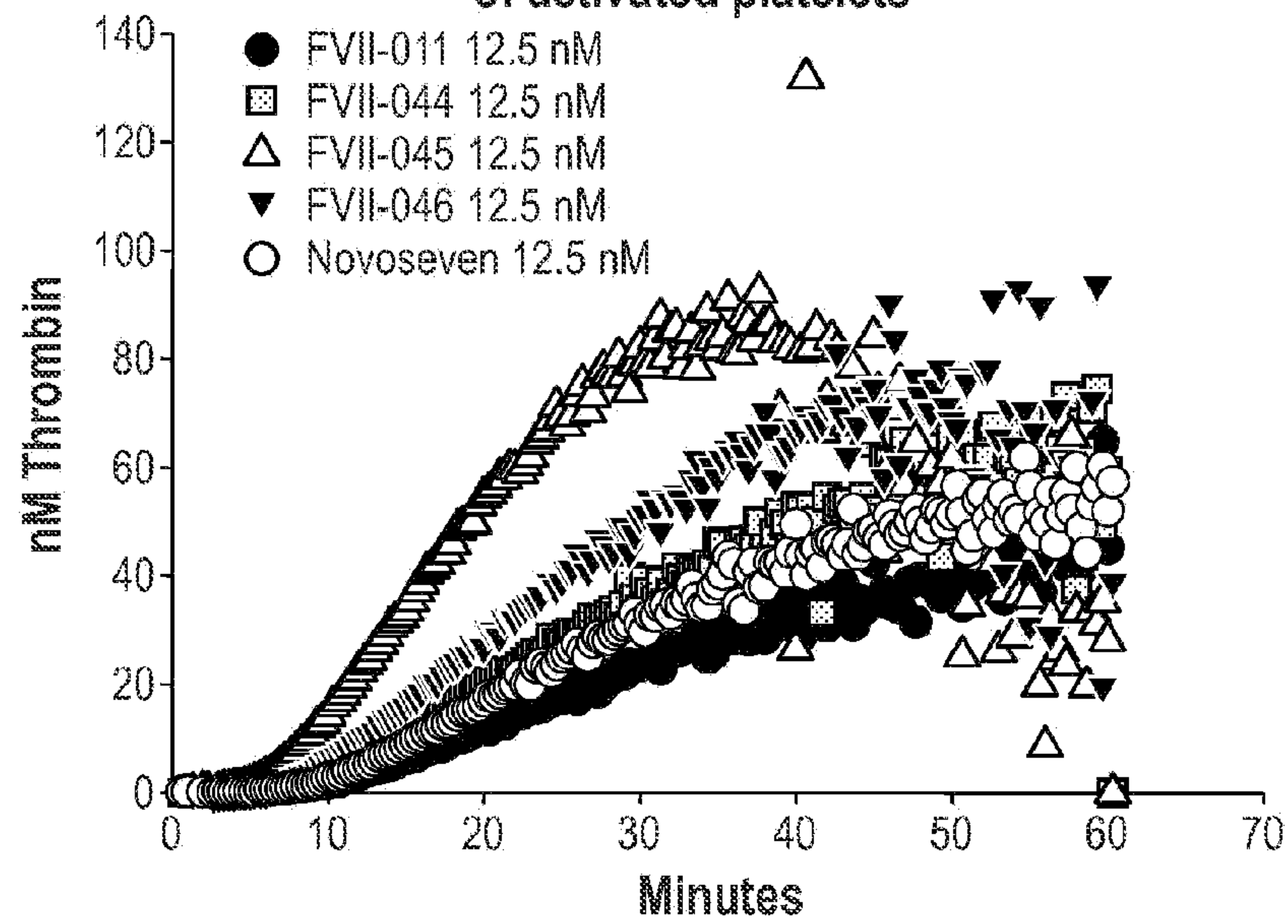


Fig. 18C

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

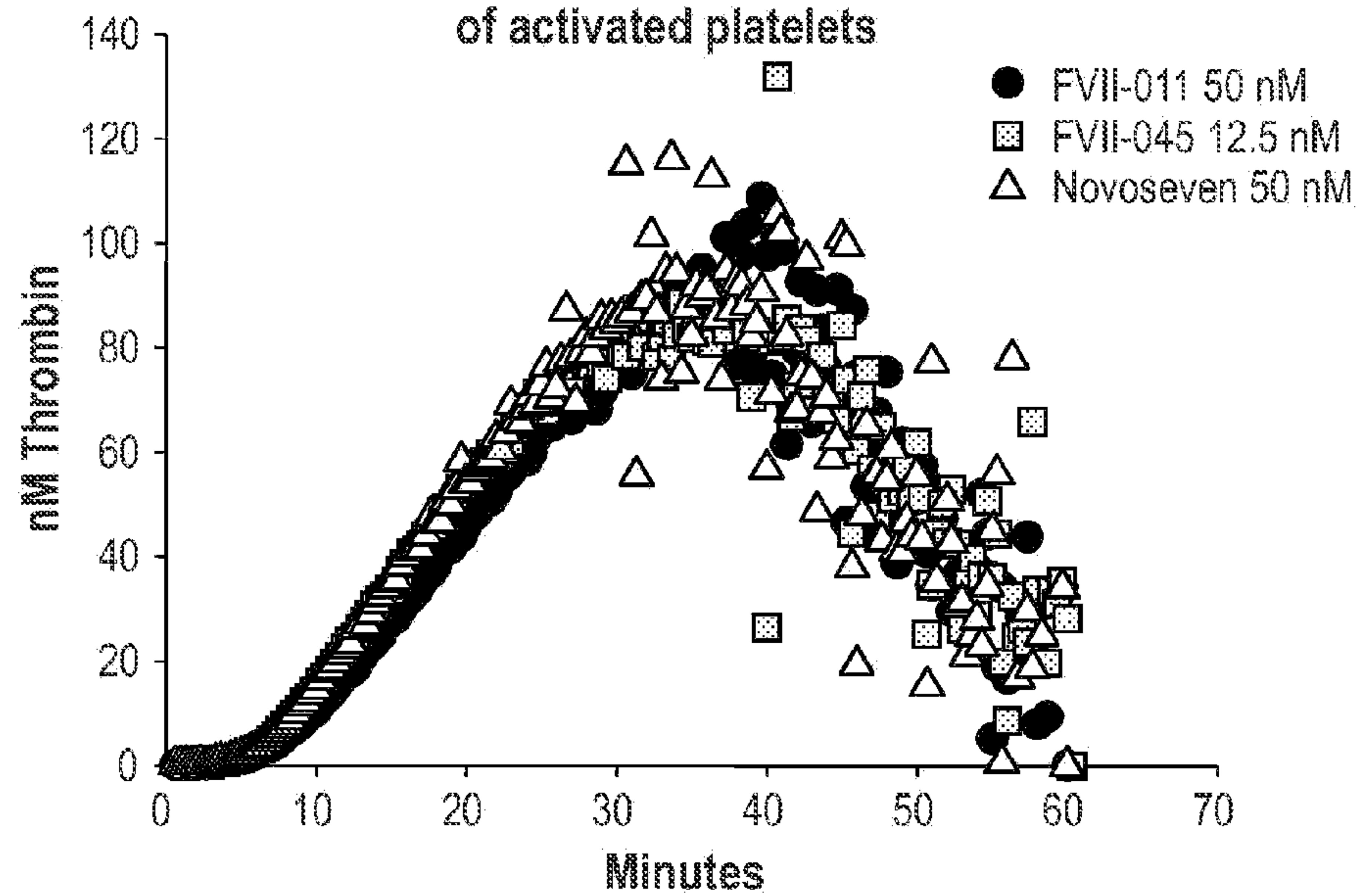
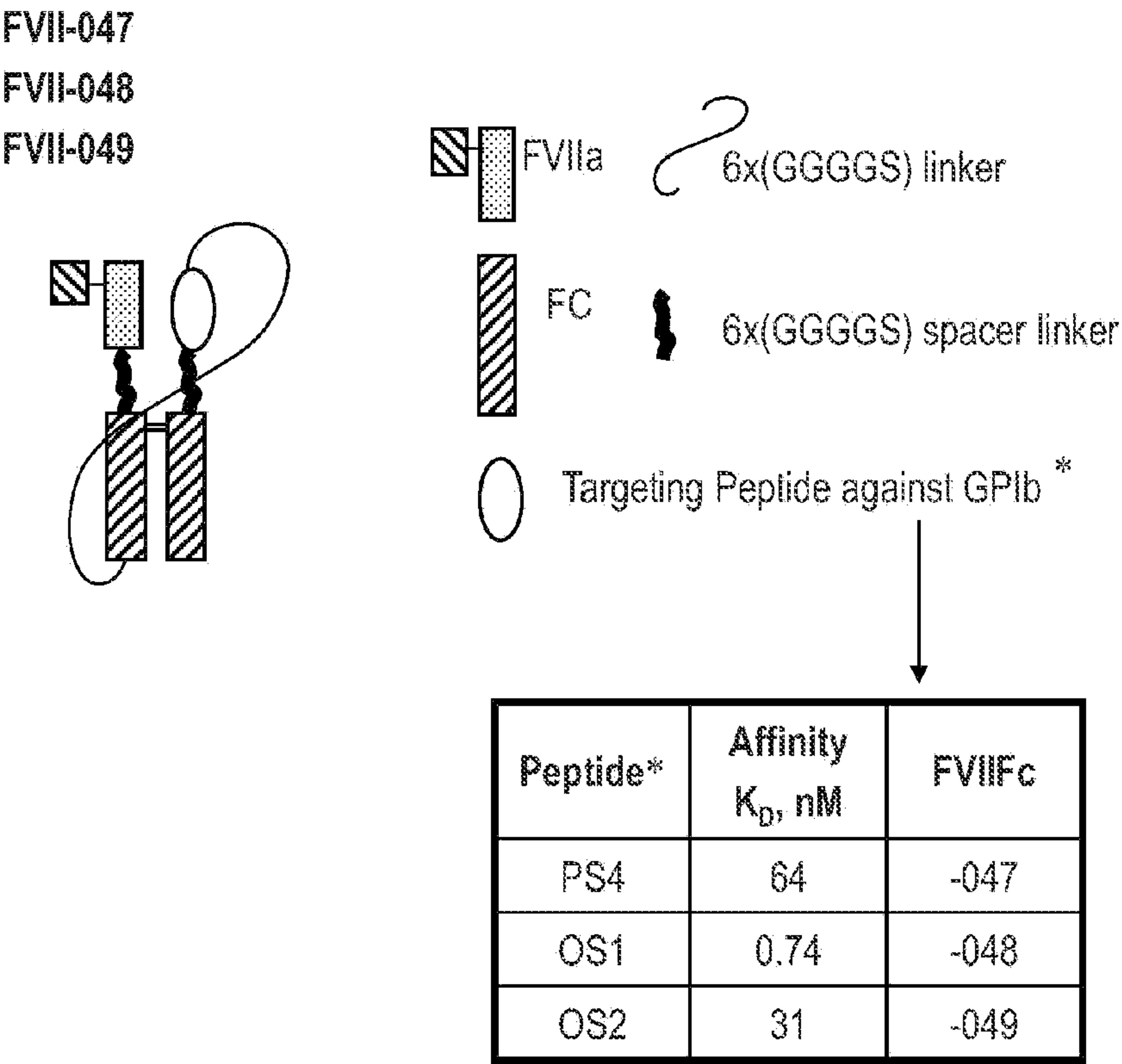


Fig. 18D

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



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Fig. 19

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

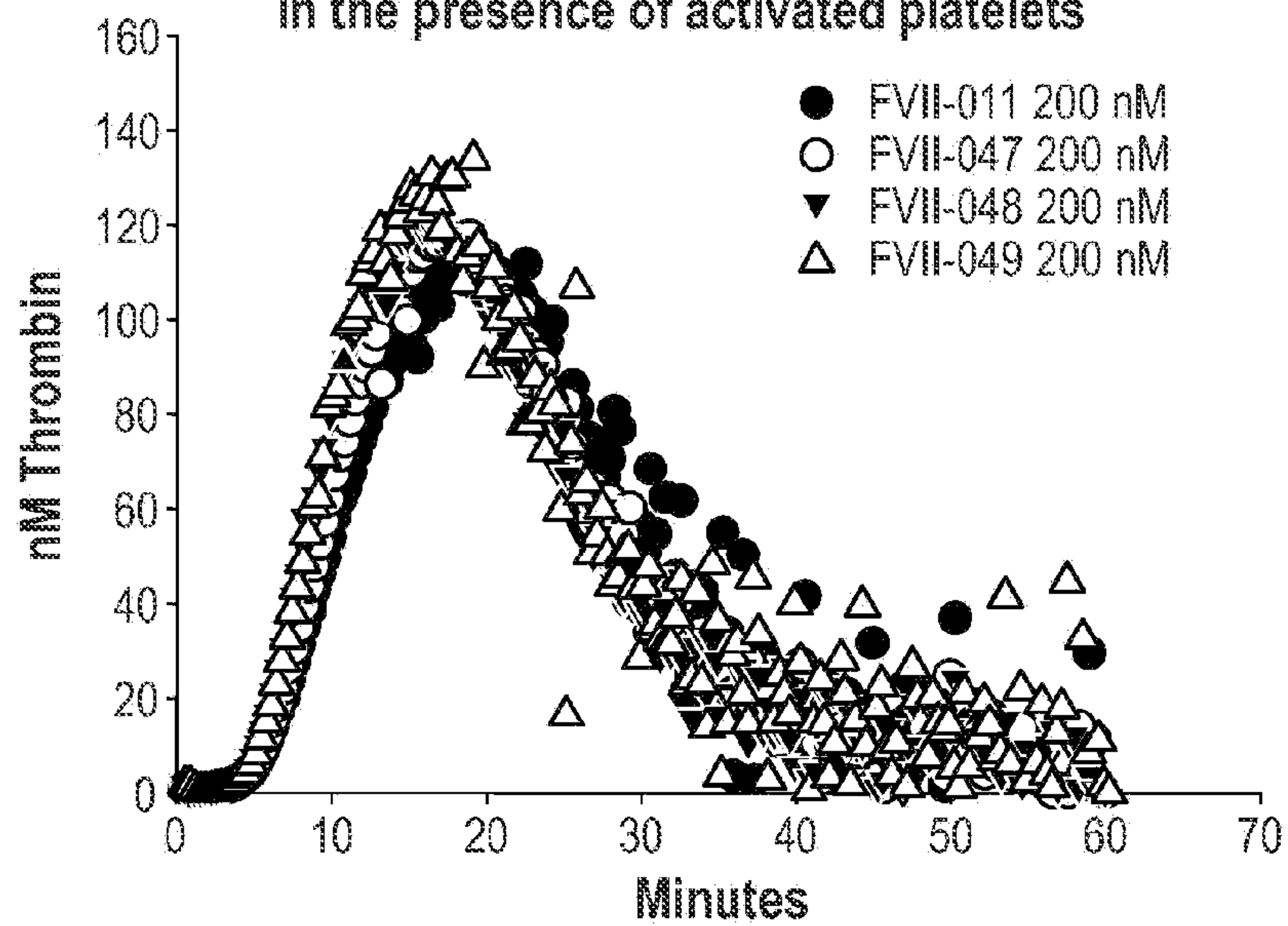


Fig. 20A

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

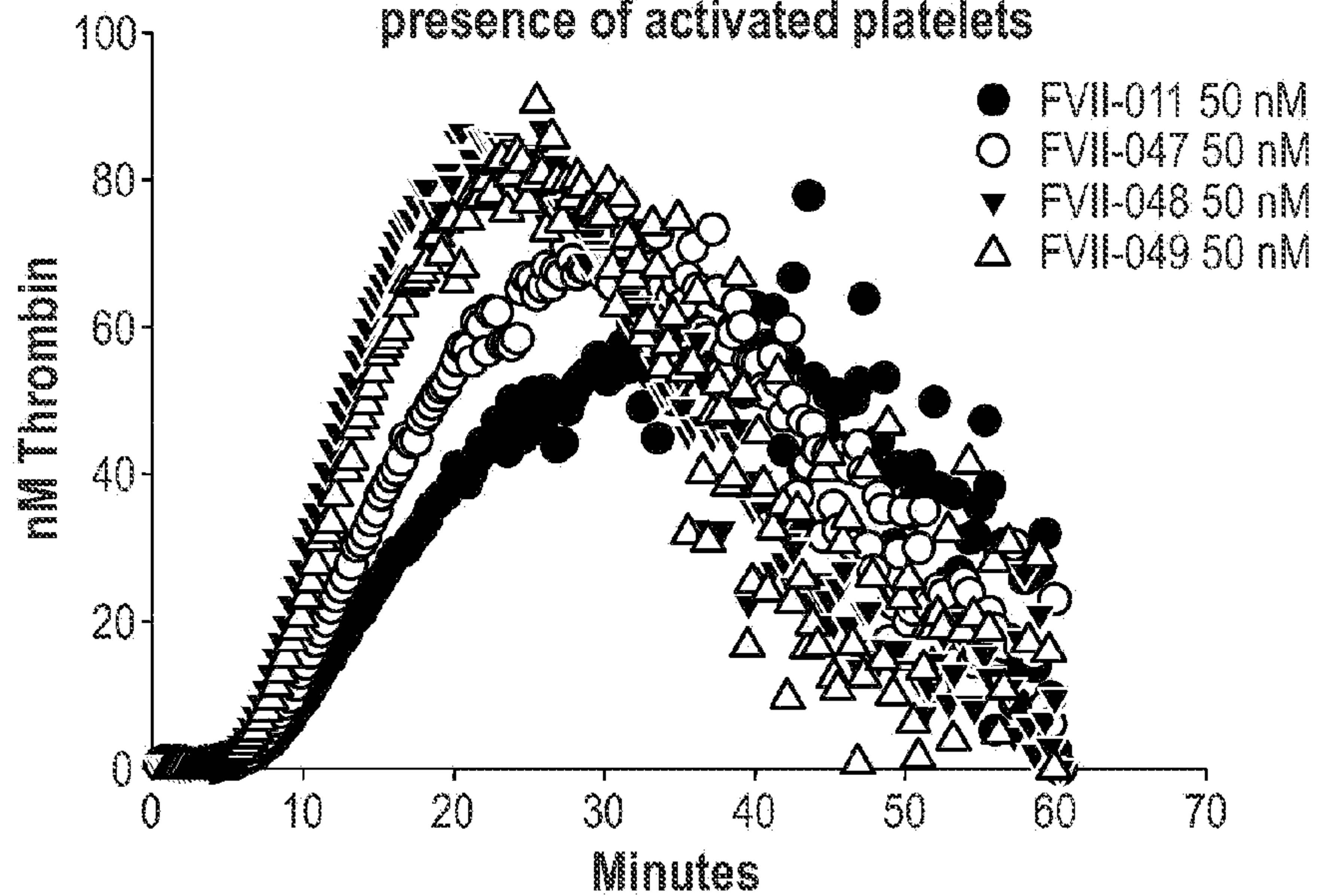


Fig. 20B

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

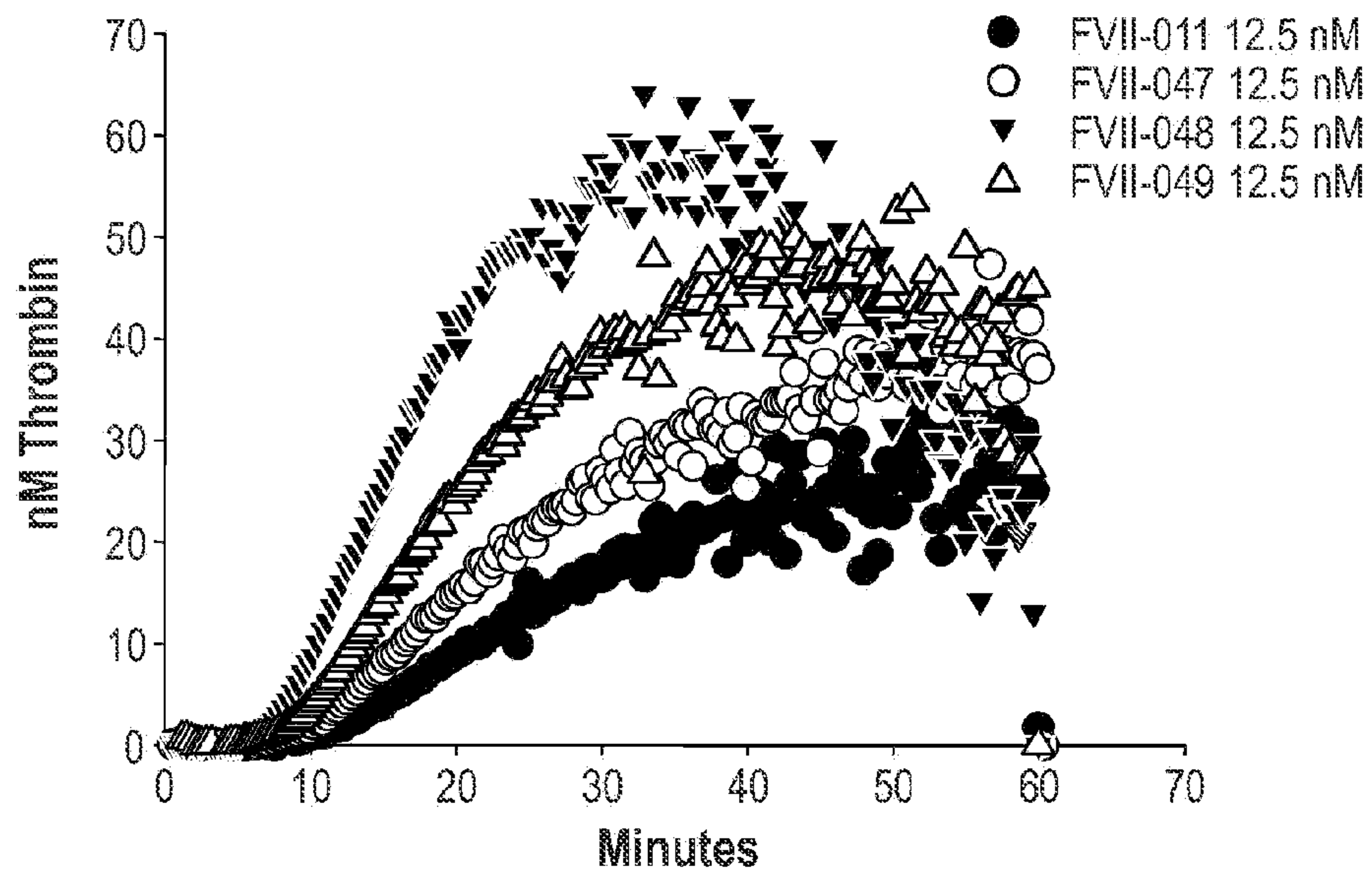


Fig. 20C

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

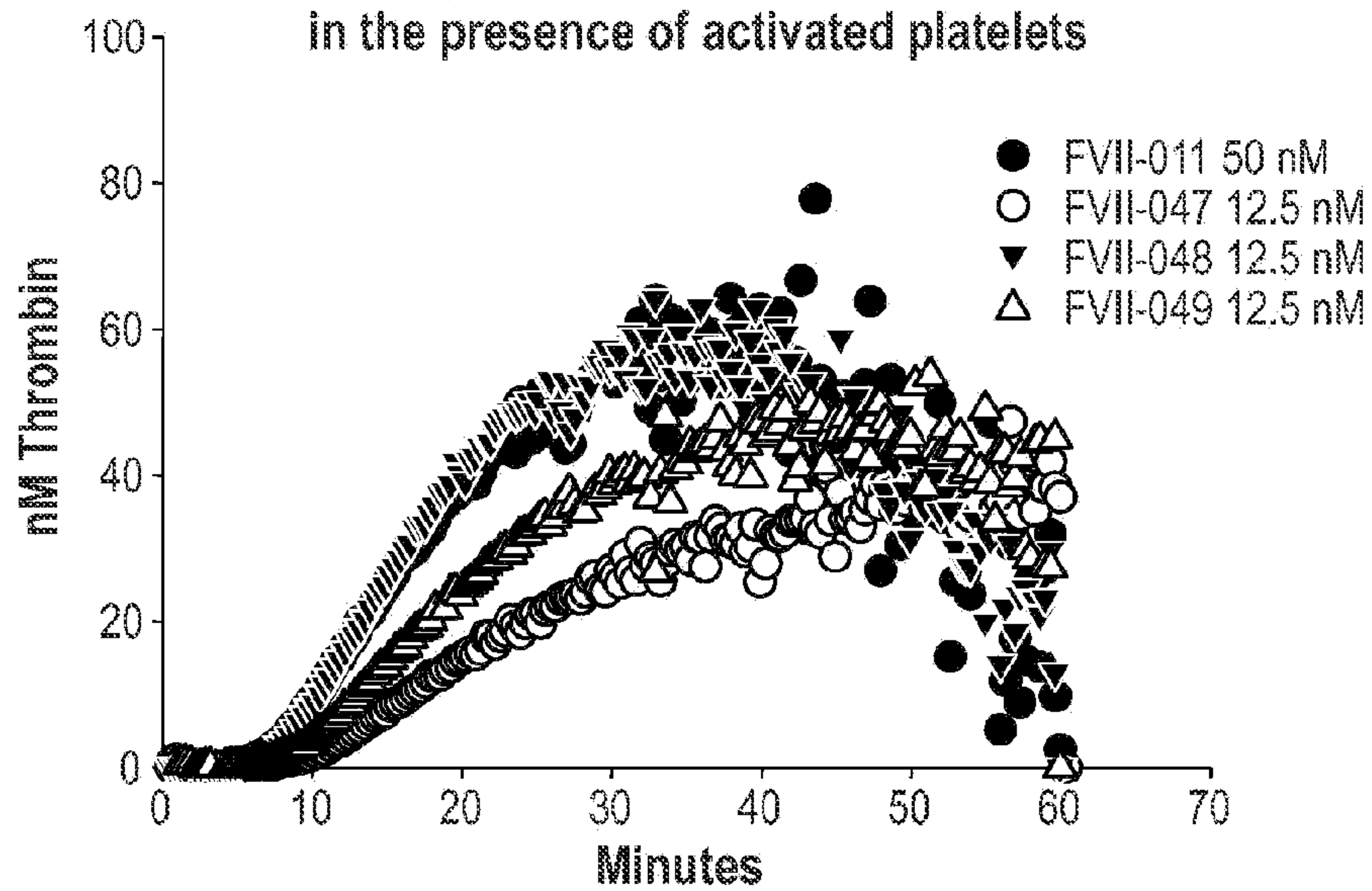


Fig. 20D

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

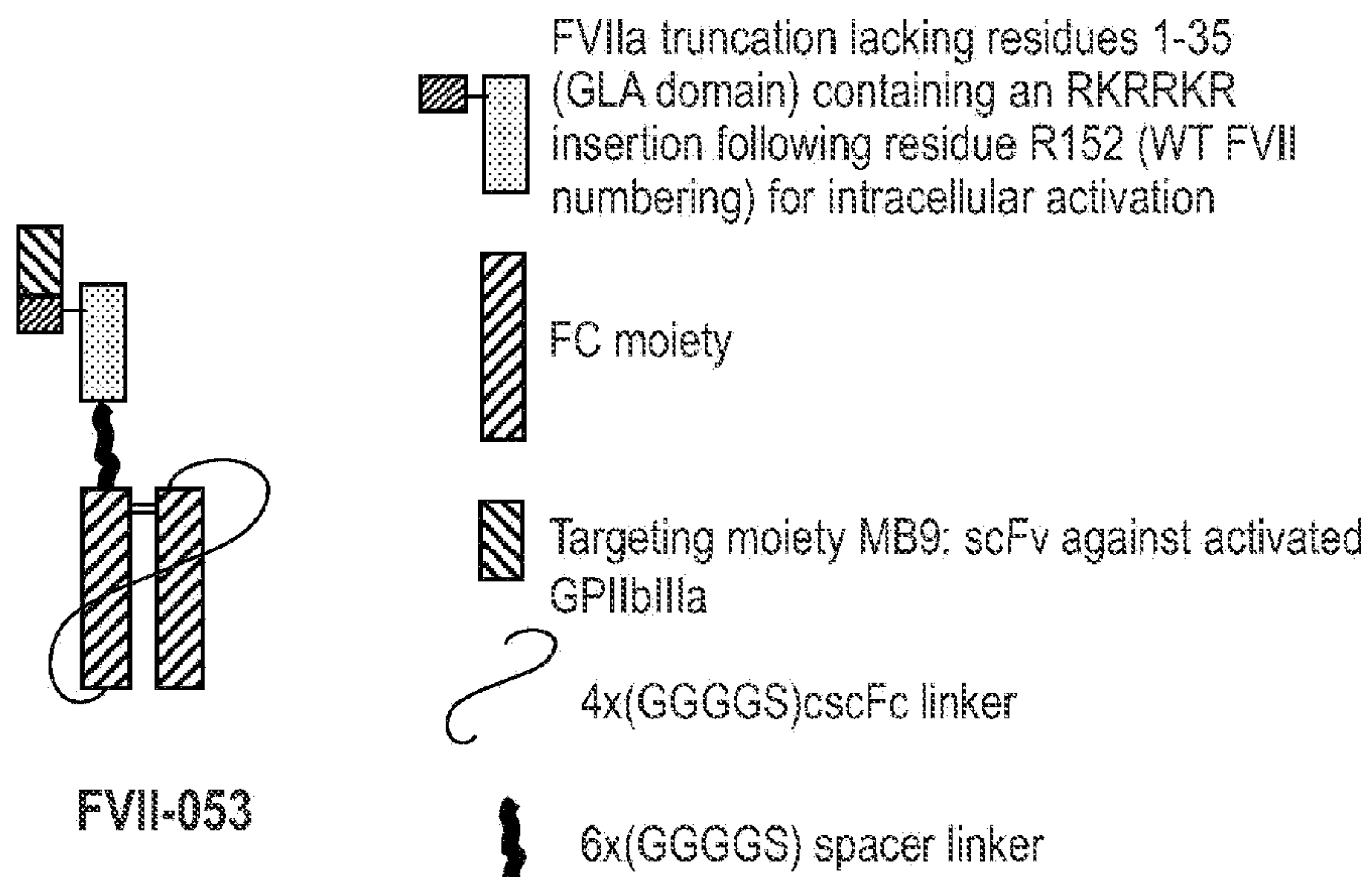


Fig. 21

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

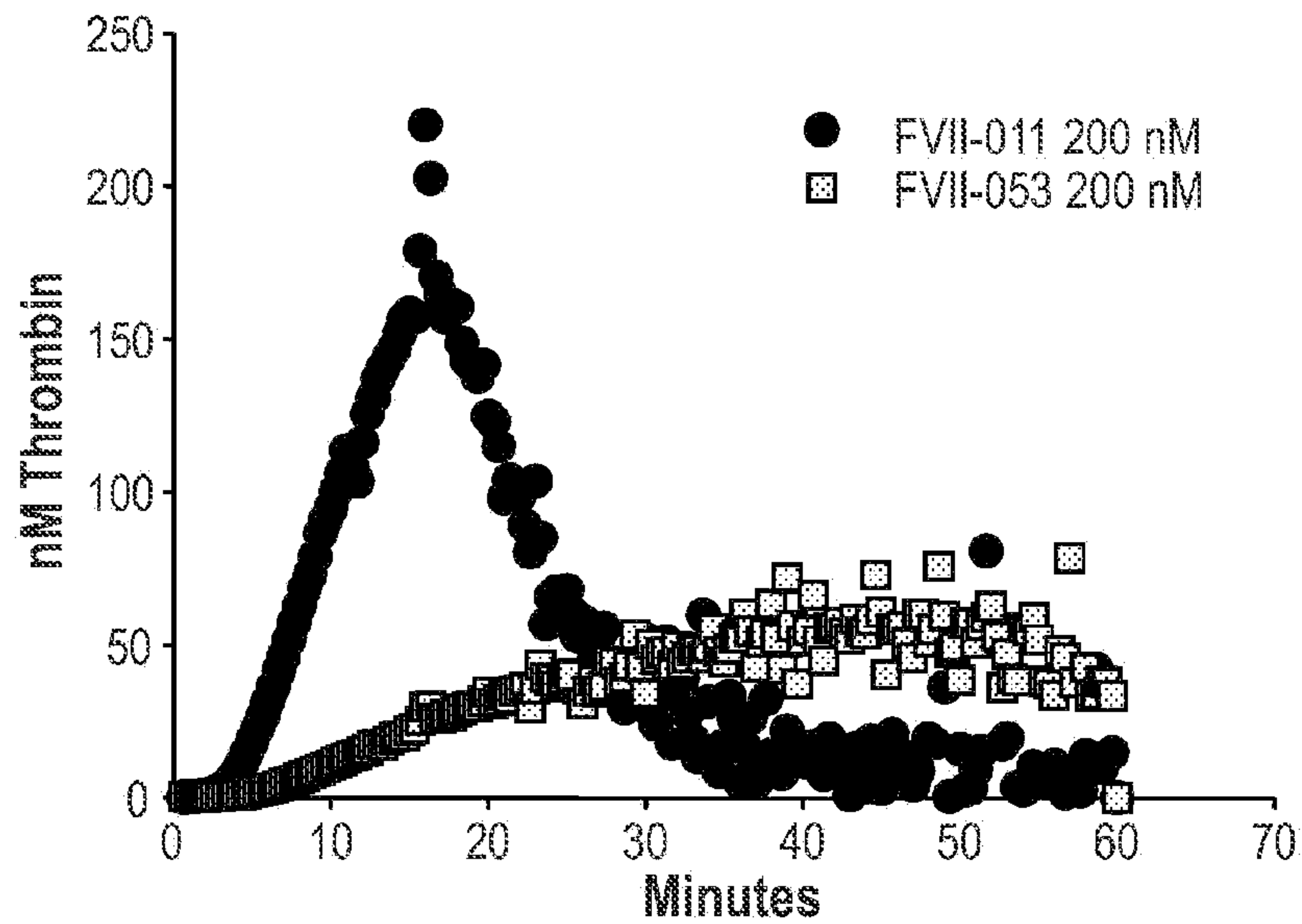


Fig. 22A

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

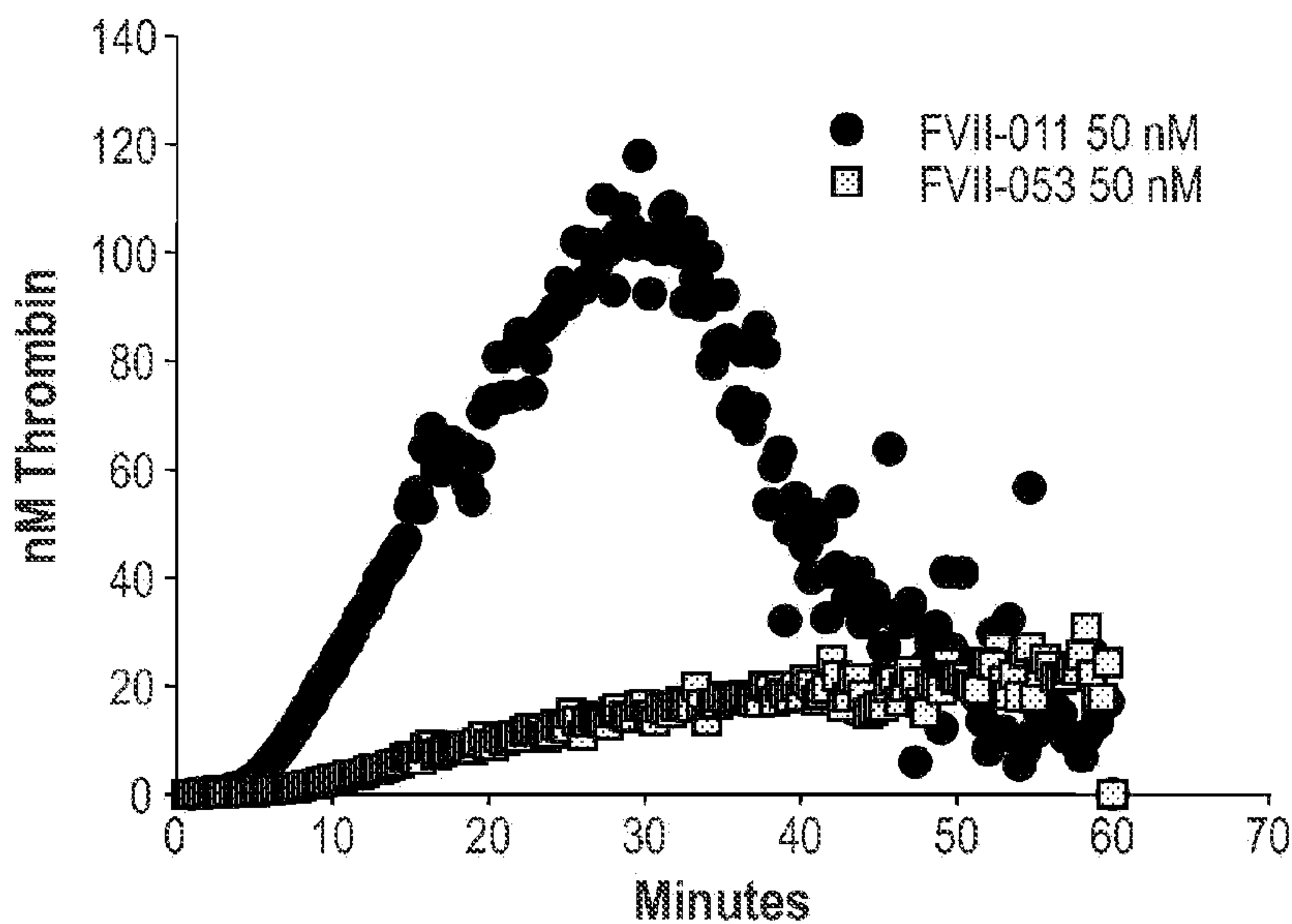


Fig. 22B

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

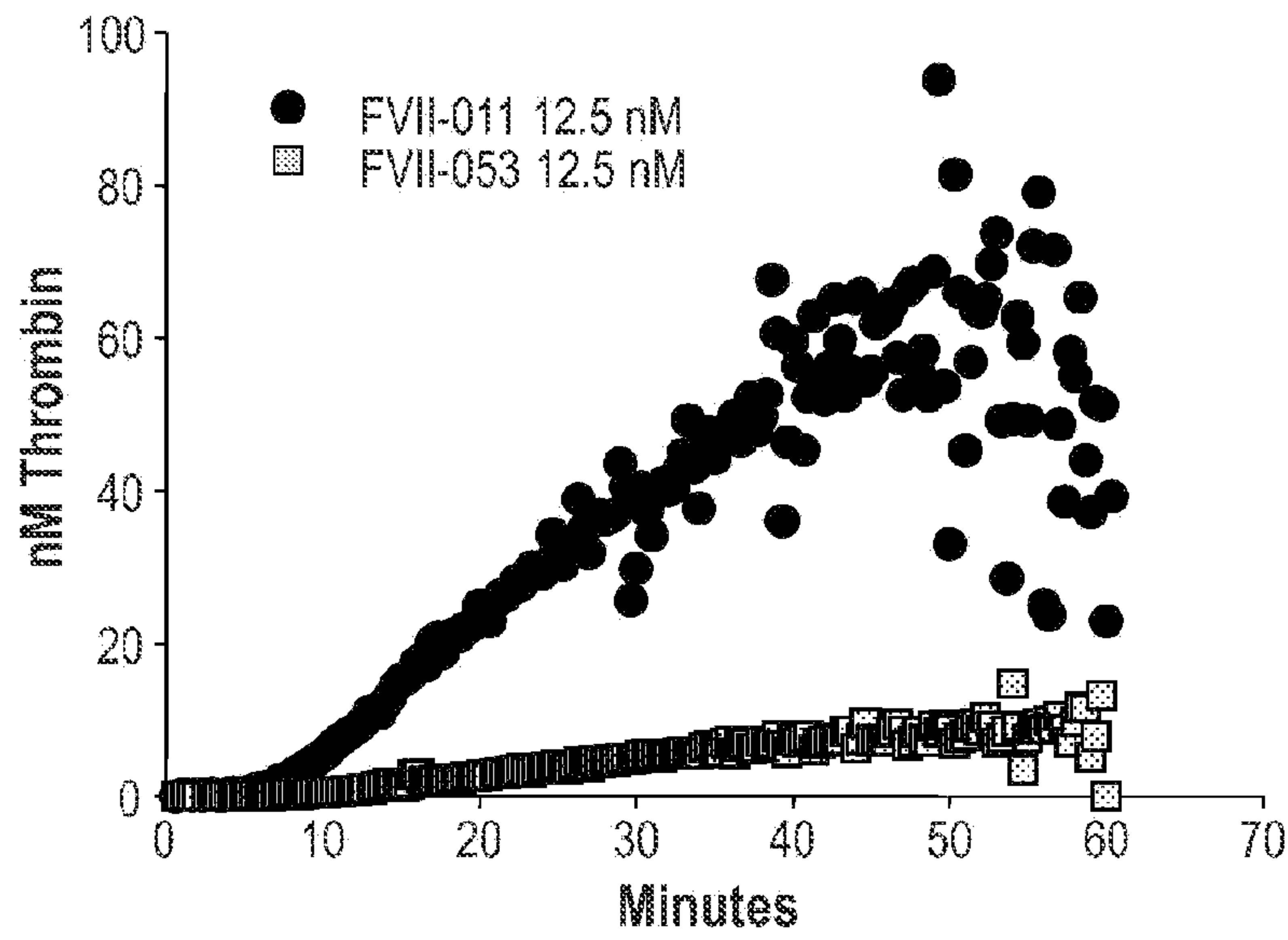


Fig. 22C

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

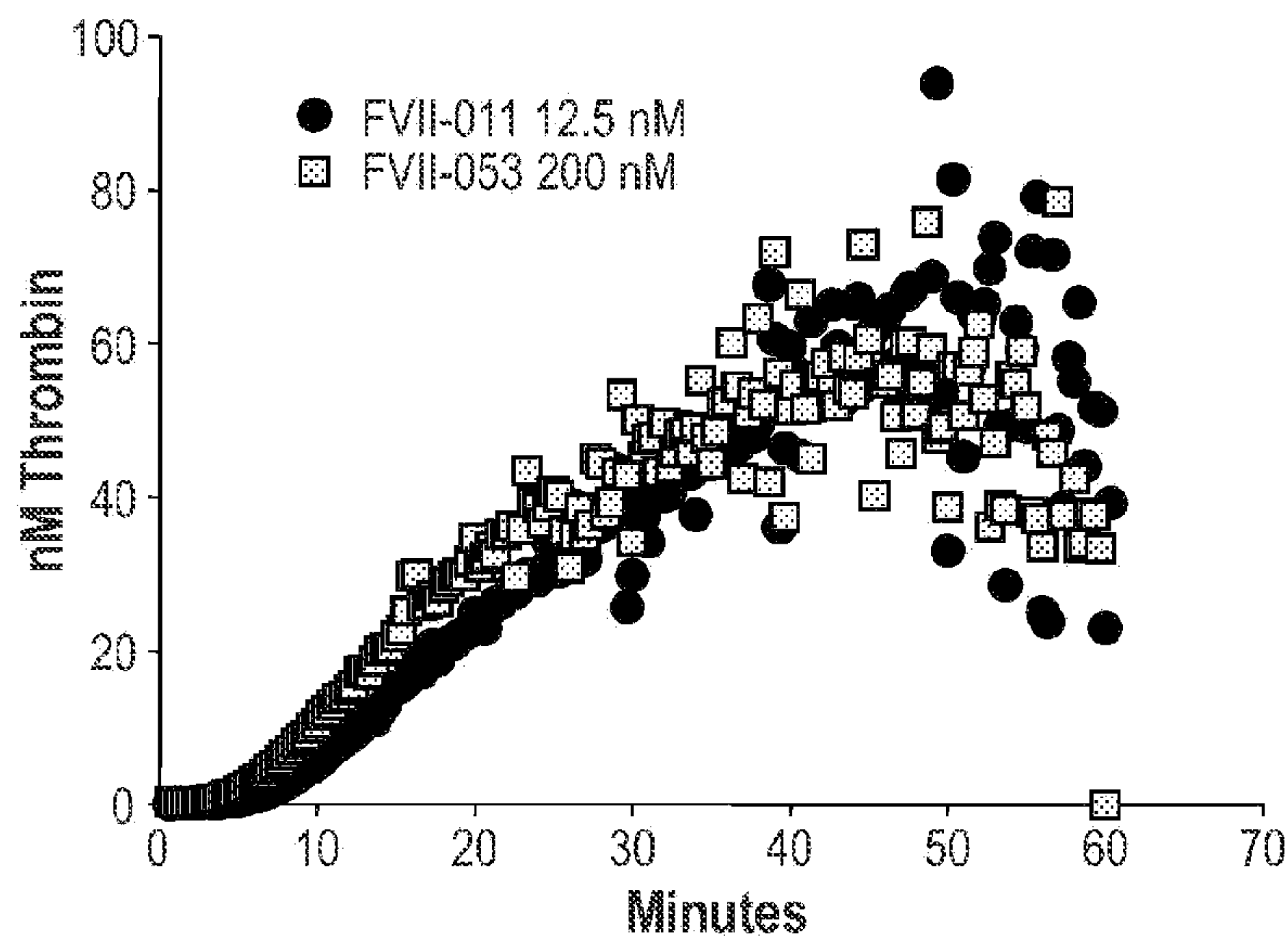
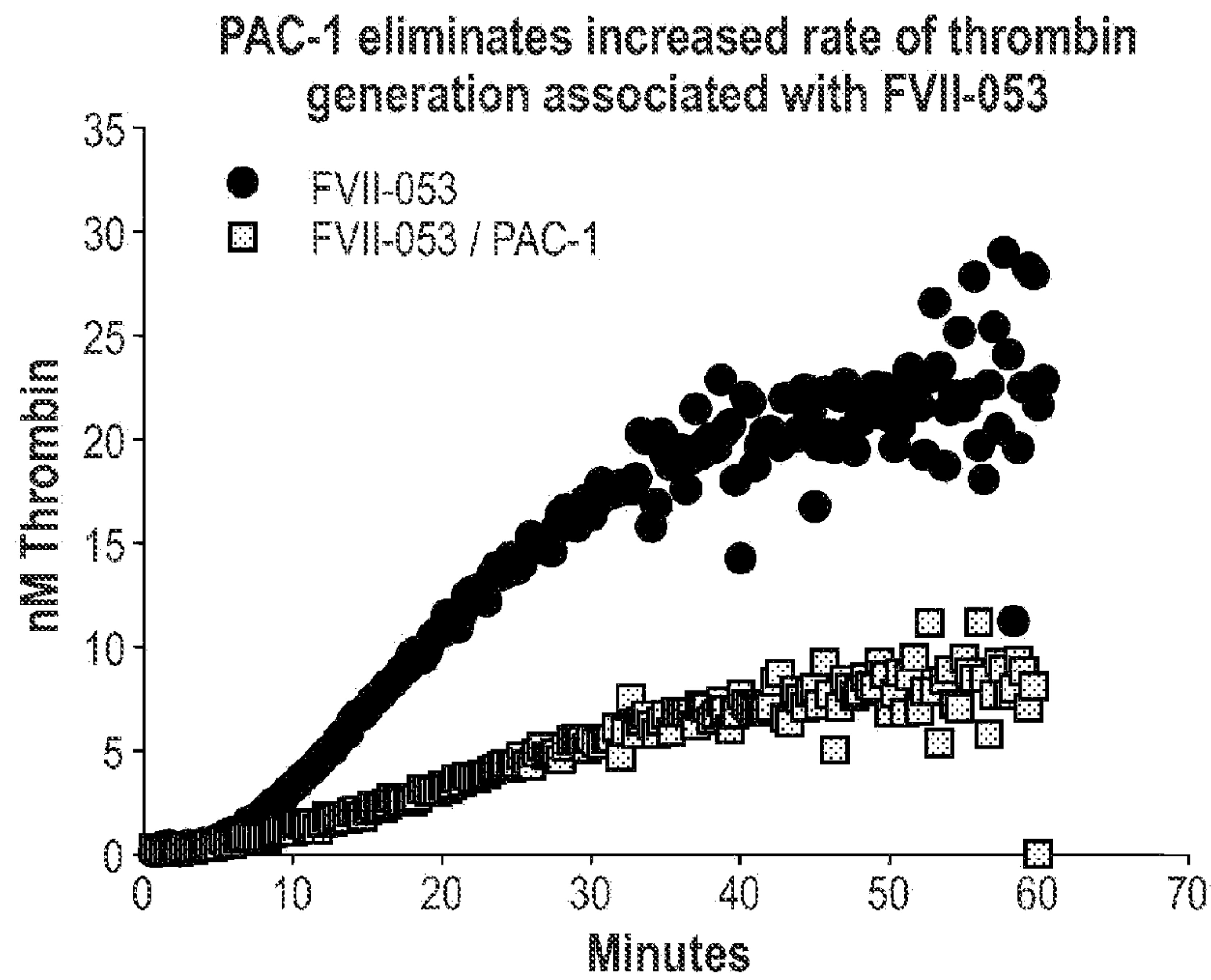
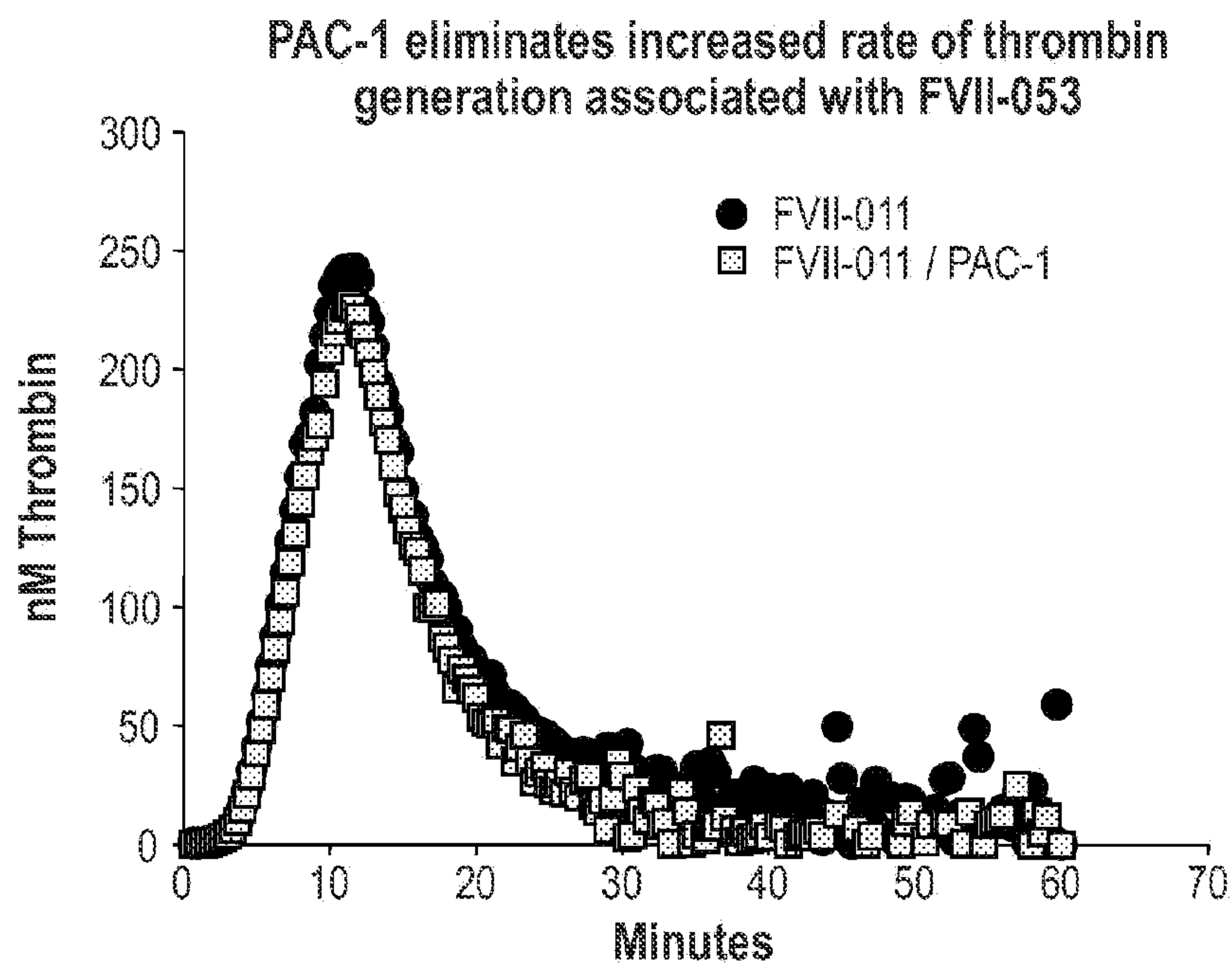


Fig. 22D

*Fig. 23A**Fig. 23B*

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

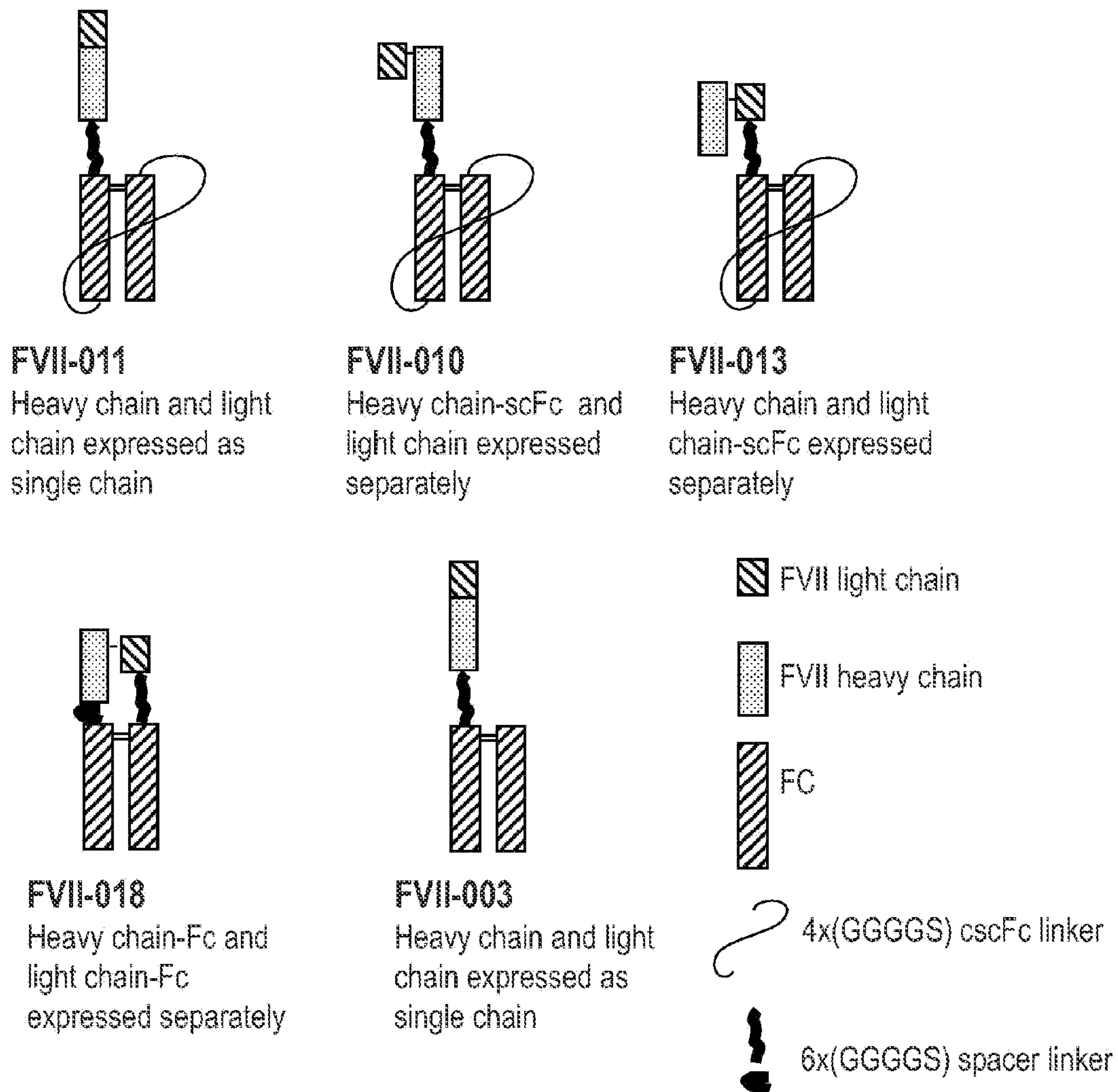


Fig. 24

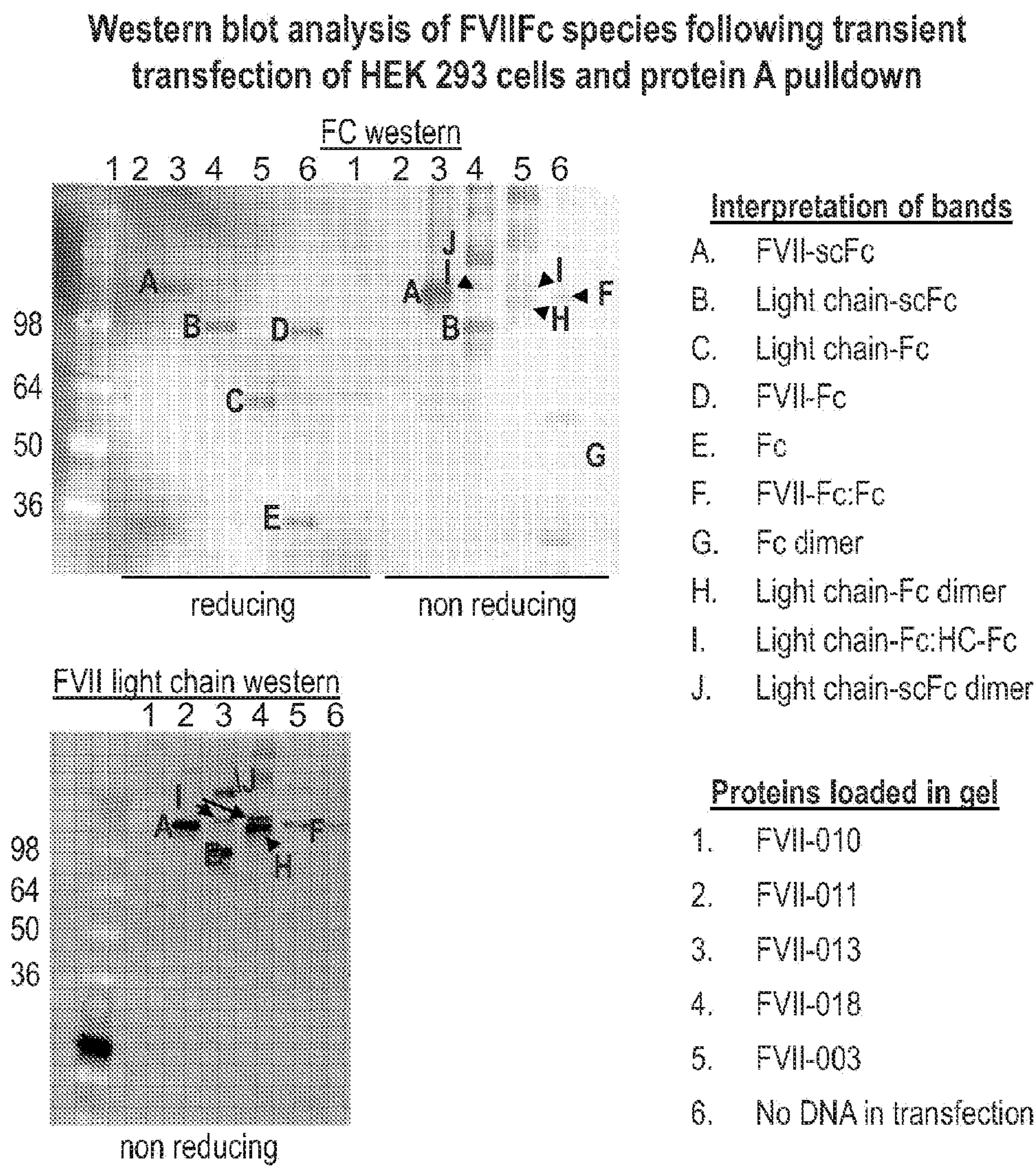


Fig. 25

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.

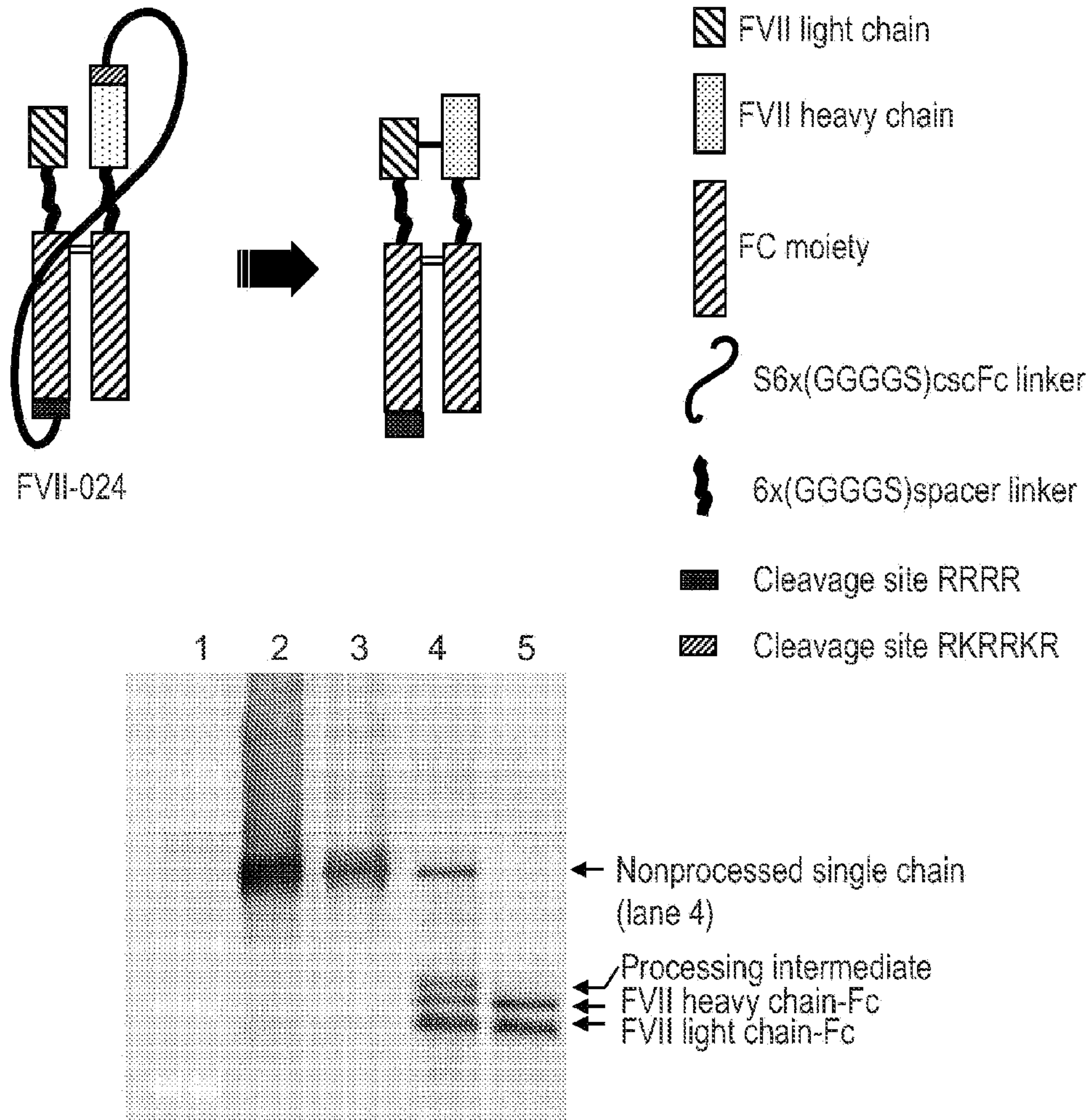


Fig. 26

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

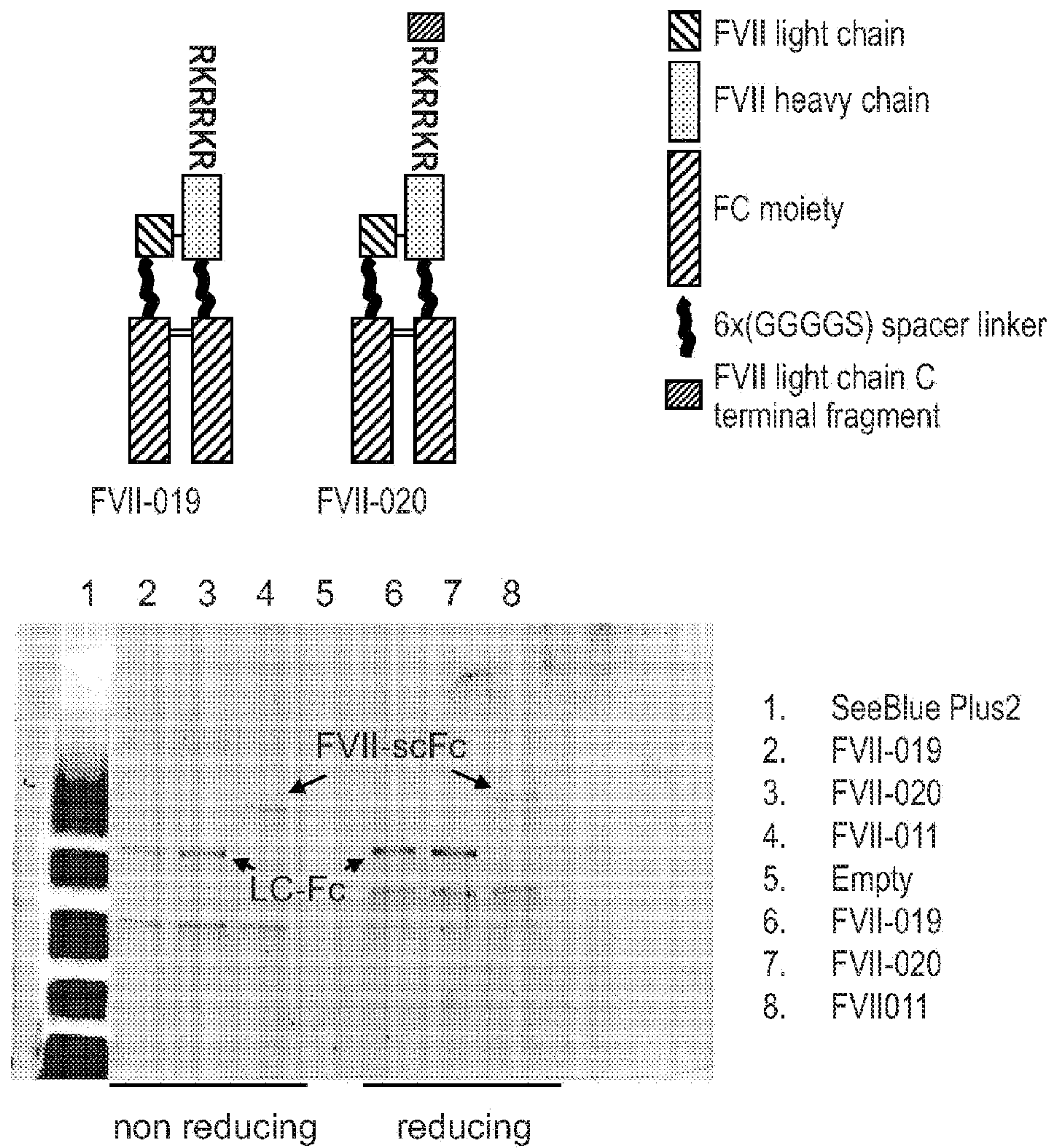
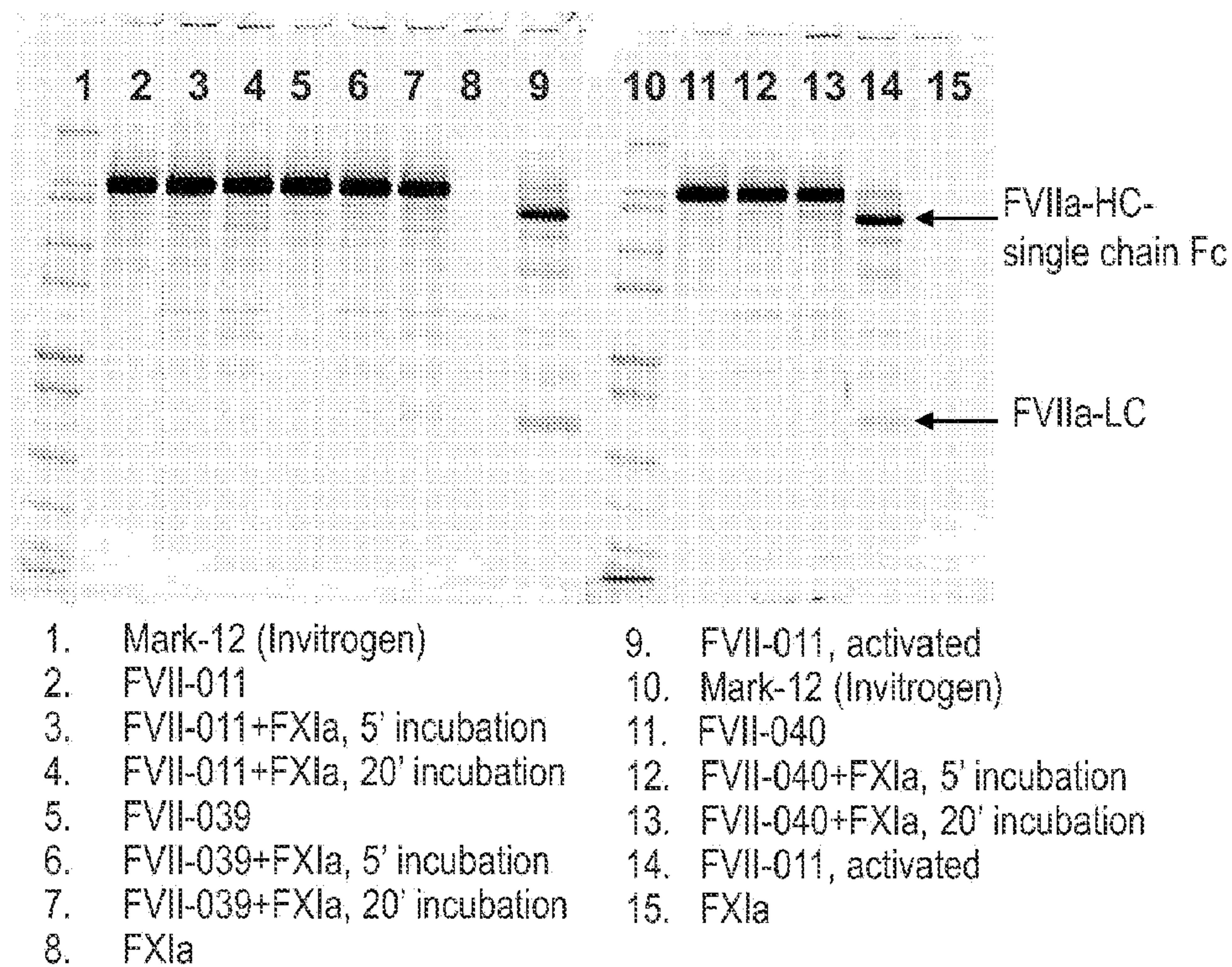
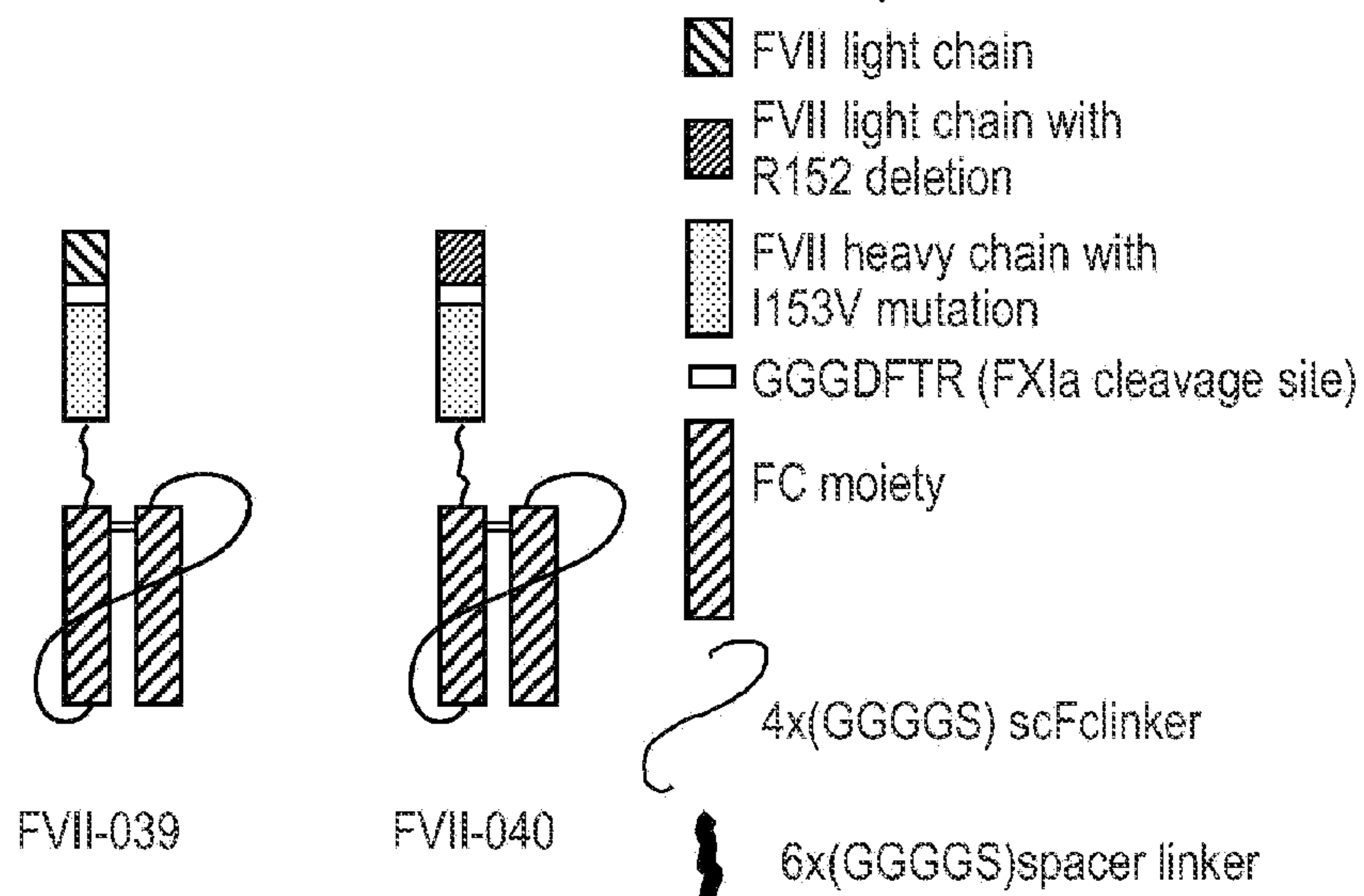
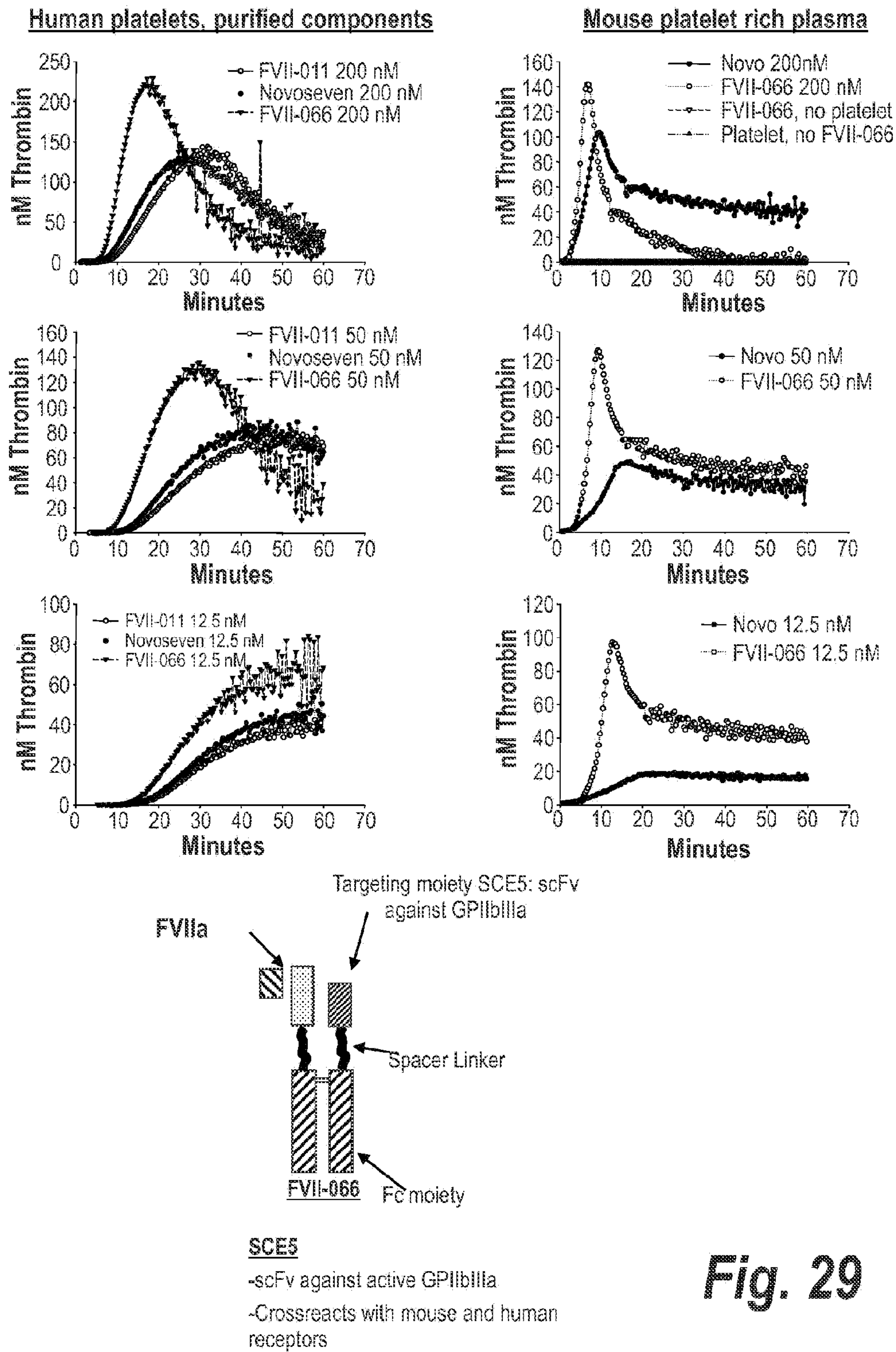


Fig. 27

FVII-039 and FVII-040 Treatment by FXIa

**Fig. 28**

**Fig. 29**

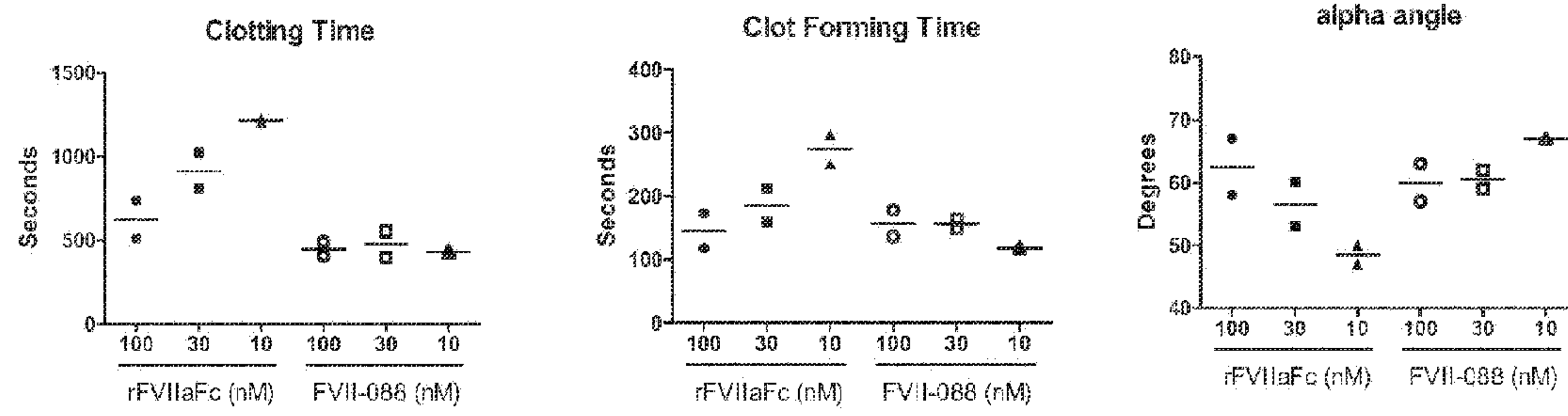


Fig. 30

Activatable ConstructsFXIa cleavage sites

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

Thrombin cleavage sites

LC-7x(GGGGS)-DFLAEGGGVR-IVGG : FVII-058

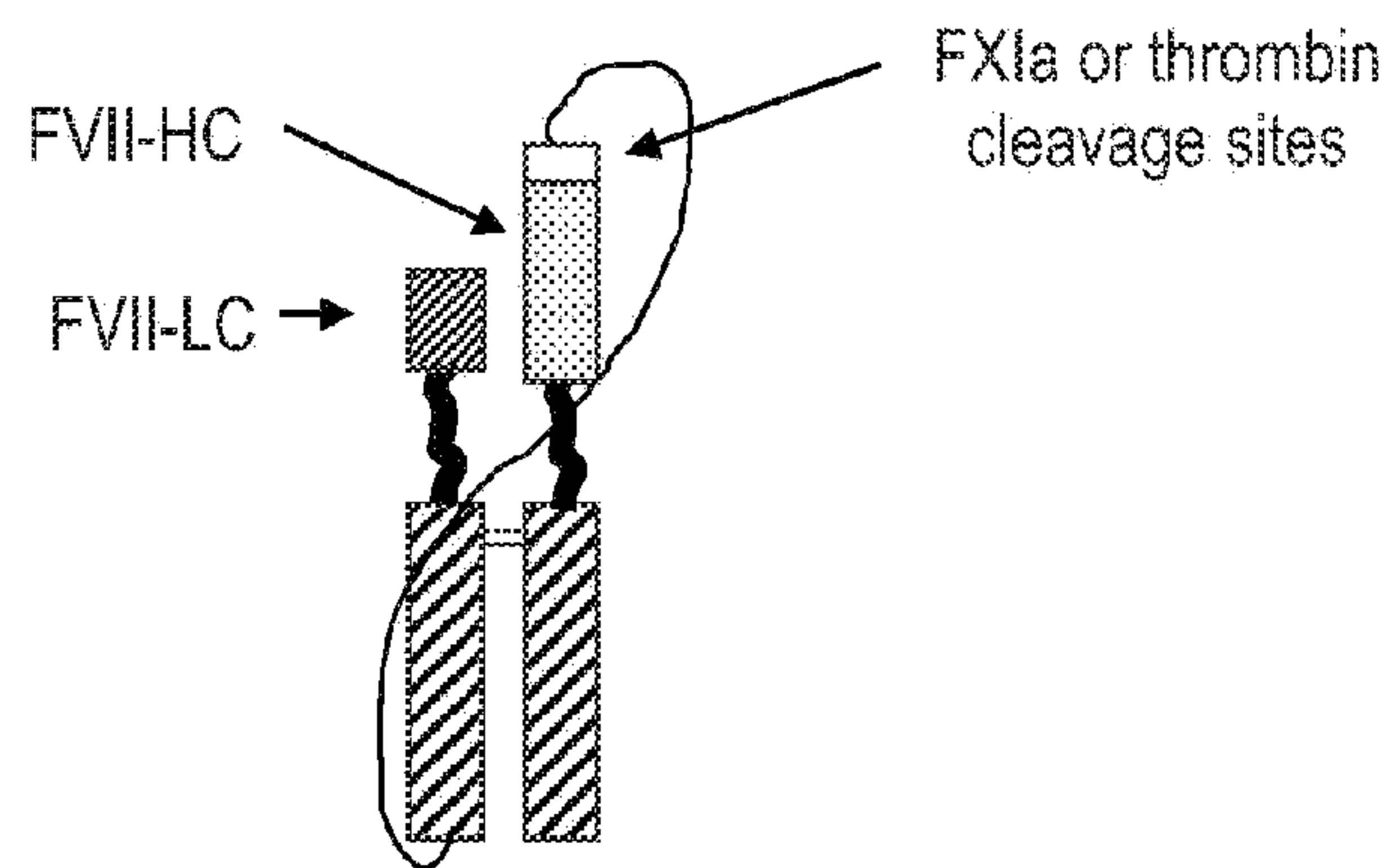
LC-7x(GGGGS)-TTKIKPR-IVGG : FVII-059

LC-7x(GGGGS)-ALRPRVVGGA-VVGG : FVII-060

LC-7x(GGGGS)-ALRPRVVGGA-IVGG : FVII-061

Negative control

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

***Fig. 31***

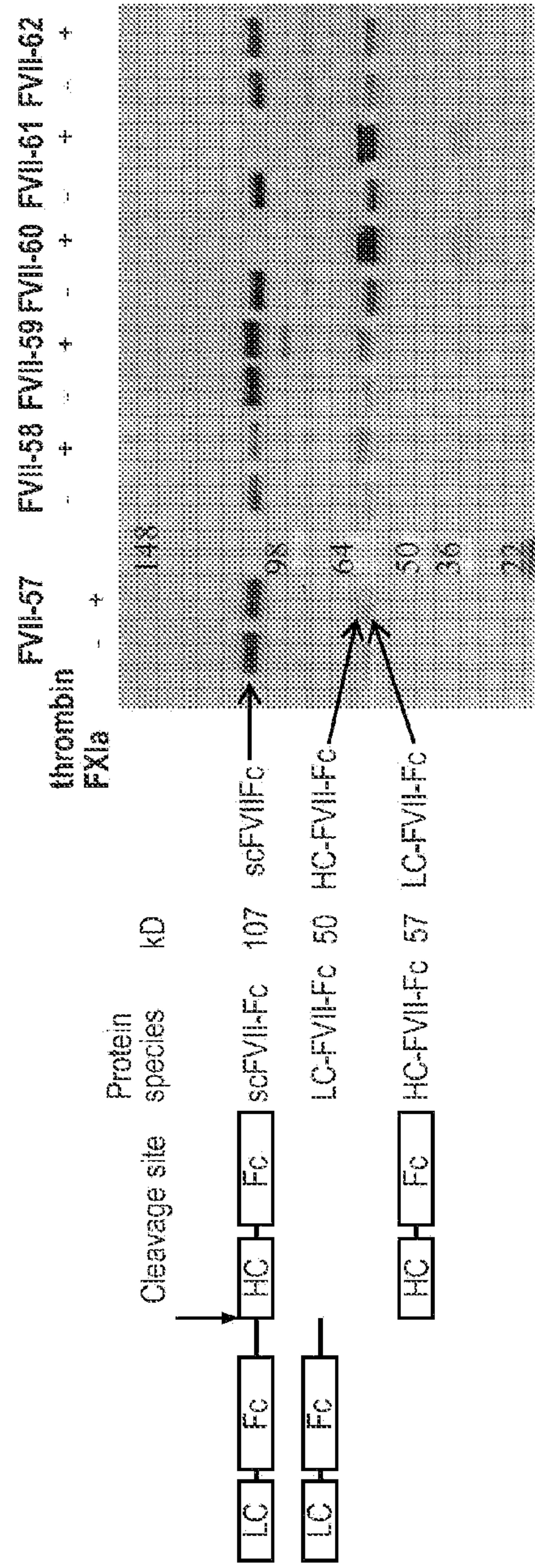


Fig. 32

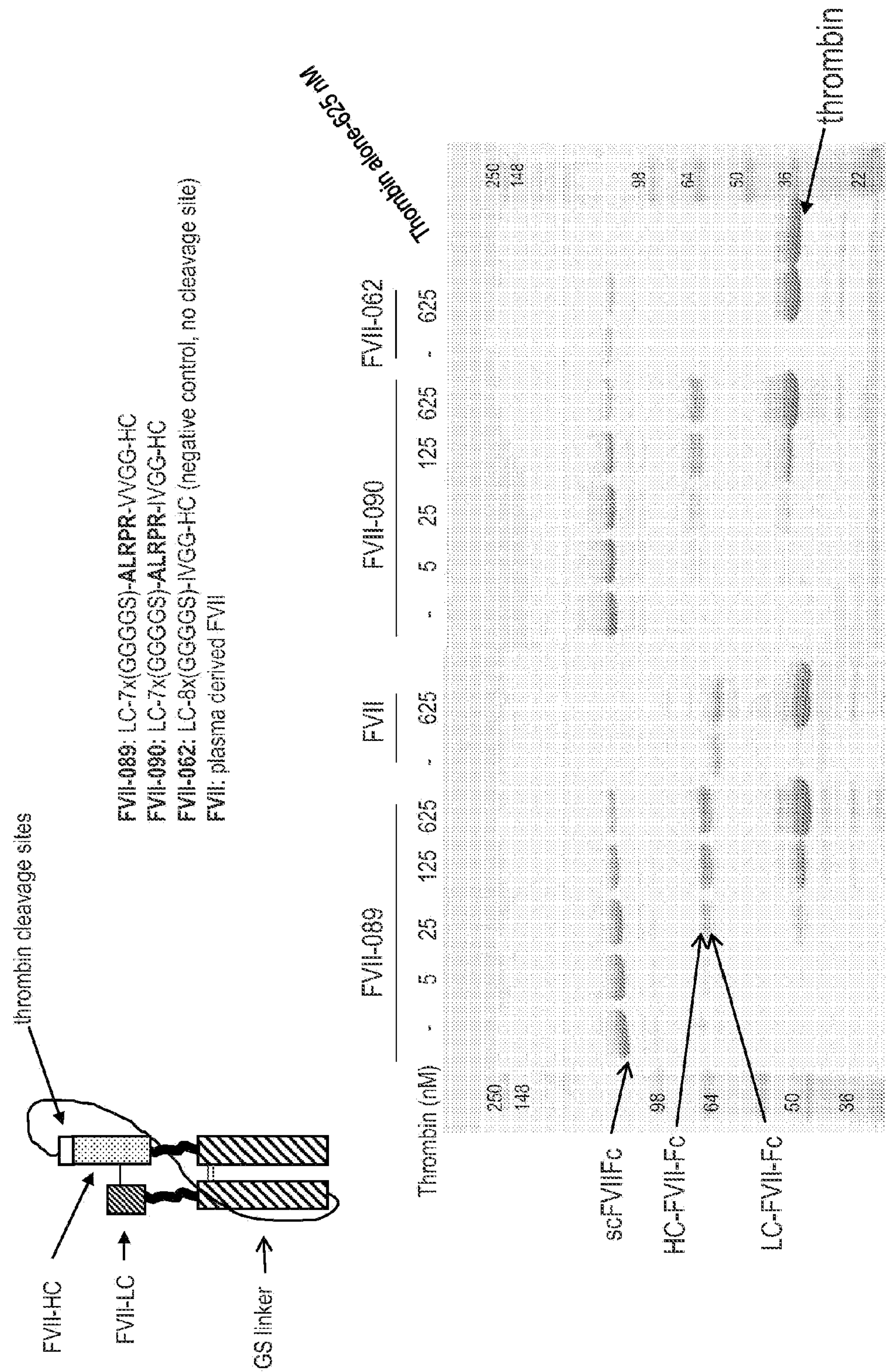
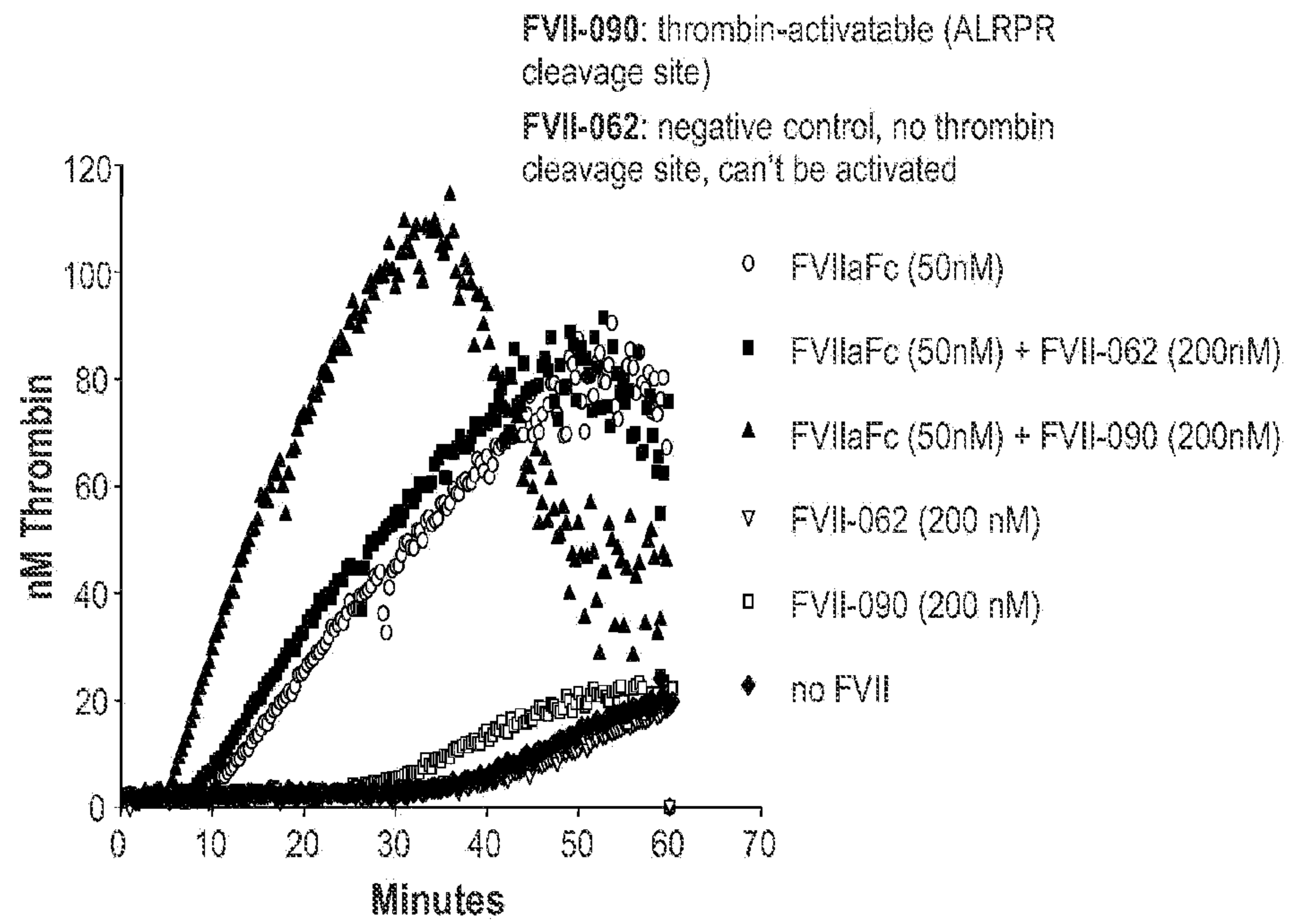


Fig. 33

**Fig. 34**

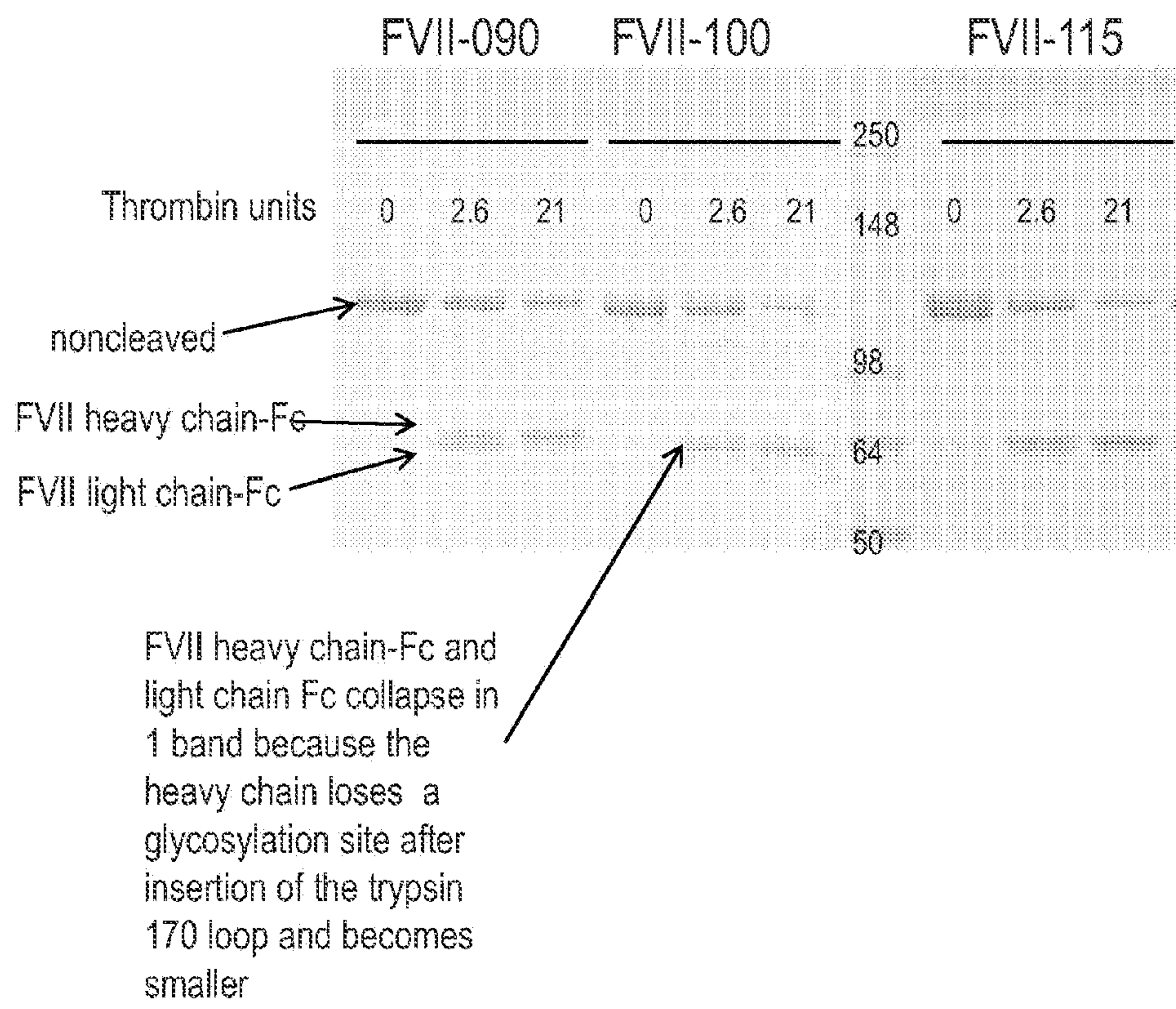
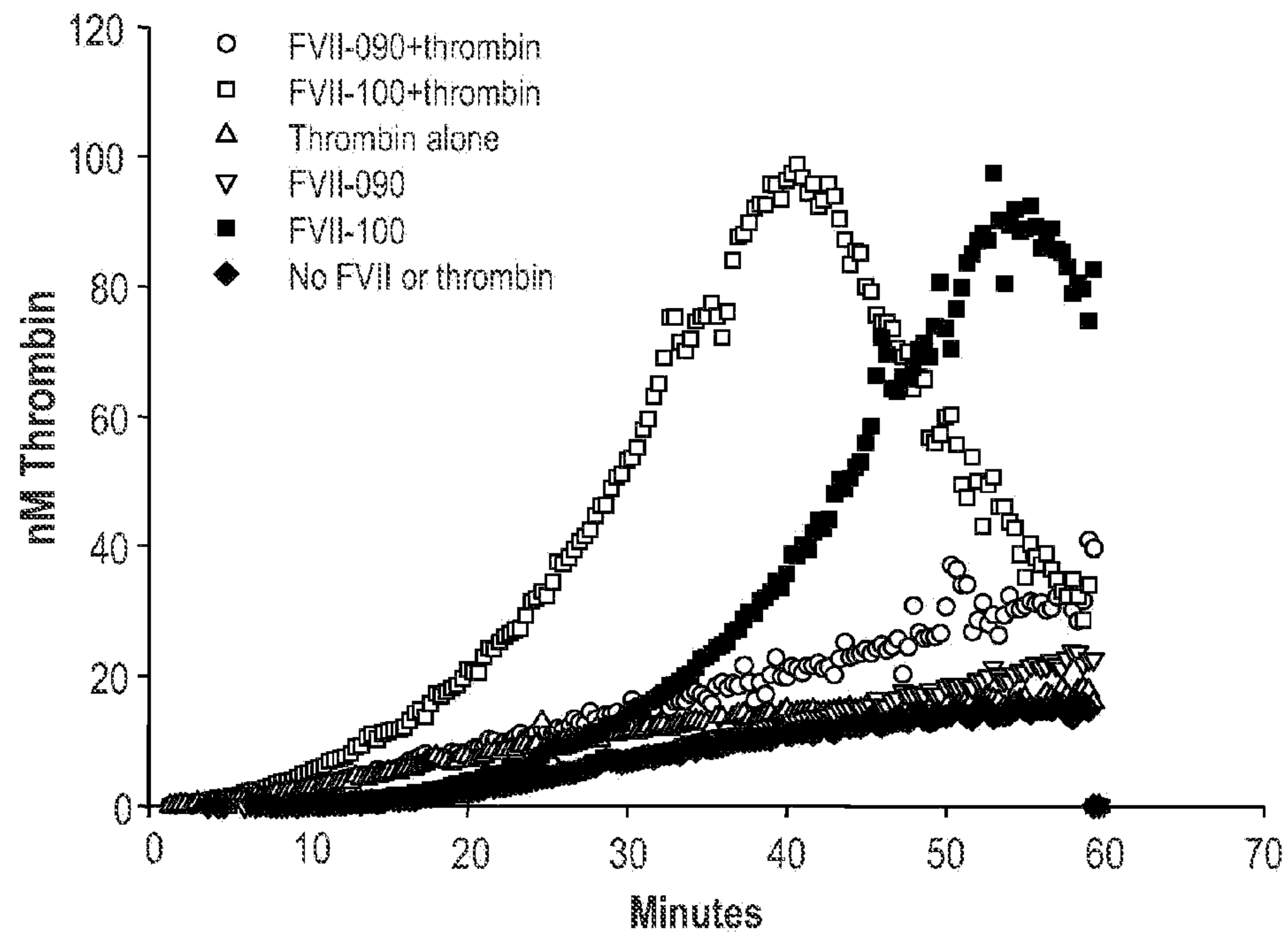
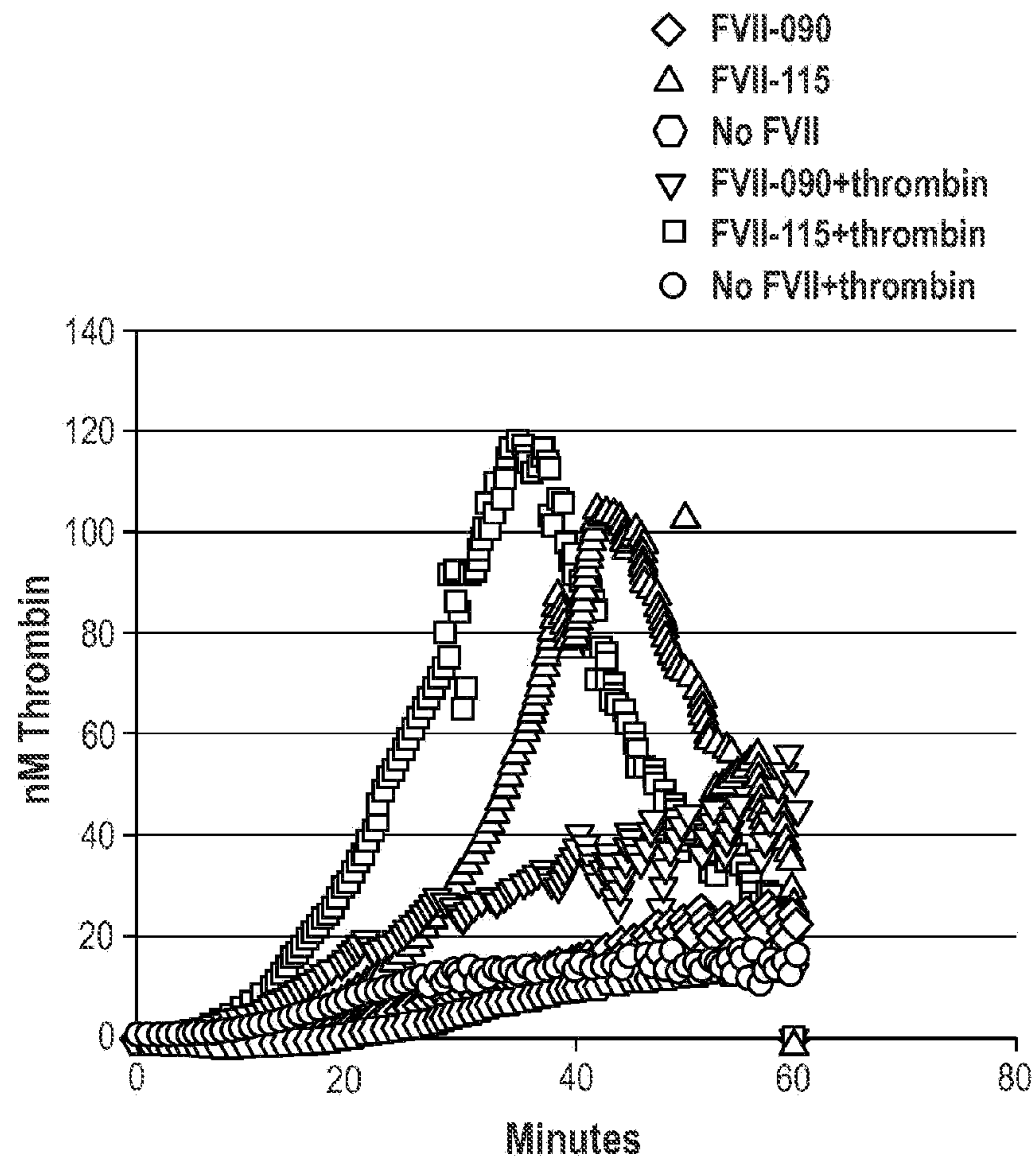
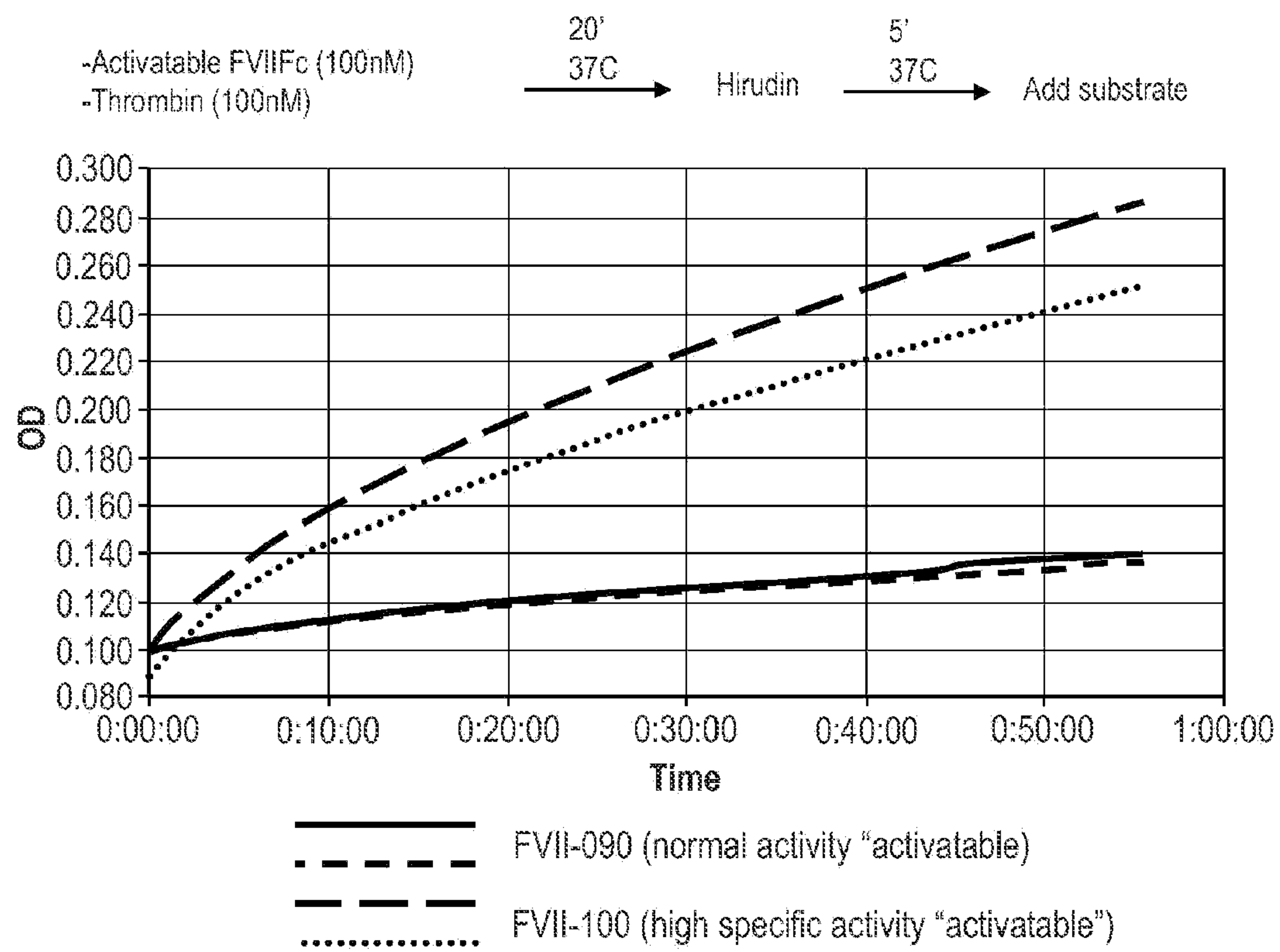


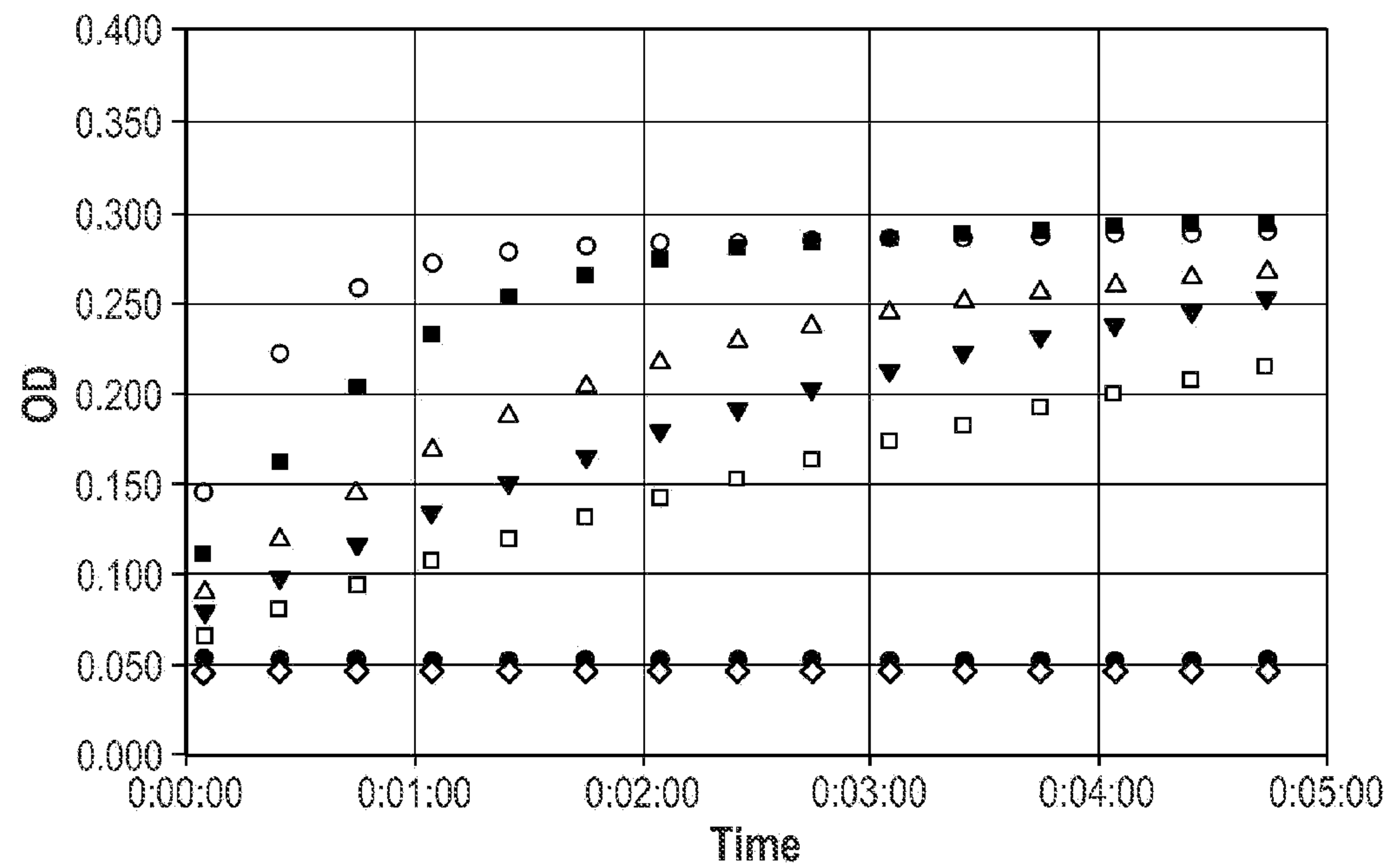
Fig. 35

*Fig. 36*

***Fig. 37***

Amidolytic activity of activatable FVIIc activated with thrombin**Fig. 38**

Activation of FX by FVIIa

10 μ M FX and FVIIa titration

- 400nM
- 200nM
- △ 100nM
- ▼ 50nM
- 25nM
- ◇ No FVIIa
- No FX, 400nM FVIIa

Fig. 39

FXa generation activity by “activatable” FVIIFc

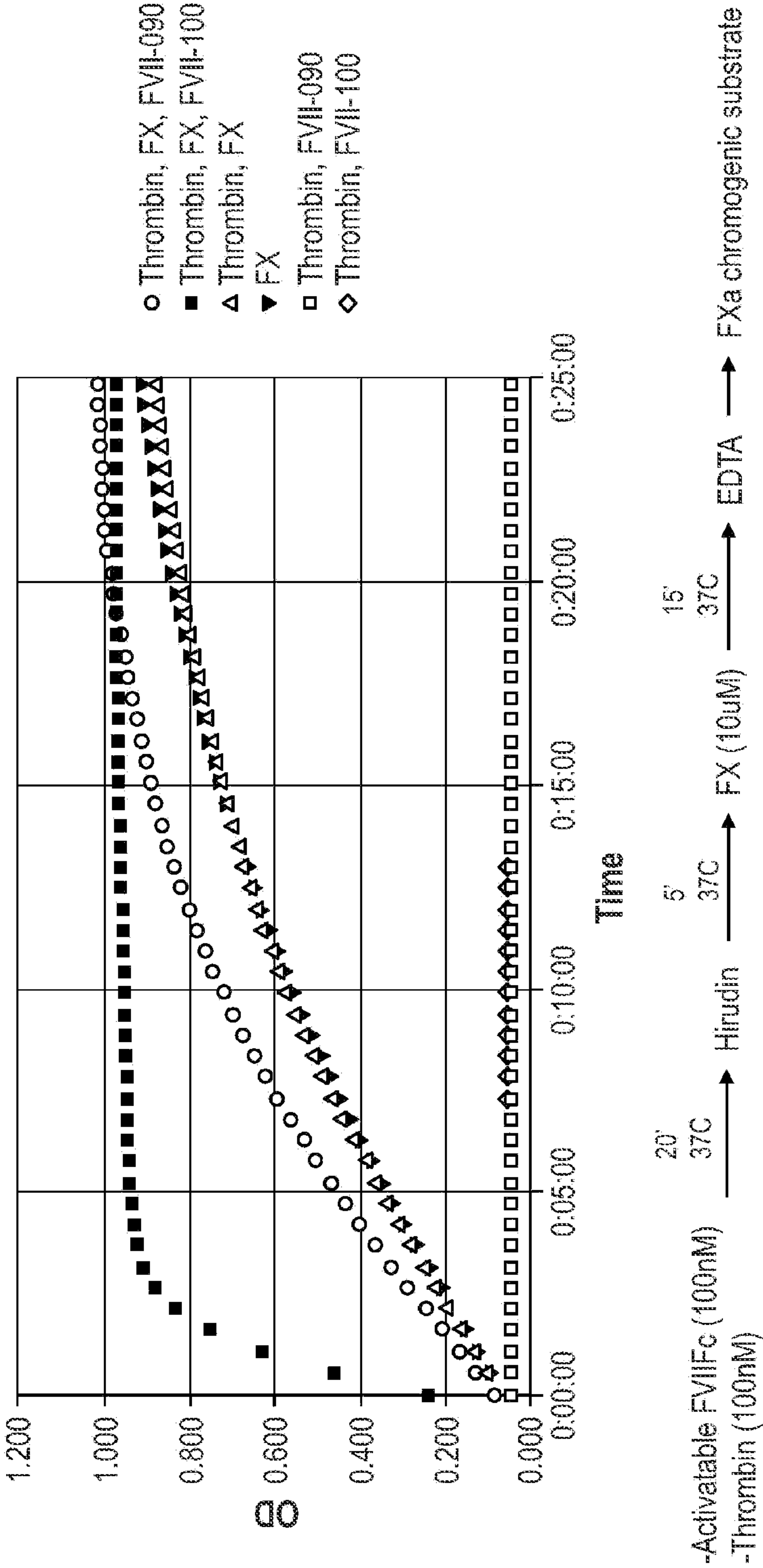


Fig. 40

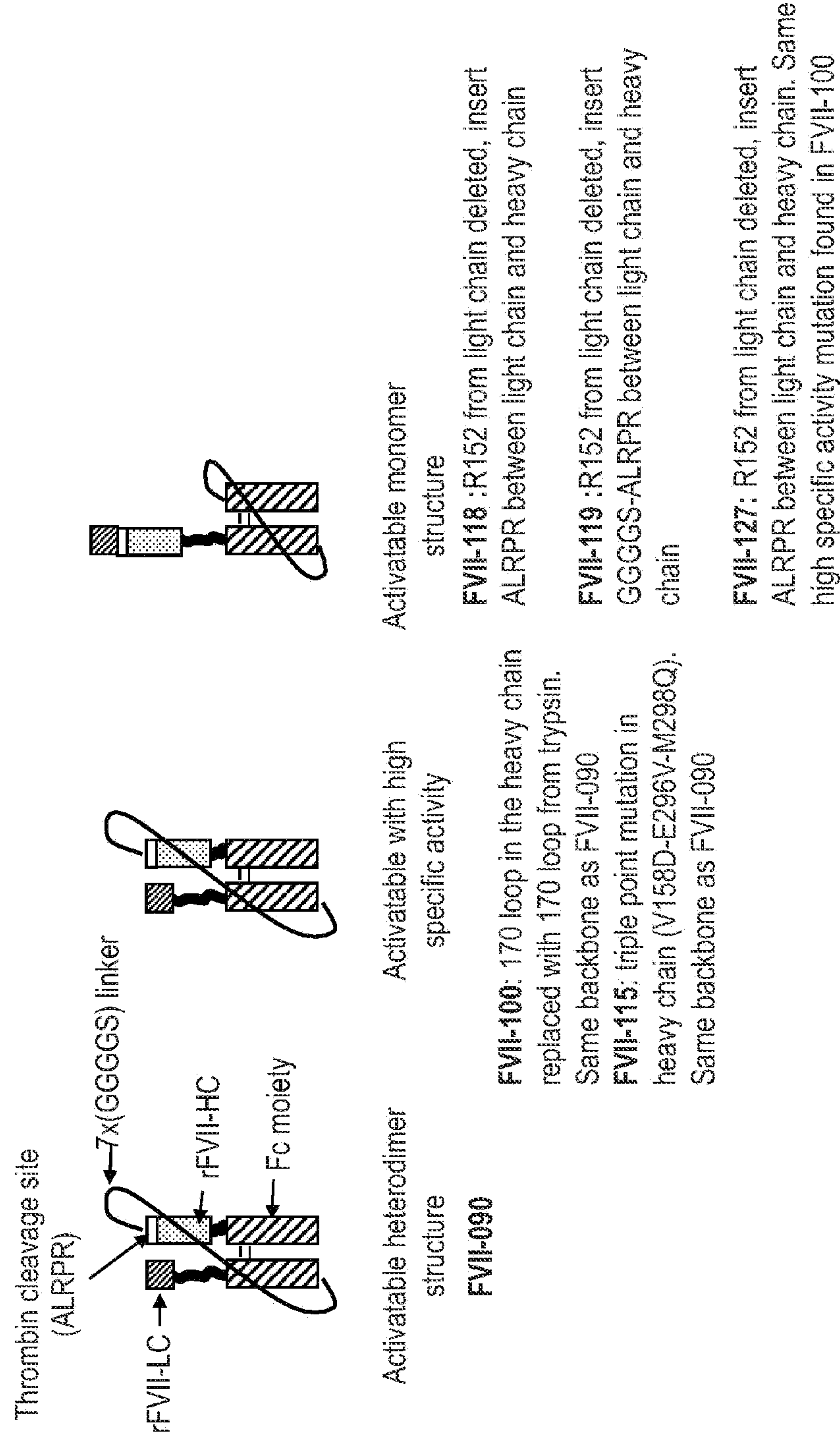


Fig. 41

Compare efficiency of thrombin cleavage of monomer (FVII-118, -119) vs heterodimer (FVII-090) activatable

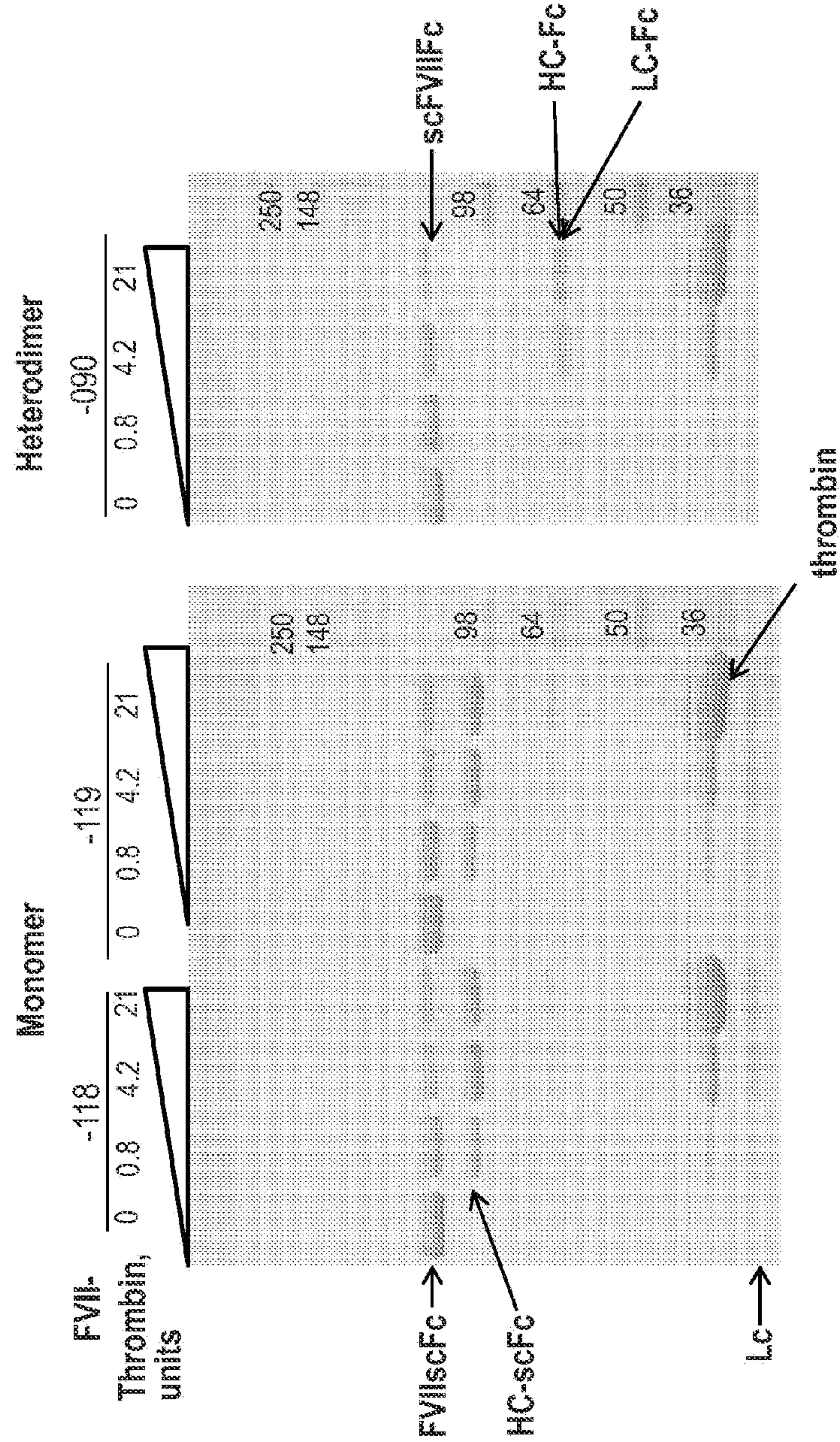


Fig. 42

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

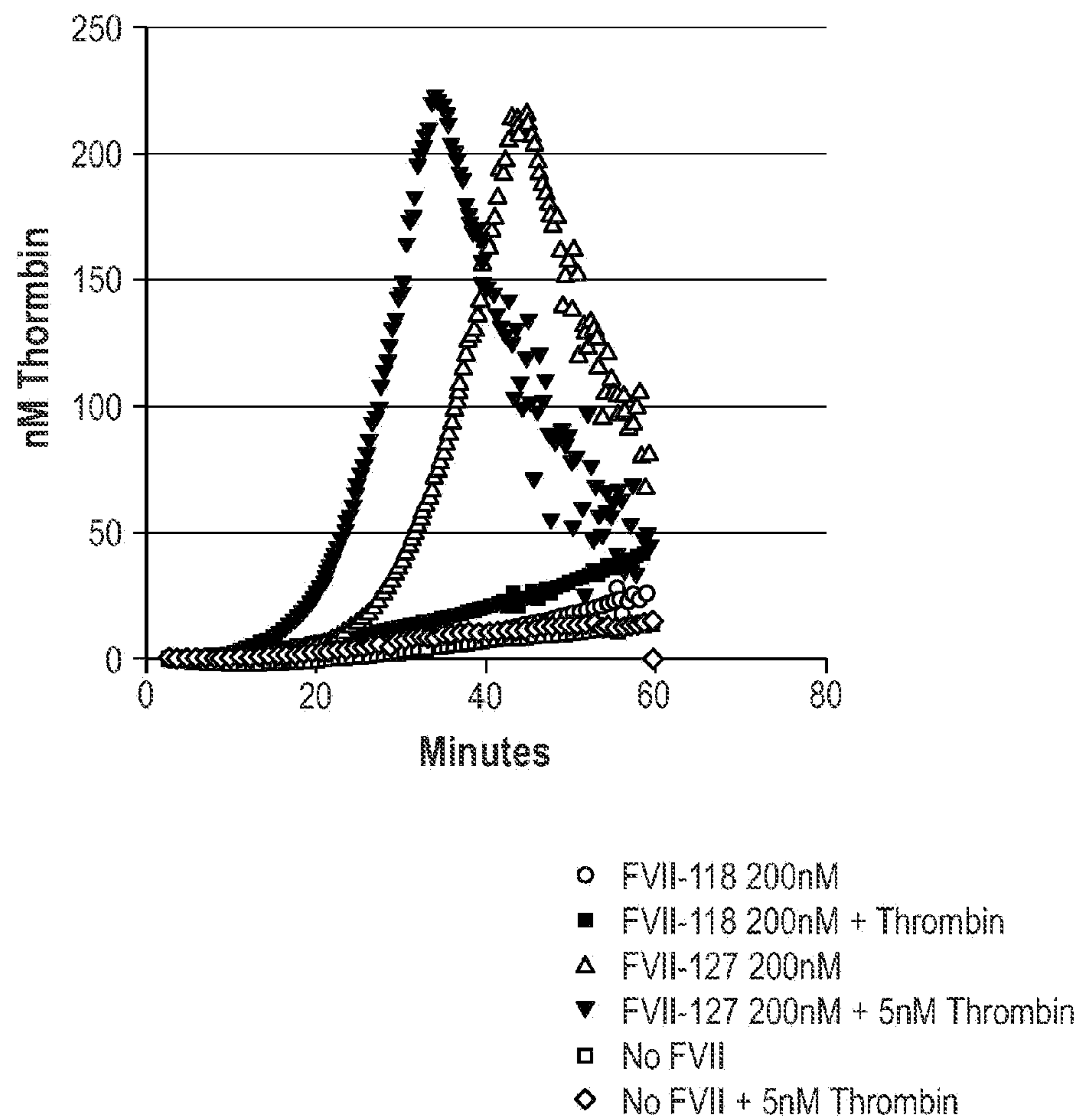
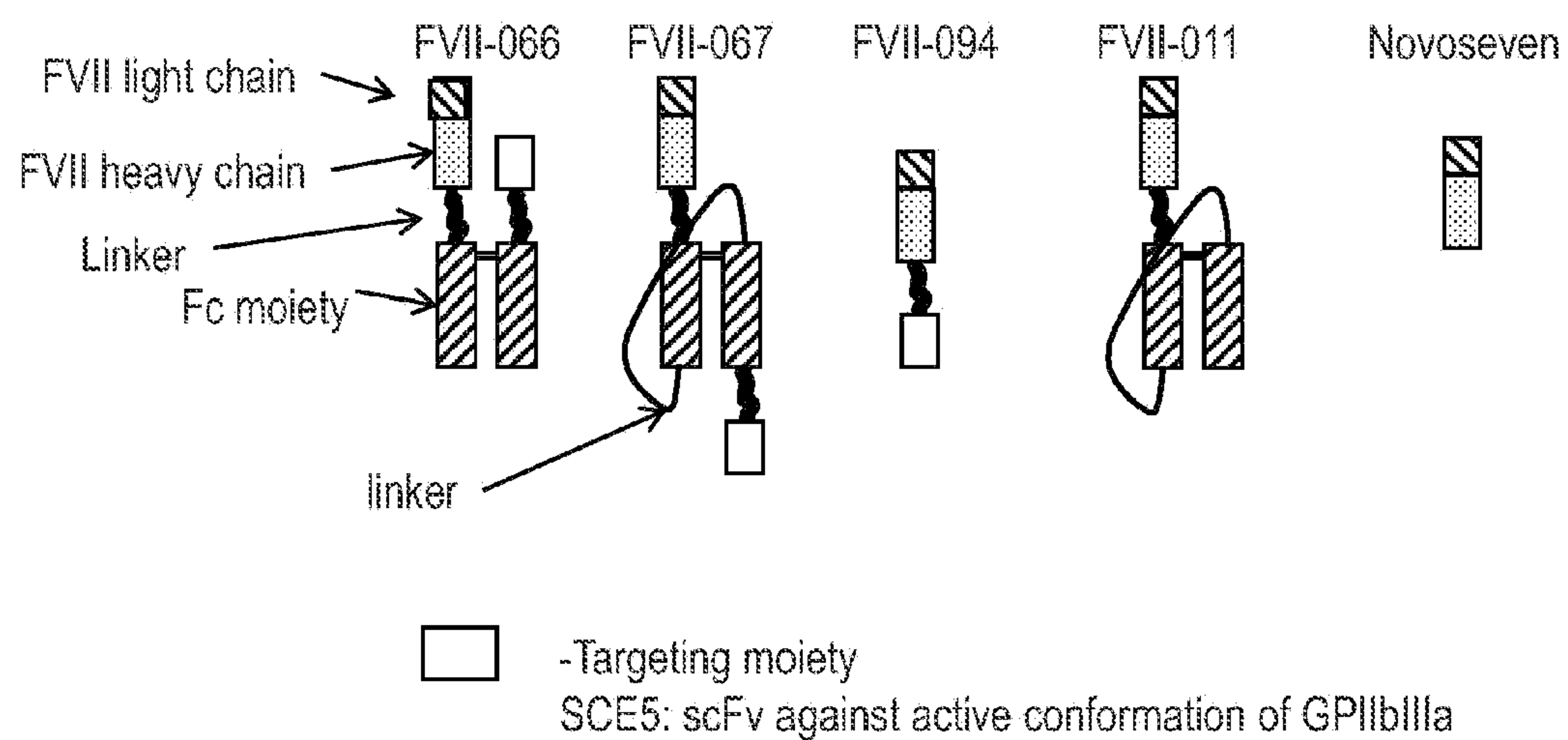


Fig. 43

Target FVIIa to active conformation of GPIIb/IIIa via scFv (SCE5)**Fig. 44A**

Thrombin generation assays in platelet-rich FVIII-deficient plasma

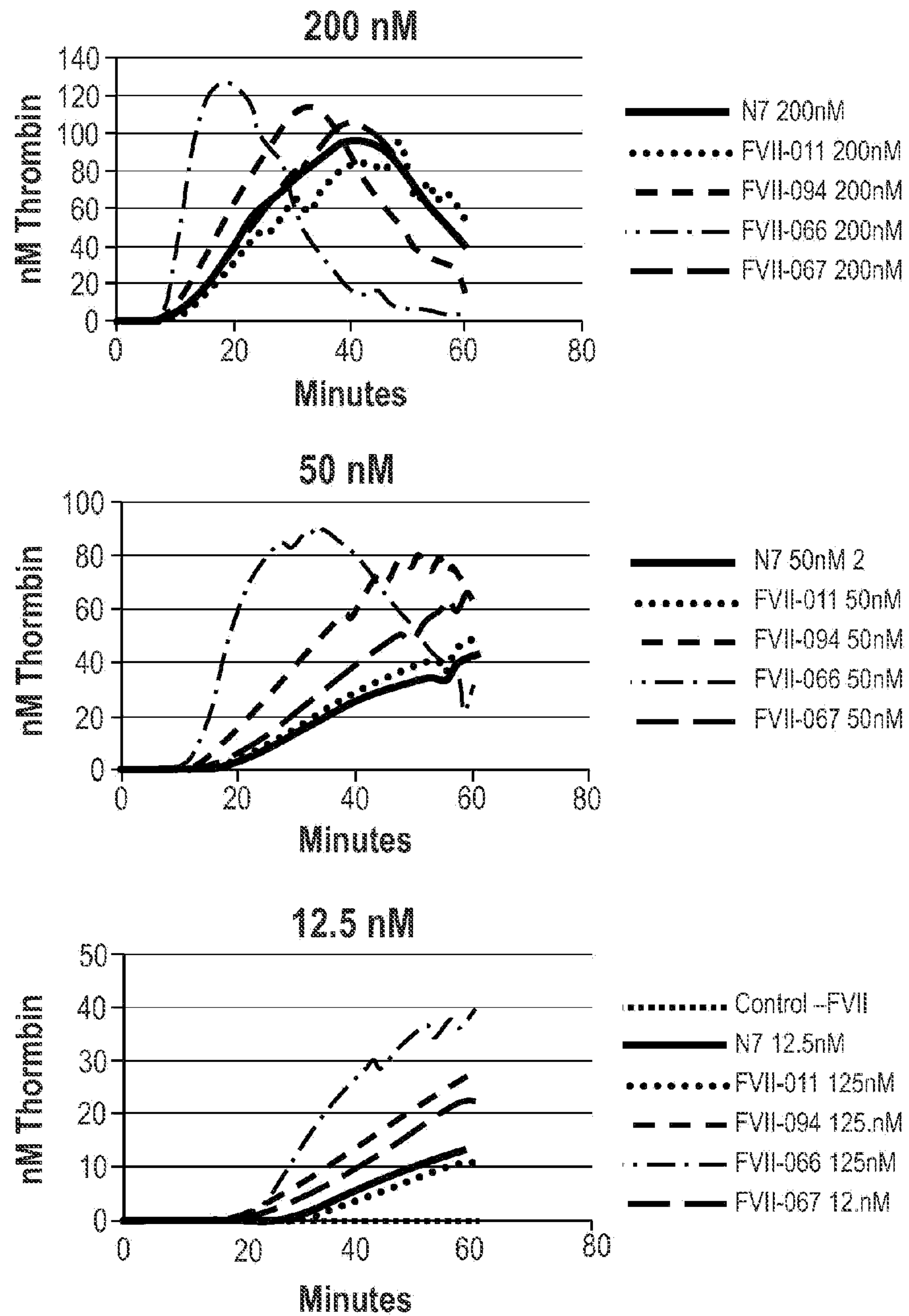
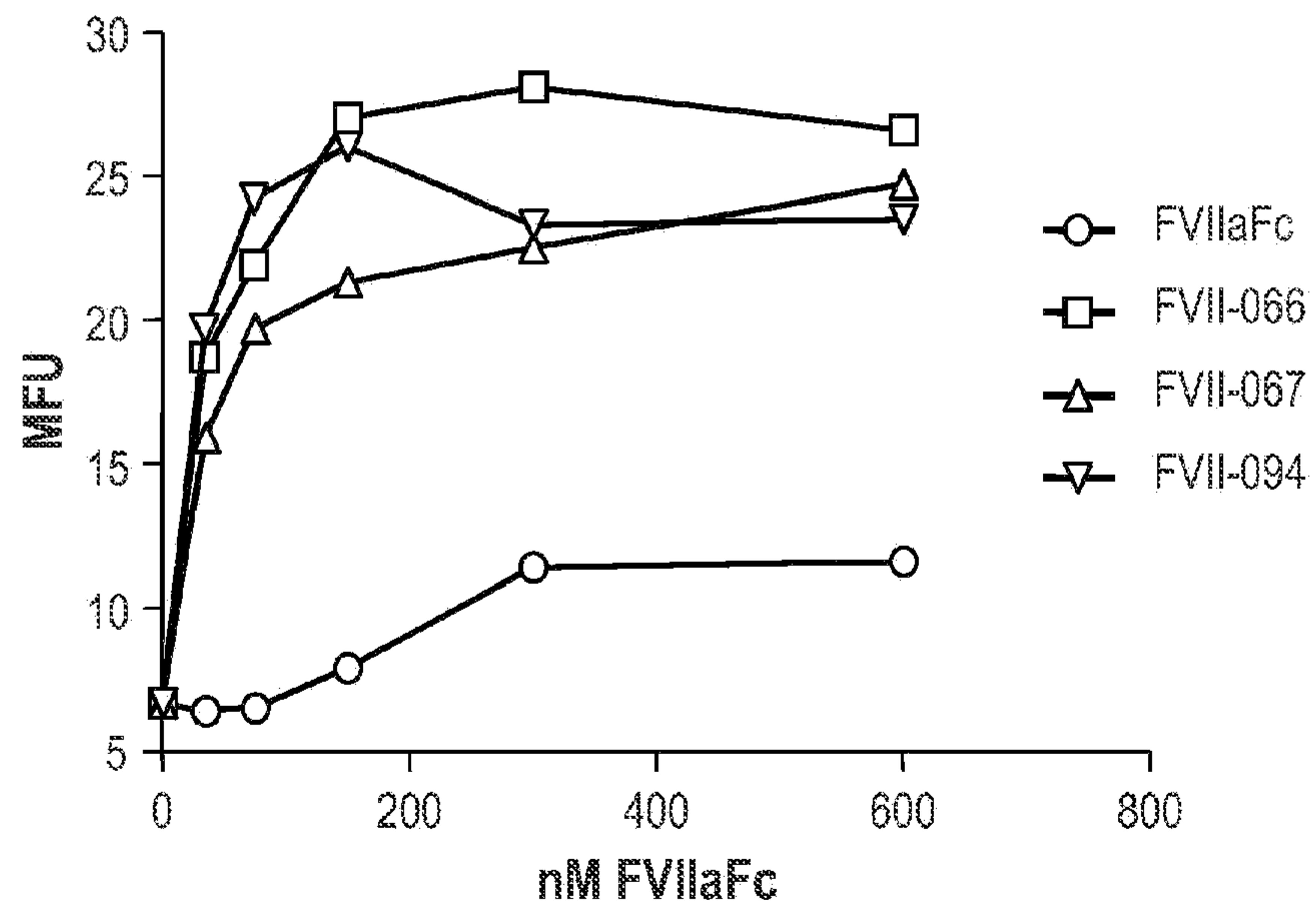
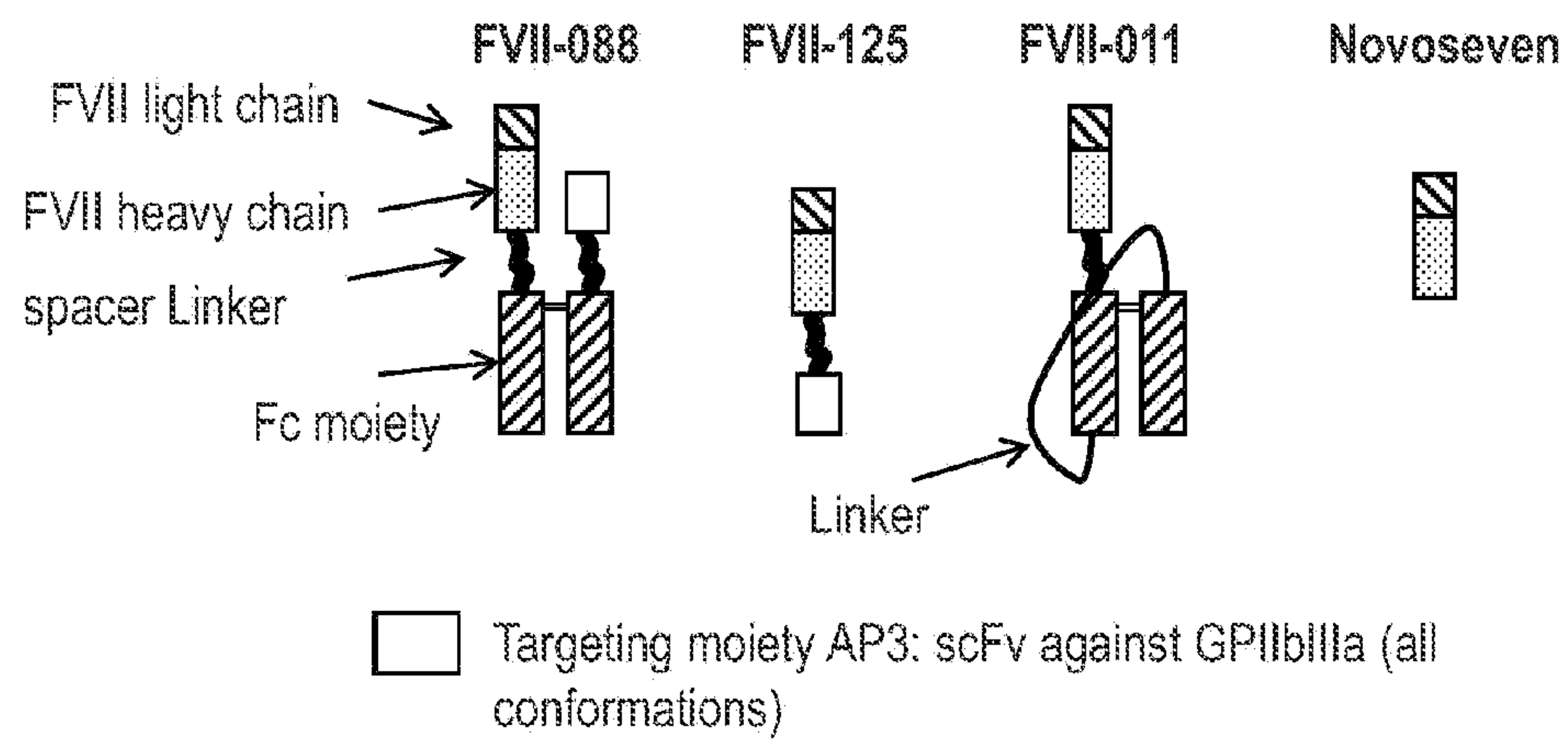


Fig. 44B

Binding of rFVIIaFc variants to platelets by FACS

**Fig. 44C**

Target FVIIa to all conformations of GPIIb/IIIa via scFv (AP3)**Fig. 45A**

Thrombin generation assays in platelet-rich
FVIII-deficient plasma

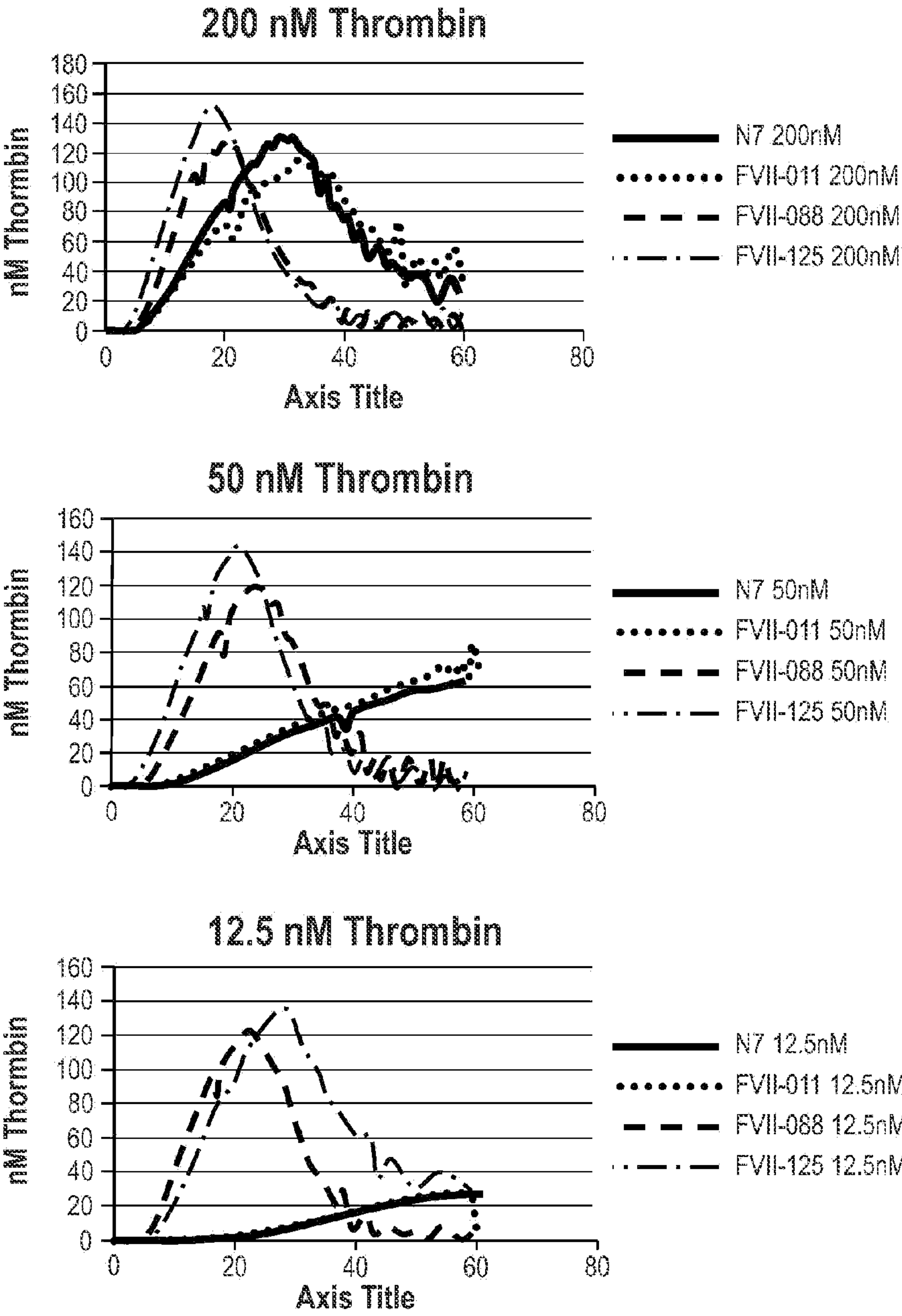
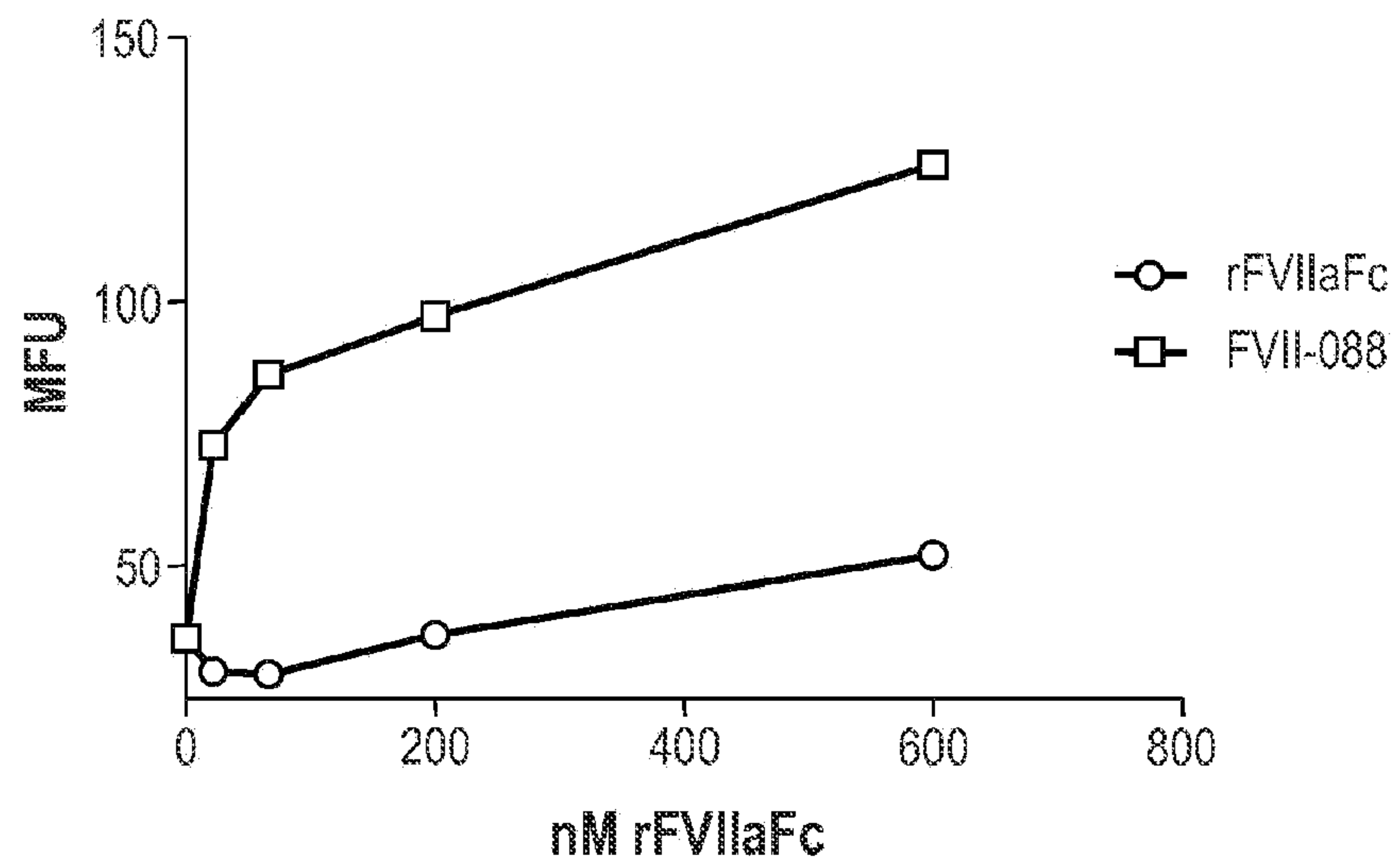
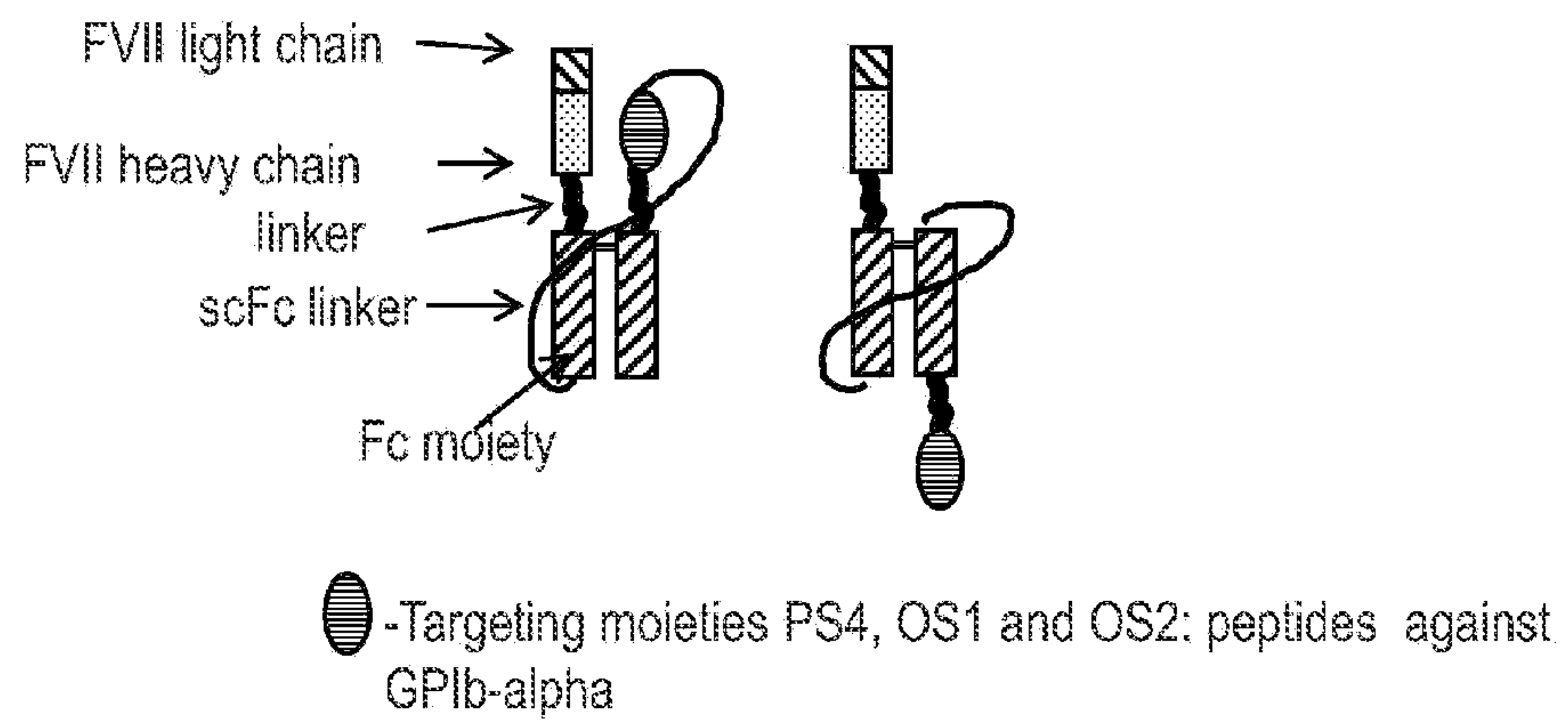


Fig. 45B

Binding of rFVIIaFc variants to platelets by FACS

*Fig. 45C*

Targeting FVIIaFc to GPIb with peptides



<u>N-terminus</u>	<u>C-terminus</u>
-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

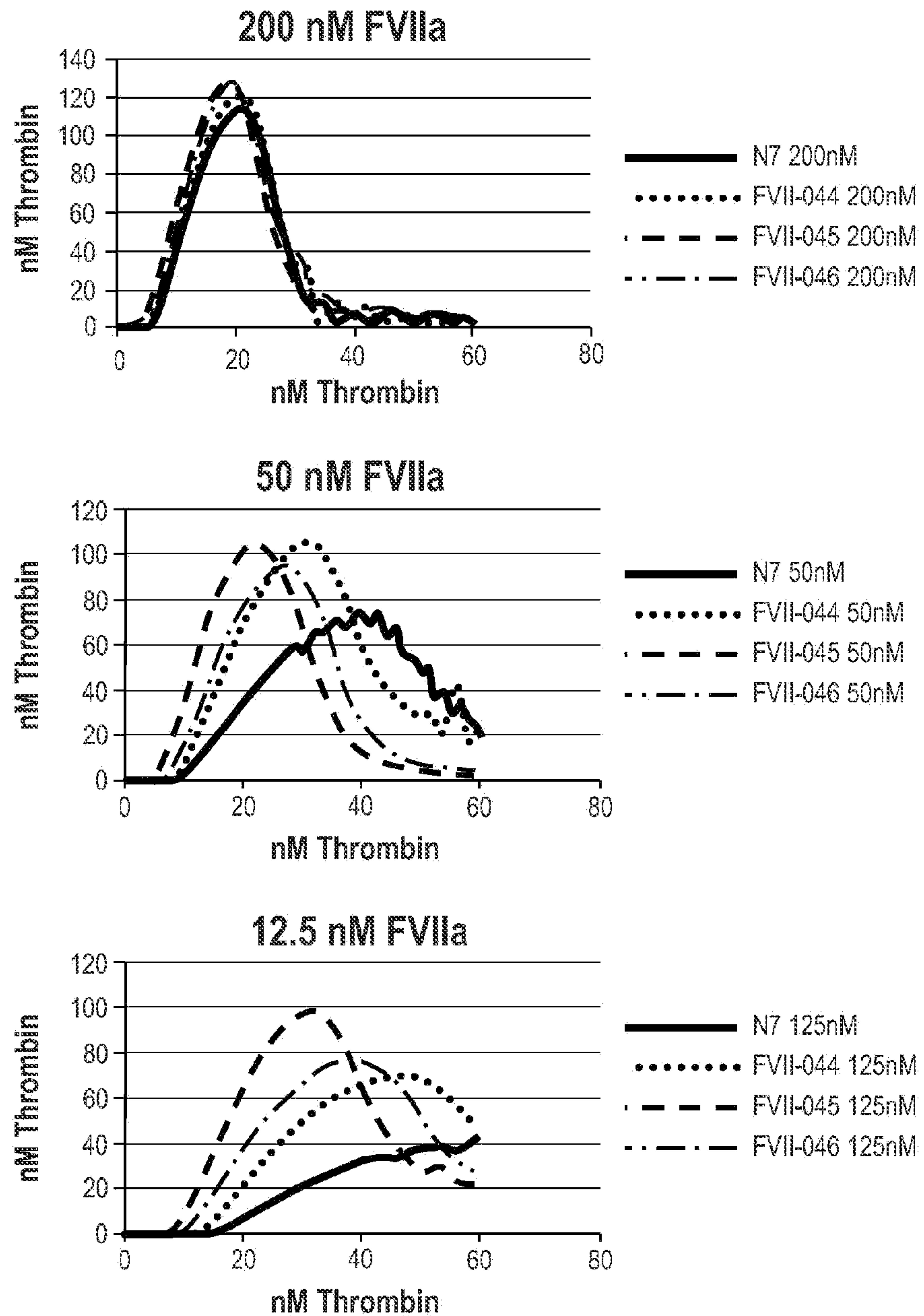


Fig. 46B

Thrombin generation assays in platelet-rich
FVIII-deficient plasma

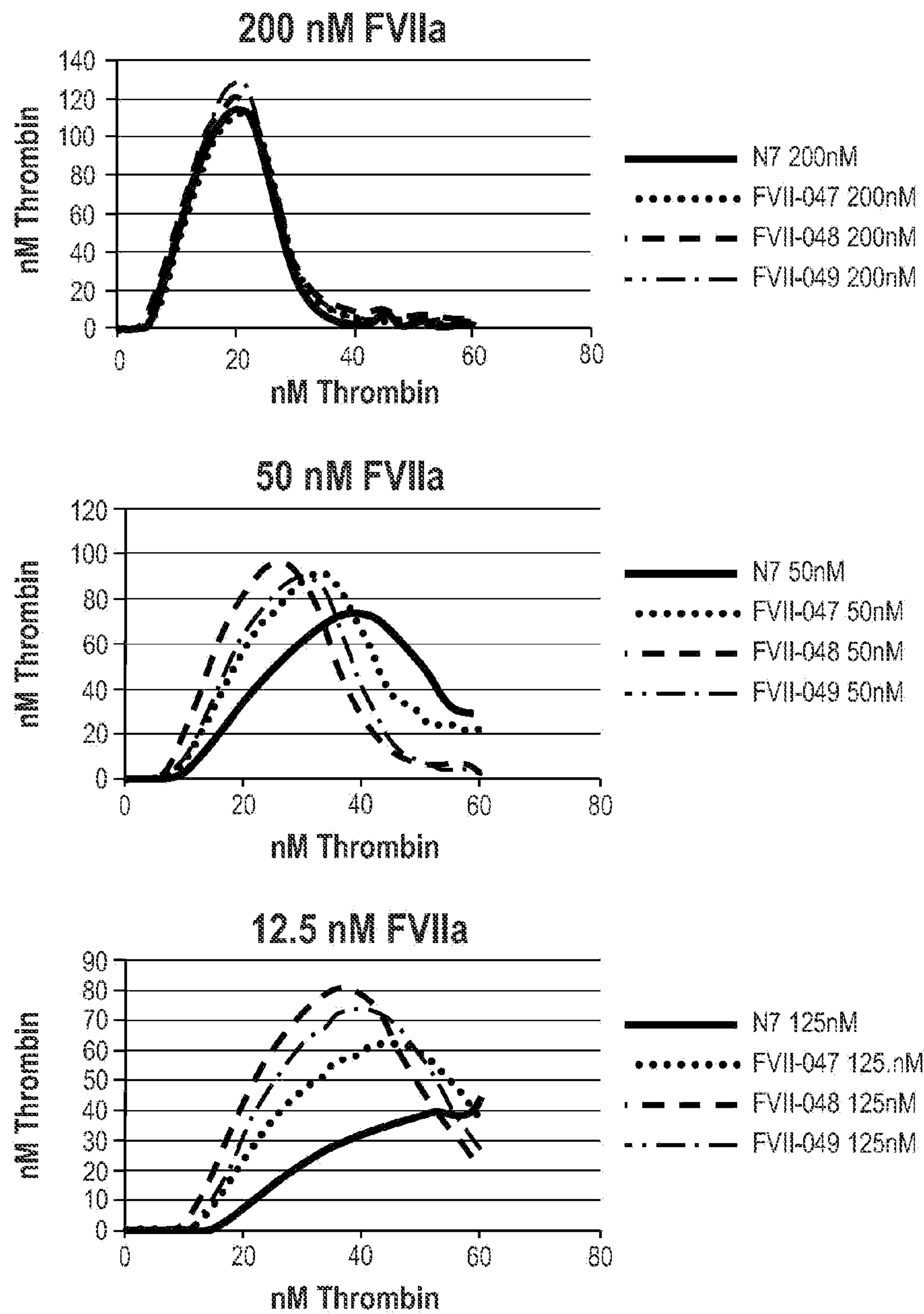


Fig. 47A

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

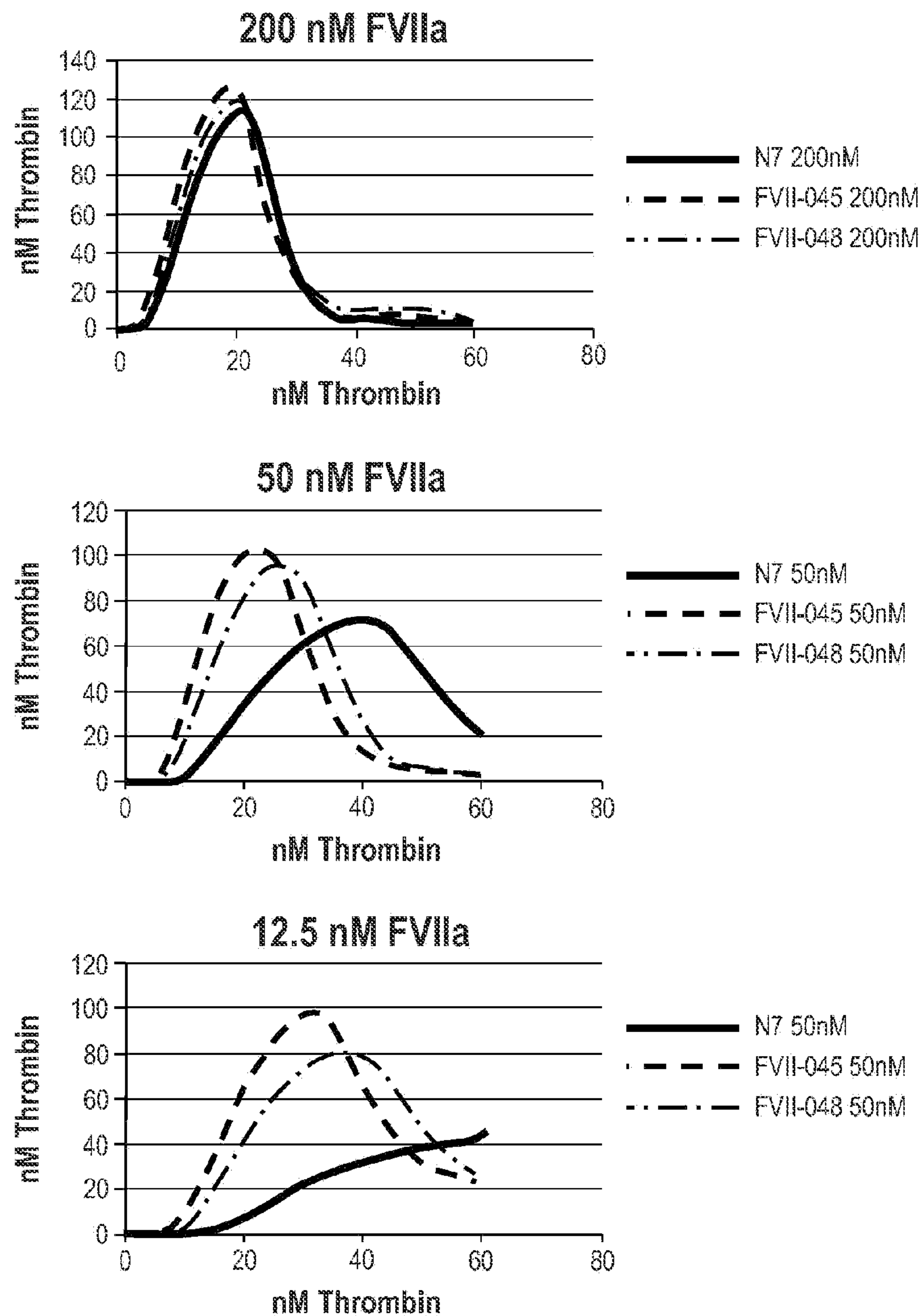
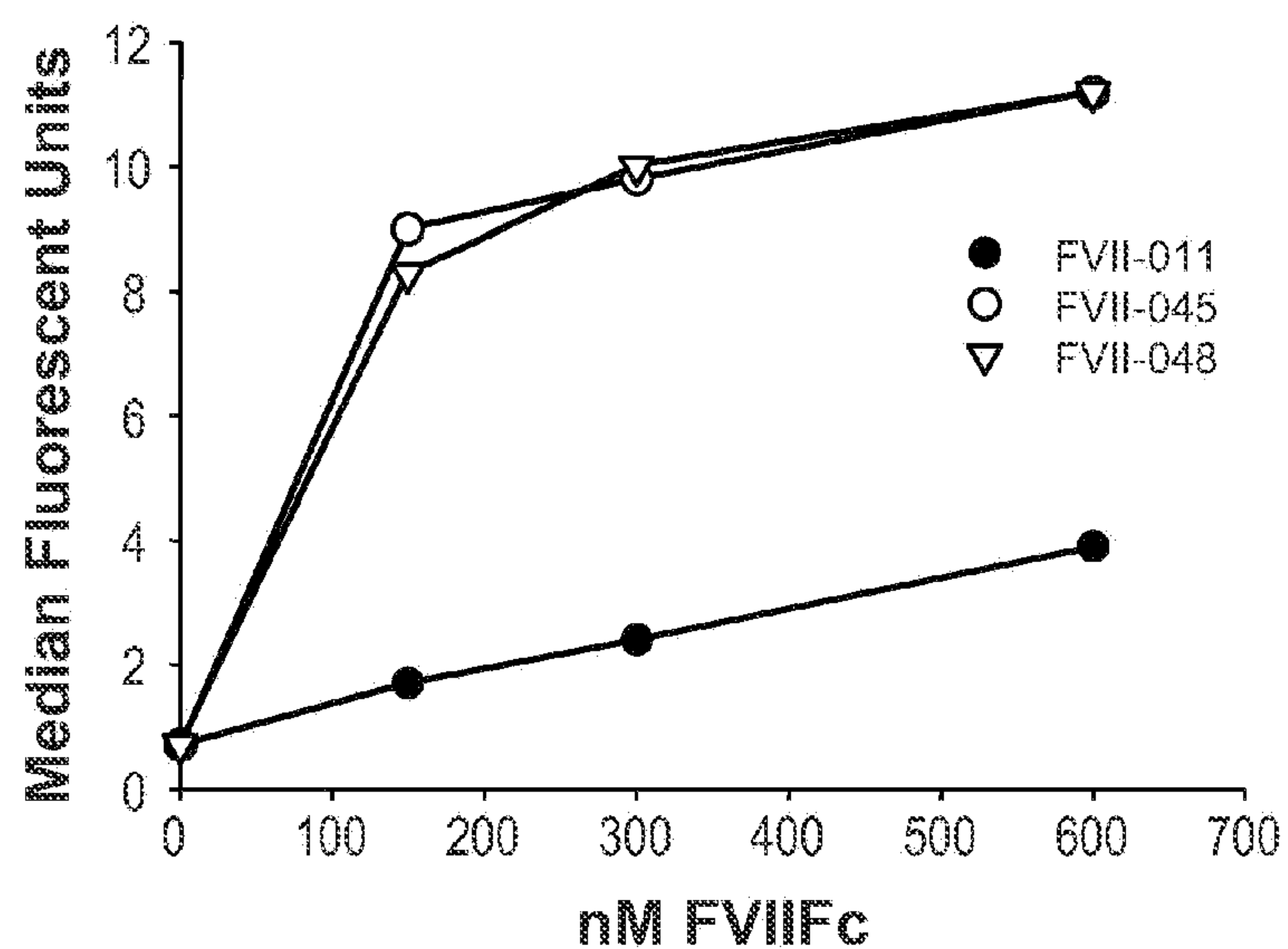


Fig. 47B

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

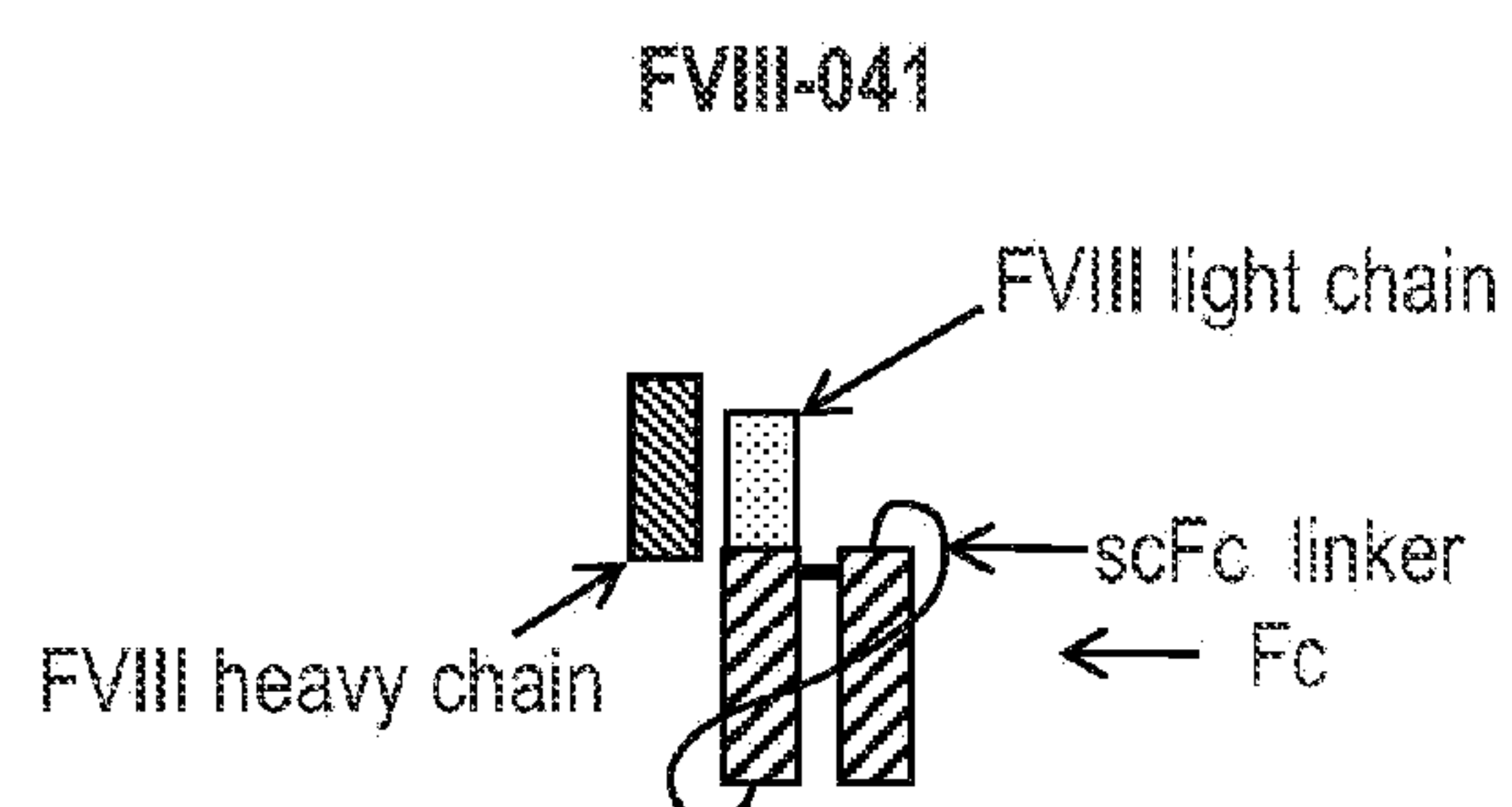
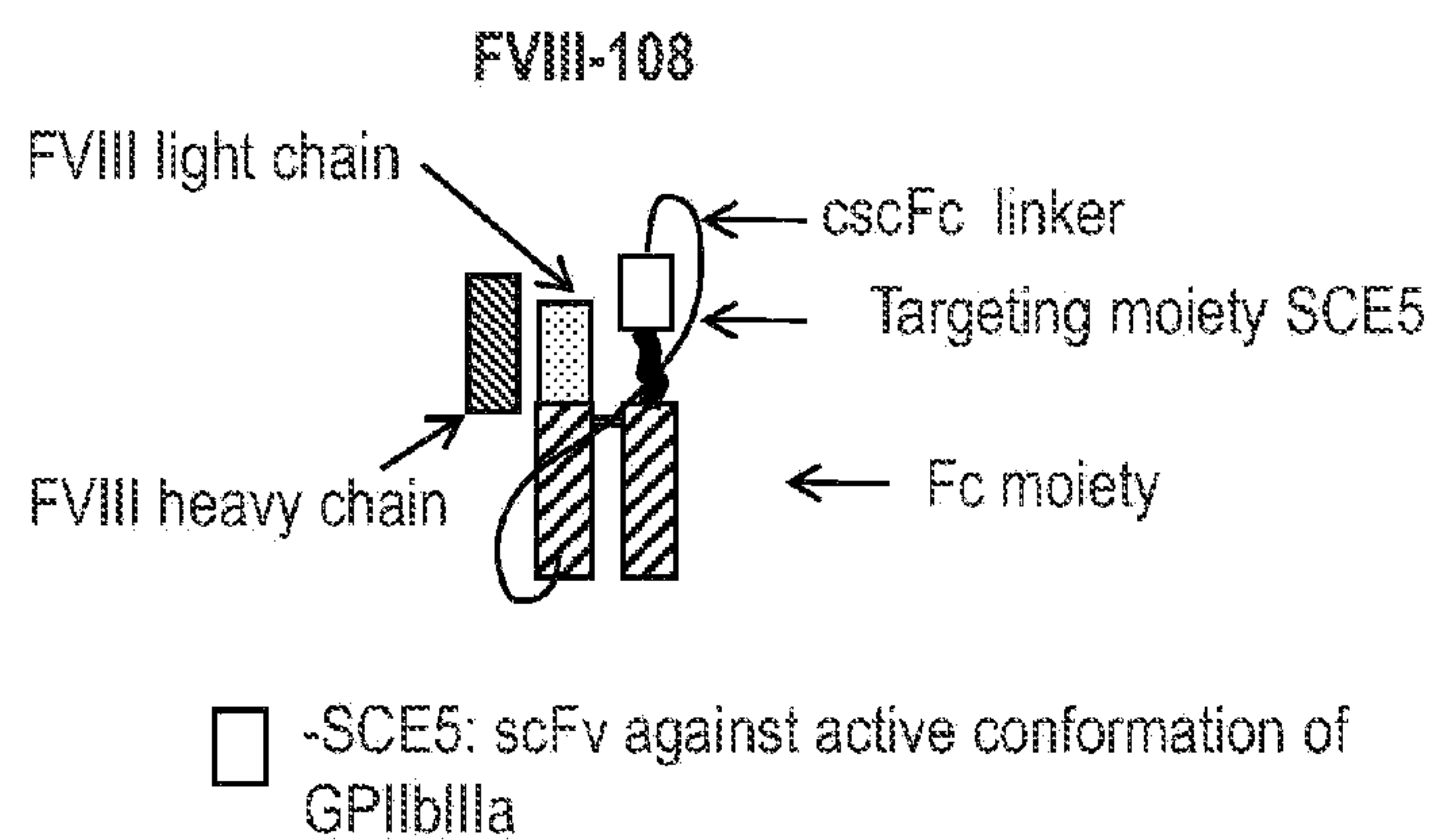


Fig. 49A

Thrombin generation assays in FVIII deficient platelet-rich plasma

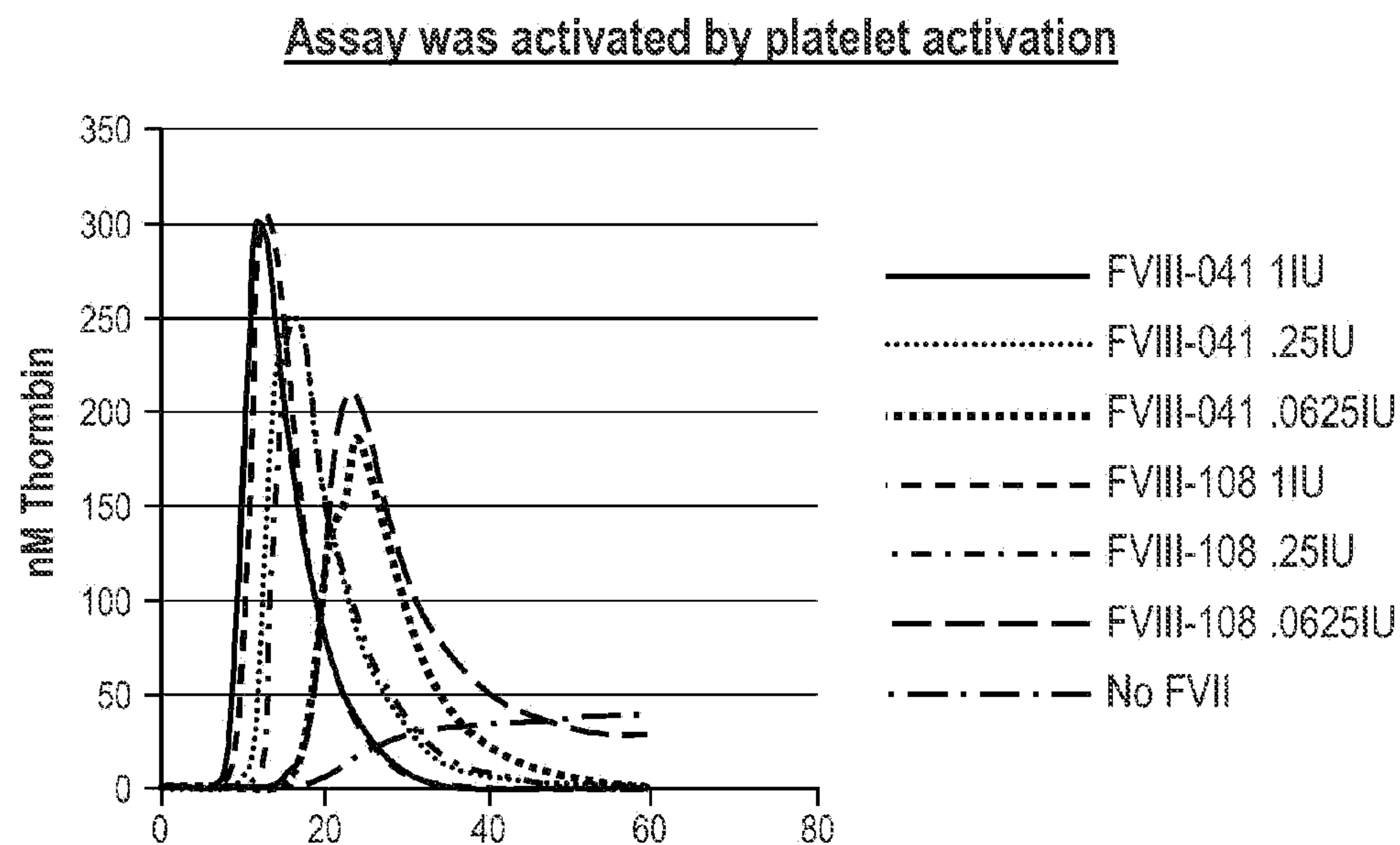
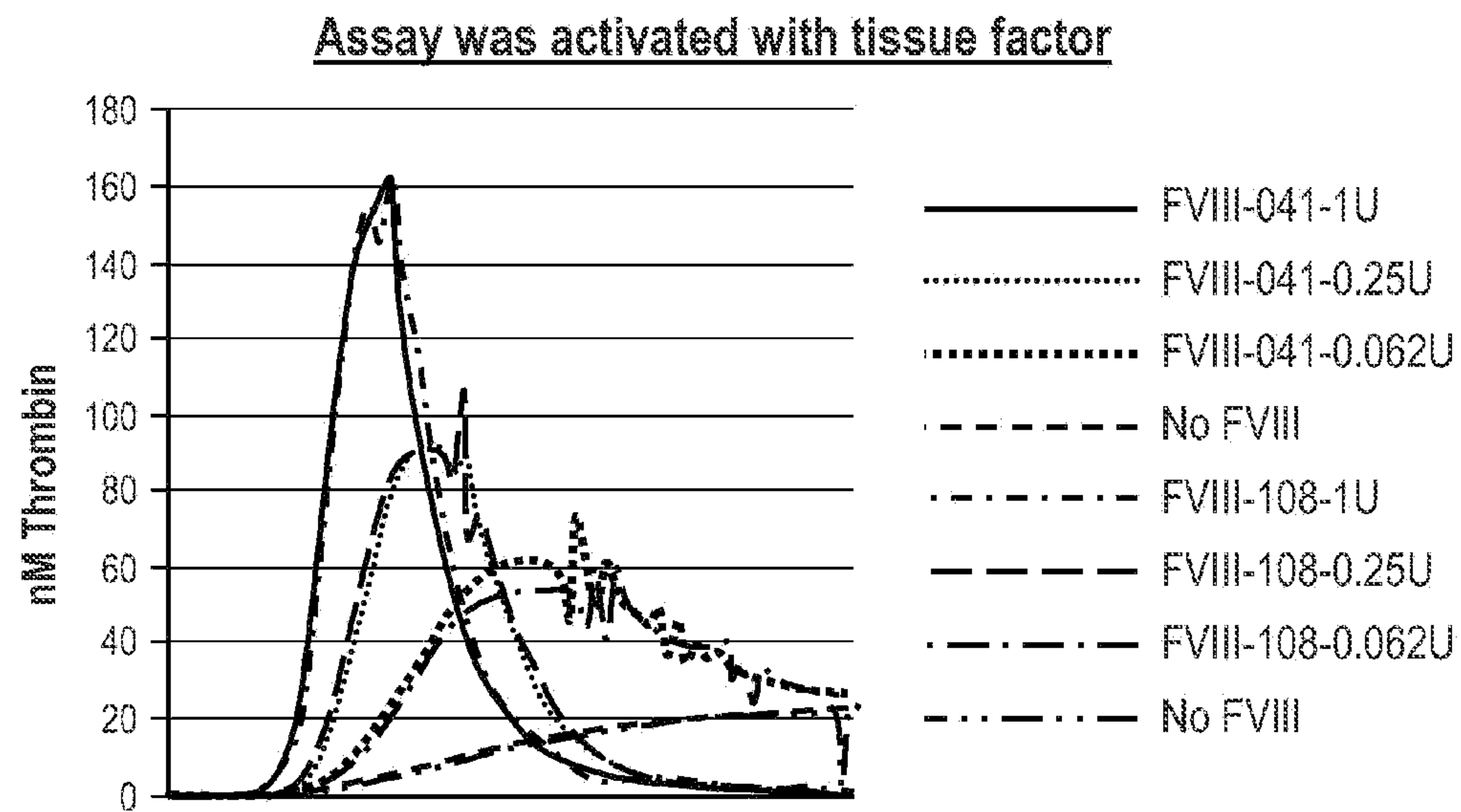
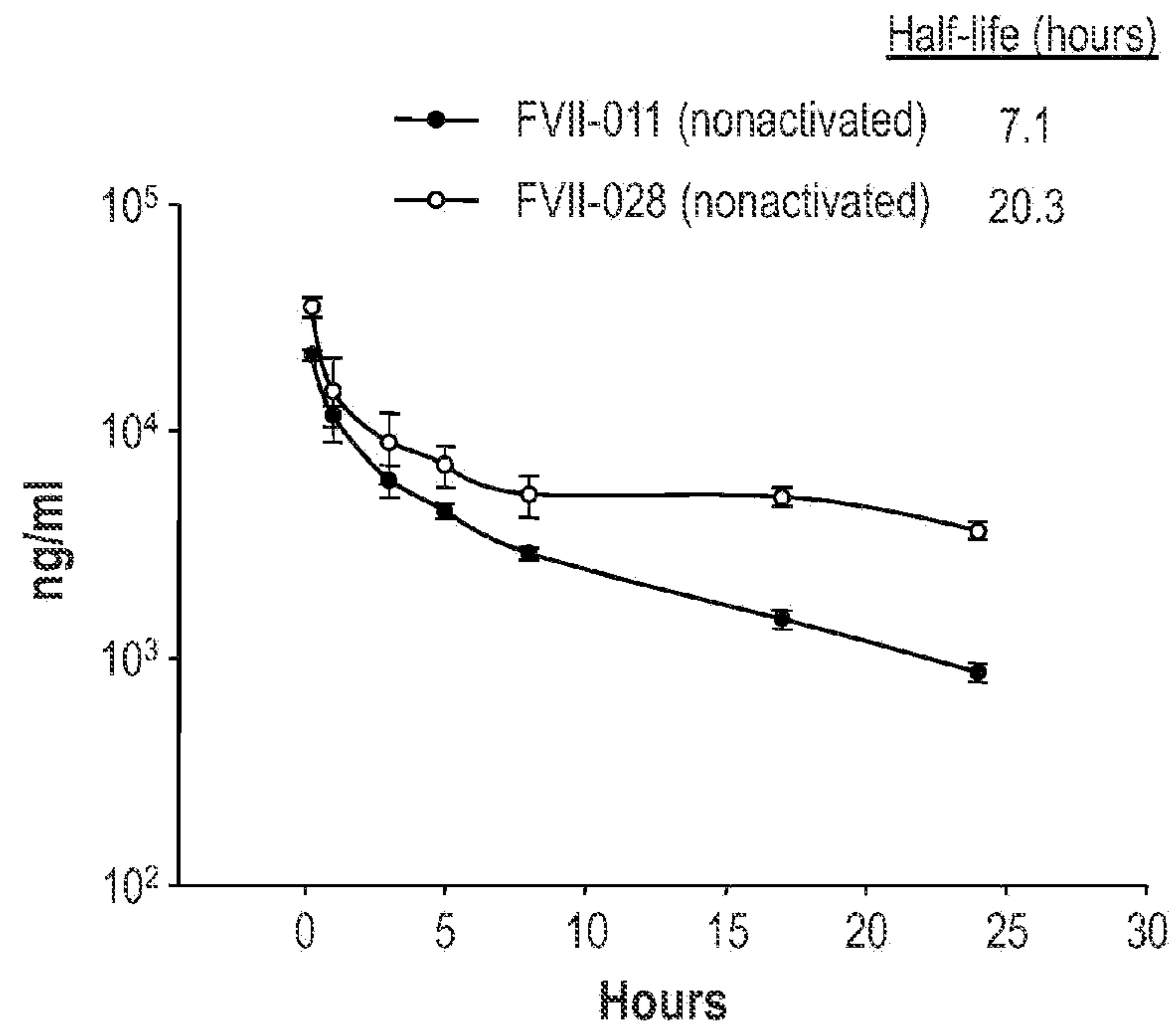


Fig. 49B

***Fig. 50***

Thrombin generation assays in platelet-rich
FIX-deficient plasma

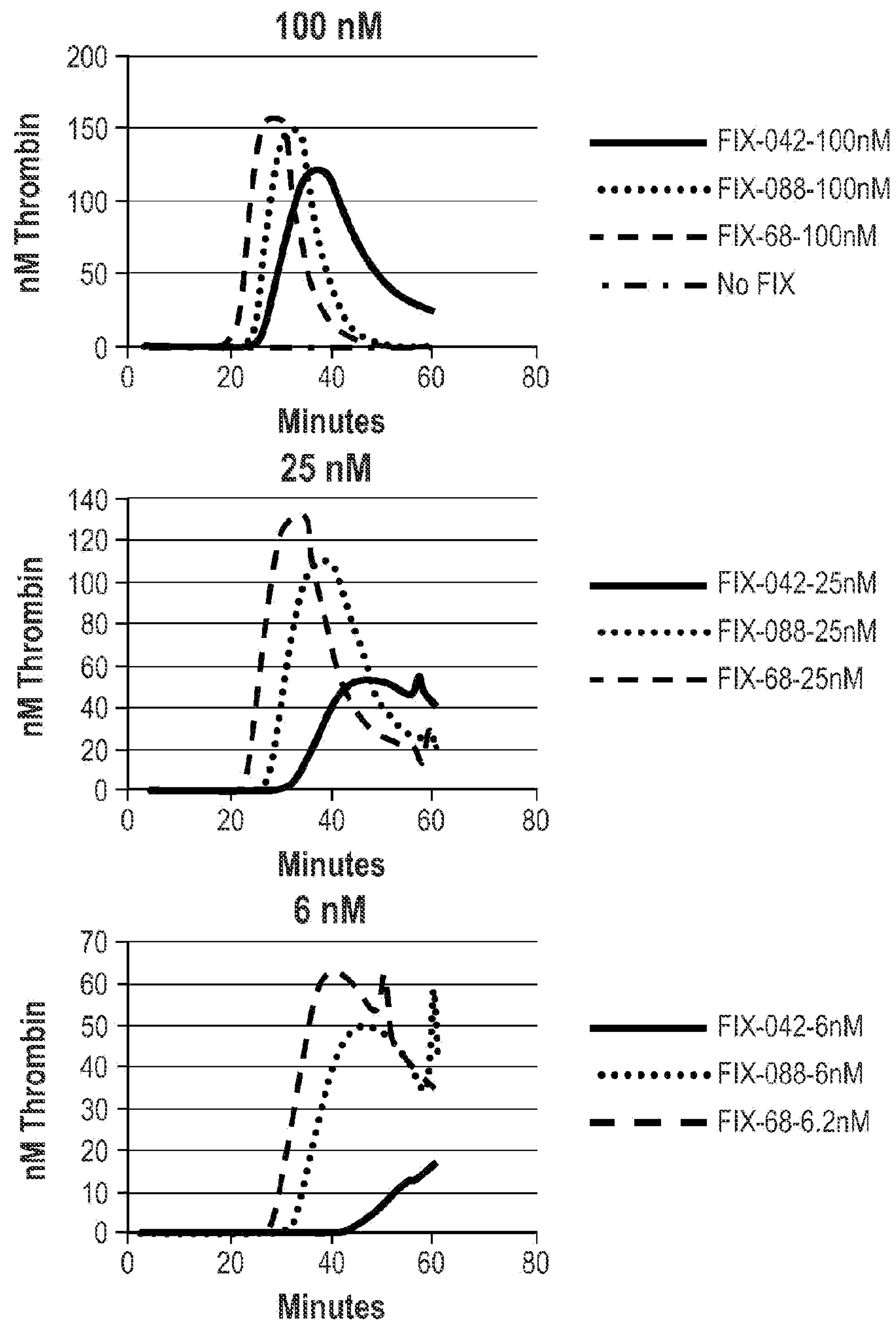


Fig. 51B

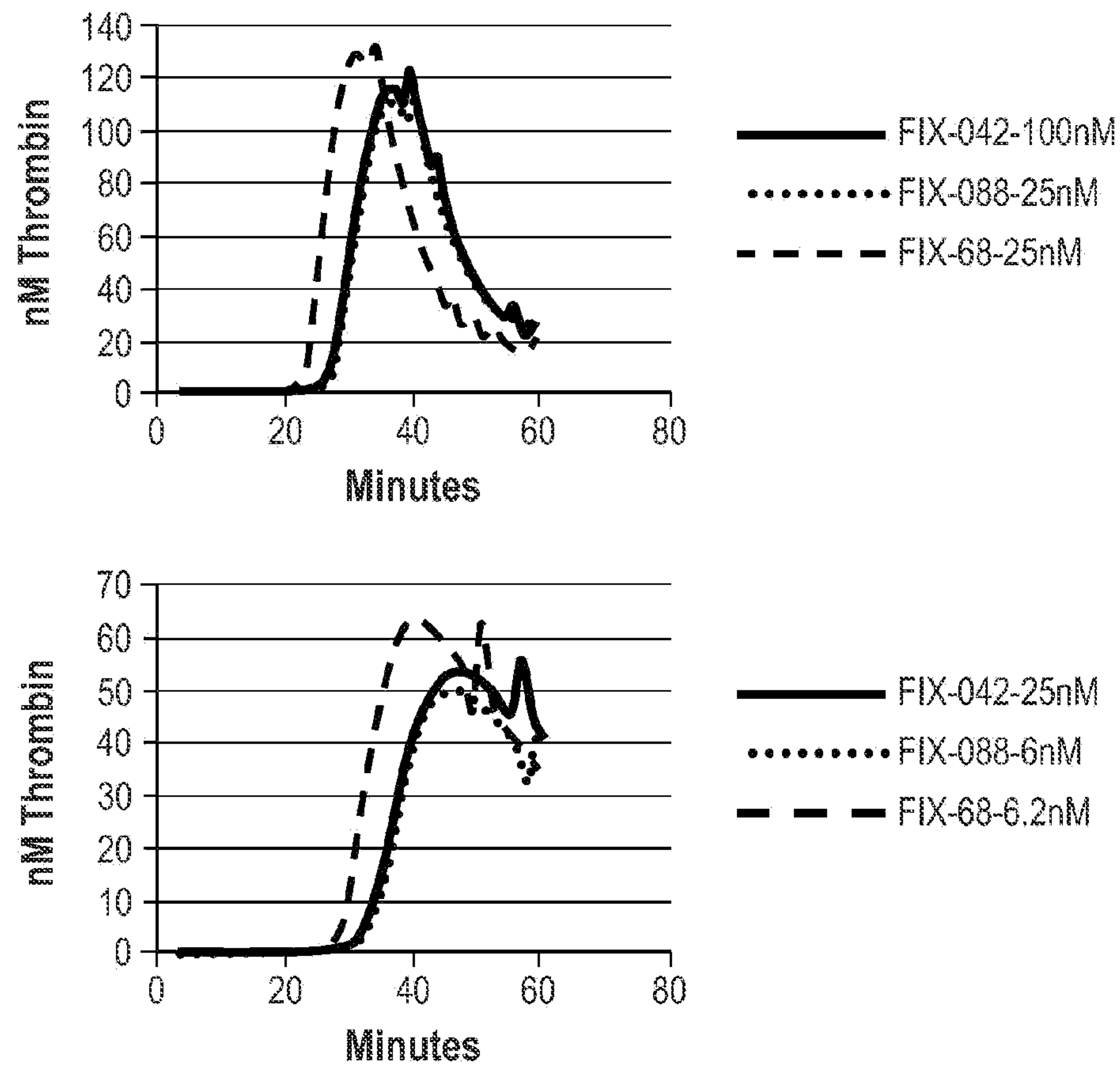
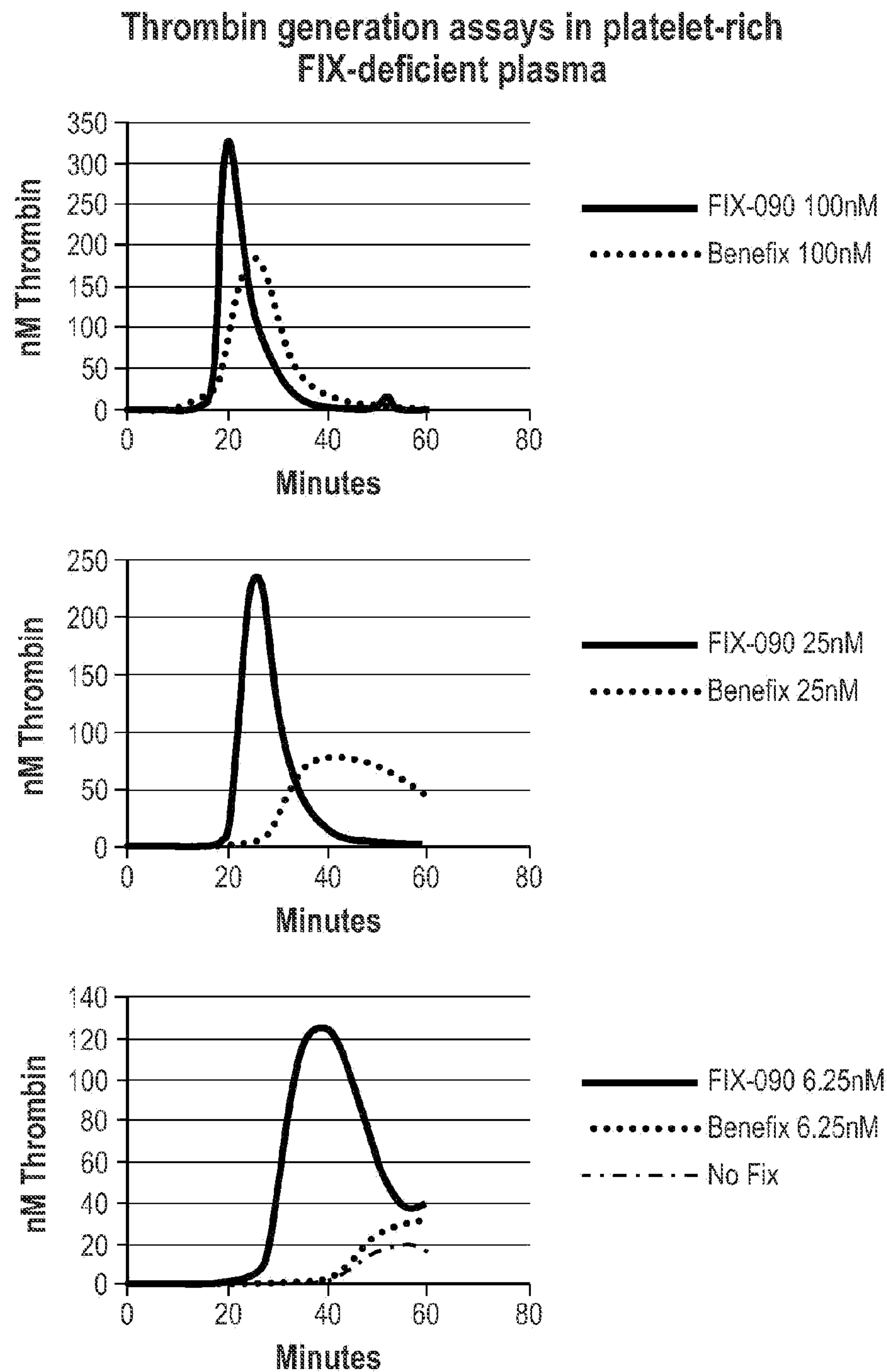


Fig. 51C

*Fig. 52A*

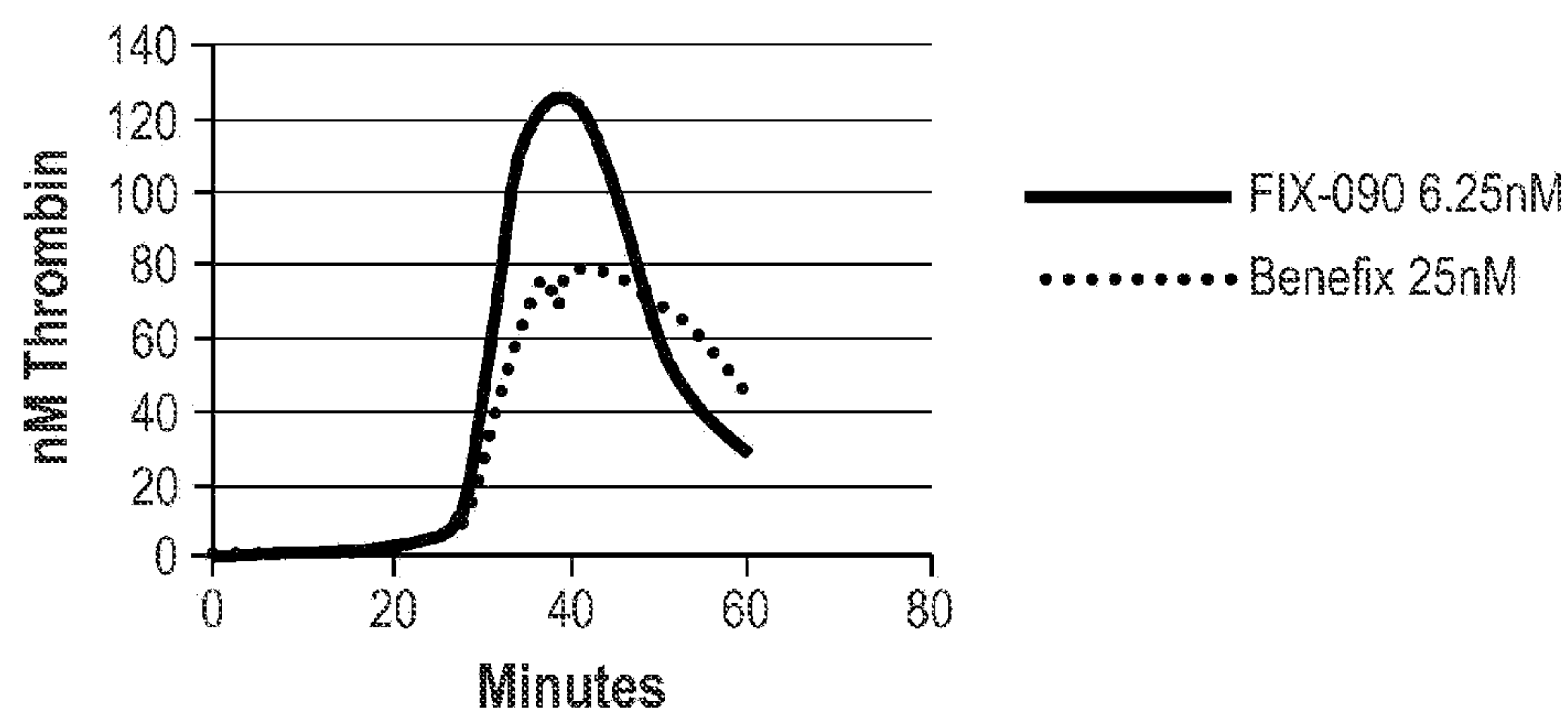


Fig. 52B

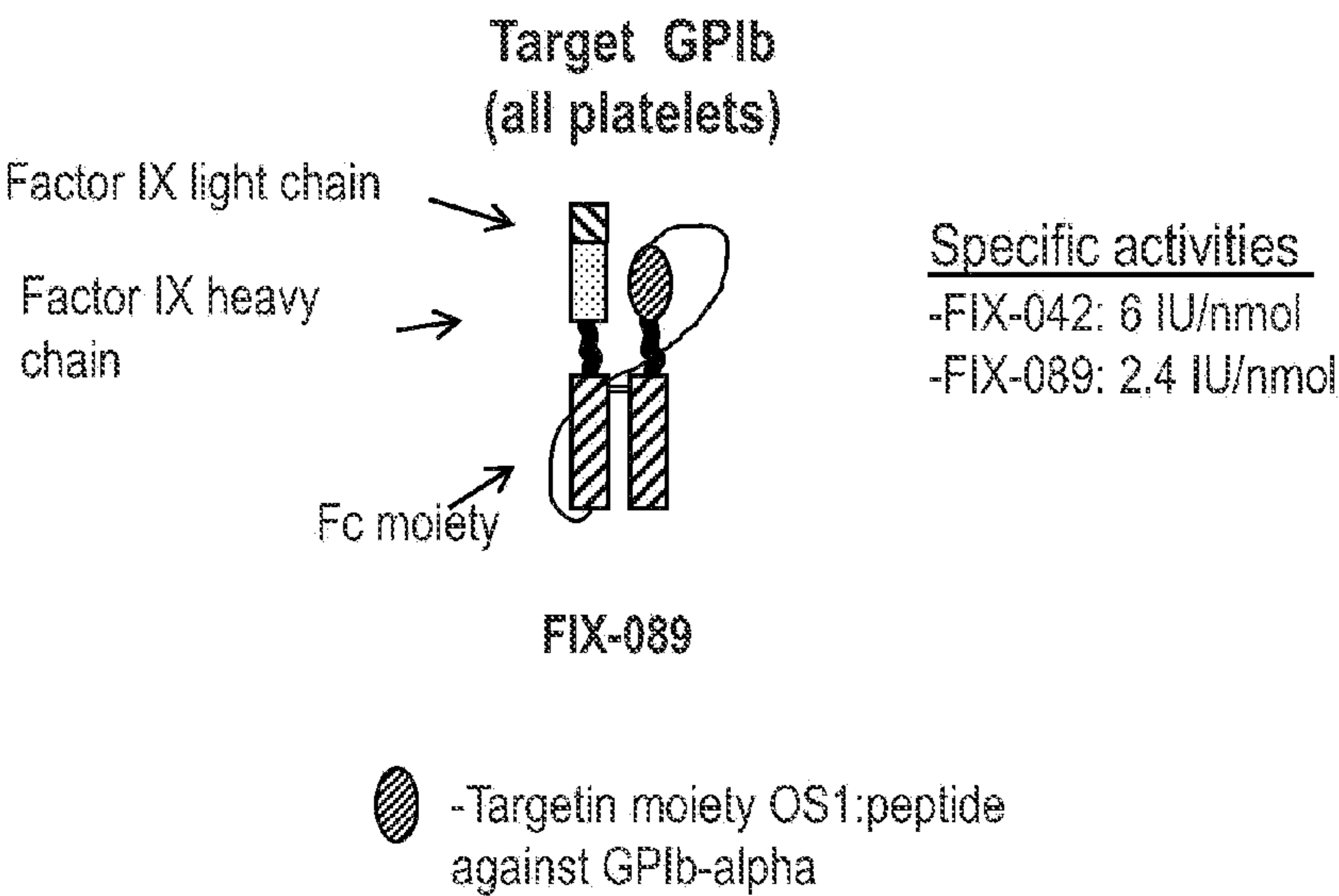


Fig. 53A

Thrombin generation assays in platelet-rich
FIX-deficient plasma

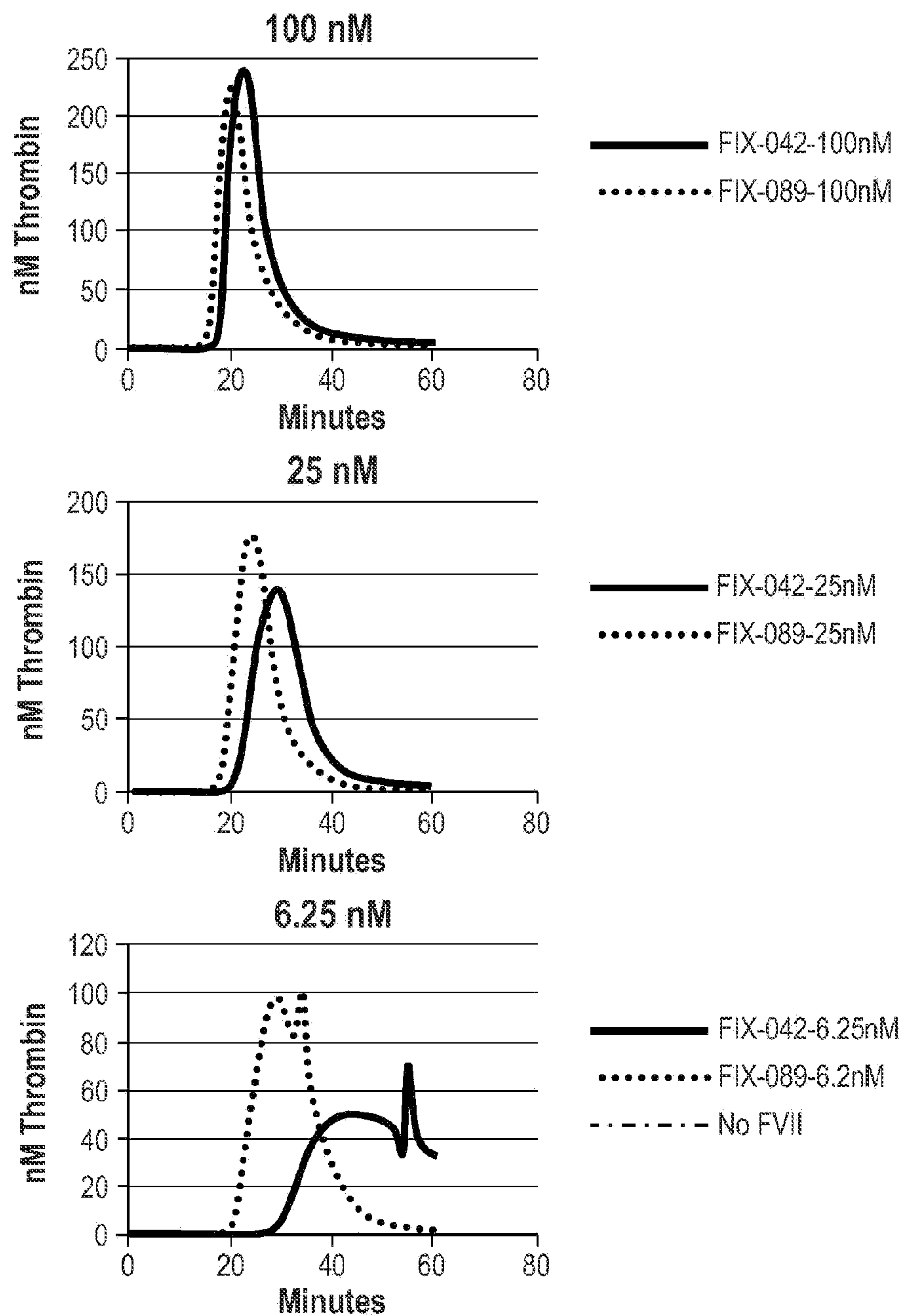


Fig. 53B

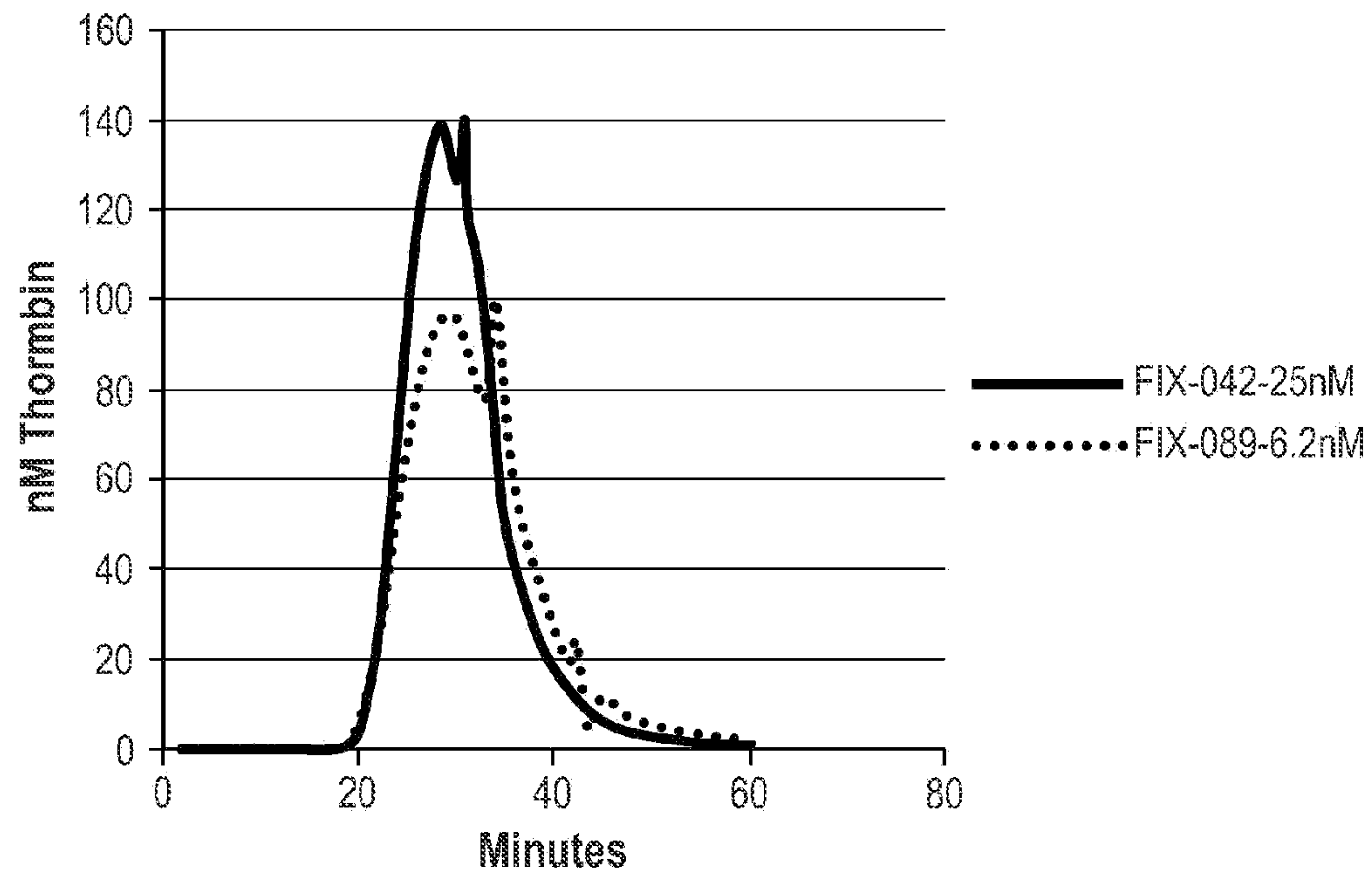


Fig. 53C