



(51) International Patent Classification:

C07K 16/28 (2006.01) *C07K 14/00* (2006.01)
A61K 38/00 (2006.01) *A61K 39/00* (2006.01)
A61K 39/40 (2006.01)

(21) International Application Number:

PCT/US2015/057187

(22) International Filing Date:

23 October 2015 (23.10.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/067,783	23 October 2014 (23.10.2014)	US
62/110,883	2 February 2015 (02.02.2015)	US
62/184,044	24 June 2015 (24.06.2015)	US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

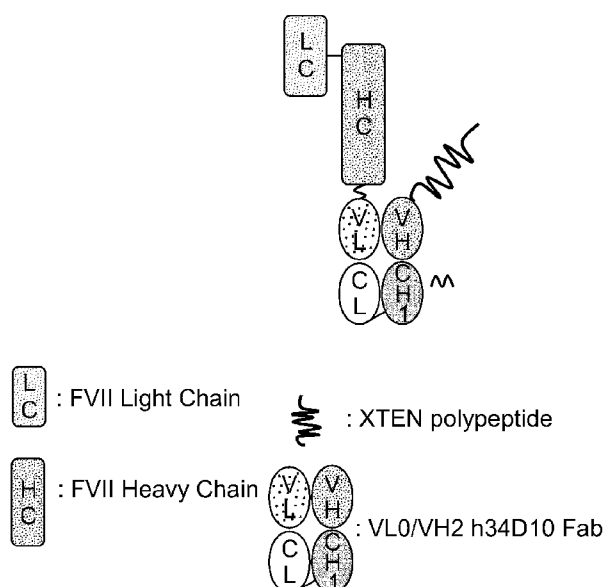
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

[Continued on next page]

(54) Title: ANTI-GPIIB/IIIA ANTIBODIES AND USES THEREOF



(57) Abstract: Antibodies and antibody fragments that specifically bind to glycoprotein IIb/IIIa (GPIIb/IIIa) are disclosed. Chimeric molecules comprising such antibodies or antigen-binding fragments are also disclosed. In addition, methods of using the disclosed antibodies, antibody fragments, and chimeric molecules, e.g., to target agents to platelets and for the treatment or prevention of diseases or disorders are provided.

FIG. 17



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- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))* — *with sequence listing part of description (Rule 5.2(a))*

ANTI-GPIIB/IIIA ANTIBODIES AND USES THEREOF

Cross-Reference to Related Applications

This patent application claims the benefit of U.S. Provisional Patent Application Nos. 62/067,783, filed October 23, 2014; 62/110,883, filed February 2, 2015; and 62/184,044, filed June 24, 2015, each of which are incorporated by reference in their entirety herein.

Sequence Listing

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 23, 2015, is named 13751-0225WO1_SL.txt and is 333,632 bytes in size.

Background

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 hemophilia A or B, deficiency of coagulation Factors XI or VII, defective platelet function, thrombocytopenia, or von Willebrand's disease.

Although such recombinant molecules are effective, there is a need for improved versions which localize the therapeutic agent to sites of coagulation, have improved pharmacokinetic properties, improved manufacturability, reduced thrombogenicity, or enhanced activity, or more than one of these characteristics.

Treatment of hemophilia by replacement therapy is targeting restoration of clotting activity. There are plasma-derived and recombinant clotting factor products available to treat bleeding episodes on-demand or to prevent bleeding episodes from occurring by treating prophylactically. Based on the half-life of these products, treatment regimens require frequent intravenous administration. Such frequent administration is painful and inconvenient. Strategies to extend the half-life of clotting factors include pegylation (Rostin J, et al., *Bioconj. Chem.*, 2000;11:387-96), glycopegylation (Stennicke HR, et al., *Thromb. Haemost.*, 2008;100:920-8), formulation with pegylated liposomes (Spira J, et al., *Blood*,

2006;108:3668-3673, Pan J, et al., *Blood*, 2009;114:2802-2811) and conjugation with albumin (Schulte S., *Thromb. Res.*, 2008;122 Suppl 4:S14-9).

Recombinant FVIIa (rFVIIa; Jurlander B et al., *Semin. Thromb. Hemost.*, 2001; 27(4):373-84) is used to treat bleeding episodes in (i) hemophilia patients with neutralizing antibodies against FVIII or FIX (inhibitors), (ii) patients with FVII deficiency, or (iii) patients with hemophilia A or B with inhibitors undergoing surgical procedures. Prior recombinant rFVIIa preparations sometimes display poor efficacy. Repeated doses of FVIIa at high concentration are often required to control a bleed, due to its low affinity for activated platelets, short half-life, and poor enzymatic activity in the absence of tissue factor. Accordingly, there is an unmet medical need for better treatment and prevention options for patients with coagulation disorders (*e.g.*, hemophilia patients with inhibitors in which the activity of the FVIIa protein is increased).

Summary

The present disclosure features antibodies and antigen-binding fragments thereof that bind to GPIIb/IIIa. These antibodies can specifically bind the GPIIb subunit and/or the GPIIb/IIIa complex. They are capable of targeting the non-active form of the GPIIb/IIIa receptor. The anti-GPIIb/IIIa antibodies and antigen-binding fragments thereof described herein can be used, for example, to target or ferry any agent of interest (*e.g.*, a therapeutic molecule such as a clotting factor) to platelets. For example, the clotting factor FVIIa has low affinity for platelets, the site of action for clot formation. Thus, one approach to increase activity of a clotting factor like FVIIa is to target this clotting factor to platelet receptors via targeting moieties (*e.g.*, Fab or scFv of an anti-GPIIb/IIIa antibody), which can increase the affinity of FVIIa for platelets thereby boosting activity. Such chimeric molecules can include a heterologous moiety to improve the pharmacokinetic parameters of the molecules such as its half-life. In addition to their use as targeting moieties, the anti-GPIIb/IIIa antibodies and antigen-binding fragments thereof of this disclosure can be used as diagnostics, for example, by conjugation to a detectable label, and also for isolating or separating platelets from a sample.

In one aspect, this disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region that is at least

75% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218. In certain embodiments, the heavy chain variable region is at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to 5×10^{-8} M (e.g., 1×10^{-8} M; 1.5×10^{-8} M; 2×10^{-8} M; 2.5×10^{-8} M; 3×10^{-8} M; 3.5×10^{-8} M; 4×10^{-8} M; 4.5×10^{-8} M; 5×10^{-8} M). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In another aspect, this disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a light chain variable region that is at least 75% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10. In certain embodiments, the light chain variable region is at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10.

In another aspect, this disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region that is at least 75% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11,

12, or 197-218, and a light chain variable region that is at least 75% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10. In certain embodiments, the heavy chain variable region is at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218. In certain embodiments, the light chain variable region is at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10. In certain embodiments, the heavy chain variable region is at least 80% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218 and the light chain variable region is at least 80% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10. In certain embodiments, the heavy chain variable region is at least 85% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218 and the light chain variable region is at least 85% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10. In some embodiments, the heavy chain variable region is at least 90% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218 and the light chain variable region is at least 90% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10. In other embodiments, the heavy chain variable region is at least 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218 and the light chain variable region is at least 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10. In yet other embodiments, the heavy chain variable region is at least 97% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218 and the light

chain variable region is at least 97% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10. In a certain embodiment, the heavy chain variable region is identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218 and the light chain variable region is identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10. In one specific embodiment, the heavy chain variable region is identical to the amino acid sequence set forth in SEQ ID NO: 7 and the light chain variable region is identical to the amino acid sequence set forth in SEQ ID NO:4. In another specific embodiment, the heavy chain variable region is identical to the amino acid sequence set forth in SEQ ID NO: 12 and the light chain variable region is identical to the amino acid sequence set forth in SEQ ID NO:8. In yet another specific embodiment, the heavy chain variable region is identical to the amino acid sequence set forth in SEQ ID NO:11 and the light chain variable region is identical to the amino acid sequence set forth in SEQ ID NO:10. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to 5 X 10⁻⁸M (*e.g.*, 1X10⁻⁸M; 1.5X10⁻⁸M; 2 X10⁻⁸M; 2.5 X10⁻⁸M; 3 X10⁻⁸M; 3.5X10⁻⁸M; 4 X10⁻⁸M; 4.5X10⁻⁸M; 5 X10⁻⁸M). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In another aspect, the disclosure features an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region that is at least 75% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 5, 7, 9, 11, 12, or 197-218, and a light chain variable region that is at least 75% identical to the amino acid sequence set forth in SEQ ID NO:4. In one embodiment, the heavy chain variable region that is at least 80% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is at least 80% identical to the amino acid sequence set forth in SEQ ID NO:4. In another embodiment, the heavy chain variable region that is at least 85% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is at least 85% identical to the amino acid sequence set forth in SEQ ID NO:4. In one embodiment, the heavy chain variable region that is at least 90% identical to the amino acid

sequence set forth in any one of SEQ ID NOs: 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:4. In another embodiment, the heavy chain variable region that is at least 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:4. In yet another embodiment, the heavy chain variable region that is at least 97% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is at least 97% identical to the amino acid sequence set forth in SEQ ID NO:4. In a specific embodiment, the heavy chain variable region that is identical to the amino acid sequence set forth in any one of SEQ ID NOs: 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is identical to the amino acid sequence set forth in SEQ ID NO:4. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to 5×10^{-8} M (*e.g.*, 1×10^{-8} M; 1.5×10^{-8} M; 2×10^{-8} M; 2.5×10^{-8} M; 3×10^{-8} M; 3.5×10^{-8} M; 4×10^{-8} M; 4.5×10^{-8} M; 5×10^{-8} M). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In another aspect, the disclosure features an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region that is at least 75% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218, and a light chain variable region that is at least 75% identical to the amino acid sequence set forth in SEQ ID NO:10. In one embodiment, the heavy chain variable region that is at least 80% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is at least 80% identical to the amino acid sequence set forth in SEQ ID NO:10. In another embodiment, the heavy chain variable region that is at least 85% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is at least 85% identical to the amino acid sequence set forth in SEQ ID NO:10. In one embodiment, the heavy chain variable region that is at least 90% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218, and the light

chain variable region is at least 90% identical to the amino acid sequence set forth in SEQ ID NO:10. In another embodiment, the heavy chain variable region that is at least 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is at least 95% identical to the amino acid sequence set forth in SEQ ID NO:10. In yet another embodiment, the heavy chain variable region that is at least 97% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is at least 97% identical to the amino acid sequence set forth in SEQ ID NO:10. In a specific embodiment, the heavy chain variable region that is identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218, and the light chain variable region is identical to the amino acid sequence set forth in SEQ ID NO:10. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to 5×10^{-8} M (e.g., 1×10^{-8} M; 1.5×10^{-8} M; 2×10^{-8} M; 2.5×10^{-8} M; 3×10^{-8} M; 3.5×10^{-8} M; 4×10^{-8} M; 4.5×10^{-8} M; 5×10^{-8} M). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In another aspect, the disclosure relates to an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region that is identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218, except for a total of 1 to 10 (*i.e.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions, deletions, or insertions. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to 5×10^{-8} M (e.g., 1×10^{-8} M; 1.5×10^{-8} M; 2×10^{-8} M; 2.5×10^{-8} M; 3×10^{-8} M; 3.5×10^{-8} M; 4×10^{-8} M; 4.5×10^{-8} M; 5×10^{-8} M). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In a further aspect, the disclosure provides an antibody antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a light chain variable region that is identical

to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10, except for a total of 1 to 10 (*i.e.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) amino acid substitutions, deletions, or insertions. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to 5×10^{-8} M (*e.g.*, 1×10^{-8} M; 1.5×10^{-8} M; 2×10^{-8} M; 2.5×10^{-8} M; 3×10^{-8} M; 3.5×10^{-8} M; 4×10^{-8} M; 4.5×10^{-8} M; 5×10^{-8} M). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In yet another aspect, the disclosure relates to an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises (i) a heavy chain variable region that is identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218, except for a total of 1 to 10 amino acid substitutions, deletions, or insertions; and (ii) a light chain variable region that is identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10, except for a total of 1 to 10 amino acid substitutions, deletions, or insertions. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to 5×10^{-8} M (*e.g.*, 1×10^{-8} M; 1.5×10^{-8} M; 2×10^{-8} M; 2.5×10^{-8} M; 3×10^{-8} M; 3.5×10^{-8} M; 4×10^{-8} M; 4.5×10^{-8} M; 5×10^{-8} M). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In certain embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:26), and GGDYGYALDY (SEQ ID NO:27), respectively.

In other embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences

AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVVKR (SEQ ID NO:26), and GGDYSYALDY (SEQ ID NO:245), respectively. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to 5 X 10⁻⁸M (e.g., 1X10⁻⁸M; 1.5X10⁻⁸M; 2 X10⁻⁸M; 2.5 X10⁻⁸M; 3 X10⁻⁸M; 3.5X10⁻⁸M; 4 X10⁻⁸M; 4.5X10⁻⁸M; 5 X10⁻⁸M). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In other embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVER (SEQ ID NO:241), and GGDYSYALDY (SEQ ID NO:245), respectively. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to 5 X 10⁻⁸M (e.g., 1X10⁻⁸M; 1.5X10⁻⁸M; 2 X10⁻⁸M; 2.5 X10⁻⁸M; 3 X10⁻⁸M; 3.5X10⁻⁸M; 4 X10⁻⁸M; 4.5X10⁻⁸M; 5 X10⁻⁸M). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In other embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSDGTTYYPDSVVKR (SEQ ID NO:242), and GGDYSYALDY (SEQ ID NO:245), respectively. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to 5 X 10⁻⁸M (e.g., 1X10⁻⁸M; 1.5X10⁻⁸M; 2 X10⁻⁸M; 2.5 X10⁻⁸M; 3 X10⁻⁸M; 3.5X10⁻⁸M; 4 X10⁻⁸M; 4.5X10⁻⁸M; 5 X10⁻⁸M). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In other embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTDYPDSVKR (SEQ ID NO:243), and GGDYGYALDY (SEQ ID NO:27), respectively. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to $5 \times 10^{-8}\text{M}$ (e.g., $1 \times 10^{-8}\text{M}$; $1.5 \times 10^{-8}\text{M}$; $2 \times 10^{-8}\text{M}$; $2.5 \times 10^{-8}\text{M}$; $3 \times 10^{-8}\text{M}$; $3.5 \times 10^{-8}\text{M}$; $4 \times 10^{-8}\text{M}$; $4.5 \times 10^{-8}\text{M}$; $5 \times 10^{-8}\text{M}$). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In other embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), GISSGGTTYYPDSVKR (SEQ ID NO:244), and GGDYGYALDY (SEQ ID NO:27), respectively. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to $5 \times 10^{-8}\text{M}$ (e.g., $1 \times 10^{-8}\text{M}$; $1.5 \times 10^{-8}\text{M}$; $2 \times 10^{-8}\text{M}$; $2.5 \times 10^{-8}\text{M}$; $3 \times 10^{-8}\text{M}$; $3.5 \times 10^{-8}\text{M}$; $4 \times 10^{-8}\text{M}$; $4.5 \times 10^{-8}\text{M}$; $5 \times 10^{-8}\text{M}$). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7.

In certain embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively. In certain embodiments, these antibodies have an apparent monovalent affinity that is improved over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ ID NO:7. In some embodiments, these antibodies have an apparent monovalent affinity that is about 1 to $5 \times 10^{-8}\text{M}$ (e.g., $1 \times 10^{-8}\text{M}$; $1.5 \times 10^{-8}\text{M}$; $2 \times 10^{-8}\text{M}$; $2.5 \times 10^{-8}\text{M}$; $3 \times 10^{-8}\text{M}$; $3.5 \times 10^{-8}\text{M}$; $4 \times 10^{-8}\text{M}$; $4.5 \times 10^{-8}\text{M}$; $5 \times 10^{-8}\text{M}$). In certain embodiments, these antibodies have an improved off-rate over an antibody comprising a VH comprising an amino acid sequence set forth in SEQ

ID NO:7. In certain embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:26), and GGDYGYALDY (SEQ ID NO:27), respectively; and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In certain embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:26), and GGDYSYALDY (SEQ ID NO:245), respectively; and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In certain embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVER (SEQ ID NO:241), and GGDYSYALDY (SEQ ID NO:245), respectively; and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In certain embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSDGTTYYPDSVKR (SEQ ID NO:242), and GGDYSYALDY (SEQ ID NO:245), respectively; and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences

RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In certain embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTDYPDSVKR (SEQ ID NO:243), and GGDYGYALDY (SEQ ID NO:27), respectively; and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In certain embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof comprises (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), GISSGGTTYYPDSVKR (SEQ ID NO:244), and GGDYGYALDY (SEQ ID NO:27), respectively; and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In one aspect, the disclosure features an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:26), and GGDYSYALDY (SEQ ID NO:245), respectively.

In another aspect, the disclosure features an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVER (SEQ ID NO:241), and GGDYSYALDY (SEQ ID NO:245), respectively.

In another aspect, the disclosure features an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSDGTYYPD SVKR (SEQ ID NO:242), and GGDYSYALDY (SEQ ID NO:245), respectively.

In another aspect, the disclosure features an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTDYPD SVKR (SEQ ID NO:243), and GGDYGYALDY (SEQ ID NO:27), respectively.

In a further aspect, the disclosure features an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), GISSGGTTYYPD SVKR (SEQ ID NO:244), and GGDYGYALDY (SEQ ID NO:27), respectively.

In yet another aspect, the disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPD SVKR (SEQ ID NO:26), and GGDYSYALDY (SEQ ID NO:245), respectively; and a light chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In yet another aspect, the disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino

acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVER (SEQ ID NO:241), and GGDYSYALDY (SEQ ID NO:245), respectively; and a light chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In yet another aspect, the disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSDGTTTYYPDSVKR (SEQ ID NO:242), and GGDYSYALDY (SEQ ID NO:245), respectively; and a light chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In yet another aspect, the disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTDYPDSVKR (SEQ ID NO:243), and GGDYGYALDY (SEQ ID NO:27), respectively; and a light chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In yet another aspect, the disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:244), and GGDYGYALDY (SEQ ID NO:27), respectively; and a light chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of

the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

In certain embodiments of the above aspects, the antibody or antigen-binding fragment thereof has an apparent monovalent affinity that is about 1 to about 5×10^{-8} M.

In certain embodiments of all of the above aspects, the antibody or the antigen-binding fragment thereof is an Fab, an Fab', an F(ab')₂, an Facb, an Fv, an Fd, a diabody, an scFv, or an sc(Fv)₂. In a specific embodiment, the antibody or the antigen-binding fragment thereof is an Fab.

In another aspect, the disclosure relates to a chimeric molecule comprising the anti-GPIIb/IIIa antibody or antigen-binding fragment thereof described herein and a heterologous moiety.

In certain embodiments of this aspect, the heterologous moiety of the chimeric molecule comprises a clotting factor. In some embodiments, the clotting factor is FVII, FIX, or FX. In some embodiments, the clotting factor is FVII zymogen (*e.g.*, A or B isoform), activatable FVII, activated FVII (FVIIa), FIX zymogen, activatable FIX, activated FIX (FIXa), FX zymogen, activatable FX, or activated FX (FXa). In one embodiment, the clotting factor comprises a single polypeptide chain. In another embodiment, the clotting factor comprises two polypeptide chains. In certain embodiments, the heterologous moiety of the chimeric molecule comprises a small molecule drug.

In certain embodiments of this aspect, the chimeric molecule further includes a linker. In some embodiments, the linker is a peptide linker. The peptide linker can comprise at least two, at least three, at least four, at least five, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, or at least 100 amino acids. In some embodiments, the peptide linker comprises a peptide having the formula [(Gly)_x-Ser_y]_z where x is from 1 to 4, y is 0 or 1, and z is from 1 to 50 (SEQ ID NO: 153).

In certain embodiments of this aspect, the chimeric molecule comprises a second heterologous moiety. In some embodiments, the second heterologous moiety comprises a half-life extending moiety. The half-life extending moiety can be, *e.g.*, an XTEN, albumin, albumin binding polypeptide or fatty acid, an Fc region, transferrin, PAS, the C-terminal

peptide (CTP) of the β subunit of human chorionic gonadotropin, polyethylene glycol (PEG), hydroxyethyl starch (HES), albumin-binding small molecules, vWF, and a clearance receptor or a fragment thereof which blocks binding of the chimeric molecule to a clearance receptor. In one embodiment, half-life extending moiety is an XTEN. In a specific embodiment, the XTEN is AE144. In another specific embodiment, the XTEN is AE288. In certain embodiments, the chimeric molecule comprises two half-life extending moieties. In certain embodiments, a linker connects the half-life extending molecule to the first heterologous moiety such as a clotting factor (e.g., Factor VII).

In a further aspect, the disclosure relates to a chimeric molecule comprising the anti-GPIIb/IIIa antibody or antigen-binding fragment thereof described herein, a Factor VII comprising a heavy chain and a light chain, and a half-life extending moiety. In certain embodiments, the antibody or antigen-binding fragment thereof is an Fab or an scFv. In one embodiment, the light chain of the Factor VII is linked to/associated with the heavy chain of the Factor VII, which in turn is linked to the half-life extending moiety, and the half-life extending moiety is linked to the anti-GPIIb/IIIa antibody or antigen-binding fragment thereof (e.g., Fab or scFv). The “linking” between these moieties can either be by direct covalent bonds between these moieties or via linkers (e.g., peptide linkers).

In another aspect, the disclosure features a chimeric molecule that has an amino acid sequence that is at least 65% identical, at least 70% identical, at least 75% identical, at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 74 or SEQ ID NO: 77. In certain embodiments, this chimeric molecule associates with an amino acid sequence that is at least 65% identical, at least 70% identical, at least 75% identical, at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at

least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 75 or SEQ ID NO: 76. In one embodiment, the chimeric molecule comprises, consists essentially of, or consists of the amino acid sequence set forth in SEQ ID NO: 74. In another embodiment, the chimeric molecule comprises, consists essentially of, or consists of the amino acid sequence set forth in or SEQ ID NO: 77. In certain embodiments, these chimeric molecules associates with an amino acid sequence comprising, consisting essentially of, or consisting of the amino acid sequence set forth in SEQ ID NO: 75. In certain embodiments, these chimeric molecules associates with an amino acid sequence comprising, consisting essentially of, or consisting of the amino acid sequence set forth in SEQ ID NO: 76. In certain embodiments, these chimeric molecules may comprise additional half-life extending moieties (*e.g.*, AE144, AE288). In certain embodiments, these chimeric molecules may comprise one or more (*e.g.*, 1, 2, 3, 4) linkers between Factor VII and the half-life extending moiety. In certain embodiments, these chimeric molecules may comprise additional linkers (*e.g.*, 2, 3, 4) between the half-life extending moiety and the light chain of the Fab.

In another aspect, the disclosure features a chimeric molecule that has an amino acid sequence that is at least 65% identical, at least 70% identical, at least 75% identical, at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 247. In certain embodiments, this chimeric molecule includes at least one, at least two, or all three of CDRs of SEQ ID NO:4. In certain embodiments, the above-described chimeric molecule associates with an amino acid sequence that is at least 65% identical, at least 70% identical, at least 75% identical, at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91%

identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 75. In one embodiment, the chimeric molecule comprises, consists essentially of, or consists of the amino acid sequence set forth in SEQ ID NO: 247. In certain embodiments, the chimeric molecule associates with an amino acid sequence comprising, consisting essentially of, or consisting of the amino acid sequence set forth in SEQ ID NO: 75. In certain embodiments, these chimeric molecules may comprise additional half-life extending moieties (*e.g.*, AE144, AE288). In certain embodiments, these chimeric molecules may comprise one or more (*e.g.*, 1, 2, 3, 4) linkers between Factor VII and the half-life extending moiety. In certain embodiments, these chimeric molecules may comprise additional linkers (*e.g.*, 2, 3, 4) between the half-life extending moiety and the light chain of the Fab.

In another aspect, the disclosure features a chimeric molecule comprising a clotting factor (*e.g.*, FVII, FIX, or FX), an anti-GPIIb/IIIa antibody or antigen-binding fragment thereof, and a half-life extending moiety (*e.g.*, XTEN). This chimeric molecule may comprise one or more linkers (*e.g.*, 6X(GGGGS) (SEQ ID NO:170)). The optional linker(s) can be between the clotting factor and the anti-GPIIb/IIIa antibody or antigen-binding fragment thereof and/or between the anti-GPIIb/IIIa antibody or antigen-binding fragment thereof and the half-life extending moiety. In certain embodiments of this aspect, the chimeric molecule comprises FVII, which may be the FVII zymogen (A or B isoform), activatable FVII, or activated FVII. In certain embodiments of this aspect, the chimeric molecule comprises FVII, a 6X(GGGGS) linker (SEQ ID NO:170), an Fab that binds GPIIb/IIIa, and an XTEN (*e.g.*, AE288). In other embodiments of this aspect, the chimeric molecule comprises FVII, a 6X(GGGGS) linker (SEQ ID NO:170), an Fab that binds GPIIb/IIIa, two XTENs (*e.g.*, AE288), and another linker. Non-limiting examples of chimeric molecules of this aspect are shown in Figs. 17 and 20. In certain embodiments of this aspect, the chimeric molecule comprises an amino acid sequence that is at least 65% identical, at least 70% identical, at least 75% identical, at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93%

identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in SEQ ID NO:74. In certain embodiments, this chimeric molecule includes at least one, at least two, or all three of the CDRs of SEQ ID NO:4. In certain embodiments of this aspect, the above-described chimeric molecule associates with a second chimeric molecule comprising an amino acid sequence that is at least 65% identical, at least 70% identical, at least 75% identical, at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in SEQ ID NO:252. In certain embodiments, this second chimeric molecule includes at least one, at least two, or all three of the CDRs of SEQ ID NO:7. In one embodiment, the chimeric molecule comprises, consists essentially of, or consists of the amino acid sequence set forth in SEQ ID NO:74 and associates with a second chimeric molecule with an amino acid sequence comprising, consisting essentially of, or consisting of the amino acid sequence set forth in SEQ ID NO:252. In certain embodiments, these chimeric molecules may comprise additional half-life extending moieties (*e.g.*, AE144, AE288). In certain embodiments, these chimeric molecules may comprise one or more (*e.g.*, 1, 2, 3, 4) linkers between Factor VII and the half-life extending moiety. In certain embodiments, these chimeric molecules may comprise additional linkers (*e.g.*, 2, 3, 4) between the half-life extending moiety and the light and/or heavy chain of the Fab. In certain embodiments of this aspect, the chimeric molecule (*e.g.*, polypeptides comprising SEQ ID NOs.: 74 and 252 that associate with each other) binds to the ectodomain of GPIIb/IIIa with a K_D of about 10^{-6} M to about 10^{-8} M. In specific embodiments, the chimeric molecule (*e.g.*, polypeptides comprising SEQ ID NOs.: 74 and 252 that associate with each other) binds to the ectodomain of GPIIb/IIIa with a K_D of 1 to 10×10^{-7} M.

In one aspect, this disclosure provides a pharmaceutical composition comprising the anti-GPIIb/IIIa antibody or antigen-binding fragment thereof (*e.g.*, Fab or scFv) and a pharmaceutically acceptable carrier. In another aspect, this disclosure provides a

pharmaceutical composition comprising the chimeric molecules described herein and a pharmaceutically acceptable carrier.

In a different aspect, methods for reducing the frequency or degree of a bleeding episode in a subject in need thereof are provided. These methods involve administering to the subject (*e.g.*, a human subject) an effective amount of a composition comprising the antibody or antigen-binding fragment thereof or the chimeric molecule described herein. In some embodiments, the subject has developed or has a tendency to develop an inhibitor against Factor VIII ("FVIII"), Factor IX ("FIX"), or both. The inhibitor against FVIII or FIX can be, *e.g.*, a neutralizing antibody against FVIII, FIX, or both. In certain embodiments, the bleeding episode is the result of or caused by hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the iliopsoas sheath, or any combinations thereof.

In another aspect, the disclosure relates to a method of treating a blood coagulation disorder in a subject in need thereof. The method involves administering to the subject (*e.g.*, a human subject) an effective amount of a composition comprising the antibody or antigen-binding fragment thereof, or the chimeric molecule described herein. In certain embodiments, the blood coagulation disorder is hemophilia A or hemophilia B.

In one aspect the disclosure provides a composition comprising the antibody or antigen-binding fragment thereof, or the chimeric molecule described herein for use in reducing the frequency or degree of a bleeding episode in a subject (*e.g.*, human) in need thereof. In some embodiments, the subject has developed or has a tendency to develop an inhibitor against Factor VIII ("FVIII"), Factor IX ("FIX"), or both. The inhibitor against FVIII or FIX can be, *e.g.*, a neutralizing antibody against FVIII, FIX, or both. In certain embodiments, the bleeding episode is the result of or caused by hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the iliopsoas sheath, or any combinations thereof.

In another aspect the disclosure provides a composition comprising the antibody or antigen-binding fragment thereof, or the chimeric molecule described herein for use in treating a blood coagulation disorder in a subject (*e.g.*, human) in need thereof. In certain embodiments, the blood coagulation disorder is hemophilia A or hemophilia B.

In a further aspect, the disclosure relates to the use of a composition comprising the antibody or antigen-binding fragment thereof, or the chimeric molecule described herein in the preparation of a medicament for use in reducing the frequency or degree of a bleeding episode in a subject (*e.g.*, human) in need thereof. In certain embodiments, the bleeding episode is the result of or caused by hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the iliopsoas sheath, or any combinations thereof.

In yet another aspect, the disclosure relates to the use of a composition comprising the antibody or antigen-binding fragment thereof, or the chimeric molecule described herein in the preparation of a medicament for use in treating a blood coagulation disorder in a subject (*e.g.*, human) in need thereof. In certain embodiments, the blood coagulation disorder is hemophilia A or hemophilia B.

In a different aspect, the disclosure features a method of detecting platelets. The method involves contacting a human blood preparation with an anti-GPIIb/IIIa antibody or antigen-binding fragment thereof described herein and detecting cells in the blood preparation to which the antibody or antigen-binding fragment thereof binds. In some embodiments, the anti-GPIIb/IIIa antibody or antigen-binding fragment thereof is linked or conjugated to a detectable label (*e.g.*, a fluorescent label, a radioactive label).

In yet another aspect, the disclosure provides a method for enriching platelets. This method comprises contacting a human blood preparation with an anti-GPIIb/IIIa antibody or antigen-binding fragment thereof described herein and enriching cells to which the antibody or antigen-binding fragment thereof are bound as compared to those cells in the blood preparation that are not bound by the antibody or antigen-binding fragment thereof.

In another aspect, the disclosure provides a method for isolating or enriching resting platelets (as opposed to activated platelets). This method comprises contacting a human blood preparation with an anti-GPIIb/IIIa antibody or antigen-binding fragment thereof described herein and enriching cells to which the antibody or antigen-binding fragment thereof are bound as compared to those cells in the blood preparation that are not bound by the antibody or antigen-binding fragment thereof.

The disclosure also provides an isolated nucleic acid comprising a nucleotide sequence that is at least 75% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 13-22, 59-68, and 219-240.

In another aspect, the disclosure provides a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence that is at least 75% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 to 12, 74-77, and 197-218.

In another aspect, the disclosure provides an isolated protein encoded by the above nucleic acid molecules. In yet another aspect, the disclosure provides a recombinant vector comprising the nucleic acids described herein. In a further aspect, the disclosure features a host cell comprising such recombinant vectors. In one embodiment, the disclosure provides an expression vector comprising a DNA comprising a nucleotide sequence that encodes the amino acid sequence set forth in SEQ ID NO:77. In another embodiment, the disclosure provides an expression vector comprising a DNA comprising a nucleotide sequence that encodes the amino acid sequence set forth in SEQ ID NO:247. In another embodiment, the

disclosure provides an expression vector comprising a DNA comprising a nucleotide sequence that encodes the amino acid sequence set forth in SEQ ID NO:75. In yet another embodiment, the disclosure provides an expression vector comprising a DNA comprising a nucleotide sequence that encodes the amino acid sequence set forth in SEQ ID NO: 76. In certain embodiments, such expression vectors are either singly transformed/transfected into a host cell (e.g., 293, CHO) or transformed together (e.g., the expression vectors encoding the amino acid sequence set forth in SEQ ID NO:77 and 75; or the expression vectors encoding the amino acid sequence set forth in SEQ ID NO:77 and 76; or the expression vectors encoding the amino acid sequence set forth in SEQ ID NO:247 and 75). In certain embodiments, the host cell is cultured under conditions that allow the expression of the polypeptides encoded by these nucleic acids and involve isolating the polypeptides. In certain instances the Factor VII that is a component of SEQ ID NO:77 is activated. In certain instances the Factor VII that is a component of SEQ ID NO:247 is activated.

In another aspect, the disclosure provides a method of preparing an anti-GPIIb/IIIa antibody or antigen-binding fragment thereof. The method comprises culturing a host cell comprising recombinant vectors comprising the nucleic acid sequences set forth in SEQ ID NOs: 14 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 15 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 16 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 17 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 18 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 13 and 22; the nucleic acid sequences set forth in SEQ ID NOs: 14 and 22; the nucleic acid sequences set forth in SEQ ID NOs: 15 and 22; the nucleic acid sequences set forth in SEQ ID NOs: 16 and 22; the nucleic acid sequences set forth in SEQ ID NOs: 17 and 22; the nucleic acid sequences set forth in SEQ ID NOs: 18 and 22; the nucleic acid sequences set forth in SEQ ID NOs: 18 and 21; the nucleic acid sequences set forth in SEQ ID NOs: 219 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 220 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 221 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 222 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 223 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 224 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 225 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 226 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 227 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 228 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 229 and 19; the nucleic acid

sequences set forth in SEQ ID NOs: 230 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 231 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 232 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 233 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 234 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 235 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 236 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 237 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 238 and 19; the nucleic acid sequences set forth in SEQ ID NOs: 239 and 19; or the nucleic acid sequences set forth in SEQ ID NOs: 240 and 19, under conditions appropriate for expression and production of the antibody or antigen-binding fragment thereof. The method further comprises isolating the antibody or antigen-binding fragment thereof. In certain embodiments, the host cell is a 293 cell, a CHO cell or a DG44i cell.

In a further aspect, the disclosure features a method of preparing a chimeric molecule described herein. For example, the method comprises culturing a host cell comprising recombinant vectors comprising the nucleic acid sequences encoding the amino acid sequences set forth in SEQ ID NOs: 74 and 75; or the nucleic acid sequences encoding the amino acid sequences set forth in SEQ ID NOs: 74 and 76; under conditions appropriate for expression and production of the chimeric molecule. The method further comprises isolating the chimeric molecule. In certain embodiments, the host cell is a 293 cell, a CHO cell or a DG44i cell.

In one aspect, the disclosure features a method of preparing a chimeric molecule described herein. For example, the method comprises culturing a host cell comprising recombinant vectors comprising the nucleic acid sequences encoding the amino acid sequences set forth in SEQ ID NOs: 77 and 75; or the nucleic acid sequences encoding the amino acid sequences set forth in SEQ ID NOs: 77 and 76; under conditions appropriate for expression and production of the chimeric molecule. The method further comprises isolating the chimeric molecule. In certain embodiments, the host cell is a 293 cell, a CHO cell or a DG44i cell.

In another aspect, the disclosure features a method of preparing a chimeric molecule described herein. For example, the method comprises culturing a host cell comprising recombinant vectors comprising the nucleic acid sequences encoding the amino acid sequences set forth in SEQ ID NOs: 247 and 75; under conditions appropriate for expression

and production of the chimeric molecule. The method further comprises isolating the chimeric molecule. In certain embodiments, the host cell is a 293 cell, a CHO cell or a DG44i cell.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the exemplary methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present application, including definitions, will control. The materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

Fig. 1 is an alignment of the variable heavy chain (VH) amino acid sequences of six humanized 34D10 VH regions with the VH region of 34D10 (*i.e.*, the murine anti-integrin GPIIb/IIIa antibody). The mutations in the humanized versions VH1 to VH5 compared to the humanized VH0 CDR graft are shown in bold, lower case font. The amino acids that differ between the 34D10 VH region and the humanized 34D10 VH CDR graft are highlighted in gray. The CDR regions (VHCDR1, VHCDR2, and VHCDR3) are underlined.

Fig. 2 is an alignment of the variable light chain (VL) amino acid sequences of four humanized 34D10 VL regions with the VL region of 34D10 (*i.e.*, the murine anti-integrin GPIIb/IIIa antibody). The mutations in the humanized versions VL1 to VL3 compared to the humanized VL0 CDR graft are shown in bold, lower case font. The amino acids that differ between the 34D10 VL region and the humanized 34D10 VL CDR graft are highlighted in gray. The CDR regions (VLCDR1, VLCDR2, and VLCDR3) are underlined.

Fig. 3 is a bar graph depicting the binding affinity for GPIIb/IIIa of humanized Fab fragments of 34D10.

Fig. 4A is a diagrammatic representation of the structure of the chimeric molecules FVII-245/Fab-033, FVII-250/Fab-036, and FVII-250/Fab-037. The “6x(GGGGS) linker” has the amino acid sequence of SEQ ID NO: 170.

Fig. 4B is a graph comparing the clotting time (CT) in seconds for different concentrations (nM) of FactorVIIa-linked Fab fragments of murine 34D10 (FVII-245/Fab-033) and humanized 34D10 (FVII-250/Fab-036 (VL0/VH5) and FVII-250/Fab-037 (VL0/VH2)).

Fig. 4C is a graph comparing the clotting time (CT) in seconds for different concentrations (nM) of FactorVIIa-linked Fab fragments of murine 34D10 (FVII-245/Fab-033) and mouse 34D10 in scFv format recombinantly fused to rFVIIa (FVII-189).

Fig. 4D is a graph comparing the clotting time (CT) in seconds for different concentrations (nM) of mouse 34D10 in scFv format recombinantly fused to rFVIIa (FVII-189) and recombinant FVIIa.

Fig. 5A is a diagrammatic representation of the structure of the chimeric molecules FVII-251/Fab-036 and FVII-251/Fab-037. The “6x(GGGGS) linker” has the amino acid sequence of SEQ ID NO: 170.

Fig. 5B is a graph comparing the clotting time (CT) in seconds for different concentrations (nM) of FactorVIIa-linked via XTEN AE288 and a linker to either Fab fragments of humanized 34D10 (FVII-251/Fab-036 (VL0/VH5) and FVII-251/Fab-037 (VL0/VH2)) or Fab fragments of murine 34D10 (FVII-200).

Fig. 5C is a graph comparing the clotting time (CT) in seconds for different concentrations (nM) of FVII-251/Fab-036 (VL0/VH5) against recombinant FVIIa.

Fig. 6 is a graphical depiction of the *ex vivo* activity measured by rotational thromboelastometry (ROTEM) of FVII-251/Fab-037 and rFVIIa in transgenic hemophilia A mice with a fully humanized α IIB subunit in the α IIB/ β 3 integrin. Mice were dosed with 10 nmol/kg of rFVIIa or FVII-251/Fab-037. The data each time point is the average $\pm$ standard deviation of 3 mice.

Figs. 7A-F show possible configurations for chimeric molecules comprising the heavy and light chains of a clotting factor (e.g., a FVII), an Fab or scFv targeting moiety

(*e.g.*, derived for GPIIb/IIIa-specific antibodies), a heterologous moiety (*e.g.*, a half-life extending moiety), and at least one optional linker.

Fig. 8 shows possible configuration for chimeric molecules comprising one or two heterologous moieties (H1 and/or H2) and scFv moieties derived for GPIIb/IIIa-specific antibodies. It is to be understood that an Fab from the humanized anti-GPIIb/IIIa antibodies can be used instead of the scFv in these chimeric molecules.

Fig. 9 depicts the plasma activity of both rFVIIa (open circles) and FVII-251/Fab-037 (black circles) as percentile of injected dose over time (hours) as determined by the VIIa-rTF VIIa-activity assay, using rFVIIa or FVII-251/Fab037 as respective matched self standards. Mean $\pm$ SD.

Fig. 10 is a graph showing the acute efficacy of FVII-251/Fab-037 compared to rFVIIa in tail clip bleeding model. Results presented are individual and median blood loss over 30 minutes for treatments and dosing as indicated. P values for vehicle versus all other treatments are indicated. Data indicate similar or improved efficacy in mice dosed with 3 nmol/kg and 10 nmol/kg FVII-251/Fab-037 compared to mice dosed with 100 nmol/kg rFVIIa.

Fig. 11 is an alignment of the variable heavy chain (VH) amino acid sequences of 22 affinity matured variants of the humanized 34D10 VH2 (**SEQ ID NO: 7**) (*i.e.*, an anti-integrin GPIIb/IIIa antibody). The mutations identified in the humanized sequence compared to the humanized VH2 are shown in bold, lower case font. The CDR regions (VHCDR1, VHCDR2, and VHCDR3) are underlined.

Fig. 12A-E shows the measurement of binding by Bio-Layer Interferometry (BLI) of the indicated yeast purified Fab to sensor-associated GPIIb/IIIa (**SEQ ID NOs.: 23 and 24**), as a function of time.

Fig. 12F is a table listing the apparent monovalent affinity (K_D) and apparent dissociation rate (K_{dis}) of the indicated yeast purified Fab.

Fig. 13 is a table listing the calculated melting temperatures for the indicated antibodies in the Fab format as performed by differential scanning fluorimetry.

Fig. 14A is a graphical depiction of the *ex-vivo* activity measured by rotational thromboelastometry (ROTEM) of FVII-265/Fab-037 and rFVIIa in human α IIb transgenic HemA mice with a fully humanized α IIb subunit in the α IIb/ β 3 integrin.

Fig. 14B depicts the *ex-vivo* activity of FVII-251/Fab-037 and rFVIIa in HemA mice, in which the murine α IIb/ β 3 integrin is not targeted by the Fab-037 moiety.

Fig. 15A is a graph showing the plasma PK as measured by soluble tissue factor (sTF)-prothrombin time (PT) activity of FVIIa and FVII-251/Fab-037 dosed at 10 nmol/kg, showing an approximately 5-fold decreased clearance of plasma levels of FVII-251/Fab-037 compared to rFVIIa.

Fig. 15B is a graph comparing the clotting time (CT) in seconds as measured in whole blood by ROTEM at the indicated time points, comparing recombinant FVII-251/Fab37 to FVIIa both dosed at 10 nmol/kg over time in human α IIb transgenic HemA mice.

Fig. 16 is a graph showing the prolonged efficacy of 10 nmol/kg FVII-265/Fab-037 compared with 100 nmol/kg rFVIIa in a modified prolonged tail clip bleeding efficacy model.

Fig. 17 shows the configuration of FVII-250 (SEQ ID NO:74)/Fab-062 (SEQ ID NO:252). The FVIIa molecule is fused to the Fab light chain VL0/CL via a 6x(GGGGS) linker (SEQ ID NO:170). VL0/CL forms a dimer with Fab-062 (Fab heavy chain) via a disulfide bond. Fab-062 comprises an XTEN fused to the N-terminus of the VH2/CH1 heavy chain.

Fig. 18 displays the results of surface plasmon resonance experiments showing the binding of FVII-250/Fab-062 to biotinylated GPIIb/IIIa immobilized on a streptavidin chip.

Fig. 19 shows the results of rotational thromboelastometry (ROTEM) experiments measuring the activities of FVII-250/Fab-062 and recombinant FVIIa in whole blood from a hemophilia donor.

Fig. 20 illustrates non-limiting examples of possible configurations for chimeric molecules comprising the heavy and light chain of a clotting factor, a Fab targeting moiety, one or two heterologous moieties (*e.g.*, half-life extension moieties), and optional linkers.

Detailed Description

This disclosure features antibodies and antigen-binding fragments that specifically bind GPIIb/IIIa, an integrin that is expressed specifically and at high levels on platelets. Upon activation, the GPIIb/IIIa receptors change from a bent low ligand affinity conformation to an extended high ligand affinity conformation. Activated GPIIb/IIIa receptor binds fibrinogen and modulates platelet aggregation. The anti-GPIIb/IIIa antibodies described herein are capable of targeting the non-active form of the receptor. The anti-GPIIb/IIIa antibodies and antigen-binding fragments derived from these antibodies do not activate platelets, and can be used, for example, to target agents (*e.g.*, therapeutic agents such as clotting factors or other molecules capable of having a pharmacological effect in platelets) to the platelet surface. In addition to their use as platelet-targeting moieties, these antibodies and antigen-binding fragments thereof can be used for diagnostics, for example, by conjugation to a detectable label, and also used for isolating and separating platelets from a sample.

This disclosure also provides chimeric molecules comprising the anti-GPIIb/IIIa antibodies and antigen-binding fragments thereof disclosed herein as targeting moieties, and one or more (*e.g.*, one, two, three, four) heterologous moieties. For example, the chimeric molecules can comprise a heterologous moiety comprising a therapeutic molecule (*e.g.*, a procoagulant molecule such as a clotting factor), and optionally a second heterologous moiety comprising, for example, a pharmacokinetic (PK) enhancing moiety (*i.e.*, a molecule which can improve various pharmacokinetic properties, *e.g.*, half-life). The heterologous moieties can optionally be connected by linkers (*e.g.*, peptide linkers). In addition the targeting moiety of the chimeric molecule (*e.g.*, an Fab or scFv of an anti-GPIIb/IIIa antibody described herein) can optionally be connected to the heterologous moiety or moieties by linkers (*e.g.*, a peptide linker). Exemplary anti-GPIIb/IIIa antibodies and antigen-binding fragments thereof, as well as exemplary constructs (chimeric molecules) comprising such antibodies and antigen-binding fragments thereof (*e.g.*, scFv or F(ab)) are illustrated in the instant description and figures. See, *e.g.*, the chimeric molecules having the structures set forth in Figures 7 and 8.

The disclosure also provides polynucleotides encoding the antibodies and antigen-binding fragments thereof as well as the chimeric molecule constructs described herein.

In addition, this disclosure relates to methods of using the anti-GPIIb/IIIa antibodies and antigen-binding fragments thereof in the treatment of coagulation deficiencies such as hemophilia well as coagulation deficiencies other than hemophilia characterized by an impaired thrombin generation and life-threatening bleeding.

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

A. Definitions

It is understood that wherever embodiments are described herein with the language "comprising," otherwise analogous embodiments described in terms of "consisting of" and/or "consisting essentially of" are also provided.

The term "antibody" means an immunoglobulin molecule that recognizes and specifically binds to a target, such as a protein (e.g., the GPIIb/IIIa receptor, a subunit thereof, or the receptor complex), polypeptide, peptide, carbohydrate, polynucleotide, lipid, or combinations of the foregoing through at least one antigen recognition site within the variable region of the immunoglobulin molecule. A typical antibody comprises at least two heavy (HC) chains and two light (LC) chains interconnected by disulfide bonds. Each heavy chain is comprised of a "heavy chain variable region" or "heavy chain variable domain" (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2, and CH3. Each light chain is comprised of a "light chain variable region" or "light chain variable domain" (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, Cl. The VH and VL regions can be further subdivided into regions of hypervariability, termed Complementarity Determining Regions (CDR), interspersed with regions that are more conserved, termed framework regions (FRs). Each VH and VL region is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. As used herein, the term "antibody" encompasses intact polyclonal antibodies, intact monoclonal antibodies, antibody fragments (such as Fab, Fab', F(ab')₂, Fd, Facb, and Fv fragments), single chain Fv (scFv), minibodies (e.g., sc(Fv)₂, diabody), multispecific antibodies such as bispecific antibodies generated from at least two intact antibodies, chimeric antibodies, humanized antibodies,

human antibodies, fusion proteins comprising an antigen determination portion of an antibody, and any other modified immunoglobulin molecule comprising an antigen recognition site so long as the antibodies exhibit the desired biological activity. Thus, the term "antibody" includes whole antibodies and any antigen-binding fragment or single chains thereof. Antibodies can be naked or conjugated to other molecules such as toxins, radioisotopes, small molecule drugs, polypeptides, etc.

The term "antigen binding fragment" refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody. It is known in the art that the antigen binding function of an antibody can be performed by fragments of a full-length antibody. Examples of antigen-binding antibody fragments include, but are not limited to Fab, Fab', F(ab')₂, Facb, Fd, and Fv fragments, linear antibodies, single chain antibodies, and multispecific antibodies formed from antibody fragments. In some instances, antibody fragments may be prepared by proteolytic digestion of intact or whole antibodies. For example, antibody fragments can be obtained by treating the whole antibody with an enzyme such as papain, pepsin, or plasmin. Papain digestion of whole antibodies produces F(ab)₂ or Fab fragments; pepsin digestion of whole antibodies yields F(ab')₂ or Fab'; and plasmin digestion of whole antibodies yields Facb fragments.

The term "Fab" refers to an antibody fragment that is essentially equivalent to that obtained by digestion of immunoglobulin (typically IgG) with the enzyme papain. The heavy chain segment of the Fab fragment is the Fd piece. Such fragments can be enzymatically or chemically produced by fragmentation of an intact antibody, recombinantly produced from a gene encoding the partial antibody sequence, or it can be wholly or partially synthetically produced. The term "F(ab')₂" refers to an antibody fragment that is essentially equivalent to a fragment obtained by digestion of an immunoglobulin (typically IgG) with the enzyme pepsin at pH 4.0-4.5. Such fragments can be enzymatically or chemically produced by fragmentation of an intact antibody, recombinantly produced from a gene encoding the partial antibody sequence, or it can be wholly or partially synthetically produced. The term "Fv" refers to an antibody fragment that consists of one NH and one N domain held together by noncovalent interactions.

As used herein the term "scFv" or "scFv molecule" includes binding molecules which consist of one light chain variable domain (VL) or a portion thereof, and one heavy chain variable domain (VH) or a portion thereof, wherein each variable domain (or a portion thereof) is derived

from the same or different antibodies. Single chain Fv molecules preferably comprise an scFv linker interposed between the VH domain and the VL domain. Exemplary scFv molecules are known in the art and are described, for example, in US Patent No. 5,892,019; Ho et al., *Gene*, 77:51 (1989); Bird et al., *Science*, 242:423 (1988); Pantoliano et al., *Biochemistry*, 30:10117 (1991); Milenic et al., *Cancer Research*, 51:6363 (1991); Takkinen et al., *Protein Engineering*, 4:837 (1991). The term "scFv linker" as used herein refers to a moiety interposed between the VL and VH domains of the scFv. The scFv linkers preferably maintain the scFv molecule in an antigen-binding conformation. In one embodiment, a scFv linker comprises or consists of an scFv linker peptide. In certain embodiments, an scFv linker peptide comprises or consists of a Gly-Ser peptide linker. In other embodiments, an scFv linker comprises a disulfide bond.

The terms "GPIIb/IIIa antibody," "anti-GPIIb/IIIa antibody," "anti-GPIIb/IIIa," "antibody that binds to GPIIb/IIIa" and any grammatical variations thereof refer to an antibody that is capable of specifically binding to the GPIIb/IIIa receptor with sufficient affinity such that the antibody is useful as a therapeutic agent or diagnostic reagent in targeting GPIIb/IIIa. The extent of binding of an anti-GPIIb/IIIa antibody disclosed herein to an unrelated, non-GPIIb/IIIa protein is less than about 10% of the binding of the antibody to GPIIb/IIIa as measured, e.g., by a radioimmunoassay (RIA), BIACORE™ (using recombinant GPIIb/IIIa as the analyte and antibody as the ligand, or vice versa), or other binding assays known in the art. In certain embodiments, an antibody that binds to GPIIb/IIIa has a dissociation constant (KD) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 50 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 10 \text{ pM}$, $\leq 1 \text{ pM}$, or $\leq 0.1 \text{ pM}$.

The term "% identical" between two polypeptide (or polynucleotide) sequences refers to the number of identical matched positions shared by the sequences over a comparison window, taking into account additions or deletions (i.e., gaps) that must be introduced for optimal alignment of the two sequences. A matched position is any position where an identical nucleotide or amino acid is presented in both the target and reference sequence. Gaps presented in the target sequence are not counted since gaps are not nucleotides or amino acids. Likewise, gaps presented in the reference sequence are not counted since target sequence nucleotides or amino acids are counted, not nucleotides or amino acids from the reference sequence. The percentage of sequence identity is calculated by determining the number of positions at which the identical amino acid residue or nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and

multiplying the result by 100 to yield the percentage of sequence identity. The comparison of sequences and determination of percent sequence identity between two sequences can be accomplished using readily available software both for online use and for download. Suitable software programs are available from various sources, and for alignment of both protein and nucleotide sequences. One suitable program to determine percent sequence identity is *bl2seq*, part of the BLAST suite of program available from the U.S. government's National Center for Biotechnology Information BLAST web site (blast.ncbi.nlm.nih.gov). *Bl2seq* performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. Other suitable programs are, e.g., Needle, Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at www.ebi.ac.uk/Tools/psa. In certain embodiments, the percentage identity "X" of a first amino acid sequence to a second sequence amino acid is calculated as $100 \times (Y/Z)$, where Y is the number of amino acid residues scored as identical matches in the alignment of the first and second sequences (as aligned by visual inspection or a particular sequence alignment program) and Z is the total number of residues in the second sequence. If the length of a first sequence is longer than the second sequence, the percent identity of the first sequence to the second sequence will be higher than the percent identity of the second sequence to the first sequence. One skilled in the art will appreciate that the generation of a sequence alignment for the calculation of a percent sequence identity is not limited to binary sequence-sequence comparisons exclusively driven by primary sequence data. Sequence alignments can be derived from multiple sequence alignments. One suitable program to generate multiple sequence alignments is ClustalW2, available from www.clustal.org (ClustalX is a version of the ClustalW2 program ported to the Windows environment). Another suitable program is MUSCLE, available from www.drive5.com/muscle. ClustalW2 and MUSCLE are alternatively available, e.g., from the EBI.

As used herein, the term "targeting moiety" refers to a moiety capable of interacting with a target molecule (e.g., the GPIIb/IIIa receptor, or a molecule comprising the α and/or β subunits of the GPIIb/IIIa receptor). Targeting moieties having limited cross-reactivity are generally preferred. In certain embodiments, suitable targeting moieties include, for example, any member of a specific binding pair, antibodies, monoclonal antibodies, or derivatives or analogs thereof, including without limitation: Fv fragments, single chain Fv (scFv) fragments, Fab' fragments, F(ab')₂ fragments, single domain antibodies, camelized antibodies and

antibody fragments, humanized antibodies and antibody fragments, and multivalent versions of the foregoing; multivalent binding reagents including without limitation: monospecific or bispecific antibodies, such as disulfide stabilized Fv fragments, scFv tandems ((scFv) fragments), diabodies, tribodies or tetrabodies, which typically are covalently linked or otherwise stabilized (i.e., leucine zipper or helix stabilized) scFv fragments; and other targeting moieties include for example, aptamers, receptors, ligands, and fusion proteins.

The terms "linked" or "fused" refers to linkage via a peptide bonds (e.g., genetic fusion), chemical conjugation, or other means known in the art. For example, one way in which molecules or moieties can be linked employs peptide linkers that link the molecules or moieties via peptide bonds.

The term "associated with" refers to a covalent or non-covalent bond formed between a first amino acid chain and a second amino acid chain. In one embodiment, the term "associated with" means a covalent, non-peptide bond or a non-covalent bond. In another embodiment, the term "associated with" refers to a covalent, non-peptide bond or a non-covalent bond that is not chemically crosslinked. In another embodiment, it means a covalent bond except a peptide bond. In some embodiments this association is indicated by a colon, i.e., (:). For example, when representing the structure of the clotting factor, "CFH:CFL " refers to a dimer comprising a heavy chain of a clotting factor (CFH) disulfide bonded to a light chain of a clotting factor (CFL) in a N-terminus to C-terminus orientation.

The term "moiety" refers to a component part or constituent of a chimeric molecule of the present disclosure.

The term "heterologous moiety" refers to a moiety genetically fused, conjugated, and/or otherwise associated to a targeting molecule (*e.g.*, GPIIb/IIIa antibody or antigen-binding molecule thereof).

The term "therapeutic agent" refers to any biological or chemical agent used in the treatment of a disease or disorder. Therapeutic agents include any suitable biologically active chemical compounds, biologically derived components such as cells, peptides, antibodies, and polynucleotides, and radiochemical therapeutic agents such as radioisotopes. In some embodiments, the therapeutic agent comprises a clotting factor.

The term "stability" refers to an art-recognized measure of the maintenance of one or more physical properties of the chimeric molecule in response to an environmental condition (*e.g.*, an elevated or lowered temperature). In certain embodiments, the physical property can be the maintenance of the covalent structure of the chimeric molecule (*e.g.*, the absence of proteolytic cleavage, unwanted oxidation or deamidation). In other embodiments, the physical property can also be the presence of the chimeric molecule in a properly folded state (*e.g.*, the absence of soluble or insoluble aggregates or precipitates). In one embodiment, the stability of the chimeric molecule is measured by assaying a biophysical property of the chimeric molecule, for example thermal stability, pH unfolding profile, stable removal of glycosylation, solubility, biochemical function (*e.g.*, ability to bind to a protein, receptor or ligand), etc., and/or combinations thereof. In another embodiment, biochemical function is demonstrated by the binding affinity of the interaction. In one embodiment, a measure of protein stability is thermal stability, *i.e.*, resistance to thermal challenge. Stability can be measured using methods known in the art, such as, HPLC (high performance liquid chromatography), SEC (size exclusion chromatography), DLS (dynamic light scattering), etc. Methods to measure thermal stability include, but are not limited to differential scanning calorimetry (DSC), differential scanning fluorimetry (DSF), circular dichroism (CD), and thermal challenge assay.

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. The term "clotting factor," as used herein encompasses clotting factors (*e.g.*, vWF, FV, FVa, FVII, FVIIa, FVIII, FVIIIa, FIX, FIXa, FX, FXa, FXI, FXIa, FXII, FXIIa, FXIII, or FXIIIa), fragments, variants, analogs, or derivatives thereof, naturally occurring, recombinantly produced, or synthetically produced which prevent or decrease the duration of a bleeding episode in a subject.

The term "activatable clotting factor" refers to a clotting factor in an inactive form (*e.g.*, in its zymogen form) that is capable of being converted to an active 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.

As used herein, the term "half-life extending moiety" refers to a heterologous moiety which increases the *in vivo* half-life of a protein, for example, a chimeric molecule. The term "half-life" refers to a biological half-life of a particular protein or polypeptide (e.g., a clotting factor or a chimeric molecule disclosed herein) *in vivo*. Half-life can 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 or chimeric molecule of the invention 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. In some embodiments, procoagulant compounds of the invention are monophasic, and thus do not have an alpha phase, but just the single beta phase. In certain embodiments, the term half-life as used herein refers to the half-life of the procoagulant compound in the β -phase. The typical β -phase half-life of a human antibody in humans is 21 days. *In vivo* half-life of a chimeric molecule can be determined by any method known to those of skill in the art. In certain embodiments, the half-life extending moiety can comprise an attachment site for a non-polypeptide moiety (e.g., PEG).

B. GPIIb/IIIa

The terms "GPIIb/IIIa" and "GPIIb/IIIa receptor" refer to glycoprotein IIb/IIIa (also known as integrin α IIb β 3), an integrin complex found on platelets. Integrins are composed of two chains, an α subunit and a β subunit, which are held together by noncovalent bonds in a calcium dependent manner. GPIIb constitutes the α subunit, which comprises divalent cation binding domains, whereas GPIIIa is a pro typical β subunit (β 3). On each circulating platelet, there are about 35,000 to 100,000 GPIIb/IIIa complexes: most are distributed on the platelet surface, while a smaller pool is found in an internal reserve. The GPIIb/IIIa complex does not interact with its plasma ligands until platelets have been activated by exogenous agonists

such as ADP or thrombin. When this occurs, an inside-out signal is generated that results in a conformational change in the extracellular portion of the complex that renders the molecule capable of binding fibrinogen and other ligands. The amino acid sequences of the two chains of this platelet receptor can be found in Uniprot entries P05106 (ITB3_HUMAN; GPIIIa; CD61; integrin beta-3; integrin $\beta 3$) and P08514 (ITA2B_HUMAN; GPIIb; CD41; integrin alpha-2b; integrin αII) as published in Universal Protein Resource (Uniprot) database release 2013_05 (May 1, 2013), which are incorporated by reference in their entirety.

The amino acid sequence of human GPIIb is provided below:

```
MARALCPLQALWLLLEWVLLLLGPCAAPPAWALNLDPVQLTFYAGPNGSQFGFSLDFHKDSHGRVAIVVGAPRTLGP
PSQEETGGVFLCPWRAEGGQCPSLLFDLRDETRNVGSQTLQTFKARQGLGASVVSWSVDVIIVACAPWQHWNVLEKT
EEAEKTPVGSCFLAQPESEGRRAEYSPCRGNTLSRIYVENDFSWDKRYCEAGFSSVVTQAGELVLGAPGGYYFLGL
LAQAPVADIFSSYRPGILLWHVSSQSLSFSSNPEYFDGYWGYSVAVGEFDGDLNTEYVVGAPTWSWTLGAVEI
LDSYYQRLHRLRGEQMASYFGHSVAVTDVNGDGRHDLVGAPLYMESRADRKLAEVGRVYFLQPRGPHALGAPS
LLLTGTQLYGRFGSAIAPLGDLDRDGYNDIAVAAPYGGPSGRGQVLVFLGQSEGLRSRPSQVLDSPFPTGSAFGF
SLRGAVDIDDNGYPDLIVGAYGANQVAVYRAQPVVKASVQLLVQDSLNPVAVKSCVLPQTKTPVSCFNIQMCVGAT
GHNI PQKLSLNAELQLDRQKPRQGRRVLLLSQAGTTLNLDLGGKHSPICHTTMAFLRDEADFRDKLSPIVLSL
NVSLPPTTEAGMAPAVVLHGDTHVQEQTTRIVLDCGEDDVCPQLQLTASVTGSPLLVGADNVLELQMDAANECEGA
YEAEALAVHLPQGAHYMRALSNVEGFERLICNQQKENETRVVLCGLNPMKKNAQIGIAMLVSVGNLEEAGESVSF
QLQIRSKNSQNPNSKIVLLDVPVRAEAQVELRGNSFPASLVVAAEEGEREQNSLDSWGPKVEHTYELHNNGPQTV
NGLHLSIHLPGQSQPSDLLYILDIQPQGLQCFPQPPVNPPLKVDWGLPIPSPSPIHPAHHKRDRRQIFLPEPEQP
SRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFVLQSHAWFNVSSLPYAVPPLSL
PRGEAQVWTQLLRALERA (SEQ ID NO: 23)
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The amino acid sequence of human GPIIIa is provided below:

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MRARPRRPLWATVLALGALAGVGVGGPNICTTRGVSSCQQCLAVSPMCAWCSDEALPLGSPRCDLKENLLKDNC
APESIEFPVSEARVLEDRPLSDKSGSDSSQVTQVSPQRIALRLRPDDSKNFSIQVRQVEDYPVDIYYLMDLSYSM
KDDLWSIQNLGTLKLTQMRKLTSLNLRIGFGAFVDKVPSPYMYISPPEALENPCYDMKTTCLPMFGYKHVLTLLTDQ
VTRFNEEVKKQSVSRNRDAPEGGFDAIMQATVCDEKIGWRNDASHLLVFTTDAKTHIALDGRLAGIVQPNQGQCH
VGSDNHYSASTTMDYPSLGLMTEKLSQKNINLI FAVTENVNLYQNYSELIPGTTVGVLMSDSSNVLQLIVDAYG
KIRSKVELEVRLDPEELSLSFNATCLNNEVI PGLKSCMGLKIGDTVFSFIEAKVRGCPQEKEKSFTIKPVGFKDS
LIVQVTFDCDCACQAQAEPNSHRCNNNGTFCGVCRCGPGWLGSQCECSEEDYRPSQQDECSREGQPVCSQRG
ECLCGQCVCHSSDFGKITGKYCECDDFSCVRYKGEMCSGHGQCSCGDCDSDWTGYCNCTTRTDTCMSNGLL
CSGRGKCECGSCVCIQPGSYGDTCEKCPCTCPDACTFKKECVECKKFDRLGALHDENTCNRYCRDEIESVKELKDTG
KDAVNCTYKNEDDCVVRFYQYEDSSGKSILYVVEEPECPKG (SEQ ID NO: 24)
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C. Anti-GPIIb/IIIa Antibodies

This disclosure provides antibodies and antigen-binding fragments thereof that specifically bind to GPIIb/IIIa. In certain embodiments, the antibodies and antigen-binding fragments thereof bind the GPIIb/IIIa receptors located on the surface of platelets. In other embodiments, the antibodies and antigen-binding fragments thereof bind the GPIIb/IIIa found within the platelets. The anti-GPIIb/IIIa antibodies and antigen binding fragments can bind the GPIIb subunit of the receptor and/or the GPIIb/IIIa complex. These antibodies do not activate the platelets and also do not compete with fibrinogen for binding to GPIIb/IIIa.

One example of an anti-GPIIb/IIIa antibody is the murine antibody, 34D10. This antibody was obtained as follows: Hybridomas were generated from BALB/C mice immunized with plasmids containing DNA sequences encoding GPIIb/IIIa according to methods known in the art. Hybridomas were then screened for binding to human and cynomolgus monkey platelets using flow cytometry, and for binding to GPIIb/IIIa using Enzyme-linked immunosorbent assays (ELISA). To determine binding to human and monkey platelets, gel-purified human or monkey (cynomolgus) platelets in Tyrode's buffer were incubated with hybridoma supernatant and antibody binding was detected by flow cytometry. The binding of supernatants from hybridomas to human GPIIb/IIIa (α Ib β) was also determined by using ELISA. The supernatants from hybridomas which tested positive in the ELISA assays were mixed with platelets and screened for platelet activation using flow cytometry. The antibodies that did not activate platelets upon binding to GPIIb/IIIa were selected. The supernatants from non-activating hybridomas were subject to additional characterization assays (i) to confirm antibody binding to human and cynomolgus platelets, (ii) to determine antibody binding specificity for the α and/or β subunit of GPIIb/IIIa, and (iii) to determine whether the antibodies can compete with fibrinogen for binding to platelets. Fibrinogen is the natural ligand of GPIIb/IIIa and its binding to GPIIb/IIIa is essential to mediate platelet aggregation. Thus, the antibodies that compete with the binding of fibrinogen to GPIIb/IIIa were excluded from the selection. 34D10 was identified in the process and determined to be an antibody that does not activate platelets, that does not compete with the binding of fibrinogen to GPIIb/IIIa, and that binds both the α subunit of GPIIb/IIIa and the GPIIb/IIIa complex. The amino acid sequences of the heavy chain variable domain (VH) and light chain variable domain (VL) of the murine anti-GPIIb/IIIa antibody, 34D10, are provided below (the CDRs according to Kabat are underlined).

34D10 VH:

VHCDR1

VHCDR2

EVKLIVESGGGLVKPGGSLKLSCAASGFTFSAYAMSWVRQTPEKRLEWVASISSGGTTYYPDSVKRRFT
IS

VHCDR3

RDNARNILYLQMSSLRSEDTAMYYCTRGGDYGALDYWGQGTSTVTVSS (SEQ ID NO:1)

34D10 VL:

VLCDR1

VLCDR2

ENVLTQSPAIMASASLGEKVTMSCRASSSVNYMYWYQQKSDASPKLWIYYTSNLAPGVPARFSGS

VLCDR3

GSNGSYSLTISSMEGEDAATYYCQQFSSSPWTFGGGTKLEIK (SEQ ID NO:2)

The 34D10 antibody was humanized as described in Example 1. This example discloses six exemplary humanized heavy chain variable regions termed VH0, VH1, VH2, VH3, VH4, and VH5, with the amino acid sequences set forth in SEQ ID NOs: 3, 5, 7, 9, 11 and 12, respectively, and four exemplary humanized light chain variable regions termed VL0, VL1, VL2, and VL3, with the amino acid sequences set forth in SEQ ID NOs: 4, 6, 8, and 10, respectively. Each of these VH chains can pair with any of the VL chains: *i.e.*, VH0 can pair with VL0, VL1, VL2, or VL3; VH1 can pair with VL0, VL1, VL2, or VL3; VH2 can pair with VL0, VL1, VL2, or VL3; VH3 can pair with VL0, VL1, VL2, or VL3; VH4 can pair with VL0, VL1, VL2, or VL3; and VH5 can pair with VL0, VL1, VL2, or VL3. Thus, the heavy chain variable region and light chain variable regions disclosed in Example 1 can form 24 different VH-VL pairs. All of these antibodies are considered part of this disclosure. In some embodiments, these antibodies can comprise a kappa light chain constant region. In other embodiments, these antibodies can comprise a lambda light chain constant region. In one embodiment, the light chain constant region comprises the following amino acid sequence:

RTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYF REAKVQWKVD NALQSGNSQE SVTEQDSKDS
TYSLSSTLTLL SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC (SEQ ID NO:56).

In other embodiments, the light chain constant region comprises an amino acid sequence that is at least 65% identical, at least 70% identical, at least 75% identical, at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81%

identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to SEQ ID NO:56.

The antibodies of this disclosure can also comprise a heavy chain constant region. In certain embodiments the heavy chain constant region is from an IgG1 or IgG4 antibody. In one embodiment, the heavy chain constant region comprises the following amino acid sequence:
AS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
YSLSSVVTVP SSSLGTQTYI CNVNHKPSNT KVDKKVEPKS C (SEQ ID NO:57).

In other embodiments, the heavy chain constant region comprises an amino acid sequence that is at least 65% identical, at least 70% identical, at least 75% identical, at least 76% identical, at least 77% identical, at least 78% identical, at least 79% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, or at least 99% identical to SEQ ID NO:57. In another embodiment, the heavy chain constant region comprises the following amino acid sequence:
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVTV
DVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVTLHQLDNLNGKEYKCKVSNKGLPSSIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
KLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPG (SEQ ID NO:58)

The amino acid sequences of the heavy and light chain CDRs 1, 2, and 3, as well as the framework regions (FRs) 1, 2, 3, 4 of the six heavy chain variable regions and the four light chain variable regions of the exemplary humanized anti-GPIIb/IIIa antibodies described in Example 1 are provided below in Table 1. The CDRs are based upon the Kabat numbering system.

TABLE 1. Humanized 34D10 (h34D10) CDR and FR Amino Acid Sequences

Domain	SEQ ID NO	Sequence
VH CDR1	25	AYAMS
VH CDR2	26	SISSGGTTYYPDSVKR
VH101 CDR2	241	SISSGGTTYYPDSVER
VH108 CDR2	242	SISSDGGTTYYPDSVKR
VH109 CDR2	243	SISSGGTTDYPDSVKR
VH112 CDR2	244	GISSGGTTYYPDSVKR
VH CDR3	27	GGDYGYALDY
VH100 CDR3	245	GGDYSYALDY
VL CDR1	28	RASSSVNYMY
VL CDR2	29	YTSNLAP
VL CDR3	30	QQFSSSPWT
VH0 FR1	31	EVQLVESGGGLVQPGGSLRLSCAASGFTFS
VH0 FR2	32	WVRQAPGKGLVWV
VH0 FR3	33	QFTISRDNAKNTLYLQMNSLRAEDMAVYYCTR
VH0 FR4	34	WGQGTLVTVSS
VH1 FR1	35	EVQLVQSGGGLVQPGESLRLSCAASGFTFS
VH1 FR2	36	WVRQAPGKGLEWVS
VH1 FR3	33	QFTISRDNAKNTLYLQMNSLRAEDMAVYYCTR
VH1 FR4	34	WGQGTLVTVSS
VH2 FR1	37	EVQLVESGGGLVKPGGSLRLSCAASGFTFS

Domain	SEQ ID NO	Sequence
VH2 FR2	38	WVRQAPGKGLVWVA
VH2 FR3	39	QFTISRDNKNTLYLQMNSLRAEDTAVYYCTR
VH2 FR4	34	WGQGTLVTVSS
VH3 FR1	40	EVQLVQSGGGLVKPGESLRLSCAASGFTFS
VH3 FR2	41	WVRQAPGKGLEWVA
VH3 FR3	42	RFTISRDNKNTLYLQMNSLRAEDTAVYYCTR
VH3 FR4	34	WGQGTLVTVSS
VH4 FR1	40	EVQLVQSGGGLVKPGESLRLSCAASGFTFS
VH4 FR2	41	WVRQAPGKGLEWVA
VH4 FR3	43	RFTISRDNKNTLYLQMNSLRAEDTAVYYCTR
VH4 FR4	34	WGQGTLVTVSS
VH5 FR1	44	EVKLVESGGGLVKPGGSLRLSCAASGFTFS
VH5 FR2	41	WVRQAPGKGLEWVA
VH5 FR3	45	RFTISRDNKNTLYLQMNSLRAEDTAVYYCTR
VH5 FR4	34	WGQGTLVTVSS
VL0 FR1	46	EIVMTQSPATLSVSPGERATLSC
VL0 FR2	47	WYQQKPGQAPRLIY
VL0 FR3	48	GIPARFSGSGSGTEFTLTISLQSEDFAVYYC
VL0 FR4	49	FGQGTKVEIK
VL1 FR1	50	EIVLTQSPATLSVSPGERATLSC

Domain	SEQ ID NO	Sequence
VL1 FR2	47	WYQQKPGQAPRLLIY
VL1 FR3	51	GVPARFSGSGSGTEFTLTISLQSEDFAVYYC
VL1 FR4	49	FGQGTKVEIK
VL2 FR1	52	EIVLTQSPATLSASPGERVMTSC
VL2 FR2	53	WYQQKPGQSPRLLIY
VL2 FR3	54	GVPARFSGSGSGTEYTLTISLQSEDFAVYYC
VL2 FR4	49	FGQGTKVEIK
VL3 FR1	55	ENVMTQSPATLSASPGERVMTSC
VL3 FR2	53	WYQQKPGQSPRLLIY
VL3 FR3	54	GVPARFSGSGSGTEYTLTISLQSEDFAVYYC
VL3 FR4	49	FGQGTKVEIK

Although the above Table discloses the CDRs according to Kabat (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)), the antibodies of this disclosure can comprise CDRs of 34D10 according to any CDR definition (*e.g.*, Kabat, Chothia, enhanced Chothia, contact, IMGT, AbM). The CDRs of an antibody according to the different CDR definitions can be determined, *e.g.*, by using the AbYsis database (www.bioinf.org.uk/abysis/sequence_input/key_annotation/key_annotation.cgi). According to the classical Kabat numbering, Kabat VH-CDR1 is at positions 31-35, VH-CDR2 is at positions 50-65, and VH-CDR3 is at positions 95-102; and, VL-CDR1, VL-CDR2, and VL-CDR3 are at positions 24-34, 50-56 and 89-97, respectively. According to the Chothia definition, VH-CDR1 is at positions 26 -32 (Chothia numbering) , VH-CDR2 is at positions 52-56, VH-CDR3 is at positions 95-102, VL-CDR1 is at positions 24-34, VL-CDR2 is at positions 50-56, and VL-CDR3 is at positions 89-97. According to the contact definition, VH-CDR1 is at positions 30-35 (Chothia numbering) , VH-CDR2 is at positions 47-58, VH-CDR3 is at positions 93-101, VL-CDR1 is at positions 30-36, VL-CDR2 is at positions 46-55, and VL-CDR3 is at positions 89-96.

According to the IMGT numbering schema VH-CDR1 is at positions 26 to 35, VH-CDR2 is at positions 51 to 57, VH-CDR3 is at positions 93 to 102, VL-CDR1 is at positions 27 to 32, VL-CDR2 is at positions 50 to 52, and VL-CDR3 is at positions 89 to 97.

The humanized antibodies can include the three CDRs of the VH of 34D10 (according to any CDR definition) in the context of any suitable heavy chain human acceptor framework. In one embodiment, a suitable heavy chain human acceptor framework is an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of the germline humIGHV3/OR16-13, with framework region 4 (FR4) from human consensus subgroup Heavy III. In one embodiment the heavy chain human acceptor framework is an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of the germline humIGHV3-15. In another embodiment the heavy chain human acceptor framework is an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of the germline humIGHV3-7. In yet another embodiment the heavy chain human acceptor framework is an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of the germline humIGHV3-53. In a further embodiment the heavy chain human acceptor framework is an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 76%, at least 77%,

at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of the germline humIGHV3-66. The humanized antibodies can include the three CDRs of the VL of 34D10 (according to any CDR definition) in the context of any suitable light chain human acceptor framework. In one embodiment, a suitable light chain human acceptor framework is an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of the germline humIGKV3-15, with framework region 4 (FR4) from human consensus subgroup Kappa I. In another embodiment the light chain human acceptor framework is an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of the germline humIGKV1-NL1. In yet another embodiment the heavy chain human acceptor framework is an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of the germline humIGKV1D-43. Antibodies or antigen-binding fragments thereof can be selected for use based on higher affinity or avidity for GPIIb or the GPIIb/IIIa complex and/or reduced immunogenicity than previously known anti- GPIIb/IIIa antibodies. Methods of determining potency, affinity or avidity, and immunogenicity of antibodies are within the skill of the ordinary artisan.

This disclosure also includes antibodies or antigen-binding fragments thereof that specifically bind GPIIb and/or the GPIIb/IIIa complex that have heavy chain variable regions that are: at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%,

at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequences set forth in any one of SEQ ID NOs.: 3, 5, 7, 9, 11, 12, or 197-218. This disclosure also provides antibodies or antigen-binding fragments thereof that specifically bind GPIIb and/or the GPIIb/IIIa complex that have heavy chain variable regions that are identical to the amino acid sequences set forth in any one of SEQ ID NOs.: 3, 5, 7, 9, 11, 12, or 197-218 except for a total of 1 to 40, 1 to 30, 1 to 20, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, or 1 amino acid substitutions, deletions, or insertions. In certain embodiments, these antibodies or antigen-binding fragments thereof have at least one, at least two, at least three, at least four, at least five, or all six of the CDRs of 34D10 (wherein the CDRs can be according to any CDR definition). In some embodiments, these antibodies or antigen-binding fragments thereof have light chain variable regions that are: at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequences set forth in any one of SEQ ID NOs.: 4, 6, 8, or 10. This disclosure also provides antibodies or antigen-binding fragments thereof that specifically bind GPIIb and/or the GPIIb/IIIa complex that have light chain variable regions that are identical to the amino acid sequences set forth in any one of SEQ ID NOs.: 4, 6, 8, or 10 except for a total of 1 to 40, 1 to 30, 1 to 20, 1 to 10, 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, or 1 amino acid substitutions, deletions, or insertions. In certain embodiments, these antibodies or antigen-binding fragments thereof have at least one, at least two, at least three, at least four, at least five, or all six of the CDRs of 34D10 (wherein the CDRs can be according to any CDR definition). In some embodiments, these antibodies or antigen-binding fragments thereof do not compete with fibrinogen for binding to GPIIb/IIIa. In some embodiments, these antibodies or antigen-binding fragments thereof do not activate platelets.

Exemplary antibodies or antigen-binding fragments thereof described herein that specifically bind GPIIb and/or the GPIIb/IIIa complex comprise amino acid sequences that are: at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at

least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequences set forth in: (i) SEQ ID NOs.: 5 and 4; (ii) SEQ ID NOs.: 7 and 4; (iii) SEQ ID NOs.: 9 and 4; (iv) SEQ ID NOs.: 11 and 4; (v) SEQ ID NOs.: 12 and 4; (vi) SEQ ID NOs.: 12 and 8; (vii) SEQ ID NOs.: 3 and 10; (viii) SEQ ID NOs.: 5 and 10; (ix) SEQ ID NOs.: 7 and 10; (x) SEQ ID NOs.: 9 and 10; (xi) SEQ ID NOs.: 11 and 10; and (xii) SEQ ID NOs.: 12 and 10. In certain embodiments, these antibodies or antigen-binding fragments thereof have at least one, at least two, at least three, at least four, at least five, or all six of the CDRs of 34D10 (wherein the CDRs can be according to any CDR definition). In some embodiments, these antibodies or antigen-binding fragments thereof do not compete with fibrinogen for binding to GPIIb/IIIa. In some embodiments, these antibodies or antigen-binding fragments thereof do not activate platelets.

The VH and or VL region of the anti-GPIIb/IIIa antibodies or antigen-binding fragments thereof described herein can be linked to a constant region (e.g., a wild-type human Fc region or an Fc region that includes one or more alterations). In some embodiments, the antibody has a light chain constant region derived from a human kappa sequence. In some embodiments, the antibody has a light chain constant region derived from a human lambda sequence. In a specific embodiment, the light chain constant region comprises a human subgroup kappa 1 sequence. In certain embodiments, the antibody has an isotype selected from the group consisting of IgG1, IgG2, IgG3, and IgG4. The heavy chain constant region can be a wild-type human Fc region, or a human Fc region that includes one or more amino acid substitutions. The antibodies can have mutations that stabilize the disulfide bond between the two heavy chains of an immunoglobulin, such as mutations in the hinge region of IgG4, as disclosed in the art (e.g., Angal et al., *Mol. Immunol.*, 30:105-08 (1993)). See also, e.g., U.S. 2005/0037000. The heavy chain constant region can also have substitutions that modify the properties of the antibody (e.g., decrease one or more of: Fc receptor binding, antibody glycosylation, deamidation, binding to complement, or methionine oxidation). In some instances, the antibodies may have mutations such as those described in U.S. Patent Nos. 5,624,821 and 5,648,260. In some embodiments, the antibody is modified to reduce or eliminate effector function. In some embodiments, the heavy chain constant region has one or more of the following mutations: S228P; N297Q; and T299A (numbering according to Kabat). The heavy chain constant region can be chimeric, e.g., the Fc region can comprise the CH1 and CH2 domains of an IgG antibody of the IgG4 isotype, and the CH3 domain from an IgG antibody of the IgG1 isotype (see, e.g., U.S. Patent Appl. No. 2012/0100140A1 which is incorporated by reference in its entirety herein). In a specific embodiment, the humanized anti-

GPIIb/IIIa antibodies described herein have a chimeric constant region comprising the CH1 and CH2 domains of an IgG antibody of the IgG4 isotype, and the CH3 domain from an IgG antibody of the IgG1 isotype and further contain the S228P and N297Q mutations (numbering according to Kabat).

Antigen-binding fragments of the anti-GPIIb/IIIa antibodies are also encompassed by this disclosure. In some embodiments, the anti-GPIIb/IIIa antibody or antigen-binding molecule thereof comprises or consists of (i) a single chain Fv ("scFv"); (ii) a diabody; (iii) an sc(Fv)₂; (iv) a polypeptide chain of an antibody; (v) F(ab')₂; or (vi) F(ab). In one embodiment, the antigen-binding fragment is an Fab molecule. The fragment antigen-binding (Fab fragment) is a region on an antibody that binds to antigens. It is composed of one constant and one variable domain of each of the heavy and the light chain. These domains shape the paratope, *i.e.*, the antigen-binding site. The enzyme papain can be used to cleave an immunoglobulin monomer into two Fab fragments and an Fc fragment. Recombinant methods can also be used to make an Fab molecule. In another embodiment, the antigen-binding fragment is a single-chain fragment variable (scFv). An scFv is comprised of the variable regions of the heavy and light chains of an antibody. It is only half the size of the Fab fragment and yet retains the original specificity of the parent immunoglobulin. Methods of making an ScFv are well known in the art (see, e.g., Ahmad et al., *Clinical and Developmental Immunology*, vol. 2012, Article ID 980250, 15 pages, 2012. doi:10.1155/2012/980250).

In certain embodiments, the anti-GPIIb/IIIa antibody or antigen-binding molecule thereof can be a targeting moiety. These targeting moieties are useful in ferrying an agent of interest (e.g., a therapeutic agent, a coagulation factor, a small molecule drug) to platelets. In some embodiments, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein can target GPIIb/IIIa located on the surface of platelets.

D. Chimeric Molecules Comprising Anti-GPIIb/IIIa Antibodies

The present disclosure also provides "chimeric molecules" comprising, for example, at least one of the GPIIb/IIIa antibodies or antigen-binding fragments thereof disclosed herein that is linked and/or conjugated and/or otherwise associated with at least one heterologous moiety. In certain embodiments, the heterologous moiety is an agent that to be ferried or delivered to a platelet or its local environment. Such an agent can be *e.g.*, a therapeutic agent such as a clotting factor (*e.g.*, rFVIIa).

A chimeric molecule disclosed herein encompasses any molecule comprising (i) a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein (*e.g.*, an Fab or scFv derived from a humanized 34D10 antibody), and (ii) at least one (*e.g.*, one two, three, four) heterologous moiety (*e.g.*, a therapeutic moiety, a clotting factor, a half-life extending moiety) and optionally including one or more linkers. In some embodiments, a chimeric molecule is a chimeric protein, *i.e.*, a chimeric molecule in which all its components (heterologous moieties and/or linkers) are polypeptides. Other chimeric molecules can comprise non-polypeptide heterologous moieties (*e.g.*, PEG, lipids, carbohydrates, nucleic acids, small molecule therapeutic agents, radionuclides, fluorescent probes, etc.) and/or non-polypeptide linkers.

In some embodiments, a chimeric molecule comprises 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 molecule can include for example, a protein derived from at least two 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 molecule 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 molecule can 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 molecule can also 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.

In some embodiments, the chimeric molecule has, for example, a formula: (i) Ab-(L)-H or (ii) H-(L)-Ab, wherein, H is a heterologous moiety; L is an optional linker; and, Ab is an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein. One or more

copies (*e.g.*, one, two, three, four) of the same heterologous moiety may be included in the chimeric molecule.

In some embodiments, the chimeric molecule further comprises a second heterologous moiety. Accordingly, in some embodiments, the chimeric molecule has a formula selected from:

- (i) H1-(L1)-Ab-(L2)-H2;
- (ii) H2-(L2)-Ab-(L1)-H1;
- (iii) H1-(L1)-H2-(L2)-Ab;
- (iv) H2-(L2)-H1-(L1)-Ab;
- (v) Ab-(L1)-H1-(L2)-H2; or,
- (vi) Ab-(L2)-H2-(L1)-H1;

wherein, Ab is an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein; H1 is a first heterologous moiety, H2 is a second heterologous moiety, L1 is a first optional linker, and L2 is a second optional linker. One or more copies (*e.g.*, one, two, three, four) of the same heterologous moiety may be included in the chimeric molecule.

In some embodiments, the first heterologous moiety and the second heterologous moiety are the same. In other embodiments, the first heterologous moiety and the second heterologous moiety are different. In some embodiments, L1 and L2 are the same. In other embodiments, L1 and L2 are different.

The chimeric molecule formulas disclosed are oriented from N-terminus (left) to C-terminus (right). One skilled in the art would understand that the chimeric molecule formulas disclosed herein are non-limiting examples of chimeric molecules comprising the disclosed anti-GPIIb/IIIa antibodies or antigen-binding fragments thereof. For example, the formulas can comprise further sequences at their N-terminal or C-terminal ends, or inserted between elements of the formula. Accordingly, a chimeric molecule can comprise one, two, three, four, five, or more than five heterologous moieties. In some embodiments, the hyphen (-) in a formula indicates a peptide bond or one or more amino acids. Exemplary chimeric molecules are presented in Figures 7 and 8.

In some embodiments, a chimeric protein comprises a first polypeptide chain and a second polypeptide chain, which are associated with each other. In some embodiments, the first polypeptide chain comprises a light chain of a clotting factor (e.g., FVII) and a heterologous moiety (e.g., a half-life extending moiety), and the second polypeptide chain comprises a heavy chain of the clotting factor (e.g., FVII) and a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein. In other embodiments, the first polypeptide chain comprises a light chain of a clotting factor (e.g., FVII) and a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, and the second polypeptide chain comprises a heavy chain of the clotting factor (e.g., FVII) and a heterologous moiety (e.g., a half-life extending moiety). In yet another embodiment, the first polypeptide chain comprises a light chain of a clotting factor (e.g., FVII) and the second polypeptide chain comprises a heavy chain of the clotting factor (e.g., FVII), a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, and a heterologous moiety (e.g., a half-life extending moiety). In some embodiments, the first polypeptide chain comprises a light chain of a clotting factor (e.g., FVII) and the second polypeptide chain comprises a heavy chain of the clotting factor (e.g., FVII), a heterologous moiety (e.g., a half-life extending moiety), and a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein. In other embodiments, the first polypeptide chain comprises a light chain of a clotting factor (e.g., FVII), a heterologous moiety (e.g., a half-life extending moiety), and a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, and the second polypeptide chain comprises a heavy chain of the clotting factor (e.g., FVII). In some embodiments, the first polypeptide chain comprises a light chain of a clotting factor (e.g., FVII), a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, and a heterologous moiety (e.g., a half-life extending moiety), and the second polypeptide chain comprises a heavy chain of the clotting factor (e.g., FVII).

In some embodiments, the chimeric molecule comprises a formula wherein:

(1) the first polypeptide chain comprises CF_L-H or H-CF_L and the second polypeptide chain comprises CF_H-Ab or Ab-CF_H;

(2) the first polypeptide chain comprises CF_L-Ab or Ab-CF_L and the second polypeptide chain comprises CF_H-H or H-CF_H;

(3) the first polypeptide chain comprises CF_L and the second polypeptide chain comprises CF_H-Ab-H or H-Ab-CF_H;

(4) the first polypeptide chain comprises CF_L and the second polypeptide chain comprises CF_H-H-Ab or Ab-H-CF_H;

(5) the first polypeptide chain comprises CF_L-H-Ab or Ab-H-CF_L and the second polypeptide chain comprises CF_H; or

(6) the first polypeptide chain comprises CF_L-Ab-H or H-Ab-CF_L and the second polypeptide chain comprises CF_H;

wherein, CF_L is a light chain of a clotting factor (e.g., FVII); CF_H is a heavy chain of the clotting factor (e.g., FVII); Ab is an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof; and H is a heterologous moiety (e.g., a half-life extending moiety). In some embodiments, the clotting factor is independently selected from the group consisting of FVII, FIX, FX, and any combinations thereof.

This disclosure also provides a chimeric molecule comprising a first polypeptide chain and a second polypeptide chain, which are associated with each other, (1) wherein the first polypeptide chain comprises a light chain of a clotting factor (e.g., FVII, FIX, or FX), and a targeting moiety, which binds to a platelet, and the second polypeptide chain comprises a heavy chain of the clotting factor (e.g., FVII, FIX, or FX) and a heterologous moiety (e.g., a half-life extending moiety); (2) wherein the first polypeptide chain comprises a light chain of a clotting factor (e.g., FVII) and a heterologous moiety (e.g., a half-life extending moiety), and the second polypeptide chain comprises a heavy chain of the clotting factor (e.g., FVII, FIX, or FX) and a targeting moiety, which binds to a platelet; (3) wherein the first polypeptide chain comprises a light chain of a clotting factor (e.g., FVII, FIX, or FX), a heterologous moiety (e.g., a half-life extending moiety), and a targeting moiety, which binds to a platelet, and the second polypeptide comprises a heavy chain of the clotting factor (e.g., FVII, FIX, or FX); or (4) wherein the first polypeptide chain comprises a light chain of a clotting factor (e.g., FVII, FIX, or FX), a targeting moiety, which binds to a platelet, and a heterologous moiety (e.g., a half-life extending moiety) and the second polypeptide chain comprises a heavy chain of the clotting factor (e.g., FVII, FIX, or FX). In some embodiments, the clotting factor is FVII, FIX, or FX.

As used herein, the phrases "which binds to a platelet," "binding to a platelet," and variants thereof generally refer to the specific binding of (i) a GPIIb/IIIa antibody or antigen-binding molecule thereof or (ii) a chimeric molecule of the present disclosure to an antigenic site on the surface of the platelet, *e.g.*, an epitope on the extracellular domains of the α and/or β subunits of the GPIIb/IIIa receptor. It is known to a person skilled in the art that GPIIb/IIIa is present in two pools, a plasma membrane pool present in the platelet's resting state and an internal pool of GPIIb/IIIa which is expressed upon platelet activation. *See, e.g.*, Quinn et al., *J. Pharmacol. Exp. Ther.*, 297:496-500 (2001). Accordingly, in some specific embodiments, and particularly for diagnostic uses where the platelet's plasma membrane can be permeabilized, the binding of an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof to platelets, or the binding of a chimeric molecule of the present disclosure to platelets can refer to binding to the plasma membrane pool and/or to the internal pool of GPIIb/IIIa.

In some embodiments, the chimeric molecule comprises a first polypeptide chain and a second polypeptide chain, which are associated with each other, (1) wherein the first polypeptide chain comprises CF_L-H or H-CF_L and the second polypeptide chain comprises CF_H-Ab or Ab-CF_H; (2) wherein the first polypeptide chain comprises CF_L-Ab or Ab-CF_L and the second polypeptide chain comprises CF_H-H or H-CF_H; (3) wherein the first polypeptide chain comprises CF_L-H-Ab or Ab-H-CF_L and the second polypeptide chain comprises CF_H; or (4) wherein the first polypeptide chain comprises CF_L-Ab-H or H-Ab-CF_L and the second polypeptide chain comprises CF_H; wherein, H is a heterologous moiety (*e.g.*, a half-life extending moiety), CF_H is a heavy chain of a clotting factor (*e.g.*, FVII), CF_L is a light chain of the clotting factor (*e.g.*, FVII, FIX, or FX), Ab is an anti-GPIIb/IIIa antibody that binds to a platelet, and L is an optional linker.

In some embodiments, the association between the first polypeptide chain and the second polypeptide chain in the chimeric molecule is a covalent bond or a non-covalent bond. Thus, in other embodiments, the association between the first polypeptide chain and the second polypeptide chain in the chimeric molecule is a covalent bond between the heavy chain and the light chain of the clotting factor (*e.g.*, FVII, FIX, or FX). In contrast, in some other embodiments, the covalent bond is a disulfide bond.

The present disclosure also provides a chimeric molecule comprising a single polypeptide chain, which comprises, from N terminus to C terminus, (i) a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX), a heterologous moiety (*e.g.*, a half-life extending

moiety), a protease cleavage site, a heavy chain of the clotting factor (e.g., FVII, FIX, or FX), and a targeting moiety (e.g., a GPIIb/IIIa antibody or antigen-binding molecule thereof) which binds to a platelet or (ii) a light chain of a clotting factor (e.g., FVII), a targeting moiety, which binds to a platelet, a protease cleavage site, a heavy chain of the clotting factor (e.g., FVII, FIX, or FX), and a heterologous moiety (e.g., a half-life extending moiety). In some embodiments, the clotting factor is FVII. In other embodiments, the clotting factor is FIX or FX. In yet other embodiments, the clotting factor is FVII, FIX, or FX. In some embodiments, the protease cleavage site is an intracellular processing site. In some embodiments, the intracellular processing site is processed by a proprotein convertase. In some embodiments, the proprotein convertase is selected from the group consisting of PC5, PACE, PC7, and any combinations thereof.

I. Heterologous Moieties

The heterologous moiety or moieties of the chimeric molecules disclosed herein can comprise, consist of, or consist essentially of, for example, prophylactic and/or therapeutic agents (e.g., clotting factors), molecules capable of improving a pharmacokinetic (PK) property (e.g., plasma half-life extending moieties), and detectable moieties (e.g., fluorescent molecules or radionuclides). In some embodiments, the heterologous moiety comprises a clotting factor (e.g., a Factor VII). In some embodiments, a heterologous moiety comprises a molecule that can modify a physicochemical property of a chimeric molecule lacking such heterologous moiety. For example, it can increase the hydrodynamic radius of a chimeric molecule. In other embodiments, the incorporation of a heterologous moiety into a chimeric molecule can improve one or more pharmacokinetic properties without significantly affecting its biological activity or function (e.g., procoagulant activity in chimeric molecules comprising a clotting factor). In other embodiments, a heterologous moiety increases stability of the chimeric molecule of the invention or a fragment thereof.

In some embodiments, the heterologous moiety is a polypeptide comprising, consisting essentially of, or consisting of at least about 10, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2500, 3000, or 4000 amino acids. In other embodiments, the heterologous moiety is a polypeptide comprising, consisting essentially of, or consisting of about 100 to about 200 amino acids, about 200 to about 300 amino acids, about 300 to about 400 amino acids, about 400 to about

500 amino acids, about 500 to about 600 amino acids, about 600 to about 700 amino acids, about 700 to about 800 amino acids, about 800 to about 900 amino acids, or about 900 to about 1000 amino acids.

Non-limiting examples of the heterologous moieties are discussed below.

1. Clotting Factors

In some embodiments, the chimeric molecules of this disclosure comprise at least one polypeptide heterologous moiety which is (i) a clotting factor, or (ii) a procoagulant peptide (e.g., a synthetic procoagulant peptide). Blood coagulation is a process that involves a complex interaction of various blood factors that eventually result in a fibrin clot. Generally, the blood factor, which participate in what has been referred to as the coagulation "cascade", are enzymatically inactive proteins (proenzymes or zymogens) that are converted to proteolytic enzymes by the action of an activator (which itself is an activated clotting factor). Coagulation factors that have undergone such a conversion are generally referred to as "active factors", and are designated by the addition of the letter "a" to the name of the coagulation factor (e.g. Factor VIIa). In some embodiments, the clotting factor is independently selected from the group consisting of factor FVII ("FVII"), factor IX ("FIX"), or factor X ("FX"), and any combinations thereof. As discussed in detail below, the clotting factor can be, for example, FVII zymogen, activatable FVII, activated FVII (FVIIa), FIX zymogen, activatable FIX, activated FIX (FIXa), FX zymogen, activatable FX, or activated FX (FXa). In some embodiments, the clotting factor can comprise a single polypeptide chain or two polypeptide chains (I the heavy chain and the light chain of FVII). In some embodiments, the chimeric molecule comprises a FVII or activated FVII (FVIIa) clotting factor. In some embodiments, the chimeric molecule of the invention comprises a FIX or activated FIX (FIXa) clotting factor. In other embodiments, the chimeric molecule comprises a FX or activated FX (FXa) clotting factor.

In some embodiments, the chimeric molecule comprises a single clotting factor, which in the chimeric molecule is represented by a formula as H, H1 or H2. In other embodiments, the chimeric molecule comprises two clotting factors. In some embodiments, the two clotting factors are the same, whereas in other embodiments, the two clotting factors are different. In some embodiments, one clotting factor is a fragment of a clotting factor (e.g., a heavy chain of a clotting factor such as FVII) and the second clotting factor is a fragment of

the same clotting factor (e.g., a light chain of a clotting factor such as FVIII). In some embodiments, the chimeric molecule comprises more than two clotting factors.

a. Factor VII

In some embodiments, the chimeric molecule comprises a clotting factor which is a mature form of Factor VII or a variant thereof. Factor VII (FVII, F7; also referred to as Factor 7, coagulation factor VII, serum factor VII, serum prothrombin conversion accelerator, SPCA, proconvertin and eptacog alpha) is a serine protease that is part of the coagulation cascade. FVII includes a Gla domain, two EGF domains (EGF-1 and EGF-2), and a serine protease domain (or peptidase S1 domain) that is highly conserved among all members of the peptidase S1 family of serine proteases, such as for example with chymotrypsin. In some embodiments, the chimeric molecule comprises a Factor VIIa. In certain embodiments, the Factor VIIa is recombinant.

FVII can occur as a single chain zymogen, an activated zymogen-like two-chain polypeptide, or a fully activated two-chain form. The zymogen composed of a single chain polypeptide is converted to a two-chain form connected by disulfide bonds by the action of Factor Xa in the presence of calcium ions and phospholipids, thrombin, or by the action of factor XIIa (without additional cofactors). This hydrolysis of Factor VII is accompanied by an at least 85-fold increase in the Factor VII coagulant activity compared to the single chain form (*see, e.g., Radcliffe et al., J. Biol. Chem., 250(2):388-395 (1975) and Handbook of Enzymes, Class 3.4 Hydrolases II: EC3.4.21-3.4.22, Volume 7, coed. By Antje Chang, 2002, (Springer, 2nd edition)*). Following vascular damage, blood clotting is triggered when factor VIIa (FVIIa) forms a complex with tissue factor (TF). In hemophilia A and B, the propagation phase of blood coagulation is disrupted due to the lack of factors VIII (FVIII) and IX (FIX), leading to excessive bleeding. However, high doses of recombinant FVIIa (rFVIIa) can bypass the FVIII/FIX deficiency and ameliorate bleeding problems.

The amino acid sequence of the B isoform of FVII zymogen is provided below (the signal sequence (boldened), propeptide sequence (underlined); the peptide bond between R and I (boldened and underlined) is cleaved to activate FVII):

1	MVSQALRLLC	<u>LLGLQGCLA</u>	AVFVTQEEAH	GVLHRRRRAN	AFLEELRPGS
51	LERECKEEQC	SFEEAREIFK	DAERTKLFWI	SYSDGDQCAS	SPCQNGGSCK
101	DQLQSYICFC	LPAFEGRNCE	THKDDQLICV	NENGGEQYC	SDHTGTRKSC
151	RCHGYSLLA	DGVSTPTVE	YPCGKIPILE	KRNASKPQGR	<u>IV</u> GGKVCPKG
201	ECPWQVLLLV	NGAQLCGGTL	INTIWVVSAA	HCFDKIKNWR	NLIAVLGEHD
251	LSEHDGDEQS	RRVAQVIIPS	TYVPGTTNHD	IALRLHQPV	VLTDHVVPLC

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301   LPERTFSERT LAFVRFSLVS GWGQLLD RGA TALELMVLNV PRLMTQDCLQ
351   QSRKVG DSPN ITEYMFCAGY SDGSKDSCKG DSGGPHATHY RGTWYLTGIV
401   SWGQGCATVG HFGVYTRVSQ YIEWLQKL MR SEPRPGVLLR APFP
      (SEQ ID NO: 79)

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It is to be understood the chimeric molecules of this disclosure can include any FVII zymogen (*e.g.*, the A or B isoforms) so long as intended results are achieved (*e.g.*, effectiveness in treatment of a coagulation or hemostatic disorder).

The amino acid sequence of the light chain of FVII is provided below:

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ANAFLEELRP GSLERECKEE QCSFEEAREI FKDAERTKLF WISYSDGDQC ASSPCQNGGS
CKDQLQSYIC FCLPAFEGRN CETHKDDQLI CVNENGGCEQ YCSDHTGTRK SCRCHEGYSL
LADGVSCTPT VEYPCGKIPI LEKRNASKPQ GR      (SEQ ID NO:80)

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The amino acid sequence of the heavy chain of FVII is provided below:

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IVGGKVC P KGECPWQVLL LVNGAQLCGG TLINTIWVVS AAHCFDKIKN WRNLI AVLGE
HDLSEHDGDE QSRRAQV VII PSTYVPGTTN HDIALRLHQ PVVLT DHVVP LCLPERTFSE
RTLAFVRFSL VSGWGQLLDR GATALELMVL NVPRLMTQDC LQQSRKVGDS PNITEYMFCA
GYSDGSKDSC KGDSGGPHAT HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV SQYIEWLQKL
MRSEPRPGVL LRAPFP (SEQ ID NO:81)

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This disclosure also encompasses any allelic variants of FVII.

Other exemplary FVII variants that are encompassed by this disclosure 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, *Semin Hematol.*, 41 (1Suppl 1):89-92 (2004); Persson *et al.*, *Proc. Natl. Acad. Sci. USA* 98:13583 (2001); Petrovan and Ruf, *J. Biol. Chem.* 276:6616 (2001); Persson *et al.*, *J. Biol. Chem.* 276:29195 (2001); Soejima *et al.*, *J. Biol. Chem.* 276:17229 (2001); Soejima *et al.*, *J. Biol. Chem.* 247:49027 (2002); and WO2002/022776.

In one embodiment, a variant form of FVII includes mutations, *e.g.*, V158D-E296V-M298Q. In another embodiment, a variant form of FVII includes a replacement of amino acids 608-619 (LQQSRKVG DSPN (SEQ ID NO:82), corresponding to the 170-loop) from the FVII mature sequence with amino acids EASYPGK (SEQ ID NO:83) from the 170-loop of trypsin. High specific activity variants of FVII are also known in the art. For example, Simioni *et al.* (*N.E. Journal of Medicine* 361:1671, 2009) describe an R338L mutation. Chang *et al.* (*J. Biol. Chem.* 273:12089, 1988) and Pierri *et al.* (*Human Gene Therapy* 20:479, 2009) describe an R338A mutation. Other mutations are known in the art and include

those described, *e.g.*, in Zogg and Brandstetter, *Structure* 17:1669 (2009); Sichler *et al.*, *J. Biol. Chem.* 278:4121 (2003); and Sturzebecher *et al.*, *FEBS Lett.* 412:295 (1997). The contents of all of the references above are incorporated herein by reference.

Full activation, which occurs upon conformational change from a zymogen-like form, occurs upon binding to its co-factor, *i.e.*, 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.

b. Factor IX

In one embodiment, the chimeric molecule comprises a clotting factor which is a mature form of Factor IX or a variant thereof. Factor IX circulates as a 415 amino acid, single chain plasma zymogen. *See*, Vysotchin *et al.*, *J. Biol. Chem.* 268:8436 (1993). The amino acid sequence of FIX zymogen is provided below (the signal sequence is underlined (1-28); the propeptide sequence (29-46) is boldened):

MQRVNMIMAESPLITICLLGYLLSAEC**TVFLDHENANKILNRPKRY**NSGKLEEFVQGNLERECMEEEK
CSFEEAREVFENTERTEFWKQYVDGDQCESNPCLNGGCKDDINSYECWCPFGFEGKNCELDVTCNI
KNGRCEQFCKNSADNKVVCSCTEGYRLAENQKSCEPAVFPFCGRVSVSQTSLTRAETVFPDVDYVNS
TEAETILDNITQSTQSFNDFTRVVGGEDAKPGQFPWQVVLNGKVDAFCGGSIVNEKWIVTAAHCVETG
VKITVVAGEHNIETEHEQKRNVIIRIIPHHYNAAINKYNHDIALLELDEPLVLNSYVTPICIADE
YTNIFLKFGSGYVSGWGRVFKGRSALVLQYLRVPLVDRATCLRSTKFTIYNMFCAGFHEGGRDSCQ
GDSGGPHVTEVEGTSFLTGIISWGEECAMKGKGYGIYTKVSRYVNWIKETKLT (SEQ ID
NO: 84)

The zymogen of FIX is activated by FXIa or by the tissue factor/FVIIa complex. Specific cleavages between arginine-alanine 145-146 and arginine-valine 180-181 result in a light chain and a heavy chain linked by a single disulfide bond between cysteine 132 and cysteine 289 (Bajaj *et al.*, *Biochemistry* 22:4047 (1983)).

The structural organization of FIX is similar to that of the vitamin K-dependent blood clotting proteins FVII, FX and protein C. The approximately 45 amino acids of the amino terminus comprise the gamma-carboxyglutamic acid, or Gla, domain. This is followed by two epidermal growth factor homology domains (EGF), an activation peptide and the catalytic "heavy chain" which is a member of the serine protease family (Vysotchin *et al.*, *J. Biol. Chem.* 268:8436 (1993); Spitzer *et al.*, *Biochemical Journal* 265:219 (1990); Brandstetter *et al.*, *Proc. Natl. Acad. Sci. USA* 92:9796 (1995)).

c. Factor X

In one embodiment, the chimeric molecule comprises a clotting factor which is a mature form of Factor X. Factor X is a vitamin-K dependent glycoprotein with a molecular weight of 58.5 kDa, which is secreted from liver cells into the plasma as a zymogen. Initially factor X is produced as a prepropeptide with a signal peptide consisting in total of 488 amino acids. The amino acid sequence of FX zymogen is provided below (the signal sequence (1-23) is underlined and the propeptide (24-40) is boldened):

MGRPLHLVLLSASLAGLLLLGES**LFIRREQANNILARV**TRANSFLEEMKKGHLERECMEETCSYEEAR
 EVFEDSDKTNEFWNKYKDGDCETSPCQNQGKCKDGLGEYTCTCLEGFEGKNCELFTRKLCSLDNGDC
 DQFCHEEQNSVVCSCARGYTLADNGKACIPTGPYPCGKQTLERRKRSVAQATSSSGEAPDSITWKPYD
 AADLDPTENPFDLLDFNQTPERGDNNLTRIVGGQECKDGECPWQALLINEENEGFCGGTILSEFYIL
 TAAHCLYQAKRFKVRVGDRNTEQEEGGEAVHEVEVVIKHNRFTEKTYDFDIAVRLKTPITFRMNVAP
 ACLPERDWAESTLMTQKTGIVSGFGRTHEKGRQSTRCLKMLEVPYVDRNSCKLSSSFIIITQNMFCAGYD
 TKQEDACQGDGSGPHVTRFKDTYFVTGIVSWGEGCARKGKYGIYTKVTAFLKWIDRSMKTRGLPKAKS
 HAPEVITSSPLK (SEQ ID NO: 85)

The signal peptide is cleaved off by signal peptidase during export into the endoplasmic reticulum. The propeptide sequence is cleaved off after gamma carboxylation took place at the first 11 glutamic acid residues at the N-terminus of the mature N-terminal chain. A further processing step occurs by cleavage between Arg182 and Ser183. This processing step also leads concomitantly to the deletion of the tripeptide Arg180-Lys181-Arg182. The resulting secreted factor X zymogen consists of an N-terminal light chain of 139 amino acids (M, 16,200) and a C-terminal heavy chain of 306 amino acids (M, 42,000) which are covalently linked via a disulfide bridge between Cys172 and Cys342. Further posttranslational processing steps include the β -hydroxylation of Asp103 as well as N- and O-type glycosylation.

It will be understood that in addition to wild type (WT) versions of these clotting factors or biologically active portions thereof, the heterologous moieties in the chimeric molecules disclosed herein can also comprise 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 FVII, such as TF binding, factor X binding, phospholipid binding, and/or coagulant activity of FVII

can 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 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 clotting factors can 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.

The "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.

The Gla domain of factor VII comprises the uncommon amino acid γ -carboxyglutamic acid (Gla), which plays a vital role in the binding of clotting factors to negatively charged phospholipid surfaces. 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. 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 (Osterlund *et al.*, *Biochem. Biophys. Res. Commun.* 337:1276 (2005)). In addition, clearance

of clotting factors can be significantly mediated through Gla interactions, *e.g.*, on liver cells and clearance receptors, *e.g.*, EPCR.

In one embodiment, the chimeric molecule comprises a heterologous moiety comprising a clotting factor 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 (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof) targets the Gla deleted clotting factor to platelets. Accordingly, in one embodiment, the chimeric molecule comprises a targeting moiety (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof) and a heterologous moiety comprising a clotting factor that 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 the exemplary clotting factors disclosed herein are known in the art. The Gla 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 can be introduced into constructs lacking a Gla domain to facilitate activation of the molecule. For example, in one embodiment, such a cleavage site can 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).

In one embodiment, a cleavage site can be introduced into chimeric molecules comprising a clotting factor that lacks a Gla domain to facilitate activation of the molecule. For example, in one embodiment, such a cleavage site can 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 known in the art. Exemplary clotting factors are those of mammalian, *e.g.*, human, origin.

2. Half-life Extending Moieties

In some embodiments, the chimeric molecule comprises at least one heterologous moiety that is a "half-life extending moiety." Half-life extending moieties, as discussed below in detail, can comprise, for example, (i) XTEN polypeptides; (ii) Fc; (iii) albumin, (iv)

albumin binding polypeptide or fatty acid, (v) the C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin, (vi) PAS; (vii) HAP; (viii) transferrin; (ix) polyethylene glycol (PEG); (x) hydroxyethyl starch (HES), (xi) polysialic acids (PSAs); (xii) a clearance receptor or fragment thereof which blocks binding of the chimeric molecule to a clearance receptor; (xiii) low complexity peptides; (xiv) vWF; or (xv) any combinations thereof. In some embodiments, the half-life extending moiety comprises an Fc region. In other embodiments, the half-life extending moiety comprises two Fc regions fused by a linker. Exemplary heterologous moieties also include, *e.g.*, FcRn binding moieties (*e.g.*, complete Fc regions or portions thereof which bind to FcRn), single chain Fc regions (scFc regions, *e.g.*, as described in U.S. Publ. No. 2008-0260738, and Intl. Publ. Nos. WO 2008-012543 and WO 2008-1439545), or processable scFc regions. In some embodiments, a heterologous moiety can include an attachment site for a non-polypeptide moiety such as polyethylene glycol (PEG), hydroxyethyl starch (HES), polysialic acid, or any derivatives, variants, or combinations of these moieties.

In certain embodiments, a chimeric molecule of the disclosure comprises at least one (*e.g.*, one, two, three, or four) half-life extending moiety which increases the *in vivo* half-life of the chimeric molecule compared with the *in vivo* half-life of the corresponding chimeric molecule lacking such heterologous moiety. *In vivo* half-life of a chimeric molecule can be determined by any method known to those of skill in the art, *e.g.*, activity assays (chromogenic assay or one stage clotting aPTT assay), ELISA, etc. In some embodiments, the presence of one or more half-life extending moiety results in the half-life of the chimeric molecule to be increased compared to the half-life of the corresponding chimeric molecule lacking such one or more half-life extending moieties. The half-life of the chimeric molecule comprising a half-life extending moiety is at least about 1.5 times, at least about 2 times, at least about 2.5 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 6 times, at least about 7 times, at least about 8 times, at least about 9 times, at least about 10 times, at least about 11 times, or at least about 12 times longer than the *in vivo* half-life of the corresponding chimeric molecule lacking such half-life extending moiety.

In one embodiment, the half-life of the chimeric molecule comprising a half-life extending moiety is about 1.5-fold to about 20-fold, about 1.5 fold to about 15 fold, or about 1.5 fold to about 10 fold longer than the *in vivo* half-life of the corresponding chimeric molecule lacking such half-life extending moiety. In another embodiment, the half-life of chimeric molecule comprising a half-life extending moiety is extended about 2-fold to about

10-fold, about 2-fold to about 9-fold, about 2-fold to about 8-fold, about 2-fold to about 7-fold, about 2-fold to about 6-fold, about 2-fold to about 5-fold, about 2-fold to about 4-fold, about 2-fold to about 3-fold, about 2.5-fold to about 10-fold, about 2.5-fold to about 9-fold, about 2.5-fold to about 8-fold, about 2.5-fold to about 7-fold, about 2.5-fold to about 6-fold, about 2.5-fold to about 5-fold, about 2.5-fold to about 4-fold, about 2.5-fold to about 3-fold, about 3-fold to about 10-fold, about 3-fold to about 9-fold, about 3-fold to about 8-fold, about 3-fold to about 7-fold, about 3-fold to about 6-fold, about 3-fold to about 5-fold, about 3-fold to about 4-fold, about 4-fold to about 6 fold, about 5-fold to about 7-fold, or about 6-fold to about 8 fold as compared to the in vivo half-life of the corresponding chimeric molecule lacking such half-life extending moiety.

(i) XTEN Polypeptides

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

The chimeric molecules of the invention can include a single XTEN polypeptide or two or more (e.g., two, three, four, five) XTEN polypeptides. In one embodiment, a chimeric molecule comprises a FVII, a first XTEN polypeptide, a second XTEN polypeptide, and an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof. The chimeric molecule thus can comprise a formula of FVII-(L1)-X1-(L2)-Ab-(L3)-X2, X2-(L1)-Ab-(L2)-X1-(L3)-FVII, FVII-(L1)-X1-(L2)-X2-(L3)-Ab, or Ab-(L3)-X2-(L2)-X1-(L1)-FVII, wherein FVII comprises FVIIa, X1 is a first XTEN polypeptide, X2 is a second XTEN polypeptide, Ab is an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof as described above, L1 is a first optional linker, L2 is a second optional linker, and L3 is a third optional linker. In another embodiment, a chimeric molecule comprises two polypeptide chains associated with each other, the first polypeptide chain comprising a light chain of FVII and a first XTEN

polypeptide the second polypeptide chain comprising a heavy chain of FVII, a second XTEN polypeptide, and a targeting moiety, which binds to a platelet, in any order. In other embodiments, a chimeric molecule comprises two polypeptide chains associated with each other, the first polypeptide chain comprising a light chain of FVII and the first XTEN polypeptide a second polypeptide chain comprising, from N-terminus to C-terminus, a heavy chain of FVII, a second XTEN polypeptide, and a targeting moiety, which binds to a platelet or a heavy chain of FVII, a targeting moiety, which binds to a platelet, and a second XTEN polypeptide.

Other embodiments within the scope of this disclosure encompass a chimeric molecule represented by the following formula: FVII-(L1)-X1-(L2)-Ab-(L3)-X2 in which FVII comprises FVIIa, L1 is a first optional linker, X1 is a first optional XTEN polypeptide, L2 is a second optional linker, Ab is an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof as described above (*e.g.*, an Fab, scFv, etc.), L3 is a third optional linker, and X2 is a second XTEN polypeptide which may be the same or different from the first optional XTEN polypeptide. In a more specific embodiment, the first XTEN polypeptide is present in the molecule. Illustrative non-limiting examples of these embodiments are shown in Figs. 17 and 20.

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

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

In some embodiments, the XTEN comprises non-overlapping sequence motifs in which about 80%, or at least about 85%, or at least about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% or about 100% of the sequence consists of multiple units of non-overlapping sequences selected from a single motif family selected from TABLE 2, resulting in a family sequence. As used herein, “family” means that the XTEN has motifs selected only from a single motif category from TABLE 2; i.e., AD, AE, AF, AG, AM, AQ, BC, or BD XTEN, and that any other amino acids in the XTEN not from a family motif are selected to achieve a needed property, such as to permit incorporation of a restriction site by the encoding nucleotides, incorporation of a cleavage sequence, or to achieve a better linkage to FVII. In some embodiments of XTEN families, an XTEN sequence comprises multiple units of non-overlapping sequence motifs of the AD motif family, or of the AE motif family, or of the AF motif family, or of the AG motif family, or of the AM motif family, or of the AQ motif family, or of the BC family, or of the BD family, with the resulting XTEN exhibiting the range of homology described above. In other embodiments, the XTEN comprises multiple units of motif sequences from two or more of the motif families of TABLE 2. These sequences can be selected to achieve desired physical/chemical characteristics, including such properties as net charge, hydrophilicity, lack of secondary structure, or lack of repetitiveness that are conferred by the amino acid composition of the motifs, described more fully below. In the embodiments hereinabove described in this paragraph, the motifs incorporated into the XTEN can be selected and assembled using the methods described herein to achieve an XTEN of about 36 to about 3000 amino acid residues. Additional, non-limiting, examples of XTENs linked to FVII are disclosed in U.S. Patent Publication No. 2012/0263701, which is incorporated herein by reference in its entirety.

TABLE 2. XTEN Sequence Motifs of 12 Amino Acids and Motif Families

Motif Family*	MOTIF SEQUENCE	SEQ ID NO:
AD	GESPGGSSGSES	86
AD	GSEGSSGPGESS	87
AD	GSSESGSSEGGP	88
AD	GSGGEPSESGSS	89
AE, AM	GSPAGSPTSTEE	90

Motif Family*	MOTIF SEQUENCE	SEQ ID NO:
AE, AM, AQ	GSEPATSGSETP	91
AE, AM, AQ	GTSESATPESGP	92
AE, AM, AQ	GTSTEPSEGSAP	93
AF, AM	GSTSESPSGTAP	94
AF, AM	GTSTPESGSASP	95
AF, AM	GTSPSGESSTAP	96
AF, AM	GSTSSTAESPGP	97
AG, AM	GTPGSGTASSSP	98
AG, AM	GSSTPSGATGSP	99
AG, AM	GSSPSASTGTGP	100
AG, AM	GASPGTSSTGSP	101
AQ	GEPAGSPTSTSE	102
AQ	GTGEPSTPASE	103
AQ	GSGPSTESAPTE	104
AQ	GSETPSGPSETA	105
AQ	GPSETSTSEPGA	106
AQ	GSPSEPTEGTSA	107
BC	GSGASEPTSTEP	108
BC	GSEPATSGTEPS	109
BC	GTSEPSTSEPGA	110
BC	GTSTEPSEPGSA	111
BD	GSTAGSETSTEA	112
BD	GSETATSGSETA	113
BD	GTSESATSESGA	114
BD	GTSTEASEGSAS	115

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

XTEN can have varying lengths. In one embodiment, the length of the XTEN polypeptide(s) is chosen based on the property or function to be achieved in the fusion

protein. Depending on the intended property or function, XTEN can be short or intermediate length sequence or longer sequence that can serve as carriers. In certain embodiments, the XTEN include short segments of about 6 to about 99 amino acid residues, intermediate lengths of about 100 to about 399 amino acid residues, and longer lengths of about 400 to about 1000 and up to about 3000 amino acid residues. Thus, the XTEN linked to FVII (*e.g.*, heavy chain or light chain) or a targeting moiety can have lengths of about 6, about 12, about 36, about 40, about 42, about 72, about 96, about 144, about 288, about 400, about 500, about 576, about 600, about 700, about 800, about 864, about 900, about 1000, about 1500, about 2000, about 2500, or up to about 3000 amino acid residues in length. In other embodiments, the XTEN sequences is about 6 to about 50, about 50 to about 100, about 100 to 150, about 150 to 250, about 250 to 400, about 400 to about 500, about 500 to about 900, about 900 to 1500, about 1500 to 2000, or about 2000 to about 3000 amino acid residues in length. The precise length of an XTEN polypeptide that can be linked to FVII (*e.g.*, light chain or heavy chain) or a targeting moiety (Ab) can vary without adversely affecting the activity of FVII. In one embodiment, one or more of the XTEN used herein has about 42 amino acids, about 72 amino acids, about 108 amino acids, about 144 amino acids, about 180 amino acids, about 216 amino acids, about 252 amino acids, about 288 amino acids, about 324 amino acids, about 360 amino acids, about 396 amino acids, about 432 amino acids, about 468 amino acids, about 504 amino acids, about 540 amino acids, about 576 amino acids, about 612 amino acids, about 624 amino acids, about 648 amino acids, about 684 amino acids, about 720 amino acids, about 756 amino acids, about 792 amino acids, about 828 amino acids, about 836 amino acids, about 864 amino acids, about 875 amino acids, about 912 amino acids, about 923 amino acids, about 948 amino acids, about 1044 amino acids, about 1140 amino acids, about 1236 amino acids, about 1318 amino acids, about 1332 amino acids, about 1428 amino acids, about 1524 amino acids, about 1620 amino acids, about 1716 amino acids, about 1812 amino acids, about 1908 amino acids, or about 2004 amino acids in length and can be selected from one or more of the XTEN family sequences; *i.e.*, AD, AE, AF, AG, AM, AQ, BC, BD, or any combinations thereof.

In some embodiments, the XTEN polypeptide used in the invention is at least 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of AE42, AG42, AE42_2, AE42_3, AE48, AM48, AE72, AE72_2, AE72_3, AG72, AE108, AG108, AE144, AF144, AE144_2, AE144_3, AG144, AE180, AG180, AE216, AG216, AE252, AG252, AE288, AG288,

AE295, AE324, AG324, AE360, AG360, AE396, AG396, AE432, AG432, AE468, AG468, AE504, AG504, AF504, AE540, AG540, AF540, AD576, AE576, AF576, AG576, AE612, AG612, AE624, AE648, AG648, AG684, AE720, AG720, AE756, AG756, AE792, AG792, AE828, AG828, AD836, AE864, AF864, AG864, AE872, AE884, AM875, AE912, AM923, AM1318, BC864, BD864, AE948, AE1044, AE1140, AE1236, AE1332, AE1428, AE1524, AE1620, AE1716, AE1812, AE1908, AE2004A, AG948, AG1044, AG1140, AG1236, AG1332, AG1428, AG1524, AG1620, AG1716, AG1812, AG1908, AG2004, and any combinations thereof. *See* US 2010-0239554 A1.

In one embodiment, the XTEN sequence is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of AE42, AE864, AE576, AE288, AE144, AG864, AG576, AG288, AG144, and any combinations thereof. In another embodiment, the XTEN sequence is selected from the group consisting of AE42, AE864, AE576, AE288, AE144, AG864, AG576, AG288, AG144, and any combinations thereof. In one embodiment, the XTEN sequence is AE144. In a specific embodiment, the XTEN sequence is AE288. The amino acid sequences for certain XTEN sequences of the invention are shown in TABLE 3.

TABLE 3. XTEN Sequences

XTEN	Amino Acid Sequence
AE42 SEQ ID NO: 116	GAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPASS
AE42_2 SEQ ID NO: 117	TGGGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPASS
AE42_3 SEQ ID NO: 118	GTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPAT
AE72 SEQ ID NO: 119	GAP TSESATPESG PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG TSTEPSEGSAPGASS
AE72_2 SEQ ID NO: 120	GTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSET PGTSESATPESGPGTSTEPSEGSAP
AE72_3 SEQ ID NO: 121	SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGP GTSTEPSEGSAPGTSTEPSEGSAPG

XTEN	Amino Acid Sequence
AE144 SEQ ID NO: 122	GSEPATSGSETPGTSESATPESGPGSEPATSGSETPGSPAGSPTSTE EGTSTEPSEG SAPGSEPATSGSETPGSEPATSGSETPGSEPATSGSETPGTSTEPSE GSAPGTSESA PESGPGSEPATSGSETPGTSTEPSEGSAP
AE144_2 SEQ ID NO: 123	GTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSET PGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPES GPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTS TEE
AE144_3 SEQ ID NO: 124	GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTE EGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSEPATSGSE TPGSEPATSGSETPGSPAGSPTSTEEGTSESATPESGPGTSTEPSEG SAP
AG144 SEQ ID NO: 125	GTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTG PGASPGTSST GSPGASPGTSSTGSPGSSTPSGATGSPGSSPSASTGTGPGASPGTSS TGSPGSSPSA STGTGPGTPGSGTASSSPGSSTPSGATGSP
AE288 SEQ ID NO: 126	GTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSET PGTSESATPESG PGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSE TPGTSESATPES GPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPE SGPGTSESATPE SGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPT STEEGTSTEPSE GSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPS EGSAP
AG288 SEQ ID NO: 127	PGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATG SPGTPGSGTASS SPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGAT GSPGSSPSASTG TGPSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGA TGSPGSSPSAST GTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGSSTPSG ATGSPGSSPSAS TGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASSSPGSSTPS GATGS

XTEN	Amino Acid Sequence
AE576 SEQ ID NO: 128	GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTE EGTSTEPSEGS PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSE TPGSPAGSPTST EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTS TEEGTSTEPSEG SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATP ESGPGSEPATSG SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESAT PESGPGSPAGSP TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEP SEGSAPGTSTEP SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTE PSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSE SATPESGPGSEP ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSP AGSPTSTEEGSP AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAP
AG576 SEQ ID NO: 129	PGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSS TPSGATG SPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGT PGSGTAS SSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPG TPSGTA SSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSP GSSTPSG ATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGATGS PGSSTPS GATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASS SPGASPG TSSTGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTAS SSPGSST PSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGA TGSPGSS TPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGTPGSGT ASSSPGS

XTEN	Amino Acid Sequence
	STPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGS
AE864 SEQ ID NO: 130	GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTST EPSEGSA PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSP AGSPTST EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGT STEPSEG SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPG SEPATSG SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESATPESGP GSPAGSP TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSA PGTSTEP SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGS APGSPAG SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPE SGPGSEP ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPT STEEGSP AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGTSESAT PESGPGS EPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEP SEGSAPG SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAG SPTSTEE GSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSE SATPESG PGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTS TEPSEGS APGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP
AG864 SEQ ID NO: 131	GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSSPGSST PSGATGS PGSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTP GSGTASS SPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGA SPGTSST GSPGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPG SSTPSGA TGSPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSP

XTEN	Amino Acid Sequence
	GTPGSGT ASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTG PGTPGSG TASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATG SPGSSTP SGATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSTPSGAT GSPGSST PSGATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTA SSSPGAS PGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGTPGSGT ASSSPGS STPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPS GATGSPG SSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGTPGS GTASSSP GSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGASP GTSSTGS PGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSASTGTGPGTP GSGTASS SPGSSTPSGATGSPGSSTPSGATGSPGASPGTSSTGSP

In some embodiments wherein the XTEN has less than 100% of its amino acids consisting of 4, 5, or 6 types of amino acid selected from glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P), or less than 100% of the sequence consisting of the sequence motifs from Table 2 or the XTEN sequences of Table 3, the other amino acid residues of the XTEN are selected from any of the other 14 natural L-amino acids, but are preferentially selected from hydrophilic amino acids such that the XTEN sequence contains at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% hydrophilic amino acids. An individual amino acid or a short sequence of amino acids other than glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) may be incorporated into the XTEN to achieve a needed property, such as to permit incorporation of a restriction site by the encoding nucleotides, or to facilitate linking to a payload component, or incorporation of a cleavage sequence. The XTEN amino acids that are not glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) are either interspersed throughout the XTEN sequence, are located within or between the sequence motifs, or are

concentrated in one or more short stretches of the XTEN sequence such as at or near the N- or C-terminus. As hydrophobic amino acids impart structure to a polypeptide, the invention provides that the content of hydrophobic amino acids in the XTEN utilized in the conjugation constructs will typically be less than 5%, or less than 2%, or less than 1% hydrophobic amino acid content. Hydrophobic residues that are less favored in construction of XTEN include tryptophan, phenylalanine, tyrosine, leucine, isoleucine, valine, and methionine. Additionally, one can design the XTEN sequences to contain less than 5% or less than 4% or less than 3% or less than 2% or less than 1% or none of the following amino acids: methionine (to avoid oxidation), asparagine and glutamine (to avoid deamidation). In other embodiments, the amino acid content of methionine and tryptophan in the XTEN component used in the conjugation constructs is typically less than 5%, or less than 2%, and most preferably less than 1%. In other embodiments, the XTEN will have a sequence that has less than 10% amino acid residues with a positive charge, or less than about 7%, or less than about 5%, or less than about 2% amino acid residues with a positive charge, the sum of methionine and tryptophan residues will be less than 2%, and the sum of asparagine and glutamine residues will be less than 5% of the total XTEN sequence.

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

A variety of methods and assays can be employed to determine the physical/chemical properties of proteins comprising the XTEN polypeptide. Such methods include, but are not limited to analytical centrifugation, EPR, HPLC-ion exchange, HPLC-size exclusion, HPLC-

reverse phase, light scattering, capillary electrophoresis, circular dichroism, differential scanning calorimetry, fluorescence, HPLC-ion exchange, HPLC-size exclusion, IR, NMR, Raman spectroscopy, refractometry, and UV/Visible spectroscopy. Additional methods are disclosed in Amau *et al.*, *Prot Expr and Purif* 48, 1-13 (2006).

Additional examples of XTEN polypeptides that can be used according to the present disclosure and are disclosed in US Pat Nos. 7,855,279 and 7,846,445, US Patent Publication Nos. 2009/0092582 A1, 2010/0239554 A1, 2010/0323956 A1, 2011/0046060 A1, 2011/0046061 A1, 2011/0077199 A1, 2011/0172146 A1, 2013/0017997 A1, or 2012/0263701 A1, International Patent Publication Nos. WO 2010091122 A1, WO 2010144502 A2, WO 2010144508 A1, WO 2011028228 A1, WO 2011028229 A1, or WO 2011028344 A2; or US 2012/0178691.

(ii) Fc and single chain Fc (scFc) Region

In certain embodiments, the chimeric molecule comprises at least one heterologous moiety comprising a Fc region. "Fc" or "Fc region" as used herein means a functional neonatal Fc receptor (FcRn) binding partner comprising an Fc domain, variant, or fragment thereof, unless otherwise specified. An FcRn binding partner is any molecule that can be specifically bound by the FcRn receptor with consequent active transport by the FcRn receptor of the FcRn binding partner. Thus, the term Fc includes any variants of IgG Fc that are functional. 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.*, *Nature*, 372:379 (1994), incorporated herein by reference in its entirety). 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. FcRn binding partners include, but are not limited to, whole IgG, the Fc fragment of IgG, and other fragments of IgG that include the complete binding region of FcRn. An Fc can comprise the CH2 and CH3 domains of an immunoglobulin with or without the hinge region of the immunoglobulin. Also included are Fc fragments, variants, or derivatives which maintain the desirable properties of an Fc region in a chimeric molecule, *e.g.*, an increase in half-life, *e.g.*, *in vivo* half-life. Myriad mutants, fragments, variants, and derivatives are described, *e.g.*, in PCT Publication Nos. WO2011/069164, WO2012/006623, WO2012/006635, or WO 2012/006633, all of which are incorporated herein by reference in their entireties. In some embodiments, the chimeric molecule comprises a clotting factor

(e.g., a FVII), a targeting moiety (e.g., a GPIIb/IIIa antibody or antigen-binding molecule thereof), and an Fc region.

In one embodiment, the chimeric molecule comprises a heterologous moiety comprising one genetically fused Fc region or a portion thereof within a single polypeptide chain (i.e., a single-chain Fc (scFc) region). An exemplary single-chain human IgG1 Fc amino acid sequence is provided below (the Gly/Ser linker is underlined):

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGSGGGSGGGSGGGSGDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO:132)

The unprocessed polypeptides comprise at least two immunoglobulin constant regions or portions thereof (e.g., 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 peptide linker. For example, in one 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). In some embodiments, the chimeric molecule comprises a clotting factor (e.g., a FVII), a targeting moiety (e.g., a GPIIb/IIIa antibody or antigen-binding molecule thereof), and an scFc region.

(iii) Albumins

In certain embodiments, the chimeric molecule comprises a heterologous moiety comprising albumin or a functional fragment thereof. Human serum albumin (HSA, or HA), a protein of 609 amino acids in its full-length form, is responsible for a significant proportion of the osmotic pressure of serum and also functions as a carrier of endogenous and exogenous ligands. The term "albumin" as used herein includes full-length albumin or a functional fragment, variant, derivative, or analog thereof. Examples of albumin or the fragments or variants thereof are disclosed in US Pat. Publ. Nos. US2008/0194481, US2008/0004206,

US2008/0161243, US2008/0261877, or US2008/0153751 or PCT Appl. Publ. Nos. WO2008/033413, WO2009/058322, or WO2007/021494, which are incorporated herein by reference in their entireties. An exemplary mature human albumin amino acid sequence is provide below (NCBI Ref. Sequence NP_000468):

RGVFRDDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENC
DKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPPLPRLVRPEVDVMCTAFHDN
EETFLKKYLYEIAARRHPYFYAPELLFFAKRYKAAFTCECQAADKAACLLPKLDELREDEGKASSAKQRL
KCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTQVHTECCHGDLLECADDRADLAKYICE
NQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLP SLAADFVESKDVCKNYAEAKDVFLGMFLYEY
ARRHPDYSVLLLRLLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCLEFEQLGEYK
FQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVS
DRVTKCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPK
ATKEQLKAVMDDFAAFVEKCKADDKETCFAEEGKKLVAASQAALGL (SEQ ID NO:133)

In some embodiments, the chimeric molecule comprises a clotting factor (e.g., a FVII), a targeting moiety (e.g., a GPIIb/IIIa antibody or antigen-binding molecule thereof), and an albumin.

(iv) Albumin Binding Polypeptides and Lipids

In certain embodiments, a heterologous moiety can comprise an albumin binding moiety, which comprises an albumin binding peptide, a bacterial albumin binding domain, an albumin-binding antibody fragment, or any combinations thereof. For example, the albumin binding protein can be a bacterial albumin binding protein, an antibody or an antibody fragment including domain antibodies (*see, e.g.*, U.S. Pat. No. 6,696,245). An albumin binding protein, for example, can be a bacterial albumin binding domain, such as the one of streptococcal protein G (Konig and Skerra (1998) *J. Immunol. Methods* 218, 73-83). Other examples of albumin binding peptides that can be used as conjugation partner are, for instance, those having a Cys-Xaa₁-Xaa₂-Xaa₃-Xaa₄-Cys consensus sequence (SEQ ID NO:134), wherein Xaa₁ is Asp, Asn, Ser, Thr, or Trp; Xaa₂ is Asn, Gln, His, Ile, Leu, or Lys; Xaa₃ is Ala, Asp, Phe, Trp, or Tyr; and Xaa₄ is Asp, Gly, Leu, Phe, Ser, or Thr as described in U.S. Pub. No. US2003/0069395 or Dennis *et al.* (2002) *J. Biol. Chem.* 277, 35035-35043.

Domain 3 from streptococcal protein G, as disclosed by Kraulis *et al.*, *FEBS Lett.*, 378:190-194 (1996) and Linhult *et al.*, *Protein Sci.*, 11:206-213 (2002) is an example of a bacterial albumin-binding domain. Examples of albumin-binding peptides include a series of

peptides having the core sequence DICLPRWGCLW (**SEQ ID NO:135**) such as: RLIEDICLPRWGCLWEDD (**SEQ ID NO:136**); QRLMEDICLPRWGCLWEDDF (**SEQ ID NO:137**); QGLIGDICLPRWGCLWGDSVK (**SEQ ID NO:138**); and GEWWEDICLPRWGCLWEEED (**SEQ ID NO:139**). See, *e.g.*, Dennis *et al.*, *J. Biol. Chem.* 2002, 277: 35035-35043 (2002). Examples of albumin-binding antibody fragments are disclosed in Muller and Kontermann, *Curr. Opin. Mol. Ther.* 9:319-326 (2007); Roovers *et al.*, *Cancer Immunol. Immunother.* 56:303-317 (2007), and Holt *et al.*, *Prot. Eng. Design Sci.*, 21:283-288 (2008), which are incorporated herein by reference in their entireties. An example of such albumin binding moiety is 2-(3-maleimidopropanamido)-6-(4-(4-iodophenyl)butanamido) hexanoate ("Albu" tag) as disclosed by Trusselet *et al.*, *Bioconjugate Chem.* 20:2286-2292 (2009). Fatty acids, in particular long chain fatty acids (LCFA) and long chain fatty acid-like albumin-binding compounds can be used to extend the *in vivo* half-life of chimeric molecules of the invention. An example of a LCFA-like albumin-binding compound is 16-(1-(3-(9-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyloxy)-methyi)-7-sulfo-9H-fluoren-2-ylamino)-3-oxopropyl)-2,5-dioxopyrrolidin-3-ylthio) hexadecanoic acid (see, *e.g.*, WO 2010/140148).

In some embodiments, the chimeric molecule comprises a clotting factor (*e.g.*, a FVII), a targeting moiety (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof), and an albumin binding polypeptide or lipid.

(v) CTP

In certain embodiments, a chimeric molecule disclosed herein comprises at least one heterologous moiety comprising one β subunit of the C-terminal peptide (CTP) of human chorionic gonadotropin or fragment, variant, or derivative thereof. The insertion of one or more CTP peptides into a recombinant protein is known to increase the *in vivo* half-life of that protein. See, *e.g.*, U.S. Pat. No. 5,712,122, incorporated by reference herein in its entirety.

Exemplary CTP peptides include DPRFQDSSSSKAPPPSLPSPSRLPGPSDTPIL (**SEQ ID NO:140**) or SSSSKAPPPSLPSPSRLPGPSDTPILPQ (**SEQ ID NO:141**). See, *e.g.*, U.S. Patent Appl. Publ. No. US 2009/0087411, incorporated by reference. In some embodiments, the chimeric molecule comprises two heterologous moieties that are CTP sequences. In some embodiments, three of the heterologous moieties are CTP sequences. In some embodiments, four of the heterologous moieties are CTP sequences. In some

embodiments, five of the heterologous moieties are CTP sequences. In some embodiments, six or more of the heterologous moieties are CTP sequences.

In some embodiments, the chimeric molecule comprises a clotting factor (*e.g.*, a FVII), a targeting moiety (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof), and a CTP.

(vi) PAS

In other embodiments, at least one heterologous moiety is a PAS sequence. A PAS sequence, as used herein, means an amino acid sequence comprising mainly alanine and serine residues or comprising mainly alanine, serine, and proline residues, the amino acid sequence forming random coil conformation under physiological conditions. Accordingly, the PAS sequence is a building block, an amino acid polymer, or a sequence cassette comprising, consisting essentially of, or consisting of alanine, serine, and proline which can be used as a part of the heterologous moiety in the chimeric molecule. Yet, the skilled person is aware that an amino acid polymer also can form random coil conformation when residues other than alanine, serine, and proline are added as a minor constituent in the PAS sequence.

The term "minor constituent" as used herein means that amino acids other than alanine, serine, and proline can be added in the PAS sequence to a certain degree, *e.g.*, up to about 12%, *i.e.*, about 12 of 100 amino acids of the PAS sequence, up to about 10%, *i.e.*, about 10 of 100 amino acids of the PAS sequence, up to about 9%, *i.e.*, about 9 of 100 amino acids, up to about 8%, *i.e.*, about 8 of 100 amino acids, about 6%, *i.e.*, about 6 of 100 amino acids, about 5%, *i.e.*, about 5 of 100 amino acids, about 4%, *i.e.*, about 4 of 100 amino acids, about 3%, *i.e.*, about 3 of 100 amino acids, about 2%, *i.e.*, about 2 of 100 amino acids, about 1%, *i.e.*, about 1 of 100 of the amino acids.

The amino acids different from alanine, serine and proline can be selected from Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Tyr, and Val.

Under physiological conditions, the PAS sequence stretch forms a random coil conformation and thereby can mediate an increased *in vivo* and/or *in vitro* stability to the chimeric molecule. Since the random coil domain does not adopt a stable structure or function by itself, the biological activity mediated by the activatable clotting factor in the chimeric molecule is essentially preserved. In other embodiments, the PAS sequences that form random coil domain are biologically inert, especially with respect to proteolysis in blood plasma, immunogenicity, isoelectric point/electrostatic behavior, binding to cell surface

receptors or internalization, but are still biodegradable, which provides clear advantages over synthetic polymers such as PEG.

Non-limiting examples of the PAS sequences forming random coil conformation comprise an amino acid sequence selected from the group consisting of ASPAAPAPASPAAPAPSAPA (**SEQ ID NO:142**), AAPASPAPAAAPSAPAPAAAPS (**SEQ ID NO:143**), APSSPSPSAPSSPSPASPSS (**SEQ ID NO:144**), APSSPSPSAPSSPSPASPS (**SEQ ID NO:145**), SSPSAPSPSSPASPSPPSPA (**SEQ ID NO:146**), AASPAAPSAPPAAASPAAPSAPPA (**SEQ ID NO:147**), and ASAAAPAAASAAASAPSAAA (**SEQ ID NO:148**), or any combinations thereof. Additional examples of PAS sequences are known from, *e.g.*, US Pat. Publ. No. 2010/0292130 and PCT Appl. Publ. No. WO2008/155134 A1.

In some embodiments, the chimeric molecule comprises a clotting factor (*e.g.*, a FVII), a targeting moiety (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof), and a PAS.

(vii) HAP

In certain embodiments, at least one heterologous moiety is a glycine-rich homo-amino-acid polymer (HAP). The HAP sequence can comprise a repetitive sequence of glycine, which has at least 50 amino acids, at least 100 amino acids, 120 amino acids, 140 amino acids, 160 amino acids, 180 amino acids, 200 amino acids, 250 amino acids, 300 amino acids, 350 amino acids, 400 amino acids, 450 amino acids, or 500 amino acids in length. In one embodiment, the HAP sequence is capable of extending half-life of a moiety fused to or linked to the HAP sequence. Non-limiting examples of the HAP sequence includes, but are not limited to (Gly)_n, (**SEQ ID NO:149**), (Gly₄Ser)_n (**SEQ ID NO:150**), or Ser(Gly₄Ser)_n (**SEQ ID NO:151**), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In one embodiment, n is 20, 21, 22, 23, 24, 25, 26, 26, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40. In another embodiment, n is 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200. See, *e.g.*, Schlapschy M *et al.*, Protein Eng. Design Selection, 20: 273-284 (2007).

In some embodiments, the chimeric molecule comprises a clotting factor (*e.g.*, a FVII), a targeting moiety (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof), and a HAP.

(viii) Transferrin

In certain embodiments, at least one heterologous moiety is transferrin or a peptide or fragment, variant, or derivative thereof. Any transferrin can be used to make the chimeric molecules of the invention. As an example, wild-type human TF (TF) is a 679 amino acid protein, of approximately 75 KDa (not accounting for glycosylation), with two main domains, N (about 330 amino acids) and C (about 340 amino acids), which appear to originate from a gene duplication. N domain comprises two subdomains, N1 domain and N2 domain, and C domain comprises two subdomains, C1 domain and C2 domain. *See* GenBank accession numbers NM001063, XM002793, M12530, XM039845, XM 039847 and S95936 (www.ncbi.nlm.nih.gov), all of which are herein incorporated by reference in their entirety. In one embodiment, the transferrin heterologous moiety includes a transferrin splice variant. In one example, a transferrin splice variant can be a splice variant of human transferrin, *e.g.*, Genbank Accession AAA61140. In another embodiment, the transferrin portion of the chimeric molecule includes one or more domains of the transferrin sequence, *e.g.*, N domain, C domain, N1 domain, N2 domain, C1 domain, C2 domain or any combinations thereof.

Transferrin transports iron through transferrin receptor (TfR)-mediated endocytosis. After the iron is released into an endosomal compartment and Tf-TfR complex is recycled to cell surface, the Tf is released back extracellular space for next cycle of iron transporting. Tf possesses a long half-life that is in excess of 14-17 days (Li *et al.*, Trends Pharmacol. Sci. 23:206-209 (2002)). Transferrin fusion proteins have been studied for half-life extension, targeted deliver for cancer therapies, oral delivery and sustained activation of proinsulin (Brandsma *et al.*, Biotechnol. Adv., 29: 230-238 (2011); Bai *et al.*, Proc. Natl. Acad. Sci. USA 102:7292-7296 (2005); Kim *et al.*, J. Pharmacol. Exp. Ther., 334:682-692 (2010); Wang *et al.*, J. Controlled Release 155:386-392 (2011)).

In some embodiments, the chimeric molecule comprises a clotting factor (*e.g.*, a FVII), a targeting moiety (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof), and a transferrin.

(ix) PEG

In some embodiments, at least one heterologous moiety is a soluble polymer known in the art, including, but not limited to, polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, or polyvinyl alcohol. In some embodiments, the chimeric molecule comprising a PEG heterologous moiety further comprises a

heterologous moiety selected from an immunoglobulin constant region or portion thereof (*e.g.*, an Fc region), a PAS sequence, HES, and albumin, fragment, or variant thereof. In still other embodiments, the chimeric molecule comprises an activatable clotting factor or fragment thereof and a PEG heterologous moiety, wherein the chimeric molecule further comprises a heterologous moiety selected from an immunoglobulin constant region or portion thereof (*e.g.*, an Fc moiety), a PAS sequence, HES, and albumin, fragment, or variant thereof. In yet other embodiments, the chimeric molecule comprises a clotting factor or fragment thereof, a second clotting factor or fragment thereof, and a PEG heterologous moiety, wherein the chimeric molecule further comprises a heterologous moiety selected from an immunoglobulin constant region or portion thereof (*e.g.*, an Fc moiety), a PAS sequence, HES, and albumin, fragment, or variant thereof.

In other embodiments, the chimeric molecule comprises a clotting factor or fragment thereof, a synthetic procoagulant polypeptide, and a PEG heterologous moiety, wherein the chimeric molecule further comprises a heterologous moiety selected from an immunoglobulin constant region or portion thereof (*e.g.*, an Fc region), a PAS sequence, HES, and albumin, fragment, or variant thereof. In other embodiments, the chimeric molecule comprises two synthetic procoagulant peptides and a PEG heterologous moiety, wherein the chimeric molecule further comprises a heterologous moiety selected from the group consisting of an immunoglobulin constant region or portion thereof (*e.g.*, an Fc region), a PAS sequence, HES, and albumin, fragment, or variant thereof. In yet another embodiment, the chimeric molecule comprises a clotting factor or fragment thereof, a clotting factor cofactor (*e.g.*, Tissue Factor if the clotting factor is Factor VII), and a PEG heterologous moiety, wherein the chimeric molecule further comprises a heterologous moiety selected from an immunoglobulin constant region or portion thereof (*e.g.*, an Fc region), a PAS sequence, HES, and albumin, fragment, or variant thereof.

The polymer can be of any molecular weight, and can be branched or unbranched. For polyethylene glycol, in one embodiment, the molecular weight is between about 1 kDa and about 100 kDa for ease in handling and manufacturing. Other sizes can be used, depending on the desired profile (*e.g.*, the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a protein or analog). For example, the polyethylene glycol can have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500,

10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

In some embodiments, the polyethylene glycol can have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Pat. No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), each of which is incorporated herein by reference in its entirety.

The number of polyethylene glycol moieties attached to each chimeric molecule of the invention (*i.e.*, the degree of substitution) can also vary. For example, the PEGylated chimeric molecule can be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al.*, *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

In some embodiments, the chimeric molecule can be PEGylated. A PEGylated chimeric molecule comprises at least one polyethylene glycol (PEG) molecule. In other embodiments, the polymer can be water-soluble. Non-limiting examples of the polymer can be poly(alkylene oxide), poly(vinyl pyrrolidone), poly(vinyl alcohol), polyoxazoline, or poly(acryloylmorpholine). Additional types of polymer-conjugation to clotting factors are disclosed in U.S. Patent No. 7,199,223. See also, Singh *et al.* *Curr. Med. Chem.* 15:1802-1826 (2008).

There are a number of PEG attachment methods available to those skilled in the art, for example Malik F *et al.*, *Exp. Hematol.* 20:1028-35 (1992); Francis, *Focus on Growth Factors* 3(2):4-10 (1992); European Pat. Pub. Nos. EP0401384, EP0154316, and EP0401384; and International Pat. Appl. Pub. Nos. WO92/16221 and WO95/34326. .

In some embodiments, the chimeric molecule comprises a clotting factor (*e.g.*, a FVII), a targeting moiety (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof), and a PEG.

(x) HES

In certain embodiments, at least one heterologous moiety is a polymer, *e.g.*, hydroxyethyl starch (HES) or a derivative thereof. Hydroxyethyl starch (HES) is a derivative of naturally occurring amylopectin and is degraded by alpha-amylase in the body. HES is a substituted derivative of the carbohydrate polymer amylopectin, which is present in corn starch at a concentration of up to 95% by weight. HES exhibits advantageous biological properties and is used as a blood volume replacement agent and in hemodilution therapy in the clinics (Sommermeyer *et al.*, *Krankenhauspharmazie*, 8(8), 271-278 (1987); and Weidler *et al.*, *Arzneim.-Forschung/Drug Res.*, 41, 494-498 (1991)).

Amylopectin contains glucose moieties, wherein in the main chain alpha-1,4-glycosidic bonds are present and at the branching sites alpha-1,6-glycosidic bonds are found. The physical-chemical properties of this molecule are mainly determined by the type of glycosidic bonds. Due to the nicked alpha-1,4-glycosidic bond, helical structures with about six glucose-monomers per turn are produced. The physico-chemical as well as the biochemical properties of the polymer can be modified via substitution. The introduction of a hydroxyethyl group can be achieved via alkaline hydroxyethylation. By adapting the reaction conditions it is possible to exploit the different reactivity of the respective hydroxy group in the unsubstituted glucose monomer with respect to a hydroxyethylation. Owing to this fact, the skilled person is able to influence the substitution pattern to a limited extent.

HES is mainly characterized by the molecular weight distribution and the degree of substitution. The degree of substitution, denoted as DS, relates to the molar substitution, is known to the skilled people. See Sommermeyer *et al.*, *Krankenhauspharmazie*, 8(8), 271-278 (1987), as cited above, in particular p. 273.

In one embodiment, hydroxyethyl starch has a mean molecular weight (weight mean) of from 1 to 300 kD, from 2 to 200kD, from 3 to 100 kD, or from 4 to 70kD. Hydroxyethyl starch can further exhibit a molar degree of substitution of from 0.1 to 3, preferably 0.1 to 2, more preferred, 0.1 to 0.9, preferably 0.1 to 0.8, and a ratio between C2:C6 substitution in the range of from 2 to 20 with respect to the hydroxyethyl groups. A non-limiting example of HES having a mean molecular weight of about 130 kD is a HES with a degree of substitution of 0.2 to 0.8 such as 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, or 0.8, preferably of 0.4 to 0.7 such as 0.4, 0.5, 0.6, or 0.7. In a specific embodiment, HES with a mean molecular weight of about 130 kD is VOLUVEN[®] from Fresenius. VOLUVEN[®] is an artificial colloid, employed, *e.g.*, for volume replacement used in the therapeutic indication for therapy and prophylaxis of hypovolemia. The characteristics of VOLUVEN[®] are a mean molecular weight of 130,000+/-20,000 D, a

molar substitution of 0.4 and a C2:C6 ratio of about 9:1. In other embodiments, ranges of the mean molecular weight of hydroxyethyl starch are, *e.g.*, 4 to 70 kD or 10 to 70 kD or 12 to 70 kD or 18 to 70 kD or 50 to 70 kD or 4 to 50 kD or 10 to 50 kD or 12 to 50 kD or 18 to 50 kD or 4 to 18 kD or 10 to 18 kD or 12 to 18 kD or 4 to 12 kD or 10 to 12 kD or 4 to 10 kD. In still other embodiments, the mean molecular weight of hydroxyethyl starch employed is in the range of from more than 4 kD and below 70 kD, such as about 10 kD, or in the range of from 9 to 10 kD or from 10 to 11 kD or from 9 to 11 kD, or about 12 kD, or in the range of from 11 to 12 kD) or from 12 to 13 kD or from 11 to 13 kD, or about 18 kD, or in the range of from 17 to 18 kD or from 18 to 19 kD or from 17 to 19 kD, or about 30 kD, or in the range of from 29 to 30, or from 30 to 31 kD, or about 50 kD, or in the range of from 49 to 50 kD or from 50 to 51 kD or from 49 to 51 kD.

In certain embodiments, the heterologous moiety can be a mixture of hydroxyethyl starches having different mean molecular weights and/or different degrees of substitution and/or different ratios of C2: C6 substitution. Therefore, mixtures of hydroxyethyl starches can be employed having different mean molecular weights and different degrees of substitution and different ratios of C2: C6 substitution, or having different mean molecular weights and different degrees of substitution and the same or about the same ratio of C2:C6 substitution, or having different mean molecular weights and the same or about the same degree of substitution and different ratios of C2:C6 substitution, or having the same or about the same mean molecular weight and different degrees of substitution and different ratios of C2:C6 substitution, or having different mean molecular weights and the same or about the same degree of substitution and the same or about the same ratio of C2:C6 substitution, or having the same or about the same mean molecular weights and different degrees of substitution and the same or about the same ratio of C2:C6 substitution, or having the same or about the same mean molecular weight and the same or about the same degree of substitution and different ratios of C2: C6 substitution, or having about the same mean molecular weight and about the same degree of substitution and about the same ratio of C2:C6 substitution.

In some embodiments, the chimeric molecule comprises a clotting factor (*e.g.*, a FVII), a targeting moiety (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof), and a HES.

(xi) PSA

In certain embodiments, at least one heterologous moiety is a polymer, *e.g.*, polysialic acids (PSAs) or a derivative thereof. Polysialic acids (PSAs) are naturally occurring unbranched polymers of sialic acid produced by certain bacterial strains and in mammals in certain cells Roth J., et al. (1993) in *Polysialic Acid: From Microbes to Man*, eds. Roth J., Rutishauser U., Troy F. A. (Birkhäuser Verlag, Basel, Switzerland), pp 335–348. They can be produced in various degrees of polymerization from n =about 80 or more sialic acid residues down to n =2 by limited acid hydrolysis or by digestion with neuraminidases, or by fractionation of the natural, bacterially derived forms of the polymer. The composition of different polysialic acids also varies such that there are homopolymeric forms *i.e.* the α -2,8-linked polysialic acid comprising the capsular polysaccharide of *E. coli* strain K1 and the group-B *meningococci*, which is also found on the embryonic form of the neuronal cell adhesion molecule (N-CAM). Heteropolymeric forms also exist—such as the alternating α -2,8 α -2,9 polysialic acid of *E. coli* strain K92 and group C polysaccharides of *N. meningitidis*. Sialic acid can also be found in alternating copolymers with monomers other than sialic acid such as group W135 or group Y of *N. meningitidis*. Polysialic acids have important biological functions including the evasion of the immune and complement systems by pathogenic bacteria and the regulation of glial adhesiveness of immature neurons during fetal development (wherein the polymer has an anti-adhesive function) Cho and Troy, *P.N.A.S., USA*, 91 (1994) 11427-11431, although there are no known receptors for polysialic acids in mammals. The α -2,8-linked polysialic acid of *E. coli* strain K1 is also known as ‘colominic acid’ and is used (in various lengths) to exemplify the present disclosure. Various methods of attaching or conjugating polysialic acids to a polypeptide have been described (for example, see U.S. Pat. No. 5,846,951; WO-A-0187922, and US 2007/0191597 A1, which are incorporated herein by reference in their entireties.

In some embodiments, the chimeric molecule comprises a clotting factor (*e.g.*, FVII), a targeting moiety (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof), and a PSA.

(xii) Clearance Receptors

In certain embodiments, the *in vivo* half-life of a chimeric molecule of the invention can be extended where the chimeric molecule comprises at least one heterologous molecule

comprising a clearance receptor, fragment, variant, or derivative thereof. In specific embodiments wherein the chimeric molecule comprises Factor X, soluble forms of clearance receptors, such as the low density lipoprotein-related protein receptor LRP1, or fragments thereof, can block binding of Factor X to clearance receptors and thereby extend its *in vivo* half-life.

LRP1 is a 600 kDa integral membrane protein that is implicated in the receptor-mediated clearance of a variety of proteins, such as FVIII or X. See, *e.g.*, Narita et al., Blood 91:555-560 (1998); Lenting *et al.*, Haemophilia 16:6-16 (2010). The amino acid sequence of an exemplary human LRP1 protein is provided below (signal peptide underlined and transmembrane segment boldened; NCBI Reference Sequence: CAA32112):

MLTPPLLLLLPLLSALVAAAIDAPKTCSPKQFACRDQITCISKGWRCDCGERDCPDGSDEAPEICPQSK
AQRCQPNEHNCLGTELCVPMSRLCNGVQDCMDGSDEGPHCRELQGNCSRLGCQHHCVPTLDGPTCYCN
SSFQLQADGKTCCKDFDECSVYGTCSQLCTNTDGSFICGVEGYLLQPDNRSCAKNEPVDPRPPVLLIA
NSQNILATYLSGAQVSTITPTSTRQTTAMDFSANETVCWVHVGDSSAAQTQLKCARMPGLKGFVDEHT
INISLSLHHVEQMAIDWLTGNFYFVDDIDDRIFVCNRRNGDTCVTLLDLELYNPKGIALDPAMGKVFFT
DYGQIPKVERCDMDGQNRTKLVDISKIVFPHGITLDLVSRLVYWADAYLDYIEVVDYEGKGRQTI IQGI
LIEHLYGLTVFENYLYATNSDNANAQQKTSVIRVNRFNSTEYQVVTRVDKGGALHIYHQRQPRVRSH
ACENDQYGKPGGCSIDICLLANSHKARTCRCSRGSFSLGSDGKSCCKPEHELFLVYGKGRPGIIRGMDMG
AKVPDEHMIPIENLMNPRALDFHAETGFIYFADTTSYLIGRQKIDGTERETILKDGHNVEGVAVDWM
GDNLWYTDGPKKTIISVARLEKAAQTRKTLIEGKMTHPRAIVVDPLNGWYWDWEEDPKDSRRGRLE
RAWMDGSHRDI FVTSKTVLWPNGLSLDI PAGRLYWVDAFYDRIETILLNGTDRKIVYEGPELNHAFGL
CHHGNLYFWTEYRSGSVYRLERGVGGAPPTVTLLRSEPPIFEIRMYDAQQQQVGTNKRNVNNGCCSS
LCLATPGSRQCACAEDQVLDADGVTCLANPSYVPPPQCQPGEFACANSRCIQRWKCDGNDCLDNDSD
EAPALCHQHTCPSDRFKCENNRCPINRWLCDGNDNCGNSEDESNATCSARTCPPNQFSCASGRCPIS
WTCDLDDDCGDRSDESASCAYPCTCFPLTQFTCNNGRCININWRCDNDNDCGNSDEAGCSHSCSSTQF
KCNSGRCPHEWTCGDNDNCGDYSDETHANCTNQAATRPGGCHTDEFQCRLDGLCIPLRWRCDGDTDC
MDSSDEKSCGEGVTHVCDPSVKFGCKDSARCISKAWVCDGNDNDCEDNSDEENCESLACRPPSHPCANNT
SVCPLPPDKLCDGNDNDCGDSDEGELCDQC SLNNGGCSHNCSVAPGEGIVCSCPLGMELGPDNHTCQIQ
SYCAKHLKCSQKCDQNKFSVKCSYEGWVLEPDGESCRSLDPFKPFIIFSNRHEIRRIDLHKGDYSVL
VPGLRNTIALDFHLSQSALYWTDVVEDKIYRGKLLDNGALTSFEVVIQYGLATPEGLAVDWIAGNIYW
VESNLDQIEVAKLDGTLRTTLLAGDIEHPRAIALDPRDGILFWTDWDASLPRIEAAASMSGAGRRTVHR
ETGSGGWPNGLTVDYLEKRILWIDARSDAIYSARYDGS GHMEVLRGHEFLSHPFVAVTLYGGEVYWTDW
RTNTLAKANKWTGHNVTVVQRTNTQPFDLQVYHPSRQPMAPNPCEANGGQGPCSHLCLINYNRTVSCA
CPHLMKLHKDNTTCYEFKKFLLYARQMEIRGVLDAPYYNYIISFTVPDIDNVTVLDYDAREQRVYWS
DVRTQAIKRAFINGTGVETVVSADLPNAHGLAVDWVSRNLFWTSYDTNKKQINVARLDGSFKNAVVG
LEQPHGLVHPLRGKLYWTDGDNISMANMDGSNRTLLFSGQKGPVGLAIDFPESKLYWISSGNHTINR
CNLDGSGLEVIDAMRSQLGKATALAIMGDKLWWADQVSEKMGTCADGSGSVLRNSTTLVMHMKVY
DESIQLDHKGTNPCSVNNGDCSQLCLPTSETTRSCMCTAGYSLRSGQQACEGVGSFLLYSVHEGIRGI
PLDPNDKSDALVPVSGTSLAVGIDFHAENDTIYWVDMGLSTISRAKRDQTWREDDVTNGIGRVEGI
DWIAGNIYWTDQGFVDIEVARLNGSFRYVVISQGLDKPRAITVHPEKGYLFWTEWGYPRIERSRLDG
TERVVLVNVSISWPNGISVDYQDGKLYWCDARTDKIERIDLETGENREVVLSNNMDMFSVSVFEDFI
YWSDRTHANGSIKRGSKDNATDSVPLRTGIGVQLKDIKVFNRDRQKGTNVCANGGCGQLCLYRGRG
QRACACAHGMLAEDGASCREYAGYLLYSERTILKSIHLSDERNLNAPVQPFEDPEHMKNVIALAFDYR

AGTSPGTPNRIFFSDIHFGNIQQINDDGSRITIVENVGSVEGLAYHRGWDTLWTSYTTSTITRHTV
DQTRPGAFAFERETVITMSGDDHPRAFLVLEDCQNLMFWTNWNEQHPSIMRAALSGANVLTLLIEKDIRTPN
GLAIDHRAEKLYFSDATLDKIERCEYDGSYHRYVILKSEPVHPPGLAVYGEHIFWTDWVRRRAVQRANKH
VGSNMKLLRVLDIPQQPMGIIAVANDTNSCELSPCRINNGGCQDLCLLTHQGHVNCSCRGGRILQDDL
CRAVNSSCRAQDEFECANGECINFSLTCDGVPHCKDKSDEKPSYCNSRRCKKTRQCSNGRCVSNMLW
CNGADDCCGDSDEIPCNKTACGVGEFRCDGTICIGNSSRCNQFVDCEDASDEMNC SATDCSSYFRLGV
KGVLFQPCERTSLCYAPSWVCDGANDCGDYSDERDCPGVKRPRCPLNYFACPSGRCIPMSWTCDEKEDD
CEHGEDETHCNKFCSEAQFECQNHRCISKQWLCGSDDCGDSDEAAHCEGKTCGPSSFSCPGTHVCV
PERWLCGDGDKCADGADESIAAGCLYNSTCDDREFMCQNRQCIPKHFVCDHHRDCADGSDESPECEYP
TCGPSEFRCANGRCLSSRQWECGDGENDCHDQSDAPKNPHCTSPHKKCNASSQFLCSSGRCAEALLC
NGQDDCGDSSDERGCHINECLSRKLSGCSQDCEDLKIGFKCRCPGFRLKDDGRTCADVDECSTTFPC
SQRCINTHGSYKCLVEGYAPRGDPHSCKAVTDEEPFLIFANRYYLRLKLNLDGSNYTLLKQGLNNAV
ALDFDYREQMIYWTDVTTQGS MIRRMHLNGSNVQVLHRTGLSNPDGLAVDWVGGNLYWCDKGRDTIEV
SKLNGAYRTVLVSSGLREPRALVVDVQNGYLYWTDWGDHSLIGRIGMDGSSRSVIVDTKITWPNGLTL
DYVTERIYWADAREDYIEFASLDGSNRHVLSQDIPHIFALTLFEDYVYWTDWETKSINRAHKTGTGN
KTLLISTLHRPMDLHVHFHALRQPDVPNHPCVNNGGCSNLCLLSPGGGHKACPTNFYLGSDGRTCVS
NCTASQFVCKNDKCI PFWWKCDTEDDCGDHSDEPPDCPEFKCRPGQFQCSTGICTNPAFICDGDNDCCQ
DNSDEANCDIHVCLPSQFKCTNTNRCIPGIFRCNGQDNCGDGEDERDCPEVTCAPNQFQCSITKRCIP
RVWVCDRDNDCVDGSDEPANCTQMTGCVDEFRCCKDSGRCIPARWKCDGEDDCGDSDEPKEECDERTC
EPYQFRCKNNRCVPGRWQCDYDNDCGDNSDEESCTPRPCSESEFSCANGRCIAGRWKCDGDHDCADGS
DEKDC TPRCDMDQFQCKSGHCIPLRWRCDADADCMGDSDEEACGTGVRTCPLDEFQCNTLCKPLAWK
CDGEDDCGDNSENPEECARFVCPNRPFRCKNDRVCLWIGRQCDGTDNCGDGTDEEDCEPPTAHTTH
CKDKKEFLCRNQRLSSSLRCNMFDDCGDSDEEDCSIDPKLTSCATNASICGDEARCVRTEKAAYCA
CRSGFHTVPGQPGCQDINECLRFGTCSQLCNNTKGGHLCSCARNFMKTHNTCKAEGSEYQVLYIADDN
EIRSLFPGHPSAYEQAFQGDSESVRIDAMDVHVKAAGRVYWTNWHTGTISYRSLPPAAPPTTSNRHRRQ
IDRGVTHLNIISGLKMPRGIAIDWVAGNVYWTDSGRDVIEVAQMKGENRKTLSIGMIDEPHAIVVDPLR
GTMYSWSDWGNHPKIETAAMDGTLRETLVQDNIQWPTGLAVDYHNERLYWADAKLSVIGSIRLNGTDPI
VAADSKRGLSHPFSDIVFEDYIYGVTYINN RVFKIHKFHGSPLVNL TGGLSHASDVVLYHQHKQPEVT
NPCDRKKCEWLCLLSPSGPVCTCPNGKRLDNGTCVPVPSPTPPDAPRPGTCNLQCFNGGSCFLNARR
QPKCRCQPRYTGDKCELDQCWEHCRNGGTCAASPSGMPTCRCPTGFTGPKCTQQVCAGYCANNSTCTV
NQGNQPQCRCLPGFLGDRCQYRQCSGYCENFGTCQMAADGSRQCRCTAYFEGSRCEVNKCSRCLEGAC
VVNKQSGDVTNCNCTDGRVAPSCLTVCVGHCSNGGSC TMNSKMMPECQCPHMTGPRCEEHVFSQQQPGH
IAS**ILIPLLLLLLLLVLVAGVFWY**KRRVQGAQGFQHQRM TNGAMNVEIGNPTYKMYEGGEPPDVGGLL
DADFALDPDKPTNFTNPVYATLYMGGHGSRHSLASTDEKRELLGRGPEDEIGDPLA (**SEQ ID**
NO: 152)

Other suitable clearance receptors are, *e.g.*, LDLR (low-density lipoprotein receptor), VLDLR (very low-density lipoprotein receptor), and megalin (LRP-2), or fragments thereof. See, *e.g.*, Bovenschen *et al.*, *Blood* 106:906-912 (2005); Bovenschen, *Blood* 116:5439-5440 (2010); Martinelli *et al.*, *Blood* 116:5688-5697 (2010).

In some embodiments, the chimeric molecule comprises a clotting factor (*e.g.*, a FVII), a targeting moiety (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof), and a clearance receptor, fragment, variant, or derivative thereof.

II. Linkers

The term "linker" or "linker moiety" (represented as L, L1, or L2 in the formulas disclosed herein) refers to a peptide or polypeptide sequence (*e.g.*, a synthetic peptide or polypeptide sequence), or a non-peptide linker for which its main function is to connect two domains in a linear amino acid sequence of a polypeptide chain, for example, two heterologous moieties in a chimeric molecule of the invention. Accordingly, in some embodiments, linkers are interposed between two heterologous moieties, between a heterologous moiety and a targeting moiety, which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein), between a clotting factor (either the heavy chain or the light chain) and a targeting moiety, which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein), or between a clotting factor (either the heavy chain or the light chain) and a heterologous moiety.

When multiple linkers are present in a chimeric molecule of the invention, each of the linkers can be the same or different. Generally, linkers provide flexibility to the chimeric molecule. Linkers are not typically cleaved; however in certain embodiments, such cleavage can be desirable. Accordingly, in some embodiments a linker can comprise one or more protease-cleavable sites, which can be located within the sequence of the linker or flanking the linker at either end of the sequence of the linker.

In some embodiments, the chimeric molecule comprises one or more linkers, wherein one or more of the linkers comprise a peptide linker. In other embodiments, one or more of the linkers comprise a non-peptide linker. In some embodiments, the peptide linker can comprise at least two amino, at least three, at least four, at least five, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, or at least 100 amino acids. In other embodiments, the peptide linker can comprise at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, or at least 1,000 amino acids. In some embodiments, the peptide linker can comprise at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 amino acids. In certain embodiments, the peptide linker can comprise 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 amino acids.

The peptide linker can comprise 1-5 amino acids, 1-10 amino acids, 1-20 amino acids, 1-30 amino acids, 5-25 amino acids, 5-30 amino acids, 10-30 amino acids, 10-50 amino acids, 50-100 amino acids, 100-200 amino acids, 200-300 amino acids, 300-400 amino acids, 400-500 amino acids, 500-600 amino acids, 600-700 amino acids, 700-800 amino acids, 800-900 amino acids, 900-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600, 1600-1700, 1700-1800, 1800-1900, or 1900-2000 amino acids.

Examples of peptide linkers are well known in the art, for example peptide linkers according to the formula $[(\text{Gly})_x\text{-Ser}_y]_z$ where x is from 1 to 4, y is 0 or 1, and z is from 1 to 50 (**SEQ ID NO:153**). In certain embodiments z is from 1 to 6. In one embodiment, the peptide linker comprises the sequence G_n , where n can be an integer from 1 to 100 (**SEQ ID NO:249**). In a specific embodiment, the specific embodiment, the sequence of the peptide linker is GGGG (**SEQ ID NO:154**). The peptide linker can comprise the sequence $(GA)_n$ (**SEQ ID NO:163**). The peptide linker can comprise the sequence $(GGS)_n$ (**SEQ ID NO:155**). In other embodiments, the peptide linker comprises the sequence $(GGGS)_n$ (**SEQ ID NO:156**). In still other embodiments, the peptide linker comprises the sequence $(GGS)_n(GGGGS)_n$ (**SEQ ID NO:157**). In these instances, n can be an integer from 1-100. In other instances, n can be an integer from 1-20, *i.e.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. Examples of linkers include, but are not limited to, GGG, SGGSGGS (**SEQ ID NO:158**), GGSGGSGGSGGSGGG (**SEQ ID NO:159**), GGSGGSGGGSGGGGS (**SEQ ID NO:160**), GGSGGSGGSGGSGGSGGS (**SEQ ID NO:161**), or GGGGSGGGSGGGGS (**SEQ ID NO:162**). In other embodiments, the linker is a poly-G sequence $(GGGG)_n$, where n can be an integer from 1-100 (**SEQ ID NO:164**).

An exemplary Gly/Ser peptide linker comprises the amino acid sequence $(\text{Gly}_4\text{Ser})_n$ (**SEQ ID NO:250**), wherein n is an integer that is the same or higher than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 46, 50, 55, 60, 70, 80, 90, or 100. In one embodiment, $n=1$, *i.e.*, the linker is (Gly_4Ser) (**SEQ ID NO:165**). In one embodiment, $n=2$, *i.e.*, the linker is $(\text{Gly}_4\text{Ser})_2$ (**SEQ ID NO:166**). In another embodiment, $n=3$, *i.e.*, the linker is $(\text{Gly}_4\text{Ser})_3$ (**SEQ ID NO:167**). In another embodiment, $n=4$, *i.e.*, the linker is $(\text{Gly}_4\text{Ser})_4$ (**SEQ ID NO:168**). In another embodiment, $n=5$, *i.e.*, the linker is $(\text{Gly}_4\text{Ser})_5$ (**SEQ ID NO:169**). In yet another embodiment, $n=6$, *i.e.*, the linker is $(\text{Gly}_4\text{Ser})_6$ (**SEQ ID NO:170**). In another embodiment, $n=7$, *i.e.*, the linker is $(\text{Gly}_4\text{Ser})_7$ (**SEQ ID NO:171**). In yet another embodiment, $n=8$, *i.e.*, the linker is $(\text{Gly}_4\text{Ser})_8$ (**SEQ ID NO:172**). In another embodiment,

$n=9$, *i.e.*, the linker is $(\text{Gly}_4\text{Ser})_9$ (**SEQ ID NO:173**). In yet another embodiment, $n=10$, *i.e.*, the linker is $(\text{Gly}_4\text{Ser})_{10}$ (**SEQ ID NO:174**).

Another exemplary Gly/Ser peptide linker comprises the amino acid sequence $\text{Ser}(\text{Gly}_4\text{Ser})_n$ (**SEQ ID NO:251**), wherein n is an integer that is the same or higher than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 46, 50, 55, 60, 70, 80, 90, or 100. In one embodiment, $n=1$, *i.e.*, the linker is $\text{Ser}(\text{Gly}_4\text{Ser})$ (**SEQ ID NO:175**). In one embodiment, $n=2$, *i.e.*, the linker is $\text{Ser}(\text{Gly}_4\text{Ser})_2$ (**SEQ ID NO:176**). In another embodiment, $n=3$, *i.e.*, the linker is $\text{Ser}(\text{Gly}_4\text{Ser})_3$ (**SEQ ID NO:177**). In another embodiment, $n=4$, *i.e.*, the linker is $\text{Ser}(\text{Gly}_4\text{Ser})_4$ (**SEQ ID NO:178**). In another embodiment, $n=5$, *i.e.*, the linker is $\text{Ser}(\text{Gly}_4\text{Ser})_5$ (**SEQ ID NO:179**). In yet another embodiment, $n=6$, *i.e.*, the linker is $\text{Ser}(\text{Gly}_4\text{Ser})_6$ (**SEQ ID NO:180**). In yet another embodiment, $n=7$, *i.e.*, the linker is $\text{Ser}(\text{Gly}_4\text{Ser})_7$ (**SEQ ID NO:181**). In yet another embodiment, $n=8$, *i.e.*, the linker is $\text{Ser}(\text{Gly}_4\text{Ser})_8$ (**SEQ ID NO:182**). In yet another embodiment, $n=9$, *i.e.*, the linker is $\text{Ser}(\text{Gly}_4\text{Ser})_9$ (**SEQ ID NO:183**). In yet another embodiment, $n=10$, *i.e.*, the linker is $\text{Ser}(\text{Gly}_4\text{Ser})_{10}$ (**SEQ ID NO:184**).

In certain embodiments, said Gly/Ser peptide linker can be inserted between two other sequences of the peptide linker (*e.g.*, any of the peptide linker sequences described herein). In other embodiments, a Gly/Ser peptide linker is attached at one or both ends of another sequence of the peptide linker (*e.g.*, any of the peptide linker sequences described herein). In yet other embodiments, two or more Gly/Ser linkers are incorporated in series in a peptide linker. In one embodiment, a peptide 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 $(\text{Gly}_4\text{Ser})_n$) (**SEQ ID NO:165**).

A particular type of linker which can be present in a heterologous moiety, for example an activatable clotting factor, is herein referred to as a "cleavable linker" which comprises a heterologous protease-cleavage site (*e.g.*, a factor XIa or thrombin cleavage site) that is not naturally occurring in the clotting factor and which can include additional linkers on either the N terminal of C terminal or both sides of the cleavage site. Exemplary locations for such sites include, *e.g.*, placement between a heavy chain of a clotting factor zymogen and a light chain of a clotting factor zymogen.

Peptide 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.

III. Protease Cleavage Site

In some embodiments, a chimeric molecule can comprise a protease cleavage site linking, for example, a light chain of a clotting factor zymogen and a heavy chain of the clotting factor zymogen (*e.g.*, FVII). A protease-cleavage site linking a light chain of a clotting factor zymogen and a heavy chain of the clotting factor zymogen can be selected from any protease-cleavage site known in the art. In one embodiment, the protease-cleavage site is cleaved by a protease selected from the group consisting of factor XIa, factor XIIa, kallikrein, factor VIIa, factor IXa, factor Xa, factor IIa (thrombin), and any combinations thereof. The protease-cleavage sites allow the light chain and the heavy chain of the clotting factor to be cleaved and dissociated from each other at the site of injury. Exemplary FXIa cleavage sites include, *e.g.*, KLTR (**SEQ ID NO:185**), DFTR (**SEQ ID NO:186**), TQSFNDFTR (**SEQ ID NO:187**) and SVSQTSKLTR (**SEQ ID NO:188**). Exemplary thrombin cleavage sites include, *e.g.*, DFLAEGGGVR (**SEQ ID NO:189**), TTKIKPR (**SEQ ID NO:190**), LVPRG (**SEQ ID NO:191**) and ALRPR (**SEQ ID NO:192**).

In some embodiments, the protease-cleavage site can be combined with an intracellular processing site for efficient cleavage and activation. For example, an activatable clotting factor in the chimeric molecule can comprise a heterodimer, which comprises a light chain of a clotting factor associated with a heavy chain of the clotting factor by a covalent bond, wherein the N-terminus of the heavy chain of the clotting factor is linked to a protease-cleavage site. The protease-cleavage site can be cleaved off at the site of coagulation, thus activating the clotting factor. Such constructs can be designed by inserting an intracellular processing site between the light chain of the clotting factor zymogen and the protease-cleavage site, which is linked to the heavy chain of the clotting factor zymogen. The intracellular processing site inserted therein can be processed (cleaved) by an intracellular processing enzyme upon expression in a host cell, thereby allowing formation of a zymogen-like heterodimer.

Examples of the intracellular processing enzymes include furin, a yeast Kex2, PCSK1 (also known as PC1/Pc3), PCSK2 (also known as PC2), PCSK3 (also known as furin or PACE), PCSK4 (also known as PC4), PCSK5 (also known as PC5 or PC6), PCSK6 (also

known as PACE4), or PCSK7 (also known as PC7/LPC, PC8, or SPC7). Other processing sites are known in the art. In constructs that include more than one processing or cleavage site, it will be understood that such sites can be the same or different.

E. Exemplary Chimeric Molecules

In one embodiment, the chimeric molecule comprises, consists essentially of, or consists of, a polypeptide that has an amino acid sequence that is at least 60% , at least 65% , at least 70%, at least 75% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in SEQ ID NO:74.

```

1   ANAFLEELRP  GSLERECKEE  QCSFEEAREI  FKDAERTKLF  WISYSDGDQC
51  ASSPCQNGGS  CKDQLQSYIC  FCLPAFEGRN  CETHKDDQLI  CVNENGGCEQ
101 YCSDHTGTKR  SCRCHGYSL  LADGVSTPT  VEYPCGKIPI  LEKRNASKPQ
151 GRIVGGKVCP  KGECPWQVLL  LVNGAQLCGG  TLINTIWVVS  AAHCFDKIKN
201 WRNLI AVLGE  HDLSEHDGDE  QSRRVAQVII  PSTYVPGETT  HDIALRLRHQ
251 PVVLT DHVVP  LCLPERTFSE  RTLAFVRFSL  VSGWGQLLDR  GATALELMVL
301 NVPRLM TQDC  LQQSRKVGDS  PNITEYMFCA  GYSDGSKDSC  KGDSGGPHAT
351 HYRG TWYLTG  IVSWGQGCAT  VGHFGVYTRV  SQYIEWLQKL  MRSEPRPGVL
401 LRAPFP GGGG  SGGGSGGGG  SGGGSGGGG  SGGGSGEIVM  TQSPATLSVS
451 PGERATLSCR  ASSSVNYMYW  YQKPGQAPR  LLIYYTSNLA  PGIPARFSGS
501 GSGTEFTLTI  SSLQSEDFAV  YYCQFSSSP  WTFGQGTKVE  IKRTVAAPSV
551 FIFPPSDEQL  KSGTASVVCL  LNNFYPREAK  VQWKVDNALQ  SGNSQESVTE
601 QDSKDSTYSL  SSTLTLSKAD  YEKHKVYACE  VTHQGLSSPV  TKSFNRGEC*

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(SEQ ID NO:74)

In certain embodiments, one or more (e.g., 1, 2, 3, 4) linkers can be introduced between the light and heavy chain of Factor VII. The linker(s) can be a peptide linker.

The Fab light chain of this chimeric molecule can associate, *e.g.*, with the Fab heavy chain comprising a polypeptide sequence that has an amino acid sequence that is at least 60% , at least 65% , at least 70%, at least 75% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97%

identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in SEQ ID Nos. 75 or 76.

In one embodiment, the chimeric molecule comprises, consists essentially of, or consists of, a polypeptide that has an amino acid sequence that is at least 60% , at least 65% , at least 70%, at least 75% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to the amino acid sequence set forth in SEQ ID NO: 77.

```

1   ANAFLEELRP  GSLERECKEE  QCSFEEAREI  FKDAERTKLF  WISYSDGDQC
51  ASSPCQNGGS  CKDQLQSYIC  FCLPAFEGRN  CETHKDDQLI  CVNENGCGEQ
101 YCSDHTGTRK  SCRCHGYSL  LADGVSTPT  VEYPCGKIPI  LEKRNASKPQ
151 GRIVGGKVCP  KGECPWQVLL  LVNGAQLCGG  TLINTIWVVS  AAHCFDKIKN
201 WRNLI AVLGE  HDLSEHDGDE  QSRRVAQVII  PSTYVPGTN  HDIALRLHQ
251 PVVLTDHVVP  LCLPERTFSE  RTLAFVRFSL  VSGWGQLLDR  GATALELMVL
301 NVPRMTQDC  LQQSRKVGDS  PNITEYMFCA  GYSDGSKDSC  KGDSGGPHAT
351 HYRGTWYLTG  IVSWGQGCAT  VGHFGVYTRV  SQYIEWLQKL  MRSEPRPGVL
401 LRAPFGSPG  TSESATPESG  PGSEPATSGS  ETPGTSESAT  PESGPGSEPA
451 TSGSETPGTS ESATPESGPG TSTEPSEGS PGSPAGSPTS TEEGTSESAT
501 PESGPGSEPA TSGSETPGTS ESATPESGPG SPAGSPTSTE EGSPAGSPTS
551 TEEGTSTEPS EGSAPGTSES ATPESGPGTS ESATPESGPG TSESATPESG
601 PGSEPATSGS ETPGSEPATS GSETPGSPAG SPTSTEEGTS TEPSEGSAPG
651 TSTEPSEGS PGSEPATSGS ETPGTSESAT PESGPGTSTE PSEGSAPGSS
701 SGGGSGGGG  SGGGSGGGG  SGGGSGGGG  SEIVMTQSPA  TLSVSPGERA
751 TLSCRASSSV NYMYWYQQKP GQAPRLIIYY TSNLAPGIPA RFSGSGSGTE
801 FTLTISLQS EDFAVYQCQ FSSSPWTFGQ GTKVEIKRTV AAPSVFIFPP
851 SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD
901 STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC*

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(SEQ ID NO: 77)

This chimeric molecule includes the light and heavy chains of Factor VII, a linker having the amino acid sequence: GSPGTSESATPESGPGSEPATSGSETP (SEQ ID NO: 195), an XTEN termed AE288 (a half-life extending moiety), a GSSS (SEQ ID NO: 196) linker, a (G4S)₆ (SEQ ID NO: 170) linker, and the Fab light chain of a humanized GPIIb/IIIa antibody described herein. In certain embodiments, one or more of the linkers noted above can be eliminated (*e.g.*, SEQ ID NOs: 195 and/or 196) from the chimeric molecule. In certain embodiments, one or more (*e.g.*, 1, 2, 3, 4) linkers can be introduced between the light and heavy chain of Factor VII. The linker(s) can be a peptide linker. In certain embodiments, the heavy chain of Factor VII can precede the light chain of Factor VII in the chimeric molecule. The Fab light chain of this chimeric molecule can associate, *e.g.*, with a

polypeptide comprising an amino acid sequence that is at least 60% , at least 65% , at least 70%, at least 75% identical, at least 80% identical, at least 81% identical, at least 82% identical, at least 83% identical, at least 84% identical, at least 85% identical, at least 86% identical, at least 87% identical, at least 88% identical, at least 89% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or 100% identical to an amino acid sequence set forth in SEQ ID Nos. 75 or 76.

The above-described chimeric molecules can be modified, *e.g.*, to include additional linkers (*e.g.*, between the Factor VII and the half-life extending moiety and between the half-life extending moiety and the anti-GPIIb/IIIa antibody or antigen-binding fragment thereof). In certain instances there can be one or more (*e.g.*, 1, 2, 3, 4) linkers between these components of the chimeric molecule. These chimeric molecules can also be modified to include one or more half-life extending moieties (*e.g.*, AE144, AE288). In addition, instead of an Fab fragment, the chimeric molecules can comprise an scFv of the anti-GPIIb/IIIa antibody, a diabody, sc(Fv)₂, or a whole anti-GPIIb/IIIa antibody. In instances where the targeting moiety is an scFv, the chimeric molecule is a two polypeptide chain comprising either (i) the light chain of Factor VII and the heavy chain of Factor VII-scFv or heavy chain of Factor VII-half-life extending moiety-scFv chimeric molecule; or (ii) the heavy chain of Factor VII and the light chain of Factor VII-scFv or light chain of Factor VII-half-life extending moiety-scFv chimeric molecule.

In certain embodiments, the Factor VII of the chimeric molecule is activated. Activation of Factor VII can occur by the cleavage of the Arg152-Ile153 peptide bond of Factor VII to create a two chain FVII polypeptide. In one embodiment, the Factor VII of the chimeric molecule is activated by concentrating the chimeric polypeptide to about 4 mg/ml at a pH of 8.0 and incubating the polypeptide at 4°C for several minutes to an hour (*e.g.*, 1, 2, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 or 60 minutes).

F. Methods of Preparation

The present disclosure also provides a nucleic acid molecule or a set of nucleic acid molecules encoding (i) a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or (ii) any of the chimeric molecules disclosed herein, or (iii) a complement thereof.

In one embodiment, the invention includes a nucleic acid molecule encoding a polypeptide chain, which comprises a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX), a heterologous moiety (*e.g.*, a half-life extending moiety), an intracellular processing site, a heavy chain of the clotting factor (*e.g.*, FVII, FIX, or FX), and a targeting moiety which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof). In another embodiment, the nucleic acid molecule of the invention encodes a polypeptide chain comprising a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX), a targeting moiety which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof), an intracellular processing site, a heavy chain of the clotting factor (*e.g.*, FVII, FIX, or FX), and a heterologous moiety (*e.g.*, a half-life extending moiety). In other embodiments, the nucleic acid molecule encodes a polypeptide chain comprising a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX), an intracellular processing site, a heavy chain of the clotting factor (*e.g.*, FVII, FIX, or FX), a heterologous moiety (*e.g.*, a half-life extending moiety), and a targeting moiety which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof). In some embodiments, the nucleic acid molecule encodes a polypeptide chain comprising a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX), an intracellular processing site, a heavy chain of the clotting factor (*e.g.*, FVII, FIX, or FX), a targeting moiety which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof), and a heterologous moiety (*e.g.*, a half-life extending moiety). In certain embodiments, the nucleic acid molecule encodes a polypeptide chain comprising a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX), a heavy chain of the clotting factor (*e.g.*, FVII, FIX, or FX), at least one (*e.g.*, one two, three, four) heterologous moiety (*e.g.*, a half-life extending moiety such as the XTEN, AE144 or AE288), and a targeting moiety which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof such as an scFv, or the light and/or heavy chain of an Fab).

In some embodiments, the nucleic acid molecule comprises a set of nucleotide sequences, a first nucleotide sequence encoding a first polypeptide chain comprising a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX) and a heterologous moiety (*e.g.*, a half-life extending moiety) and a second nucleotide sequence encoding a second polypeptide chain comprising a heavy chain of the clotting factor (*e.g.*, FVII, FIX, or FX) and a targeting moiety which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof). In other embodiments, the nucleic acid molecule comprises a set of nucleotide sequences, a first nucleotide sequence encoding a first polypeptide chain

comprising a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX) and a targeting moiety which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof) and a second nucleotide sequence encoding a second polypeptide chain comprising a heavy chain of the clotting factor (*e.g.*, FVII, FIX, or FX) and a heterologous moiety (*e.g.*, a half-life extending moiety). In other embodiments, the nucleic acid molecule comprises a set of nucleotide sequences, a first nucleotide sequence encoding a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX) and a second nucleotide sequence encoding a heavy chain of the clotting factor (*e.g.*, FVII, FIX, or FX), a heterologous moiety (*e.g.*, a half-life extending moiety), and a targeting moiety which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof). In some embodiments, the nucleic acid molecule comprises a set of nucleotide sequences, a first nucleotide sequence encoding a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX) and a second nucleotide sequence encoding a heavy chain of the clotting factor (*e.g.*, FVII, FIX, or FX), a targeting moiety which binds to a platelet (*e.g.*, an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof), and a heterologous moiety (*e.g.*, a half-life extending moiety). In other embodiments, the nucleic acid molecule comprises a set of nucleotide sequences, a first nucleotide sequence encoding a first polypeptide chain comprising a light chain of a clotting factor (*e.g.*, FVII, FIX, or FX), a heavy chain of the clotting factor (*e.g.*, FVII, FIX, or FX), at least one (*e.g.*, one two, three, four) heterologous moiety (*e.g.*, a half-life extending moiety such as the XTEN, AE144 or AE288), and either the light chain or the heavy chain of an Fab of an anti-GPIIb/IIIa antibody described herein; and a second nucleotide sequence encoding the corresponding heavy or light chain of the Fab of the anti-GPIIb/IIIa antibody. It is to be understood that by “heavy chain of the Fab” is meant the VH region attached to CH1 of the heavy chain of the antibody.

Also provided are a vector or a set of vectors comprising such nucleic acid molecule or the set of the nucleic acid molecules or a complement thereof, as well as a host cell comprising the vector.

The instant disclosure also provides a method for producing a GPIIb/IIIa antibody or antigen-binding molecule thereof or chimeric molecule disclosed herein, such method comprising culturing the host cell disclosed herein and recovering the antibody, antigen-binding molecule thereof, or the chimeric molecule from the culture medium.

A variety of methods are available for recombinantly producing a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or a chimeric molecule disclosed

herein. 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.

In one embodiment a first expression vector comprising a DNA comprising a nucleic acid encoding the amino acid sequence of the chimeric polypeptide set forth in SEQ ID NO:77 is transfected into a host cell (*e.g.*, 293, CHO, COS) and the host cell is cultured under conditions that allow for the expression of the chimeric polypeptide. The chimeric polypeptide is recovered from the cell or culture medium. A second expression vector comprising a DNA comprising a nucleic acid encoding the amino acid sequence of the heavy chain of the Fab set forth in SEQ ID NOs. 75 or 76 is transfected into a host cell (*e.g.*, 293, CHO, COS) and the host cell is cultured under conditions that allow for the expression of the heavy chain of the Fab. The heavy chain of the Fab is recovered from the cell or culture medium. The chimeric polypeptide and the heavy chain of the Fab are contacted together to permit the heavy chain of the Fab to associate with the light chain of the Fab in the chimeric polypeptide. In another embodiment, a host cell (*e.g.*, 293, CHO, COS) is co-transfected with the first and second expression vectors described above and the host cell is cultured under conditions that allow for the expression of the chimeric polypeptide and the heavy chain of the Fab. The chimeric polypeptide and the heavy chain are isolated from the cell or culture medium. In certain instances, the heavy chain of the Fab is already associated with the light chain of the Fab in the chimeric polypeptide when the polypeptides are isolated from the cell or culture medium. In other instances, the heavy chain of the Fab is not already associated with the light chain of the Fab in the chimeric polypeptide when the polypeptides are isolated from the cell or culture medium and an additional step is required to facilitate their association. In certain embodiments, the Factor VII of the chimeric molecule is activated. Activation of Factor VII can occur by the cleavage of the Arg152-Ile153 peptide bond of Factor VII to create a two chain FVII polypeptide. In one embodiment, the Factor VII of the chimeric molecule is activated by concentrating the chimeric polypeptide (with or without the heavy chain Fab that associates with the light chain Fab of the chimeric polypeptide) to about 4 mg/ml at a pH of 8.0 and incubating the polypeptide at 4°C for several minutes to an hour (*e.g.*, 1, 2, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 or 60 minutes).

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 a GPIIb/IIIa antibody variant). 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 GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed herein.

For recombinant production, a polynucleotide sequence encoding a polypeptide (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed herein) 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.

The nucleic acid encoding the polypeptide (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed herein) 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 can be utilized to express the polypeptides described herein (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed 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 polypeptide is expressed in a eukaryotic cell, the DNA encoding the polypeptide (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed herein) can also code for a signal sequence that will permit the polypeptide to be secreted. One skilled in the art will understand that while the polypeptide is translated, the signal sequence is cleaved by the cell to form the mature chimeric molecule. Various signal sequences are known in the art, *e.g.*, native FVII signal sequence, native FIX signal sequence, native FX signal sequence, native GPIIb signal

sequence, native GPIIIa signal sequence, and the mouse IgK light chain signal sequence. Alternatively, where a signal sequence is not included, the polypeptide (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed herein) can be recovered by lysing the cells.

The GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed herein 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: 267).

The expression vectors can encode for tags that permit for easy purification or identification of the recombinantly produced polypeptide. Examples include, but are not limited to, vector pUR278 (Ruther *et al.* 1983, *EMBO J.* 2:1791) in which the polypeptide (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed herein) coding sequence can be ligated into the vector in frame with the lac z coding region so that a hybrid polypeptide is produced; pGEX vectors can 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.*, for PreCission Protease (Pharmacia, Peapack, N. J.) for easy removal of the tag after purification.

Numerous expression vector systems can 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 can 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 can

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.

Commonly used 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.*, U.S. Patent No. 4,704,362). Cells which have integrated the DNA into their chromosomes can be selected by introducing one or more markers which allow selection of transfected host cells. The marker can 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.

An exemplary expression vector is NEOSPLA (U.S. Patent No. 6,159,730). This vector contains the cytomegalovirus promoter/enhancer, the mouse beta globin major promoter, the SV40 origin of replication, the bovine growth hormone polyadenylation sequence, neomycin phosphotransferase exon 1 and exon 2, the dihydrofolate reductase gene and leader sequence. This vector has been found to result in very high level expression of antibodies upon incorporation of variable and constant region genes, transfection in cells, followed by selection in G418 containing medium and methotrexate amplification. Vector systems are also taught in U.S. Pat. Nos. 5,736,137 and 5,658,570, each of which is incorporated by reference in its entirety herein. This system provides for high expression levels, *e.g.*, > 30 pg/cell/day. Other exemplary vector systems are disclosed *e.g.*, in U.S. Patent No. 6,413,777.

In other embodiments, polypeptides of the invention (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed herein) can be expressed using polycistronic constructs. In these expression systems, multiple gene products of interest such as multiple polypeptides of multimer binding protein can be produced from a single polycistronic construct. These systems advantageously use an internal ribosome entry site (IRES) to provide relatively high levels of polypeptides of the invention in eukaryotic host cells. Compatible IRES sequences are disclosed in U.S. Pat. No. 6,193,980 which is also incorporated herein. Those skilled in the art will appreciate that

such expression systems can be used to effectively produce the full range of polypeptides disclosed in the instant application.

More generally, once the vector or DNA sequence encoding a polypeptide has been prepared, the expression vector can be introduced into an appropriate host cell. That is, the host cells can 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), flow cytometry, immunohistochemistry, and the like.

As used herein, the term "transformation" refers in a broad sense to the introduction of DNA into a recipient host cell that changes the genotype and consequently results in a change in the recipient cell. 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" can mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells.

In one embodiment, a host cell endogenously expresses an enzyme (or the enzymes) necessary to cleave a 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 can 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 a scFc linker occurs or is improved.

In one embodiment an enzyme which can 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). 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. U.S. Pat. No. 5,935,815, 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 PCSK1 (also known as PC1/Pc3), PCSK2 (also known as PC2), PCSK3 (also known as furin or PACE), PCSK4 (also known as PC4), PCSK5 (also known as PC5 or PC6), PCSK6 (also known as PACE4), or PCSK7 (also known as PC7/LPC, PC8, or SPC7). 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 can 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.

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 *et al.* (1993) *J. Biol. Chem.* 268:8458-65; Rehemtulla *et al.* (1993) *Biochemistry.* 32:11586-90. U.S. Pat. No. 5,840,529, 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.

Nucleotide and amino acid sequences of rodent PC5 were first described as PC5 by Lusson *et al.* (1993) Proc Natl Acad Sci USA 90:6691-5 and as PC6 by Nakagawa *et al.* (1993) J Biochem (Tokyo) 113:132-5. U.S. Pat. No. 6,380,171 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.

Genes encoding the polypeptides of the invention (*e.g.*, a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed herein) 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.

In addition to prokaryotes, eukaryotic microbes can 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* can 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.

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.*, U.S. Patent Nos. 5,741,957; 5,304,489; and 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.

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 (SEQ ID NO: 246)) can optionally be attached or included within the polypeptide sequence to facilitate downstream purification.

Once expressed, the chimeric molecules 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.

G. Pharmaceutical Compositions

The present disclosure also provides pharmaceutical compositions comprising one or more of:

- (i) a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein;
- (ii) a chimeric molecule disclosed herein;
- (iii) a nucleic acid molecule or the set of nucleic acid molecules disclosed herein; or
- (iv) a vector or set of vectors disclosed herein,

and a pharmaceutically acceptable carrier.

In some embodiments, administering (i) a chimeric molecule disclosed herein, (ii) a nucleic acid molecule or a set of nucleic acid molecules disclosed herein, (iii) a vector or a set of vectors disclosed herein, or (iii) a pharmaceutical composition disclosed herein, can be used, for example, to reduce the frequency or degree of a bleeding episode in a subject in need, and/or reducing or preventing an occurrence of a bleeding episode in a subject in need thereof. In some embodiments, the subject has developed or has a tendency to develop an inhibitor against treatment with FVIII, FIX, or both. In some embodiments, the inhibitor against FVIII or FIX is a neutralizing antibody against FVIII, FIX, or both. In some embodiments, the bleeding episode can be caused by a blood coagulation disorder, for example, hemophilia A or hemophilia B. In other embodiments, the bleeding episode can be the result of hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the iliopsoas sheath, or any combinations thereof. In certain embodiments, the subject is a human subject.

A pharmaceutical composition may include a “therapeutically effective amount” of an agent described herein. Such effective amounts can be determined based on the effect of the administered agent, or the combinatorial effect of agents if more than one agent is used. A therapeutically effective amount of an agent may also vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual, e.g., amelioration of at least one disorder parameter or amelioration of at least one symptom of the disorder. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects.

In one embodiment, the pharmaceutical composition (e.g., a composition comprising the polypeptide(s) or nucleic acid molecule(s) encoding the polypeptide(s)) is one in which the clotting factor is present in activatable form when administered to a subject. Such an activatable molecule can be activated in vivo at the site of clotting after administration to a subject.

H. Methods of Treatment

The antibodies, antigen-binding fragments thereof and chimeric molecules of the disclosure can be useful in methods of treating a subject with a disease or condition. The disease or condition can include, but is not limited to, hemostatic or coagulation disorders.

For example, this disclosure provides a method of treating, ameliorating, or preventing a hemostatic disorder to a subject comprising administering a therapeutically effective amount of a chimeric molecule of the disclosure which comprises a clotting factor. The treatment, amelioration, and prevention by the chimeric molecule can be a bypass therapy. The subject in the bypass therapy can have already developed an inhibitor to a clotting factor, e.g., FVIII or FIX, or is subject to developing a clotting factor inhibitor. In one embodiment, a chimeric molecule composition of the invention is administered in combination with at least one other agent that promotes hemostasis. As an example, but not as a limitation, hemostatic agent can include a FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII, prothrombin, or fibrinogen or activated forms of any of the preceding. The clotting factor or hemostatic agent can also include anti-fibrinolytic drugs, e.g., epsilon-amino-caproic acid, tranexamic acid.

The chimeric molecules of the invention treat or prevent a hemostatic disorder by promoting the formation of a fibrin clot. The chimeric molecule 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. A chimeric molecule 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 molecule. The hemostatic disorders that can be treated by administration of the chimeric molecule 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.

In one embodiment, the hemostatic disorder is an inherited disorder. In one embodiment, the subject has hemophilia A, and the chimeric molecule comprises activated or protease-activatable FVII linked to or associated with a GPIIb/IIIa antibody or antigen-binding molecule thereof and a half-life extending heterologous moiety. In another embodiment, the subject has hemophilia A and the chimeric molecule comprises activated or protease-activatable FVII linked to or associated with an Fab or scFv of an GPIIb/IIIa

antibody and a half-life extending heterologous moiety. In other embodiments, the subject has hemophilia B and the chimeric molecule comprises activated or protease-activatable FVII or FX linked to or associated with a GPIIb/IIIa antibody or antigen-binding molecule thereof and a half-life extending heterologous moiety. In some embodiments, the subject has inhibitory antibodies to FVIII or FVIIIa and the chimeric molecule comprises activated or protease-activatable FVII linked to or associated with a GPIIb/IIIa antibody or antigen-binding molecule thereof and a half-life extending heterologous moiety. In yet other embodiments, the subject has inhibitory antibodies against FIX or FIXa and the chimeric molecule comprises activated or protease-activatable FVII linked to or associated with a GPIIb/IIIa antibody or antigen-binding molecule thereof and a half-life extending heterologous moiety. In still other embodiments, the subject has inhibitory antibodies to FVIII or FVIIIa and the chimeric molecule comprises activated or protease-activatable FX linked to or associated with a GPIIb/IIIa antibody or antigen-binding molecule thereof and a half-life extending heterologous moiety. In certain embodiments, the subject has inhibitory antibodies against FIX or FIXa and the chimeric molecule comprises activated or protease-activatable FX linked to or associated with a GPIIb/IIIa antibody or antigen-binding molecule thereof and a half-life extending heterologous moiety.

Chimeric molecules of the invention comprising a clotting factor (*e.g.*, FVII) can be used to prophylactically treat a subject with a hemostatic or coagulation disorder. Chimeric molecules of the invention comprising a clotting factor (*e.g.*, FVII) can be used to treat an acute bleeding episode in a subject with a hemostatic disorder.

In one embodiment, the hemostatic disorder is the result of a deficiency in a clotting factor, *e.g.*, FVII, FIX, or FVIII. In another embodiment, the hemostatic disorder can be the result of a defective clotting factor. 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).

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 molecule 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 molecule of the invention can be administered prior to or after surgery as a prophylactic. The

chimeric molecule 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. In another embodiment, the chimeric molecule 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.

I. Administration

The antibodies, antigen-binding fragments thereof, chimeric molecules, or nucleic acids encoding same of the disclosure 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 molecule can be implanted within or linked to a biopolymer solid support that allows for the slow release of the chimeric molecule to the desired site. The route and/or mode of administration of the antibody or antigen-binding fragment thereof can also be tailored for the individual case, *e.g.*, by monitoring the subject,

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. For buccal and sublingual administration the composition can take the form of tablets, lozenges or fast dissolving films according to conventional protocols. For administration by inhalation, the chimeric molecules for use according to the present disclosure are conveniently delivered in the form of an aerosol spray from a pressurized pack or nebulizer (*e.g.*, in PBS), with a suitable propellant.

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 can 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.

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 can also be present such as for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like.

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.

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.

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 can 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.

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.

Effective doses of the compositions of the present disclosure, 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 can be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

In one embodiment, the dose of a biologically active moiety (*e.g.*, comprising FVII), can range from about 90 to 270 $\mu\text{g/kg}$ or 0.090 to 0.270 mg/kg . In another embodiment, the dose of a biologically active moiety (*e.g.*, comprising FX), can range from about 1 $\mu\text{g/kg}$ to 400 mg/kg .

Dosages can range from 1000 µg/kg to 0.1 ng/kg body weight. In one embodiment, the dosing range is 1 µg/kg to 100 µg/kg. The protein can be administered continuously or at specific timed intervals. *In vitro* assays can 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 can be extrapolated from dose-response curves obtained from animal models, *e.g.*, a hemophiliac dog (Mount *et al.* 2002, *Blood* 99: 2670).

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 can be administered simultaneously, in which case the dosage of each polypeptide administered falls within the ranges indicated.

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.

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.

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).

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 can 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.

It will further be appreciated that the polypeptides of the instant invention can 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 can be combined include agents that represent the current standard of care for a particular disorder being treated. Such agents can 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.

The amount of agent to be used in combination with the polypeptides of the instant invention can vary by subject or can 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 ((Hardman *et al.*, eds., 9th ed. 1996). In another embodiment, an amount of such an agent consistent with the standard of care is administered.

As previously discussed, the polypeptides of the present disclosure, can 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 disclosure 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 disclosure can be administered in single or multiple doses to provide for a pharmaceutically effective amount of the polypeptide.

In one embodiment, a chimeric molecule of the invention is 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.

In keeping with the scope of the present disclosure, the chimeric molecule of the invention can 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.

J. Other Methods of Use

The instant disclosure also provides a method to target or deliver a therapeutic or prophylactic agent (*e.g.*, a clotting factor such as FVII) to the surface of platelets, wherein the method comprises fusing the agent to one of the GPIIb/IIIa antibodies or antigen-binding fragments thereof disclosed herein.

In addition, the disclosure provides a method to increase the activity of a therapeutic or prophylactic agent (*e.g.*, a clotting factor such as FVII) comprising fusing the agent to a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein.

Further, the disclosure provides a method to improve the pharmacokinetic properties of a clotting factor comprising fusing the clotting factor to the GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein.

In some embodiments, these methods further comprise fusing or conjugating a clotting factor and/or the GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein to a half-life extending moiety. In some embodiments, the therapeutic or prophylactic agent is a FVII, a FIX, or a FX.

The present disclosure also provides a method of measuring the level of platelets in plasma of a subject in need thereof comprising contacting the GPIIb/IIIa antibody or antigen binding molecule thereof disclosed herein with the plasma from the subject and measuring the level of platelets in plasma. This method can further comprise fusing or conjugating the clotting factor and/or the GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein to a detectable heterologous moiety, for example, a fluorescent molecule or a radionuclide.

This disclosure also provides a method of isolating or separating platelets from other cells in a sample (*e.g.*, a blood sample). The method comprises contacting the sample with an GPIIb/IIIa antibody or antigen binding molecule thereof disclosed herein and separating the

cells that have bound to the GPIIb/IIIa antibody or antigen binding molecule thereof from the unbound fraction.

In addition, the disclosure also provides a method of detecting platelets in a sample (e.g., blood sample) of a subject comprising contacting the sample with a detectably labeled GPIIb/IIIa antibody or antigen binding molecule. The detectable label can be, for example, a fluorescent molecule or a radionuclide.

The following examples are included for purposes of illustration only and are not intended to be limiting of the invention. All patents and publications referred to herein are expressly incorporated by reference in their entireties.

EXAMPLES

Example 1: Humanized Anti-GPIIb/IIIa Antibody Designs

The CDRs (CDR-H1, CDR-H2, and CDR-H3 for the heavy chain region and CDR-L1, CDR-L2, and CDR-L3 for the light chain region) of the murine anti-integrin GPIIb/IIIa antibody, 34D10, were grafted onto human acceptor frameworks to create CDR-grafted variable heavy chain, VH0, and variable light chain, VL0, respectively. Five additional heavy chain regions (VH1 to 5) and three additional light chain regions (VL1 to 3) were created by combining several mutations in the human acceptor frameworks of the CDR grafts compared to the CDR-grafted chains (*i.e.*, VH0 and VL0). The majority of the mutations that were made in the human acceptor frameworks were backmutations to the amino acid of the mature murine framework to help maintain the structure of the 34D10 CDRs.

The germline humIGKV3-15 with framework region FR4 from human consensus subgroup Kappa I was chosen as the light chain acceptor framework. As the humIGKV3-15 germline sequence lacks the final framework region, FR4, the VL0 CDR graft employed the FR4 region from the corresponding human consensus subgroup, Kappa III, which is the same as the FR4 sequence of the human consensus subgroup most similar to the mature murine, Kappa I, namely FGQGTKVEIK (**SEQ ID NO:49**). This FR4 sequence differs at two positions from 34D10's FR4, FGGGTKLEIK (**SEQ ID NO: 193**).

The germline humIGHV3/OR16-13 with framework region FR4 from human consensus subgroup Heavy III was chosen as the heavy chain acceptor framework. As the humIGHV3/OR16-13 germline sequence lacks the final framework region FR4, the VH0 CDR graft used the FR4 region from the human consensus that best matched 34D10 VH, human consensus subgroup Heavy III. This framework region 4 sequence is WGQGT LVT VSS (**SEQ ID NO: 34**), differing from murine only by having that L108, which is S in the 34D10 VH and the murine consensus Heavy III(D).

The sequences of the 34D10 VH and VL regions, as well as the six humanized 34D10 variable heavy chain regions and four humanized 34D10 variable light chain regions are shown below. CDRs 1, 2, and 3 are underlined in each amino acid sequence.

Variable Heavy Chain Sequences:

34D10 VH Variable Heavy Chain Amino Acid Sequence

EVKLVESGGGLVKPGGSLKLSCAASGFTFSSAYAMSWVRQTPEKRLEWVASISSGGT
TYYPDSVKRRFTISRDNARNILYLQMSSLRSEDTAMYYCTRGGDYGYALDYWGQGT
 SVTVSS (**SEQ ID NO:1**)

h34D10 VH0 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSAYAMSWVRQAPGKGLVWVSSISSGGT
TYYPDSVKRQFTISRDNAKNTLYLQMNSLR AEDMAVYYCTRGGDYGYALDYWGQ
 GTLVT VSS (**SEQ ID NO:3**)

h34D10 VH0 Variable Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGCAGC TGGTGGAGTC TGGAGGAGGC TTGGTACAGC CTGGAGGGTC
      CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
      CCGCCAGGCT
121    CCAGGGAAGG GGCTGGTGTG GGTCTCAAGC ATTAGTAGTG GTGGTACCAC
      ATACTACCCA
181    GACTCCGTGA AGAGGCAGTT CACCATCTCC AGAGACAATG CCAAGAACAC
      GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACATG GCCGTATATT ACTGTACCAG
      AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC CTCA
      (SEQ ID NO:13)
  
```

h34D10 VH0 Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGCAGC TGGTGGAGTC TGGAGGAGGC TTGGTACAGC CTGGAGGGTC
      CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
      CCGCCAGGCT
121    CCAGGGAAGG GGCTGGTGTG GGTCTCAAGC ATTAGTAGTG GTGGTACCAC
      ATACTACCCA
181    GACTCCGTGA AGAGGCAGTT CACCATCTCC AGAGACAATG CCAAGAACAC
      GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACATG GCCGTATATT ACTGTACCAG
      AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC
      CTCAGCCTCC
361    ACCAAGGGCC CATCGGTCTT CCCGCTAGCA CCCTCCTCCA AGAGCACCTC
      TGGGGGCACA
421    GCGGCCCTGG GCTGCCTGGT CAAGGACTAC TTCCCCGAAC CGGTGACGGT
      GTCGTGGAAC
481    TCAGGCGCCC TGACCAGCGG CGTCCACACC TTCCCGGCTG TCCTACAGTC
      TAGCGGACTC
541    TACTCCCTCA GCAGCGTAGT GACCGTGCCC TCTTCTAGCT TGGGCACCCA
      GACCTACATC
601    TGCAACGTGA ATCACAAGCC CAGCAACACC AAGGTGGACA AGAAAGTTGA
      GCCCAAATCT
661    TGTTAG (SEQ ID NO:59)

```

h34D10 VH1 Variable Heavy Chain Amino Acid Sequence

EVQLVQSGGGLVQPGESLRLSCAASGFTFSAYAMSWVRQAPGKGLEWVSSISSGGT
 TYYPDSVKRRFTISRDNAKNTLYLQMNSLRAEDMAVYYCTRGGDYGYALDYWGQ
 GTLVTVSS (SEQ ID NO:5)

h34D10 VH1 Variable Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGCAGC TGGTGCAGTC TGGAGGAGGC TTGGTACAGC CTGGAGAGTC
      CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
      CCGCCAGGCT
121    CCAGGGAAGG GGCTGGAGTG GGTCTCAAGC ATTAGTAGTG GTGGTACCAC
      ATACTACCCA
181    GACTCCGTGA AGAGGAGATT CACCATCTCC AGAGACAATG CCAAGAACAC
      GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACATG GCCGTATATT ACTGTACCAG
      AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC CTCA
      (SEQ ID NO:14)

```

h34D10 VH1 Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGCAGC TGGTGCAGTC TGGAGGAGGC TTGGTACAGC CTGGAGAGTC
   CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
   CCGCCAGGCT
121    CCAGGGAAGG GGCTGGAGTG GGTCTCAAGC ATTAGTAGTG GTGGTACCAC
   ATACTACCCA
181    GACTCCGTGA AGAGGAGATT CACCATCTCC AGAGACAATG CCAAGAACAC
   GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACATG GCCGTATATT ACTGTACCAG
   AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC
   CTCAGCCTCC
361    ACCAAGGGCC CATCGGTCTT CCCGCTAGCA CCCTCCTCCA AGAGCACCTC
   TGGGGGCACA
421    GCGGCCCTGG GCTGCCTGGT CAAGGACTAC TTCCCCGAAC CGGTGACGGT
   GTCGTGGAAC
481    TCAGGCGCCC TGACCAGCGG CGTCCACACC TTCCCGGCTG TCCTACAGTC
   TAGCGGACTC
541    TACTCCCTCA GCAGCGTAGT GACCGTGCCC TCTTCTAGCT TGGGCACCCA
   GACCTACATC
601    TGCAACGTGA ATCACAAGCC CAGCAACACC AAGGTGGACA AGAAAGTTGA
   GCCCAAATCT
661    TGTTAG (SEQ ID NO:60)

```

h34D10 VH2 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGT
TYYPDSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGYALDYWGQG
 TLVTVSS (SEQ ID NO:7)

h34D10 VH2 Variable Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGCAGC TGGTGGAGTC TGGAGGAGGC TTGGTAAAGC CTGGAGGATC
   CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
   CCGCCAGGCT
121    CCAGGGAAGG GGCTGGTCTG GGTCTGCTAGC ATTAGTAGTG GTGGTACCAC
   ATACTACCCA
181    GACTCCGTGA AGAGGCAGTT CACCATCTCC AGAGACAATG CCAAGAACAC
   GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACACA GCCGTATATT ACTGTACCAG
   AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC CTCA
   (SEQ ID NO:15)

```

h34D10 VH2 Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGCAGC TGGTGGAGTC TGGAGGAGGC TTGGTAAAGC CTGGAGGATC
   CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
   CCGCCAGGCT
121    CCAGGGAAGG GGCTGGTCTG GGTGCTAGC ATTAGTAGTG GTGGTACCAC
   ATACTACCCA
181    GACTCCGTGA AGAGGCAGTT CACCATCTCC AGAGACAATG CCAAGAACAC
   GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACACA GCCGTATATT ACTGTACCAG
   AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC
   CTCAGCCTCC
361    ACCAAGGGCC CATCGGTCTT CCCGCTAGCA CCCTCCTCCA AGAGCACCTC
   TGGGGGCACA
421    GCGGCCCTGG GCTGCCTGGT CAAGGACTAC TTCCCCGAAC CGGTGACGGT
   GTCGTGGAAC
481    TCAGGCGCCC TGACCAGCGG CGTCCACACC TTCCCGGCTG TCCTACAGTC
   TAGCGGACTC
541    TACTCCCTCA GCAGCGTAGT GACCGTGCCC TCTTCTAGCT TGGGCACCCA
   GACCTACATC
601    TGCAACGTGA ATCACAAGCC CAGCAACACC AAGGTGGACA AGAAAGTTGA
   GCCCAAATCT
661    TGTTAG (SEQ ID NO: 61)

```

h34D10 VH3 Variable Heavy Chain Amino Acid Sequence

EVQLVQSGGGLVKPGESLRLSCAASGFTFSAYAMSWVRQAPGKGLEWVASISSGGT
 TYYPDSVKRRFTISRDNKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQG
 TLVTVSS (SEQ ID NO:9)

h34D10 VH3 Variable Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGCAGC TGGTGCAGTC TGGAGGAGGC TTGGTAAAGC CTGGAGAGTC
   CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
   CCGCCAGGCT
121    CCAGGGAAGG GGCTGGAGTG GGTGCTAGC ATTAGTAGTG GTGGTACCAC
   ATACTACCCA
181    GACTCCGTGA AGAGGAGATT CACCATCTCC AGAGACAATG CCAAGAACAC
   GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACACA GCCGTATATT ACTGTACCAG
   AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC CTCA
   (SEQ ID NO:16)

```

h34D10 VH3 Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGCAGC TGGTGCAGTC TGGAGGAGGC TTGGTAAAGC CTGGAGAGTC
      CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
      CCGCCAGGCT
121    CCAGGGAAGG GGCTGGAGTG GGTCTGCTAGC ATTAGTAGTG GTGGTACCAC
      ATACTACCCA
181    GACTCCGTGA AGAGGAGATT CACCATCTCC AGAGACAATG CCAAGAACAC
      GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACACA GCCGTATATT ACTGTACCAG
      AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC
      CTCAGCCTCC
361    ACCAAGGGCC CATCGGTCTT CCCGCTAGCA CCCTCCTCCA AGAGCACCTC
      TGGGGGCACA
421    GCGGCCCTGG GCTGCCTGGT CAAGGACTAC TTCCCCGAAC CGGTGACGGT
      GTCGTGGAAC
481    TCAGGCGCCC TGACCAGCGG CGTCCACACC TTCCCGGCTG TCCTACAGTC
      TAGCGGACTC
541    TACTCCCTCA GCAGCGTAGT GACCGTGCCC TCTTCTAGCT TGGGCACCCA
      GACCTACATC
601    TGCAACGTGA ATCACAAGCC CAGCAACACC AAGGTGGACA AGAAAGTTGA
      GCCCAAATCT
661    TGTTAG (SEQ ID NO: 62)

```

h34D10 VH4 Variable Heavy Chain Amino Acid Sequence

EVQLVQSGGGLVKPGESLRRLSCAASGFTFSAYAMSWVRQAPGKGLEWVASISSGGT
 TYYPDSVKRRFTISRDNSTNTLYLQMNSLRRAEDTAVYYCTRGGDYGYALDYWGQG
 TLVTVSS (SEQ ID NO:11)

h34D10 VH4 Variable Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGCAGC TGGTGCAGTC TGGAGGAGGC TTGGTAAAGC CTGGAGAGTC
      CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
      CCGCCAGGCT
121    CCAGGGAAGG GGCTGGAGTG GGTCTGCTAGC ATTAGTAGTG GTGGTACCAC
      ATACTACCCA
181    GACTCCGTGA AGAGGAGATT CACCATCTCC AGAGACAATA GTCGCAACAC
      GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACACA GCCGTATATT ACTGTACCAG
      AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC CTCA
      (SEQ ID NO:17)

```

h34D10 VH4 Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGCAGC TGGTGCAGTC TGGAGGAGGC TTGGTAAAGC CTGGAGAGTC
      CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
      CCGCCAGGCT
121    CCAGGGAAGG GGCTGGAGTG GGTGCTAGC ATTAGTAGTG GTGGTACCAC
      ATACTACCCA
181    GACTCCGTGA AGAGGAGATT CACCATCTCC AGAGACAATA GTCGCAACAC
      GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACACA GCCGTATATT ACTGTACCAG
      AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC
      CTCAGCCTCC
361    ACCAAGGGCC CATCGGTCTT CCCGCTAGCA CCCTCCTCCA AGAGCACCTC
      TGGGGGCACA
421    GCGGCCCTGG GCTGCCTGGT CAAGGACTAC TTCCCCGAAC CGGTGACGGT
      GTCGTGGAAC
481    TCAGGCGCCC TGACCAGCGG CGTCCACACC TTCCCGGCTG TCCTACAGTC
      TAGCGGACTC
541    TACTCCCTCA GCAGCGTAGT GACCGTGCCC TCTTCTAGCT TGGGCACCCA
      GACCTACATC
601    TGCAACGTGA ATCACAAGCC CAGCAACACC AAGGTGGACA AGAAAGTTGA
      GCCCAAATCT
661    TGTTAG (SEQ ID NO: 63)

```

h34D10 VH5 Variable Heavy Chain Amino Acid Sequence

EVKLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLEWVASISSGGT
TYYPDSVKRRRFTISRDNARNTLYLQMNSLRAEDTAVYYCTRGGDYGYALDYWGQG
 TLVTVSS (SEQ ID NO:12)

h34D10 VH5 Variable Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGAAGC TGGTGGAGTC TGGAGGAGGC TTGGTAAAGC CTGGAGGCTC
      CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
      CCGCCAGGCT
121    CCAGGGAAGG GGCTGGAGTG GGTGCTAGC ATTAGTAGTG GTGGTACCAC
      ATACTACCCA
181    GACTCCGTGA AGAGGAGATT CACCATCTCC AGAGACAATG CTCGCAACAC
      GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACACA GCCGTATATT ACTGTACCAG
      AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC CTCA
      (SEQ ID NO:18)

```

h34D10 VH5 Heavy Chain Nucleic Acid Sequence

```

1      GAGGTGAAGC TGGTGGAGTC TGGAGGAGGC TTGGTAAAGC CTGGAGGCTC
      CCTGAGACTC
61     TCCTGTGCAG CCTCTGGATT CACCTTTAGC GCCTATGCCA TGAGCTGGGT
      CCGCCAGGCT
121    CCAGGGAAGG GGCTGGAGTG GGTCGCTAGC ATTAGTAGTG GTGGTACCAC
      ATACTACCCA
181    GACTCCGTGA AGAGGAGATT CACCATCTCC AGAGACAATG CTCGCAACAC
      GCTGTATCTG
241    CAAATGAACA GCCTGAGAGC CGAGGACACA GCCGTATATT ACTGTACCAG
      AGGAGGGGAT
301    TATGGCTACG CTCTCGACTA CTGGGGCCAG GGAACCCTGG TCACCGTCTC
      CTCAGCCTCC
361    ACCAAGGGCC CATCGGTCTT CCCGCTAGCA CCCTCCTCCA AGAGCACCTC
      TGGGGGCACA
421    GCGGCCCTGG GCTGCCTGGT CAAGGACTAC TTCCCCGAAC CGGTGACGGT
      GTCGTGGAAC
481    TCAGGCGCCC TGACCAGCGG CGTCCACACC TTCCCGGCTG TCCTACAGTC
      TAGCGGACTC
541    TACTCCCTCA GCAGCGTAGT GACCGTGCCC TCTTCTAGCT TGGGCACCCA
      GACCTACATC
601    TGCAACGTGA ATCACAAGCC CAGCAACACC AAGGTGGACA AGAAAGTTGA
      GCCCAAATCT
661    TGTTAG (SEQ ID NO: 64)

```

Variable Light Chain Sequences:**34D10 VL Variable Light Chain Amino Acid Sequence**

ENVLTQSPA~~IM~~SASLGEKVTMSCRASSSVNYMYWYQQKSDASPKLWIYYTSN~~LAP~~G
 VPARFSGSGSGNSYSLSITSMIEGDAATYYCQQFSSSPWTFGGGTKLEIK (SEQ ID
 NO:2)

h34D10 VL0 Variable Light Chain Amino Acid Sequence

EIVMTQSPATLSVSPGERATLSCRASSSVNYMYWYQQKPGQAPRLLIYYTSN~~LAP~~GIP
 ARFSGSGSGTEFTLTISSLQSEDFAVYYCQQFSSSPWTFGQGTKVEIK (SEQ ID NO:4)

h34D10 VL0 Variable Light Chain Nucleic Acid Sequence

```

1      GAAATTGTAA TGACACAGTC TCCAGCCACC CTGTCTGTGT CTCCTGGCGA
      AAGAGCCACC
61     CTCTCCTGCC GCGCCAGTAG CAGTGTTAAC TACATGTACT GGTATCAACA
      GAAACCTGGC
121    CAGGCTCCCA GGCTCCTCAT CTATTACACA TCCAACCTGG CCCCTGGCAT
      CCCAGCCAGG
181    TTCAGTGGCA GTGGGTCTGG GACAGAGTTC ACTCTCACCA TCAGCAGCCT
      ACAGAGCGAA
241    GATTTTGCAG TTTATTACTG TCAGCAGTTC AGCAGTTCAC CTTGGACGTT
      CGGCCAAGGG

```

301 ACCAAGGTGG AAATCAAA (SEQ ID NO: 19)

h34D10 VL0 Light Chain Nucleic Acid Sequence (with signal sequence: MDMRVPAQLL LLLLLWLPGARC (SEQ ID NO:194); the nucleic acid sequence encoding the mature light chain sequence is underlined)

```

1      ATGGACATGA GGGTCCCCGC TCAGCTCCTG GGGCTCCTTC TGCTCTGGCT
      CCTGGAGCA
61     CGATGTGAAA TTGTAATGAC ACAGTCTCCA GCCACCCTGT CTGTGTCTCC
      TGGCGAAAAGA
121    GCCACCCTCT CCTGCCGCGC CAGTAGCAGT GTTAACTACA TGTACTGGTA
      TCAACAGAAA
181    CCTGGCCAGG CTCCCAGGCT CCTCATCTAT TACACATCCA ACTTGGCCCC
      TGGCATCCCA
241    GCCAGGTTCA GTGGCAGTGG GTCTGGGACA GAGTTCAC TCACCATCAG
      CAGCCTACAG
301    AGCGAAGATT TTGCAGTTTA TTACTGTCAG CAGTTCAGCA GTTCACCTTG
      GACGTTCCGGC
361    CAAGGGACCA AGGTGGAAAT CAAACGTACG GTGGCTGCAC CATCTGTCTT
      CATCTTCCCG
421    CCATCTGATG AGCAGTTGAA ATCTGGAAC GCCTCTGTTG TGTGCCTGCT
      GAATAACTTC
481    TATCCCAGAG AGGCCAAAGT ACAGTGAAG GTGGATAACG CCCTCCAATC
      GGGTAACTCC
541    CAGGAGAGTG TCACAGAGCA GGACAGCAAG GACAGCACCT ACAGCCTCAG
      CAGCACCTTG
601    ACGCTGAGCA AAGCAGACTA CGAGAAACAC AAAGTCTACG CCTGCGAAGT
      CACCCATCAG
661    GGCCTGAGCT CGCCCGTCAC AAAGAGCTTC AACAGGGGAG AGTGTTAG
      (SEQ ID NO: 65)

```

h34D10 VL1 Variable Light Chain Amino Acid Sequence

EIVLTQSPATLSVSPGERATLSCRASSSVNYMYWYQQKPGQAPRLLIYYTSNLAPGV
PARFSGSGSGTEFTLTISSLQSEDFAVYYCQQFSSSPWTFFGQGTKVEIK (SEQ ID
NO:6)

h34D10 VL1 Variable Light Chain Nucleic Acid Sequence

```

1      GAAATTGTAC TCACACAGTC TCCAGCCACC CTGTCTGTGT CTCCTGGCGA
      AAGAGCCACC
61     CTCTCCTGCC GCGCCAGTAG CAGTGTTAAC TACATGTACT GGTATCAACA
      GAAACCTGGC
121    CAGGCTCCCA GGCTCCTCAT CTATTACACA TCCAATTGG CCCCTGGCGT
      TCCAGCCAGG
181    TTCAGTGGCA GTGGGTCTGG GACAGAGTTC ACTCTACCA TCAGCAGCCT
      ACAGAGCGAA
241    GATTTTGCAG TTTATTACTG TCAGCAGTTC AGCAGTTCAC CTTGGACGTT
      CGGCCAAGGG
301    ACCAAGGTGG AAATCAAA (SEQ ID NO: 20)

```

h34D10 VL1 Light Chain Nucleic Acid Sequence

```

1      GAAATTGTAC TCACACAGTC TCCAGCCACC CTGTCTGTGT CTCCTGGCGA
   AAGAGCCACC
61     CTCTCCTGCC GCGCCAGTAG CAGTGTTAAC TACATGTACT GGTATCAACA
   GAAACCTGGC
121    CAGGCTCCCA GGCTCCTCAT CTATTACACA TCCAAC TTGG CCCCTGGCGT
   TCCAGCCAGG
181    TTCAGTGGCA GTGGGTCTGG GACAGAGTTC ACTCTCACCA TCAGCAGCCT
   ACAGAGCGAA
241    GATTTTGCAG TTTATTACTG TCAGCAGTTC AGCAGTTCAC CTTGGACGTT
   CGGCCAAGGG
301    ACCAAGGTGG AAATCAAACG TACGGTGGCT GCACCATCTG TCTTCATCTT
   CCCGCCATCT
361    GATGAGCAGT TGAAATCTGG AACTGCCTCT GTTGTGTGCC TGCTGAATAA
   CTTCTATCCC
421    AGAGAGGCCA AAGTACAGTG GAAGGTGGAT AACGCCCTCC AATCGGGTAA
   CTCCAGGAG
481    AGTGTACAG AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC
   CCTGACGCTG
541    AGCAAAGCAG ACTACGAGAA ACACAAAGTC TACGCCTGCG AAGTCACCCA
   TCAGGGCCTG
601    AGTCGCCCCG TCACAAAGAG CTTCAACAGG GGAGAGTGTT AG (SEQ ID NO: 66)

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h34D10 VL2 Variable Light Chain Amino Acid Sequence

EIVLTQSPATLSASPGERVMTSCRASSSVNYMYWYQQKPGQSPRLLIYYTSN LAPGV
 PARFSGSGSGTEYTLTISSLQSEDFAVYYCQQFSSSPWTFGGQGTKVEIK (SEQ ID
 NO:8)

h34D10 VL2 Variable Light Chain Nucleic Acid Sequence

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1      GAAATTGTAC TCACACAGTC TCCAGCCACC CTGTCTGCCT CTCCTGGCGA
   AAGAGTGACC
61     ATGTCTTGCC GCGCCAGTAG CAGTGTTAAC TACATGTACT GGTATCAACA
   GAAACCTGGC
121    CAGTCACCCA GGCTCCTCAT CTATTACACA TCCAAC TTGG CCCCTGGCGT
   TCCAGCCAGG
181    TTCAGTGGCA GTGGGTCTGG GACAGAGTAC ACTCTCACCA TCAGCAGCCT
   ACAGAGCGAA
241    GATTTTGCAG TTTATTACTG TCAGCAGTTC AGCAGTTCAC CTTGGACGTT
   CGGCCAAGGG
301    ACCAAGGTGG AAATCAAA (SEQ ID NO: 21)

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h34D10 VL2 Light Chain Nucleic Acid Sequence

```

1      GAAATTGTAC TCACACAGTC TCCAGCCACC CTGTCTGCCT CTCCTGGCGA
   AAGAGTGACC
61     ATGTCTTGCC GCGCCAGTAG CAGTGTTAAC TACATGTACT GGTATCAACA
   GAAACCTGGC
121    CAGTCACCCA GGCTCCTCAT CTATTACACA TCCAAC TTGG CCCCTGGCGT
   TCCAGCCAGG

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181   TTCAGTGGCA GTGGGTCTGG GACAGAGTAC ACTCTCACCA TCAGCAGCCT
      ACAGAGCGAA
241   GATTTTGCAG TTTATTACTG TCAGCAGTTC AGCAGTTCAC CTTGGACGTT
      CGGCCAAGGG
301   ACCAAGGTGG AAATCAAACG TACGGTGGCT GCACCATCTG TCTTCATCTT
      CCGCCATCT
361   GATGAGCAGT TGAAATCTGG AACTGCCTCT GTTGTGTGCC TGCTGAATAA
      CTTCTATCCC
421   AGAGAGGCCA AAGTACAGTG GAAGGTGGAT AACGCCCTCC AATCGGGTAA
      CTCCCAGGAG
481   AGTGTCACAG AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC
      CCTGACGCTG
541   AGCAAAGCAG ACTACGAGAA ACACAAAGTC TACGCCTGCG AAGTCACCCA
      TCAGGGCCTG
601   AGCTCGCCCG TCACAAAGAG CTTCAACAGG GGAGAGTGTT AG (SEQ ID NO: 67)

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h34D10 VL3 Variable Light Chain Amino Acid Sequence

ENVMTQSPATLSASPGERVMTMSCRASSSVNYMYWYQQKPGQSPRLLIYYTSNLAPG
 VPARFSGSGSGTEYTLTISSLQSEDFAVYYCQQFSSSPWTFGQGTKVEIK (SEQ ID
 NO:10)

h34D10 VL3 Variable Light Chain Nucleic Acid Sequence

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1     GAAAACGTAA TGACACAGTC TCCAGCCACC CTGTCTGCCT CTCCTGGCGA
      AAGAGTGACC
61    ATGTCTTGCC GCGCCAGTAG CAGTGTTAAC TACATGTACT GGTATCAACA
      GAAACCTGGC
121   CAGTCACCCA GGCTCCTCAT CTATTACACA TCCAATTGG CCCCTGGCGT
      TCCAGCCAGG
181   TTCAGTGGCA GTGGGTCTGG GACAGAGTAC ACTCTCACCA TCAGCAGCCT
      ACAGAGCGAA
241   GATTTTGCAG TTTATTACTG TCAGCAGTTC AGCAGTTCAC CTTGGACGTT
      CGGCCAAGGG
301   ACCAAGGTGG AAATCAAA (SEQ ID NO: 22)

```

h34D10 VL3 Light Chain Nucleic Acid Sequence

```

1     GAAAACGTAA TGACACAGTC TCCAGCCACC CTGTCTGCCT CTCCTGGCGA
      AAGAGTGACC
61    ATGTCTTGCC GCGCCAGTAG CAGTGTTAAC TACATGTACT GGTATCAACA
      GAAACCTGGC
121   CAGTCACCCA GGCTCCTCAT CTATTACACA TCCAATTGG CCCCTGGCGT
      TCCAGCCAGG
181   TTCAGTGGCA GTGGGTCTGG GACAGAGTAC ACTCTCACCA TCAGCAGCCT
      ACAGAGCGAA
241   GATTTTGCAG TTTATTACTG TCAGCAGTTC AGCAGTTCAC CTTGGACGTT
      CGGCCAAGGG
301   ACCAAGGTGG AAATCAAACG TACGGTGGCT GCACCATCTG TCTTCATCTT
      CCGCCATCT
361   GATGAGCAGT TGAAATCTGG AACTGCCTCT GTTGTGTGCC TGCTGAATAA
      CTTCTATCCC
421   AGAGAGGCCA AAGTACAGTG GAAGGTGGAT AACGCCCTCC AATCGGGTAA
      CTCCCAGGAG

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481   AGTGTACACAG AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC
      CCTGACGCTG
541   AGCAAAGCAG ACTACGAGAA ACACAAAGTC TACGCCTGCG AAGTCACCCA
      TCAGGGCCTG
601   AGCTCGCCCG TCACAAAGAG CTTCAACAGG GGAGAGTGTT AG (SEQ ID NO: 68)

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Alignments of the amino acid sequences of 34D10 VH and VL with the six humanized 34D10 variable heavy chain regions and four humanized 34D10 variable light chain regions are shown in Figures 1 and 2, respectively.

Example 2: Flow Cytometry Studies to Determine the Binding of the Humanized Fabs to Human Platelets and Surface Plasmon Resonance Studies to Determine the Affinity of Humanized Fabs for Purified GPIIb/IIIa

Human platelets were purified from platelet-rich plasma (PRP) using a Sepharose CL-2B column (GE Healthcare) in platelet buffer (15 mM HEPES, 138 mM NaCl, 5 mM CaCl₂, 2.7 mM KCl, 1 mM MgCl₂, 5.5 mM dextrose, 1 mg/ml BSA, pH 7.4) following methods known in the art. Humanized Fab at different increasing concentrations was incubated with the gel-purified platelets for 20 minutes at room temperature. Next platelets were washed with citrate buffer (5.4 mM trisodium citrate, 146 mM NaCl, 5.5 mM dextrose, pH 6.8) and resuspended in platelet buffer. R-phycoerythrin-labeled anti-human Fab polyclonal antibody (Southern Biotech) was added to the samples and incubated for 20 minutes at room temperature in the dark. Finally the cells were fixed with paraformaldehyde (final concentration 1%) and binding of the Fab to platelets was measured by standard flow cytometry techniques. EC₅₀ was calculated as the Fab concentration that shows a mean fluorescence signal (MFI) equal to one half of the maximal mean fluorescence signal (maximal signal observed at saturation of Fab binding). As a control, binding of the mouse Fab (34D10) was also measured. With the exception of Fab L0/H0, all Fabs with L0 light chain showed binding comparable to the mouse Fab (Figure 3). L0/H2 and L0/H5 showed the best binding. All the Fabs with the L3 light chain also showed binding comparable to the mouse Fab. In general, Fabs with the L1 or L2 light chains showed weaker binding.

To confirm the binding profiles observed in the flow cytometry experiments, binding assays were performed using surface plasmon resonance (SPR) technology. For this purpose biotinylated human GPIIb/IIIa ectodomain protein was generated as described in Zhu et al. *Molecular Cell*, 32(6): 849-861 (2008). The GPIIb/IIIa ectodomain protein was immobilized

on an SPR chip coated with streptavidin (GE Healthcare). Next, the association and dissociation rates of Fab binding to GPIIb/IIIa at sequentially increasing concentrations of the Fab were measured following methods known in the art. The kinetic parameters were derived from a 1:1 binding model. Fab L0/H2 ($K_D=2.8$ nM) and L0/H5 ($K_D=5.3$ nM) displayed affinities comparable to the mouse 34D10 Fab ($K_D=2.8$ nM), while Fab L1/H3 displays significantly lower affinity (110 nM) (data not shown). These results are consistent with the flow cytometry binding data and show that the humanized Fabs L0/H2 and L0/H5 and the mouse Fab 34D10 bind to GPIIb/IIIa with similar affinity.

Example 3: Generation of FVII-245/Fab033, FVII-250/Fab-036, FVII-250/Fab-037, FVII-251/Fab-036, FVII-251-Fab-037 and FVII-200

As shown above, the binding of humanized Fabs L0/H2 and L0/H5 and mouse Fab (34D10) to platelets and purified GPIIb/IIIa was similar. To determine if the properties of the humanized Fabs and the mouse Fab were similar when fused to FVIIa, a number of FVIIa fusion proteins were generated. Specifically, the following chimeric proteins were generated: (i) mouse Fab fused to FVIIa (FVII-245/Fab-033, Figure 4A), (ii) humanized Fab L0/H2 fused to FVIIa to generate FVII-250/Fab-037 or (iii) humanized Fab L0/H5 fused to FVIIa to generate FVII-250/Fab-036. In all of these fusions, the N-terminus of the light chain variable domain of the Fab was recombinantly fused to the C-terminus of heavy chain FVIIa via a linker(Gly₄Ser)₆ (SEQ ID NO:170), and the light chain of rFVIIa associates with the heavy chain FVIIa while the heavy chain component of the Fab associates with the light chain component of the Fab.

The amino acid sequence of the light chain (underlined) followed by the heavy chain (boldened) of FVII are provided below:

1	<u>ANAFLEELRP</u>	<u>GSLERECKEE</u>	<u>QCSFEEAREI</u>	<u>FKDAERTKLF</u>	<u>WISYSDGDQC</u>
51	<u>ASSPCQNGGS</u>	<u>CKDQLQSYIC</u>	<u>FCLPAFEGRN</u>	<u>CETHKDDQLI</u>	<u>CVNENGGCEQ</u>
101	<u>YCSDHTGTRK</u>	<u>SCRHEGYSL</u>	<u>LADGVSTPT</u>	<u>VEYPCGKIPI</u>	<u>LEKRNASKPQ</u>
151	<u>GRIVGGKVCP</u>	KGECPWQVLL	LVNGAQLCGG	TLINTIWVVS	AAHCFDKIKN
201	WRNLI AVLGE	HDLSEHDGDE	QSRRVAQVII	PSTYVPGTTN	HDIALLR LHQ
251	PVVLTDHVVP	LCLPERTFSE	RTLAFVRFSL	VSGWGQLLDR	GATALELMVL
301	NVPRLM TQDC	LQQSRKVGDS	PNITEYMFCA	GYSDGSKDSC	KGDSGGPHAT
351	HYRG TWYLTG	IVSWGQGCAT	VGHFGVYTRV	SQYIEWLQKL	MRSEPRPGVL
401	LRAPFP	(SEQ ID NO: 69)			

The amino acid sequence of the Fab light chain in FVII-245 that associates with the Fab heavy chain of Fab-033 (the VL domain is underlined and the constant region boldened) is provided below:

ENVLTQSPAIMASASLGEKVTMSCRASSSVNYMYWYQQKSDASPKLWIYYTSNLAPGVPARFSGSGSGN
SYSLTISSMEGEDAATYYCQQFSSSPWTFGGG**TKLEIKRADAAPTVSIFFPSSEQLTSGGASVVCFLN**
NFYPKDINV**KWKIDG**SERQNGVLNSWTDQDSK**STYSMSSTLT**TLTKDEYERHNSYTCEATHKTSTSPI
VKSFNRNEC (SEQ ID NO:70)

The amino acid sequence of FVII-245 (*i.e.*, the fusion of the light and heavy chain of FVII via a linker (underlined) to the light chain domain of 34D10 (variable domain boldened; constant region italicized) is provided below:

1	ANAFLEELRP	GS	LERECKEE	QCS	FEEAREI	FKDAERTKLF	WISYSDGDQC
51	ASSPCQNGGS	CKD	QLQSYIC	FCL	PAFEGRN	CETHKDDQLI	CVNENGCGEQ
101	YCS	DHTG	TKR	SCR	HEGYSL	LADGV	SCTPT
151	GRIVGGKVCP	KG	EC	PWQVLL	LV	NGAQLCGG	TLINTIWVVS
201	WRNLI	AVLGE	HDL	SEHDGDE	QS	RVAQVII	PSTYVPGTTN
251	PVVLTDHVVP	LCL	P	ERTFSE	RTL	AFVRFSL	VSGWGQLDR
301	NVPR	LMTQDC	LQ	SRKVGDS	PN	ITEYMFC	GYSDGSKDSC
351	HYRGTWYLTG	IV	SWGQGCAT	VGH	FGVYTRV	SQYIEWLQKL	MRSEPRPGVL
401	LRAPFP	GGGG	SGGGG	SGGGG	SGGGG	SGGGG	ENVL
451	LGEKVTM SCR	ASSSVNYMYW	YQQKSD ASP	LWIYYT SNLA	PGV PARFSGS		
501	GSGNSYS LT I	SSMEGEDAAT	YYCQQFSSSP	WTFGGG TKLE	IKR ADAAPTV		
551	<i>SIFPPS</i> SEQL	<i>TSGGAS</i> VVCF	<i>LNNFY</i> PKDIN	<i>VKWKIDG</i> SER	<i>QNGVLN</i> SWTD		
601	<i>QDSK</i> STYSM	<i>SSTLT</i> TLTKDE	<i>YERHNS</i> YTCE	<i>ATHKT</i> STSPI	<i>VKS</i> FNNEC*		

(SEQ ID NO:71)

The amino acid sequence of the Fab heavy chain of Fab-033 (the VH domain is underlined; the constant region is boldened) is provided below:

1	<u>EVKL</u> VESGGG	<u>LVK</u> PGGSLKL	<u>SCAAS</u> GFTFS	<u>AYAM</u> SWVRQT	<u>PEKR</u> LEWVAS
51	<u>ISSG</u> GTTYYP	<u>DSV</u> KRRFTIS	<u>RDNAR</u> NILYL	<u>QMSS</u> LRSED	<u>AMYY</u> CTRGGD
101	<u>YGYAL</u> DYWQ	<u>GTSV</u> TVSSAK	TTAP SVYPLA	PVC GD TTGSS	VT LGCL VKG Y
151	FPEP VT LTWN	SGSL SSGVHT	FPAV LQSDLY	TLSS SVTVTS	STWP SQSITC
201	NVAH PASSTK	VDK KIEPR			

(SEQ ID NO:72)

This Fab heavy chain associates with the Fab light chain in FVII-245.

The amino acid sequence of the L0 Fab light chain in FVII-250 that associates with the Fab heavy chains of Fab-036 and Fab-037 (the VL0 domain is underlined, the constant region of the Fab is boldened) is provided below:

1	<u>EIVMTQ</u> SPAT	<u>LSV</u> SPGERAT	<u>LSCR</u> ASSSVN	<u>YMYW</u> YQQKPG	<u>QAPR</u> LLIYYT
51	<u>SNLAP</u> GIPAR	<u>FSGS</u> SGS	<u>GTEF</u>	<u>TLTI</u> SSLQSE	<u>DFAV</u> YYCQQF

SSSPWTFGGQ

101 TKVEIKRTVA **APSVFIFPPS** **DEQLKSGTAS** **VVCLLNNFYF** **REAKVQWKVD**
 151 **NALQSGNSQE** **SVTEQDSKDS** **TYSLSSTLTTL** **SKADYEKHKV** **YACEVTHQGL**
 201 **SSPVTKSFNR** **GEC*** (SEQ ID NO: 73)

The amino acid sequence of FVII-250 (*i.e.*, the fusion of the light and heavy chain of FVII via a linker (underlined) to the L0 Fab light chain (boldened)) is provided below:

1 ANAFLEELRP GSLERECKEE QCSFEEAREI FKDAERTKLF WISYSDGDQC
 51 ASSPCQNGGS CKDQLQSYIC FCLPAFEGRN CETHKDDQLI CVNENGGCEQ
 101 YCSDHTGTRK SCRCHEGYSL LADGVSTPT VEYPCGKIPI LEKRNASKPQ
 151 GRIVGGKVCP KGECPWQVLL LVNGAQLCGG TLINTIWWVS AAHCFDKIKN
 201 WRNLI AVLGE HDLSEHDGDE QSRRVAQVII PSTYVPGTTN HDIALLRHLHQ
 251 PVVLT DHVVP LCLPERTFSE RTLAFVRFSL VSGWGQLLDR GATALELMVL
 301 NVPRLMTQDC LQSRKVGDS PNITEYMFCG GYSDGSKDSC KGDSGGPHAT
 351 HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV SQYIEWLQKL MRSEPRPGVL
 401 LRAPFPGGGG SGGGGSGGGG SGGGGSGGGG SGGGGSEIVM **TQSPATLSVS**
 451 **PGERATLSCR** **ASSSVNYMYW** **YQKPGQAPR** **LLIYYTSNLA** **PGIPARFSGS**
 501 **GSgteftlti** **SSLQSEDFAV** **YYCQFSSSP** **WTFGQGTKVE** **IKRTVAAPSV**
 551 **FIFPPSDEQL** **KSGTASVVCL** **LNNFYPREAK** **VQWKVDNALQ** **SGNSQESVTE**
 601 **QDSKDSTYSL** **SSTLTLSKAD** **YEKHKVYACE** **VTHQGLSSPV** **TKSFNRGEC***
 (SEQ ID NO: 74)

The amino acid sequence of the H2 chain of Fab-037 (the VH2 domain is underlined) is provided below:

1 EVQLVESGGG LVKPGGSLRL SCAASGFTFS AYAMSWVRQA PGKGLVWVAS
 51 ISSGGTTYYP DSVKRQFTIS RDNAKNTLYL QMNSLRAEDT AVYYCTRGGD
 101 YGYALDYWGQ GTLVTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY
 151 FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI
 201 CNVNHKPSNT KVDKKVEPKS C* (SEQ ID NO: 75)

The amino acid sequence of the H5 chain of Fab-036 (the VH5 domain is underlined) is provided below:

1 EVKLVESGGG LVKPGGSLRL SCAASGFTFS AYAMSWVRQA PGKGLEWVAS
 51 ISSGGTTYYP DSVKRRFTIS RDNARNTLYL QMNSLRAEDT AVYYCTRGGD
 101 YGYALDYWGQ GTLVTVSSAS TKGPSVFPLA PSSKSTSGGT AALGCLVKDY
 151 FPEPVTVSWN SGALTSGVHT FPAVLQSSGL YSLSSVVTVP SSSLGTQTYI
 201 CNVNHKPSNT KVDKKVEPKS C* (SEQ ID NO: 76)

The above two Fab heavy chains associate with the Fab light chain in FVII-250.

A schematic diagram of these chimeric constructs is depicted in Figure 4A.

In addition the same Fabs were fused to FVIIa-XTEN (AE288) as shown in Figure 5A to generate FVII-251/Fab-037 and FVII-251/Fab-036. FVII-200, was also generated with the same structural organization as FVII-251, but where the targeting moiety was an scFv version of mouse 34D10 (instead of an Fab).

The amino acid sequence of FVII-251 (*i.e.*, the fusion of the light and heavy chain of FVII via a linker (SEQ ID NO:195) to an XTEN (AE288) (italicized and underlined) and linkers (SEQ ID NO:196 and 170) (bold italics (SEQ ID NO:196) and underlined (SEQ ID NO:170)) to the Fab light chain L0 (boldened)) is provided below:

```

1   ANAFLEELRP  GSLERECKEE  QCSFEEAREI  FKDAERTKLF  WISYSDGDQC
51  ASSPCQNGGS  CKDQLQSYIC  FCLPAFEGRN  CETHKDDQLI  CVNENGGCEQ
101 YCSDHTGTRK  SCRCHGYSL  LADGVSTPT  VEYPCGKIPI  LEKRNASKPQ
151 GRIVGGKVCP  KGECPWQVLL  LVNGAQLCGG  TLINTIWVVS  AAHCFDKIKN
201 WRNLI AVLGE  HDLSEHDGDE  QSRRVAQVII  PSTYVPGTTN  HDIALLR LHQ
251 PVVLT DHVVP  LCLPERTFSE  RTLAFVRFSL  VSGWGQLLDR  GATALELMVL
301 NVPR LMTQDC  LQQSRKVGDS  PNITEYMFCA  GYSDGSKDSC  KGDSGGPHAT
351 HYRG TWYLTG  IVSWGQGCAT  VGHFGVYTRV  SQYIEWLQKL  MRSEPRPGVL
401 LRAP FPSPG  TSESATPESG  PGSEPATSGS  ETPGTSESAT  PESGPGSEPA
451 TSGSETPGTS  ESATPESGPG  TSTEPSEGSA  PGSPAGSPTS  TEEGTSESAT
501 PESGPGSEPA  TSGSETPGTS  ESATPESGPG  SPAGSPTSTE  EGSPAGSPTS
551 TEEGTSTEPS  EGSAPGTSES  ATPESGPGTS  ESATPESGPG  TSESATPESG
601 PGSEPATSGS  ETPGSEPATS  GSETPGSPAG  SPTSTEEGTS  TEPSEGSAPG
651 TSTEPSEGSA  PGSEPATSGS  ETPGTSESAT  PESGPGTSTE  PSEGSAPGSS
701 SGGGGSGGGG  SGGGGSGGGG  SGGGGSGGGG  SEIVMTQSPA  TLSVSPGERA
751 TLSCRASSSV  NYMYWYQQKP  GQAPRLLIYY  TSNLAPGIPA  RFSGSGSGTE
801 FTLTISSLQS  EDFAVYQCQQ  FSSSPWTFGQ  GTKVEIKRTV  AAPSVFIFPP
851 SDEQLKSGTA  SVVCLLNIFY  PREAKVQWKV  DNALQSGNSQ  ESVTEQDSKD
901 STYSLSSTLT  LSKADYEKHK  VYACEVTHQG  LSSEPVTKSFN  RGEC*
      (SEQ ID NO:77)

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The two Fab heavy chains discussed above (Fab -037 and Fab-036) associate with the Fab light chain in FVII-251.

The amino acid sequence of FVII-200 is provided below. The structure of this construct is as follows: light chain of FVII followed by heavy chain of FVII fused to a linker (SEQ ID NO: 195) followed by XTEN followed by a GSSS linker (SEQ ID NO:196) and a (G4S)₆ (SEQ ID NO: 170) linker fused to the VL domain of 34D10 fused to (G4S)₄ (SEQ ID NO:168) linker fused to the VH domain of 34D10 (the XTEN, AE288 is italicized and underlined; the G4S (SEQ ID NO:165) linkers are underlined; the VL of the 34D10 scFv is boldened and the VH of the 34D10 scFv is italicized):

```

1  ANAFLEELRP  GSLERECKEE  QCSFEEAREI  FKDAERTKLF  WISYSDGDQC
51  ASSPCQNGGS  CKDQLQSYIC  FCLPAFEGRN  CETHKDDQLI  CVNENGGCEQ
101 YCSDHTGTKR  SCRCHGYSL  LADGVSTPT  VEYPCGKIPI  LEKRNASKPQ
151 GRIVGGKVCP  KGECPWQVLL  LVNGAQLCGG  TLINTIWVVS  AAHCFDKIKN
201 WRNLI AVLGE  HDLSEHDGDE  QSRRVAQVII  PSTYVPGTTN  HDIALRLHQ
251 PVVLT DHVVP  LCLPERTFSE  RTLAFVRFSL  VSGWGQLLDR  GATALELMVL
301 NVPRLM TQDC  LQQSRKVGDS  PNITEYMFCA  GYSDGSKDSC  KGDSGGPHAT
351 HYRG TWYLTG  IVSWGQGCAT  VGHFGVYTRV  SQYIEWLQKL  MRSEPRPGVL
401 LRAPFP GSPG  TSESATPESG  PGSEPATSGS  ETPGTSESAT  PESGPGSEPA
451 TSGSETPGTS ESATPESGPG TSTEPSEGS PGSPAGSPTS TEEGTSESAT
501 PESGPGSEPA TSGSETPGTS ESATPESGPG SPAGSPTSTE EGSPAGSPTS
551 TEEGTSTEPS EGSAPGTSES ATPESGPGTS ESATPESGPG TSESATPESG
601 PGSEPATSGS ETPGSEPATS GSETPGSPAG SPTSTEEGTS TEPSEGSAPG
651 TSTEPSEGS PGSEPATSGS ETPGTSESAT PESGPGTSTE PESGAPGSS
701 SGGGGSGGGG SGGGGSGGGG SGGGGSGGGG SENVLTQSPA IMSASLGEKV
751 TMSCRASSV NMYWYQKS DASPKLWIYY TSNLAPGVPA RFSGSGSGNS
801 YSLTISSMEG EDAATYQCQ FSSSPWTFGG GTKLEIKRGG GGSGGGSGG
851 GGSGGGGSEV KLVESGGGLV KPGGSLKLSC AASGFTFSAY AMSWVRQTPE
901 KRLEWVASIS SGGTYYPD VKRRFTISR NARNILYLQM SSLRSEDAM
951 YYCTRGGDYG  YALDYWGQGT  SVTVSS (SEQ ID NO:78)

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To generate all these fusion proteins, DNA encoding the protein sequences were synthesized, cloned into an expression vector, and expressed in HEK 293 cells by transient transfection following methods known in the art. Protein was purified from the conditioned media following methods known in the art.

Example 4: FVIIa-Humanized Fab And FVIIa-Mouse Fab Fusion Proteins Show Comparable Activity In Whole Blood Assays

These experiments were directed at determining whether the activity associated with platelet-targeted FVIIa was similar for variants fused to the mouse 34D10 Fab and the humanized Fabs. The activity of FVII-245/Fab-033, FVII-250/Fab-037 and FVII-250/Fab-036 was determined by rotational thromboelastometry (ROTEM) assays using whole blood from hemophilia donors. The activity in whole blood is platelet-dependent, and coagulation was initiated by recalcification of the blood. In these assays all three proteins showed similar clotting times at all the concentrations tested (Figure 4B), indicating that the humanized Fabs maintain their properties after humanization.

FVII-245//Fab-033 and FVII-189 (mouse 34D10 in scFv format recombinantly fused to rFVIIa) also showed comparable activity by ROTEM (Figure 4C), demonstrating that the targeting moiety works equally well as an scFv or Fab format.

In addition, FVII-189 displays much higher activity than rFVIIa by ROTEM (Figure 4D) and from this it can be inferred that FVII-250 with the humanized Fab also displays activity by ROTEM much greater than rFVIIa.

The same experiments were performed with the FVIIa-XTEN fusion proteins (Figure 5A). First, FVII-251/Fab-037 and FVII-251/Fab-036 were compared to FVII-200 carrying the mouse version of the targeting moiety. Figure 5B shows that the activity of all three proteins is similar, indicating that the humanized and mouse targeting moieties have similar properties. Next FVII-251/Fab-037 was compared to recombinant FVIIa (rFVIIa) (Figure 5C). FVII-251/Fab-037 displays greater activity than rFVIIa, demonstrating that the humanized targeting moiety (L0/H2) can target FVIIa to platelets and increase its activity.

Example 5: Ex Vivo activity of Humanized 34D10 Fab Linked to Factor VIIa Compared With Recombinant Factor VIIa (rFVIIa) in Transgenic Hemophilia A Mice

Transgenic hemophilia A mice with a fully humanized α IIB subunit in the α IIB/ β 3 integrin were dosed with 10 nmol/kg of rFVIIa or FVII-251/Fab-037 (*i.e.*, Factor VIIa linked to XTEN linked to the VL0/VH2 h34D10 Fab). For each molecule and time point 3 mice were dosed. At different times post-dosing (Figure 6), mice were euthanized and blood was collected. Three hundred microliters of blood were analyzed by rotational thromboelastometry (ROTEM) assay, and coagulation was initiated by recalcification of the blood. FVII-251/Fab-037, showed shorter clotting times at 5 minutes post-dosing, indicating increased acute ex vivo activity. FVII-251/Fab-037 displayed shorter clotting times than rFVIIa at all the timepoints, and the clotting time for FVII-251/Fab-037 at 6 hours post-dosing was comparable to the clotting time of rFVIIa at 1 hour post-dosing, suggesting a 6-fold improvement in the prolonged *ex vivo* efficacy for FVII-251/Fab-037 compared with rFVIIa.

Example 6: Improved Plasma Pharmacokinetics of FVII-251/Fab-037 in Human α IIB Transgenic Hemophilia A Mice

Plasma samples from Example 5, in which transgenic hemophilia A mice with a fully humanized α IIB subunit in the α IIB/ β 3 integrin were dosed with 10 nmol/kg of rFVIIa (n=3) or FVII-251/Fab-037 (n=4) were taken. For each molecule and time point 3-4 mice were dosed. At different times post-dosing (Figure 9), mice were euthanized and blood was

collected. One fraction of the citrated whole blood was used for *ex-vivo* rotational thromboelastometry (ROTEM) assay as described in Example 5. From the remaining blood, plasma was isolated by centrifugation and the FVIIa plasma activity levels of rFVIIa or FVII-251/Fab037 were determined using the StacLOT VIIa-rTF assay (Diagnostica Stago) on a Sysmex CA-1500, using the respective dosing material as self-standard.

In these experiments, rFVIIa and FVII-251/Fab-037 showed comparable plasma recovery at 5 minutes post-dosing and FVII-251/Fab-037 showed decreased clearance and higher plasma activity levels compared to equal molar dosed rFVIIa over all time points measured, consistent with improved pharmacokinetic properties.

Example 7: FVII-251/Fab-037 is Efficacious in Acute Efficacy Murine Bleeding Models

Factor VIII deficient mice expressing only human alphaIIb in place of murine alphaIIb on platelets (HemA-Tg-hu-alphaIIb mice), were created by crossing hemophilia A (HemA) knock-out mice (exon 16, Bi et al., *Nat. Genet.*, 10(1):119–121, 1995) with mouse alphaIIb knock-out mice (Emambokus et al., *Immunity*, 19(1):33–45, 2003), which were transgenic for human alphaIIb integrin expression (Thornton et al., *Blood*, 100(10):3588–3596, 2002). For experiments the mice were crossed to homogeneity, resulting in HemA mice, expressing human aIIb, murine beta3 integrin heterodimers on all platelets.

Acute efficacy was studied in a blinded murine tail-clip bleeding model, in which total blood loss in dosed mice is measured after tail tip amputation, as described previously (Dumont et al., *Blood*, 119(13):3024–3030, 2012). Briefly, male HemA-Tg hu-aIIb mice (8–13 wks) were anesthetized with a cocktail of 50 mg/kg ketamine and 0.5 mg/kg dexmedetomidine. The tails were immersed in 37 °C saline for 10 minutes, to dilate the lateral vein followed by intravenous tail vein injection of either vehicle (20 mM 2-amino-2-hydroxymethyl-propane-1, 3-diol (TRIS), pH 8.0; 150 mM NaCl; 3% human serum albumin (n=13)), rFVIIa at 100 nmol/kg (n=10) or FVII-251/Fab-037 at 3 nmol/kg (n=8) or 10 nmole/kg, (n=8). Five minutes post-dosing, the 4 mm distal tip of the tail was clipped and submerged into a pre-weighted tube containing 11 mL saline for the period of 30 minutes. Blood loss was quantified by weight. Statistical significance was calculated using unpaired two-tailed t-test in GraphPad Prism 6. Such two tailed t-tests showed that the 3 and 10 nmol/kg doses of FVII-251/ Fab-037 and rFVIIa were significantly different from vehicle (p-

value < 0.001), but not significantly different from each other (p-value > 0.9) (see, Figure 10).

These results demonstrate equal or improved acute efficacy for FVII-251/Fab-037 compared to rFVIIa in this bleeding model.

Example 8: Affinity Maturation of a Humanized 34D10 Antibody

The nucleic acid sequence encoding humanized 34D10 VH2 (the amino acid sequence is set forth in **SEQ ID NO: 7**) was subjected to multiple cycles of error-prone polymerase chain reaction (PCR) according to published methods (Zaccolo et al., *J. Mol. Biol.*, 255(4):589-603, 1996; Van Deventer and Wittrup, *Methods Mol. Biol.*, 1131:151-81, 2014). The pool of mutated 34D10 VH2 nucleic acid sequences and a nucleic acid sequence encoding 34D10 VL0 (**SEQ ID NO: 19**) were then introduced into the Adimab platform, resulting in an Adimab expression library size of approximately 10^6 antibodies (see, US Patent Publications 20100056386 and 20090181855 to Adimab, Inc. as well as references cited therein). For comparison purposes, a nucleic acid sequence encoding 34D10 VH2 (**SEQ ID NO: 15**) and a nucleic acid sequence encoding 34D10 VL0 (**SEQ ID NO: 19**) were introduced into the Adimab yeast platform. To identify anti-GPIIb/IIIa antibodies with improvements in affinity (over the parental VH – *i.e.*, h34D10 VH2), expression libraries were screened in accordance with the methodologies disclosed in the US Patent Publications 20100056386 and 20090181855. After iterative rounds of selective pressure towards the target antigen, GPIIb/IIIa (**SEQ ID NOs: 23 and 24**), and efforts to improve antibody off-rate, colonies were sequenced to identify unique antibodies, according to methods known in the art. 45 unique VH sequences were discovered and subsequently expressed and purified from yeast by protein A purification followed by standard Fab generation, according to methods known in the art.

To identify antibodies discovered from our selections that displayed improvements in affinity and/or off-rate when compared to the parental VH (amino acid sequence: **SEQ ID NO:7**; nucleic acid sequence: **SEQ ID NO:15**), the antibodies purified from yeast were screened for binding to target antigen (hGPIIb-**SEQ ID NO:23** and hGPIIIa-**SEQ ID NO:24**) using Bio-Layer Interferometry (BLI) in a monovalent assay format. BLI was performed on the OctetRed94 instrument, manufactured by ForteBio, according to standard procedures. The present disclosure identifies 22 unique VH sequences with improvements in affinity

and/or off-rate when compared to the parental VH (amino acid sequence: **SEQ ID NO:7**; nucleic acid sequence: **SEQ ID NO: 15**) (Figure 11). The BLI binding profiles of all VH (Figure 12A) as well as examples of individual affinity matured VH sequences (**SEQ ID NOs: 197, 202, 205**) in comparison to the parental clone (amino acid sequences: **SEQ ID NOs: 7 and 4**; nucleic acid sequences: **SEQ ID NOs: 15 and 19**) are disclosed herein (Figures 12B-E). A table listing the apparent monovalent affinity and dissociation rates of the 22 disclosed VH sequences paired with the parental 34D10 VL0 (amino acid sequence: **SEQ ID NO:4**; nucleic acid sequence: **SEQ ID NO: 19**) purified from yeast, as determined by BLI in the monovalent format, is depicted in Figure 12F.

To determine if mutations introduced into the humanized 34D10 VH2 sequence (amino acid sequence: **SEQ ID NO:7**; nucleic acid sequence: **SEQ ID NO: 15**) during error-prone PCR had adverse effects on protein stability, yeast purified Fab of the 22 unique VH (Figure 11) with parental VL0 were subjected to thermal denaturation by differential scanning fluorimetry (DSF). Measurements were conducted on an Mx3005p real-time PCR system (Agilent Technologies) in a 96-well format using 10 µg of Fab in 50 µl PBS (at pH 7.0) supplemented with SYPRO orange fluorophor. Derivation of the melting temperature (T_m) was performed as described in Pepinsky et al., *Protein Sci.*, 19(5):954-662010. A table listing the calculated T_m is provided in Figure 13.

The amino acid and nucleic acid sequences of the 22 VH sequences obtained by the affinity maturation of h34D10 VH2 by the methods described above are provided below:

VH100 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFGAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYSYALDYWGQGTLVTVSS (**SEQ ID NO: 197**)

VH100 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTGGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCTGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATAGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(**SEQ ID NO: 219**)

VH101 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYSYALDYWGQGTLLTVSS (SEQ
ID NO: 198)

VH101 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGGAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATAGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 220)

VH102 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYSYALDYWGQGTLLTVSF (SEQ
ID NO: 199)

VH102 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATAGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTTCAGC
(SEQ ID NO: 221)

VH103 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQVNSLRAEDTAVYYCTRGGDYSYALDYWGQGTLLTVSS (SEQ
ID NO: 200)

VH103 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAGTGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG

GATTATAGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 222)

VH104 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYSYALDYWGRGTLVTVSS (SEQ
ID NO: 201)

VH104 Variable Heavy Chain Amino Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATT
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATAGCTACGCTCTCGACTACTGGGGCCGGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 223)

VH105 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYSYALDYWGQGTTLTVTVSS (SEQ
ID NO: 202)

VH105 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATAGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 224)

VH106 Variable Heavy Chain Amino Acid Sequence

EVQLVECGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYSYALDYWGQGTTLTVTVSS (SEQ
ID NO: 203)

VH106 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTGTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATAGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 225)

VH107 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGESLRLSCAASGFTFSAYAMS~~WVRQAPG~~GLVWVASISSGGTTYYPDS
VKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYSYALDYWGQGLTVTVSS (SEQ
ID NO: 204)

VH107 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGAATCCCTGAGACT
CTCCTGTGCAGCCTCGGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGGAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATAGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 226)

VH108 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMS~~WVRQAPG~~KGLVWVASISSDGTYYYPD
SVKRQFTISRDNARNNTLYLQMNSLRAEDTAVYYCTRGGDYSYALDYWGQGLTVTVSS (SEQ
ID NO: 205)

VH108 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGATGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAGGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GACTATAGCTACGCTCTCGACTACTGGGGCCAGGGGACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 227)

VH109 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTDYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGTLVTVSS
(SEQ ID NO: 206)

VH109 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACAGACTACC
CAGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTAT
CTGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGG
GGATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 228)

VH110 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFNAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGTLVTVSS
(SEQ ID NO: 207)

VH110 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAACGCCTATGCAATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 229)

VH111 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFNAYAMSWVRQAPGEGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGTLVTVSS
(SEQ ID NO: 208)

VH111 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAACGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGGAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGCACCAGAGGAGGG
GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 230)

VH112 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGEGLVWVAGISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGTLVTVSS
(SEQ ID NO: 209)

VH112 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGGAGGGGCTGGTCTGGGTCTGCTGGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 231)

VH113 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVAGISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGTLVTVSS
(SEQ ID NO: 210)

VH113 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCTGCTGGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 232)

VH114 Variable Heavy Chain Amino Acid Sequence

EMQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGTLVTVSS
(SEQ ID NO: 211)

VH114 Variable Heavy Chain Nucleic Acid Sequence

GAGATGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCTGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG

GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 233)

VH115 Variable Heavy Chain Amino Acid Sequence

GVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDDAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGTLLTVSS
(SEQ ID NO: 212)

VH115 Variable Heavy Chain Nucleic Acid Sequence

GGGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACGATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACTAGAGGGGGG
GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 234)

VH116 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDDAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGTLLTVSS
(SEQ ID NO: 213)

VH116 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACGATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 235)

VH117 Variable Heavy Chain Amino Acid Sequence

EAQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGTLLTVSS
(SEQ ID NO: 214)

VH117 Variable Heavy Chain Nucleic Acid Sequence

GAGGCGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTGAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC

TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 236)

VH118 Variable Heavy Chain Amino Acid Sequence

GVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGLVTVSS
(SEQ ID NO: 215)

VH118 Variable Heavy Chain Nucleic Acid Sequence

GGGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAGCCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 237)

VH119 Variable Heavy Chain Amino Acid Sequence

GVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGLVTVSS
(SEQ ID NO: 216)

VH119 Variable Heavy Chain Nucleic Acid Sequence

GGGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
TCCAGGGAAGGGGCTGGTCTGGGTCGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
(SEQ ID NO: 238)

VH120 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVEPGGSLRLSCAASGFTFSAYAMSWVRQAPGKGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGALDYWGQGLVTVSS
(SEQ ID NO: 217)

VH120 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAGAGCCTGGAGGATCCCTGAGACT
 CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTACGCCATGAGCTGGGTCCGCCAGGC
 TCCAGGGAAGGGGCTGGTCTGGGTCTGCTAGCATTAGTAGTGGCGGTACCACATACTACC
 CAGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTAT
 CTGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGG
 GGATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCAACCGTCTCCTCA
 (SEQ ID NO: 239)

VH121 Variable Heavy Chain Amino Acid Sequence

EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWVRQAPGEGLVWVASISSGGTTYYPD
SVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGGDYGYALDYWGQGLVTVSS
 (SEQ ID NO: 218)

VH121 Variable Heavy Chain Nucleic Acid Sequence

GAGGTGCAGCTGGTGGAGTCTGGAGGAGGCTTGGTAAAGCCTGGAGGATCCCTGAGACT
 CTCCTGTGCAGCCTCTGGATTACCTTTAGCGCCTATGCCATGAGCTGGGTCCGCCAGGC
 TCCAGGGGAGGGGCTGGTCTGGGTCTGCTAGCATTAGTAGTGGTGGTACCACATACTACCC
 AGACTCCGTGAAGAGGCAGTTCACCATCTCCAGAGACAATGCCAAGAACACGCTGTATC
 TGCAAATGAACAGCCTGAGAGCCGAGGACACAGCCGTATATTACTGTACCAGAGGAGGG
 GATTATGGCTACGCTCTCGACTACTGGGGCCAGGGAACCCTGGTCAACCGTCTCCTCA
 (SEQ ID NO: 240)

Example 9: Generation of FVII-265

FVII-265 was generated to remove a GSSS (SEQ ID NO: 196) linker sequence in FVII-251 (*see*, Example 3) located between the XTEN sequence and the (Gly4Ser)₆ (SEQ ID NO:170) linker. The amino acid sequence of FVII-265 is provided below (from N terminus to C-terminus: the amino acid sequence of the light chain of FVII is underlined; this is followed by the amino acid sequence of the heavy chain of FVII, which is boldened and italicized; this is followed by a linker having the amino acid sequence set forth in SEQ ID NO:195; which is followed by the XTEN (AE288) sequence which is both italicized and underlined; followed by a (Gly4Ser)₆ (SEQ ID NO:170) linker that is double underlined; and which is followed by the Fab light chain L0 (boldened)):

1 ANAFLEELRP GSLERECKEE QCSFEEAREI FKDAERTKLF WISYSDGDQC
 51 ASSPCQNGGS CKDQLQSYIC FCLPAFEGRN CETHKDDQLI CVNENGGCEQ
 101 YCSDHTGTKR SCRCHGYSL LADGVSTPT VEYPCGKIPI LEKRNASKPQ
 151 GRIVGGKVCP KGECPWQVLL LVNGAQLCGG TLINTIWWVS **AAHCFDKIKN**

201 *WRNLIAVLGE HDLSEHDGDE QSRRVAQVII PSTYVPGTTN HDIALLRHQ*
 251 *PVVLTDHVVP LCLPERTFSE RTLAFVRFSL VSGWGQLLDR GATALEIMVL*
 301 *NVPRMTQDC LQSRKVGDS PNITEYMFCA GYSDGSKDSC KGDSGGPHAT*
 351 *HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV SQYIEWLQKL MRSEPRPGVL*
 401 *LRAPFP*GPSG TSESATPESG PGSEPATSGS ETPGTSESAT *PESGPGSEPA*
 451 *TSGSETPGTS ESATPESGPG TSTEPSEGSA PGSPAGSPTS TEEGTSESAT*
 501 *PESGPGSEPA TSGSETPGTS ESATPESGPG SPAGSPTSTE EGSPAGSPTS*
 551 *TEEGTSTEPS EGSAPGTSES ATPESGPGTS ESATPESGPG TSESATPESG*
 601 *PGSEPATSGS ETPGSEPATS GSETPGSPAG SPTSTEEGTS TEPSEGSAPG*
 651 *TSTEPSEGSA PGSEPATSGS ETPGTSESAT PESGPGTSTE PSEGSAPGGG*
 701 *GSGGGGSGGG GSGGGGSGGG GSGGGGSEIV MTQSPATLSV SPGERATLSC*
 751 *RASSSVNYMY WYQOKPGQAP RLLIYYTSNL APGIPARFSG SGSGETFTLT*
 801 *ISSLQSEDA VYYCQQFSSS PWTFGQGTKV EIKRTVAAPS VFIFPPSDEQ*
 851 *LKSGTASVVC LLNNFYPREA KVQWKVDNAL QSGNSQESVT EQDSKDSTYS*
 901 *LSSTLTLSKA DYEKHKVYAC EVTHQGLSSP VTKSFNRGEC * (SEQ ID NO:247)*

The Fab heavy chain discussed above in Example 3, Fab-037 (SEQ ID NO:75) associates with the Fab light chain in FVII-265.

Example 10: FVII-265/Fab-037 Shows More Potent ROTEM Activity than rFVIIa in a Platelet Targeting-Dependent Manner

Human α IIB transgenic HemA mice were dosed with the indicated doses of 0.3, 1, 3, 10, 30, and 100 nmol/kg of FVII-265/Fab-037 (solid dots in Figures 14A and B) or 3, 10, 30, 100 nmol/kg of rFVIIa (open circles in Figure 14) or vehicle (open triangle in Figures 14A and 14B). Five minutes post-dosing mice were euthanized and blood was collected from the vena cava. Whole blood clotting times were analyzed by ROTEM as described in Example 5 above. FVII-265/Fab-037 showed increased clotting activity in a platelet targeting-dependent manner compared to FVIIa (Figure 14A), whereas, in HemA mice in which the murine α IIB/ β 3 integrin is not targeted by FVII-251/Fab-037, similar clotting times of FVII-251/Fab-037 and FVIIa were measured. The data for each time point is the average $\pm$ standard deviation of 3 to 4 mice (Figure 14B).

Example 11: Improved Pharmacokinetics of FVII-251/Fab-037 Compared With rFVIIa in Human α IIb Transgenic HemA Mice Correlates with Prolonged and Improved ROTEM Clotting Time Efficacy

Human α IIb-transgenic HemA mice were dosed with either 10 nmol/kg rFVIIa, FVII-251/Fab-037 or vehicle. Blood was collected from the vena cava at t=5 min, 1, 3, 6, 9, 17, or 24 hours post dosing with FVII-251/Fab37, or at t= 5 min, 1, or 3 hours for FVIIa. Plasma levels of FVII-251/Fab-037 or FVIIa were determined by soluble tissue factor (sTF)-prothrombin time (PT) activity using dosing material as activity standards. In Figure 15A, plasma activity is plotted as % of injected dose. Mean Residence Time (MRT) and other pharmacokinetic (PK) parameters were calculated using Phoenix WinNonLin 6.2.1 (Pharsight, Certara) by NCA analysis. Clotting times in the freshly isolated whole blood samples were determined in a ROTEM machine (Figure 15B). As indicated in Figure 15B, FVII-251/Fab-037 shows, when dosed at equal molar doses, improved clotting times compared to FVIIa over a longer time interval, which correlates with the improved plasma PK profile of FVII-251/Fab-037.

Example 12: FVII-265/Fab-037 is Efficacious in a Prolonged Tail-Clip Bleeding Efficacy Model in Tg-HemA Mice

Prolonged bleeding efficacy was studied in a blinded murine tail-clip bleeding model in human α IIb-transgenic HemA mice (Figure 16). Prolonged protection was determined in a modified version of the acute tail-clip amputation efficacy model as described in Example 7. In Example 7, blood loss over a 30 minute time frame was measured after a tail-tip amputation applied 5 minutes post-dosing. In the novel prolonged modified model, the tail-tip amputations are performed at a later time points post-dosing, blood loss is again measured over a 30 minute period. After the dosing of 10 nmol/kg FVII-265/Fab-037, tail-tip amputations were performed at 2, 4 and 6 hours post-dosing. Similarly, blood loss was measured at 5 min and 2 hours-post dosing of 100 nmol/kg intravenous dosed FVIIa. Blood loss is indicated as % blood loss, compared to blood loss as measured in vehicle dosed mice at similar time points post-dosing. Indicated are the median blood loss and the statistical significance. The latter was calculated using unpaired two-tailed t-test in GraphPad Prism 6. Such two tailed t-tests showed that the 100 nmol/kg dose of FVIIa significant reduces blood loss at 5 minutes post-dosing and is not advantageous at 2 hours post-dosing. In contrast, a

ten-fold lower dose of 10 nmol/kg of FVII-265/Fab-037 shows a similar and efficacious reduction in blood loss 2 hours post-dosing as 100 nmol/kg FVIIa at five minutes post-dosing. In addition, significant reduction in blood loss compared to vehicle treated mice was observed at 4 and 6 hours post-dosing.

Example 13. Generation of FVII-250/Fab-062

In this configuration the Fab light chain within FVII-250 (described in Example 3; SEQ ID NO:74) was dimerized to the Fab heavy chain Fab-062, which comprises an XTEN (AE288) moiety fused to the N-terminus of the Fab heavy chain as illustrated in Figure 17.

The amino acid sequence of the Fab-062 is provided below. The XTEN sequence is in bold (note that a glutamic acid (E) is added at the N-terminus of AE288) and the VH2 domain of the Fab is underlined.

1	EGTSESATPE	SGPGSEPATs	GSETPGTSES	ATPESGPGSE	PATSGSETPG
51	TSESATPESG	PGTSTEPSEG	SAPGSPAGSP	TSTEEGTSES	ATPESGPGSE
101	PATSGSETPG	TSESATPESG	PGSPAGSPTS	TEEGSPAGSP	TSTEEGTSTE
151	PSEGSAPGTS	ESATPESGPG	TSESATPESG	PGTSESATPE	SGPGSEPATs
201	GSETPGSEPA	TSGSETPGSP	AGSPTSTEEG	TSTEPSEGSa	PGTSTEPSEG
251	SAPGSEPATs	GSETPGTSES	ATPESGPGTS	TEPSEGSAPe	<u>VQLVESGGGL</u>
301	VKPGGSLRLS	CAASGFTFSA	YAMSWVRQAP	GKGLVWVASI	<u>SSGGTTYYPD</u>
351	SVKRQFTISR	DNAKNTLYLQ	MNSLRAEDTA	VYYCTRGGDY	<u>GYALDYWGQG</u>
401	TLVTVSSAST	KGPSVFPLAP	SSKSTSGGTA	ALGCLVKDYF	<u>PEPVTVSWNS</u>
451	GALTSGVHTF	PAVLQSSGLY	SLSSVTVPS	SSLGTQTYIC	<u>NVNHKPSNTK</u>
501	VDKKVEPKSC	*	(SEQ ID NO:252)		

To generate this fusion protein, DNA encoding the above protein sequence was synthesized, cloned into an expression vector, and expressed in HEK 293 cells by transient transfection. The fusion protein was then purified from the conditioned media.

Example 14. Surface Plasmon Resonance Studies to Determine the Affinity of FVII-250/Fab-062 for Purified GPIIb/IIIa

To measure the affinity of FVII-250/Fab-062 for GPIIb/IIIa, binding assays were performed using surface plasmon resonance (SPR) technology. For this purpose, biotinylated human GPIIb/IIIa ectodomain protein was generated as described in Zhu et al. *Molecular Cell*, 32(6): 849-861 (2008). The GPIIb/IIIa ectodomain protein was immobilized on an SPR chip coated with streptavidin (GE Healthcare). Next, the association and dissociation rates of FVII-250/Fab-062 binding to GPIIb/IIIa at sequentially increasing concentrations of FVII-

250/Fab-062 were measured. The kinetic parameters were derived from a 1:1 binding model, and FVII-250/Fab-062 displayed a $K_D = 7.3 \times 10^{-7} \text{M}$ for its association with GPIIb/IIIa (Figure 18). These results indicate that Fab-250/Fab-062 can associate with GPIIb/IIIa.

Example 15. FVII-250/Fab-062 Shows Improved Activity Compared to FVIIa in Whole Blood Assays

The activity of FVII-250/Fab-062 was determined by rotational thromboelastometry (ROTEM) assays using whole blood from hemophilia donors. The activity in whole blood is platelet-dependent, and coagulation was initiated by recalcification of the blood. FVII-250/Fab-062 displays enhanced activity compared to FVIIa, indicated by faster clotting times for FVII-250/Fab-062 than FVIIa at equivalent concentrations (Figure 19). These data demonstrate that this Fab targeting moiety, with an XTEN moiety at the N-terminus of the Fab heavy chain, can target FVIIa to platelets and increase its potency.

OTHER EMBODIMENTS

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region that is at least 75% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218.
2. The antibody or antigen-binding fragment thereof of claim 1, wherein the heavy chain variable region is at least 80% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218.
3. The antibody or antigen-binding fragment thereof of claim 1, wherein the heavy chain variable region is at least 85% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218.
4. The antibody or antigen-binding fragment thereof of claim 1, wherein the heavy chain variable region is at least 90% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218.
5. The antibody or antigen-binding fragment thereof of claim 1, wherein the heavy chain variable region is at least 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218.
6. The antibody or antigen-binding fragment thereof of claim 1, wherein the heavy chain variable region is at least 97% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218.
7. The antibody or antigen-binding fragment thereof of claim 1, wherein the heavy chain variable region is identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218.
8. An antibody or antigen-binding fragment thereof that specifically binds to GPIIb/IIIa, wherein the antibody or the antigen-binding fragment thereof comprises a light chain variable region that is at least 75% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10.

9. The antibody or antigen-binding fragment thereof of claim 8, wherein the light chain variable region is at least 80% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10.
10. The antibody or antigen-binding fragment thereof of claim 8, wherein the light chain variable region is at least 85% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10.
11. The antibody or antigen-binding fragment thereof of claim 8, wherein the light chain variable region is at least 90% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10.
12. The antibody or antigen-binding fragment thereof of claim 8, wherein the light chain variable region is at least 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10.
13. The antibody or antigen-binding fragment thereof of claim 8, wherein the light chain variable region is at least 97% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10.
14. The antibody or antigen-binding fragment thereof of claim 8, wherein the light chain variable region is identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10.
15. An antibody or antigen-binding fragment thereof that specifically binds to GPIIb/IIIa, wherein the antibody or the antigen-binding fragment thereof comprises: (i) a heavy chain variable region that is at least 75% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218; and (ii) a light chain variable region that is at least 75% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10.
16. The antibody or antigen-binding fragment thereof of claim 15, wherein: (i) the heavy chain variable region is at least 80% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11, 12, or 197-218; and (ii) the light chain variable region is at least 80% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 4, 6, 8, or 10.

17. The antibody or antigen-binding fragment thereof of claim 15, wherein: (i) the heavy chain variable region is at least 85% identical to the amino acid sequence set forth in any one of SEQ ID NOs:3, 5, 7, 9, 11, 12, or 197-218; and (ii) the light chain variable region is at least 85% identical to the amino acid sequence set forth in any one of SEQ ID NOs:4, 6, 8, or 10.

18. The antibody or antigen-binding fragment thereof of claim 15, wherein: (i) the heavy chain variable region is at least 90% identical to the amino acid sequence set forth in any one of SEQ ID NOs:3, 5, 7, 9, 11, 12, or 197-218; and (ii) the light chain variable region is at least 90% identical to the amino acid sequence set forth in any one of SEQ ID NOs:4, 6, 8, or 10.

19. The antibody or antigen-binding fragment thereof of claim 15, wherein: (i) the heavy chain variable region is at least 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs:3, 5, 7, 9, 11, 12, or 197-218; and (ii) the light chain variable region is at least 95% identical to the amino acid sequence set forth in any one of SEQ ID NOs:4, 6, 8, or 10.

20. The antibody or antigen-binding fragment thereof of claim 15, wherein: (i) the heavy chain variable region is at least 97% identical to the amino acid sequence set forth in any one of SEQ ID NOs:3, 5, 7, 9, 11, 12, or 197-218; and (ii) the light chain variable region is at least 97% identical to the amino acid sequence set forth in any one of SEQ ID NOs:4, 6, 8, or 10.

21. The antibody or antigen-binding fragment thereof of claim 15, wherein: (i) the heavy chain variable region is identical to the amino acid sequence set forth in any one of SEQ ID NOs:3, 5, 7, 9, 11, 12, or 197-218; and (ii) the light chain variable region is identical to the amino acid sequence set forth in any one of SEQ ID NOs:4, 6, 8, or 10.

22. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:26), and GGDYGYALDY (SEQ ID NO:27), respectively.

23. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:26), and GGDYSYALDY (SEQ ID NO:245), respectively.
24. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVER (SEQ ID NO:241), and GGDYSYALDY (SEQ ID NO:245), respectively.
25. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSDGTTYYPDSVKR (SEQ ID NO:242), and GGDYSYALDY (SEQ ID NO:245), respectively.
26. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTDYPDSVKR (SEQ ID NO:243), and GGDYGYALDY (SEQ ID NO:27), respectively.
27. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), GISSGGTTYYPDSVKR (SEQ ID NO:244), and GGDYGYALDY (SEQ ID NO:27), respectively.
28. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises a light chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

29. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises: (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:26), and GGDYGYALDY (SEQ ID NO:27), respectively, and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

30. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises: (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:26), and GGDYSYALDY (SEQ ID NO:245), respectively, and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

31. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises: (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVER (SEQ ID NO:241), and GGDYSYALDY (SEQ ID NO:245), respectively, and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

32. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises: (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSDGTTYYPDSVKR (SEQ ID NO:242), and GGDYSYALDY (SEQ ID NO:245), respectively, and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid

sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

33. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises: (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), SISSGGTTDYPDSVKR (SEQ ID NO:243), and GGDYGYALDY (SEQ ID NO:27), respectively, and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

34. The antibody or antigen-binding fragment thereof of any one of claims 1 to 21, wherein the antibody or the antigen-binding fragment thereof comprises: (i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences AYAMS (SEQ ID NO:25), GISSGGTTYYPDSVKR (SEQ ID NO:244), and GGDYGYALDY (SEQ ID NO:27), respectively, and (ii) a light chain variable region comprising CDR1, CDR2, and CDR3, consisting of the amino acid sequences RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

35. An antibody or antigen-binding fragment thereof that specifically binds to GPIIb/IIIa, comprising the heavy chain variable region and light chain variable region amino acid sequences set forth in (i) SEQ ID NO:7 and 4, respectively; (ii) SEQ ID NO:12 and 4, respectively; (iii) SEQ ID NO:197 and 4, respectively; (iv) SEQ ID NO:198 and 4, respectively; (v) SEQ ID NO:199 and 4, respectively; (vi) SEQ ID NO:200 and 4, respectively; (vii) SEQ ID NO:201 and 4, respectively; (viii) SEQ ID NO:202 and 4, respectively; (ix) SEQ ID NO:203 and 4, respectively; (x) SEQ ID NO:204 and 4, respectively; (xi) SEQ ID NO:205 and 4, respectively; (xii) SEQ ID NO:206 and 4, respectively; (xiii) SEQ ID NO:207 and 4, respectively; (xiv) SEQ ID NO:208 and 4, respectively; (xv) SEQ ID NO:209 and 4, respectively; (xvi) SEQ ID NO:210 and 4, respectively; (xvii) SEQ ID NO:211 and 4, respectively; (xviii) SEQ ID NO:212 and 4, respectively; (xix) SEQ ID NO:213 and 4, respectively; (xx) SEQ ID NO:214 and 4, respectively; (xxi) SEQ ID NO:215 and 4, respectively; (xxii) SEQ ID NO:216 and 4,

respectively; (xxiii) SEQ ID NO:217 and 4, respectively; or (xxiv) SEQ ID NO:218 and 4, respectively.

36. An antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences:

(a) AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:26), and GGDYSYALDY (SEQ ID NO:245), respectively;

(b) AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVER (SEQ ID NO:241), and GGDYSYALDY (SEQ ID NO:245), respectively;

(c) AYAMS (SEQ ID NO:25), SISSDGTTYYPDSVKR (SEQ ID NO:242), and GGDYSYALDY (SEQ ID NO:245), respectively;

(d) AYAMS (SEQ ID NO:25), SISSGGTTDYPDSVKR (SEQ ID NO:243), and GGDYGYALDY (SEQ ID NO:27), respectively; or

(e) AYAMS (SEQ ID NO:25), GISSGGTTYYPDSVKR (SEQ ID NO:244), and GGDYGYALDY (SEQ ID NO:27), respectively.

37. An antibody or antigen-binding fragment thereof that specifically binds to glycoprotein IIb/IIIa (GPIIb/IIIa), wherein the antibody or the antigen-binding fragment thereof comprises:

(i) a heavy chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences:

(a) AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVKR (SEQ ID NO:26), and GGDYSYALDY (SEQ ID NO:245), respectively;

(b) AYAMS (SEQ ID NO:25), SISSGGTTYYPDSVER (SEQ ID NO:241), and GGDYSYALDY (SEQ ID NO:245), respectively;

(c) AYAMS (SEQ ID NO:25), SISSDGTTYYPDSVKR (SEQ ID NO:242), and GGDYSYALDY (SEQ ID NO:245), respectively;

(d) AYAMS (SEQ ID NO:25), SISSGGTTDYPDSVKR (SEQ ID NO:243), and GGDYGYALDY (SEQ ID NO:27), respectively; or

(e) AYAMS (SEQ ID NO:25), GISSGGTTYYPDSVKR (SEQ ID NO:244), and GGDYGYALDY (SEQ ID NO:27), respectively; and

(ii) a light chain variable region comprising complementarity determining region (CDR)1, CDR2, and CDR3, consisting of the amino acid sequences:

(f) RASSSVNYMY (SEQ ID NO:28), YTSNLAP (SEQ ID NO:29), and QQFSSSPWT (SEQ ID NO:30), respectively.

38. The antibody or antigen-binding fragment thereof of claim 36 or 37, wherein the antibody or antigen-binding fragment thereof has an apparent monovalent affinity that is about 1 to about 5×10^{-8} M.

39. The antibody or the antigen-binding fragment thereof of any one of claims 1 to 38, wherein the antigen-binding fragment is selected from the group consisting of an Fab, an Fab', an F(ab')₂, an Fv, an Fd, a diabody, an scFv, and an sc(Fv)₂.

40. A chimeric molecule comprising (i) the antibody or antigen-binding fragment thereof of any one of claims 1 to 39, and (ii) a heterologous moiety.

41. The chimeric molecule of claim 40, wherein the heterologous moiety comprises a clotting factor.

42. The chimeric molecule of claim 41, wherein the clotting factor is FVII, FIX, or FX.

43. The chimeric molecule of claim 41, wherein the clotting factor is FVII zymogen, activatable FVII, activated FVII (FVIIa), FIX zymogen, FX zymogen, activatable FX, or activated FX (FXa).

44. The chimeric molecule of any one of claims 41 to 43, wherein the clotting factor comprises a single polypeptide chain or two polypeptide chains.

45. The chimeric molecule of any one of claims 40 to 44, further comprising a linker.

46. The chimeric molecule of claim 45, wherein the linker is a peptide linker.

47. The chimeric molecule of claim 46, wherein the peptide linker comprises at least two, at least three, at least four, at least five, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, or at least 100 amino acids.

48. The chimeric molecule according to claim 46 or 47, wherein the peptide linker comprises a peptide having the formula $[(\text{Gly})_x\text{-Ser}_y]_z$ where x is from 1 to 4, y is 0 or 1, and z is from 1 to 50 (SEQ ID NO:153).

49. The chimeric molecule of any one of claims 40 to 48, further comprising a second heterologous moiety.

50. The chimeric molecule according to claim 49, wherein the second heterologous moiety comprises a half-life extending moiety.

51. The chimeric molecule of claim 50, wherein the half-life extending moiety is a low-complexity polypeptide.

52. The chimeric molecule according to claim 50, wherein the half-life extending moiety is selected from the group consisting of albumin, albumin binding polypeptide or fatty acid, an Fc region, transferrin, PAS, the C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin, polyethylene glycol (PEG), hydroxyethyl starch (HES), albumin-binding small molecules, vWF, and a clearance receptor or a fragment thereof which blocks binding of the chimeric molecule to a clearance receptor.

53. A chimeric molecule comprising (i) the antibody or antigen-binding fragment thereof of any one of claims 1 to 39, (ii) a Factor VII (*e.g.*, a recombinant Factor VIIa) comprising a heavy chain and a light chain, and (iii) a half-life extending moiety.

54. The chimeric molecule of claim 53, wherein the antibody or antigen-binding fragment thereof is an Fab or an scFv.

55. The chimeric molecule of claim 53 or 54, wherein the heavy chain of the Factor VII (*e.g.*, recombinant Factor VIIa) is linked to the half-life extending moiety and the half-life extending moiety is linked to the antibody or antigen-binding fragment thereof.

56. The chimeric molecule of claim 55, wherein the Factor VII (*e.g.*, recombinant Factor VIIa) is linked to the half-life extending moiety via a first peptide linker and the half-life extending moiety is linked to the antibody or antigen-binding fragment thereof via a second peptide linker.

57. The chimeric molecule of claim 56, wherein the heavy chain of the Factor VII (*e.g.*, recombinant Factor VIIa) is linked to the half-life extending moiety via a first peptide linker and the half-life extending moiety is linked to the light chain of the antibody or antigen-binding fragment thereof via a second peptide linker.

58. The chimeric molecule of claim 56 or 57, wherein the first and second peptide linkers comprise a peptide having the formula $[(\text{Gly})_x\text{-Ser}_y]_z$ where x is from 1 to 4, y is 0 or 1, and z is from 1 to 5 (SEQ ID NO:248).

59. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1 to 39 or the chimeric molecule of any one of claims 40 to 58, and a pharmaceutically acceptable carrier.

60. A method of reducing the frequency or degree of a bleeding episode in a subject in need thereof, comprising administering to the subject an effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1 to 39, the chimeric molecule of any one of claims 40 to 58, or the pharmaceutical composition of claim 59.

61. The method of claim 60, wherein the subject has developed or has a tendency to develop an inhibitor against Factor VIII ("FVIII"), Factor IX ("FIX"), or both.

62. The method of claim 61, wherein the inhibitor against FVIII or FIX is a neutralizing antibody against FVIII, FIX, or both.

63. The method according to any one of claims 60 to 62, wherein the bleeding episode is a result of hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis, gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, bleeding in the iliopsoas sheath, or any combinations thereof.

64. A method of treating a blood coagulation disorder in a subject in need thereof, comprising administering to the subject an effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1 to 39, the chimeric molecule of any one of claims 40 to 58, or the pharmaceutical composition of claim 59.

65. The method of claim 64, wherein the blood coagulation disorder is hemophilia A or hemophilia B.

66. The method of any one of claims 60 to 65, wherein the subject is a human subject.

67. A method of detecting platelets, comprising:

contacting a human blood preparation with the antibody or antigen-binding fragment thereof of any one of claims 1 to 39; and

detecting cells in the blood preparation to which the antibody or antigen-binding fragment thereof binds.

68. A method for enriching platelets, comprising:

contacting a human blood preparation with the antibody or antigen-binding fragment thereof of any one of claims 1 to 39; and

enriching cells to which the antibody or antigen-binding fragment thereof are bound as compared to those cells in the blood preparation that are not bound by the antibody or antigen-binding fragment thereof.

69. An isolated nucleic acid comprising a nucleotide sequence that is at least 80% identical to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 13-22, 59-68, and 219-240.

70. The isolated nucleic acid of claim 69, comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 13-22, 59-68, and 219-240.

71. An isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence that is at least 75% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 to 12, 74 to 77, and 197-218.

72. The isolated nucleic acid of claim 71, comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 to 12, 74 to 77, and 197-218.

73. An isolated protein encoded by the nucleic acid of any one of claims 69 to 72.

74. A recombinant vector comprising the nucleic acid of any one of claims 69 to 72.

75. A host cell comprising the recombinant vector of claim 74.

76. A method of preparing an antibody or antigen-binding fragment thereof, the method comprising culturing a host cell comprising recombinant vectors comprising:

the nucleic acid sequences set forth in SEQ ID NOs: 14 and 19;

the nucleic acid sequences set forth in SEQ ID NOs: 15 and 19;

the nucleic acid sequences set forth in SEQ ID NOs: 16 and 19;

the nucleic acid sequences set forth in SEQ ID NOs: 17 and 19;

the nucleic acid sequences set forth in SEQ ID NOs: 18 and 19;

the nucleic acid sequences set forth in SEQ ID NOs: 13 and 22;

the nucleic acid sequences set forth in SEQ ID NOs: 14 and 22;

the nucleic acid sequences set forth in SEQ ID NOs: 15 and 22;

the nucleic acid sequences set forth in SEQ ID NOs: 16 and 22;

the nucleic acid sequences set forth in SEQ ID NOs: 17 and 22;

the nucleic acid sequences set forth in SEQ ID NOs: 18 and 22;

the nucleic acid sequences set forth in SEQ ID NOs: 18 and 21;

the nucleic acid sequences set forth in SEQ ID NOs: 219 and 19;

the nucleic acid sequences set forth in SEQ ID NOs: 220 and 19;

the nucleic acid sequences set forth in SEQ ID NOs: 221 and 19;

the nucleic acid sequences set forth in SEQ ID NOs: 222 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 223 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 224 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 225 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 226 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 227 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 228 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 229 and 19;
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the nucleic acid sequences set forth in SEQ ID NOs: 235 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 236 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 237 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 238 and 19;
the nucleic acid sequences set forth in SEQ ID NOs: 239 and 19; or
the nucleic acid sequences set forth in SEQ ID NOs: 240 and 19,

under conditions appropriate for expression and production of the antibody or antigen-binding fragment thereof.

77. The method of claim 76, further comprising isolating the antibody or antigen-binding fragment thereof.

78. A method of preparing a chimeric molecule, the method comprising culturing a host cell comprising recombinant vectors comprising:

the nucleic acid sequences encoding the amino acid sequences set forth in SEQ ID NOs: 77 and 75; or

the nucleic acid sequences encoding the amino acid sequences set forth in SEQ ID NOs: 77 and 76;

under conditions appropriate for expression and production of the chimeric molecule.

79. The method of claim 76 to 78, wherein the host cell is a 293 cell, a CHO cell or a DG44i cell.

80. A method of preparing a chimeric molecule, the method comprising culturing a host cell comprising a recombinant vector or vectors comprising the nucleic acid sequences encoding the amino acid sequences set forth in SEQ ID NOs: 247 and 75 under conditions appropriate for expression and production of the chimeric molecule.

81. The method of claim 80, wherein the host cell is a 293 cell, a CHO cell or a DG44i cell.

34D10 VH: EVKLVESGGGLVKPGGSLKLSCAASGFTFSAYAMSWRQTPKEKRL~~EWASISSGGTTYPDVSVKRFTTISRDNAKNILYLQMNLSRSED~~TAMVYCTRGDYGALDYWGQGLTVTVSS (SEQ ID NO:1)

h34D10 VH0: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQTPK~~GLVMSISSGGTTYPDVSVKRFTTISRDNAKNILYLQMNLSRAED~~MAVYCTRGDYGALDYWGQGLTVTVSS (SEQ ID NO:3)
(CDR graft)

h34D10 VH1: EVQLVQS~~GGGLVKPGES~~RLSCAASGFTFSAYAMSWRQAPGKGL~~EWASISSGGTTYPDVSVKRFTTISRDNAKNILYLQMNLSRAED~~MAVYCTRGDYGALDYWGQGLTVTVSS (SEQ ID NO:5)

h34D10 VH2: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVW~~ASISSGGTTYPDVSVKRFTTISRDNAKNILYLQMNLSRAED~~tAVYCTRGDYGALDYWGQGLTVTVSS (SEQ ID NO:7)

h34D10 VH3: EVQLVQS~~GGGLVKPGES~~RLSCAASGFTFSAYAMSWRQAPGKGL~~EWASISSGGTTYPDVSVKRFTTISRDNAKNILYLQMNLSRAED~~tAVYCTRGDYGALDYWGQGLTVTVSS (SEQ ID NO:9)

h34D10 VH4: EVQLVQS~~GGGLVKPGES~~RLSCAASGFTFSAYAMSWRQAPGKGL~~EWASISSGGTTYPDVSVKRFTTISRDNAKNILYLQMNLSRAED~~tAVYCTRGDYGALDYWGQGLTVTVSS (SEQ ID NO:11)

h34D10 VH5: EVKLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGL~~EWASISSGGTTYPDVSVKRFTTISRDNAKNILYLQMNLSRAED~~tAVYCTRGDYGALDYWGQGLTVTVSS (SEQ ID NO:12)

FIG. 1

34D10 VL: ENVLTQSPAIMSASLGKVTMSGRASSVNMVYWQQKSDASPKLWIIYVTSNLAGV¹PARFSGSGGNSYSLTISSMEGEDAATYYCQQFSSSPWTFGGGTKLEIK (SEQ ID NO:2)

34D10 VL0: ²ETIMVTS³PATLS⁴SPGERATLS⁵GRASSVNMVYWQQKPGQAPRLIIYVTSNLAGV⁶PARFSGSGGTEFTLTIS⁷SLQSEDFAVYYCQQFSSSPWTFGGGTKMEIK (SEQ ID NO:4)
(CDR graft)

34D10 VL1: EIVITQSPATLSVSPGERATLS⁸GRASSVNMVYWQQKPGQAPRLIIYVTSNLAGV⁹PARFSGSGGTEFTLTIS¹⁰SLQSEDFAVYYCQQFSSSPWTFGGGTKVEIK (SEQ ID NO:6)

34D10 VL2: EIVITQSPATLSaSPGERvTmsGRASSVNMVYWQQKPGQsPRLIIYVTSNLAGV¹¹PARFSGSGGTEyTLTISSLQSEDFAVYYCQQFSSSPWTFGGGTKVEIK (SEQ ID NO:8)

34D10 VL3: EnVITQSPATLSaSPGERvTmsGRASSVNMVYWQQKPGQsPRLIIYVTSNLAGV¹²PARFSGSGGTEyTLTISSLQSEDFAVYYCQQFSSSPWTFGGGTKVEIK (SEQ ID NO:10)

FIG. 2

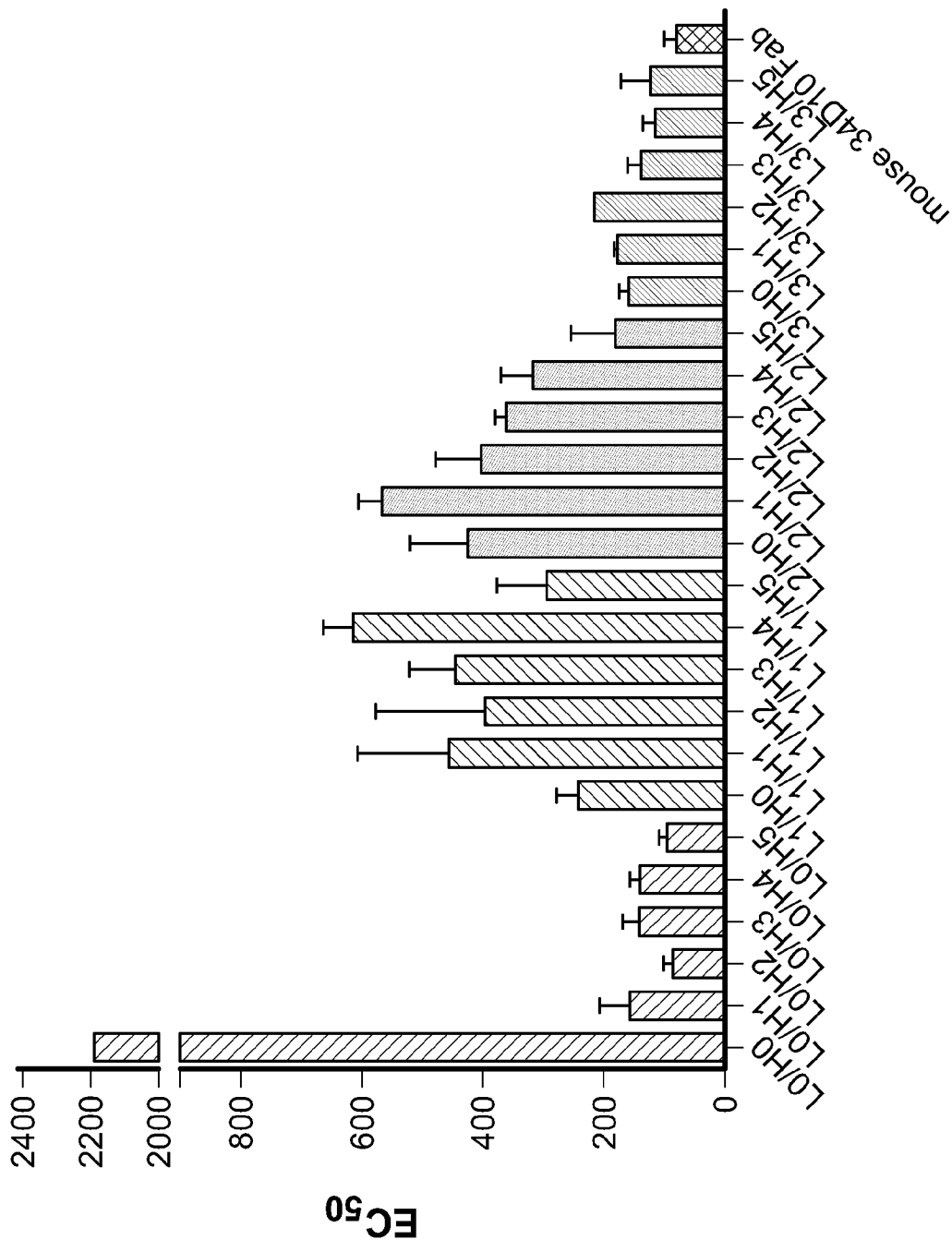


FIG. 3

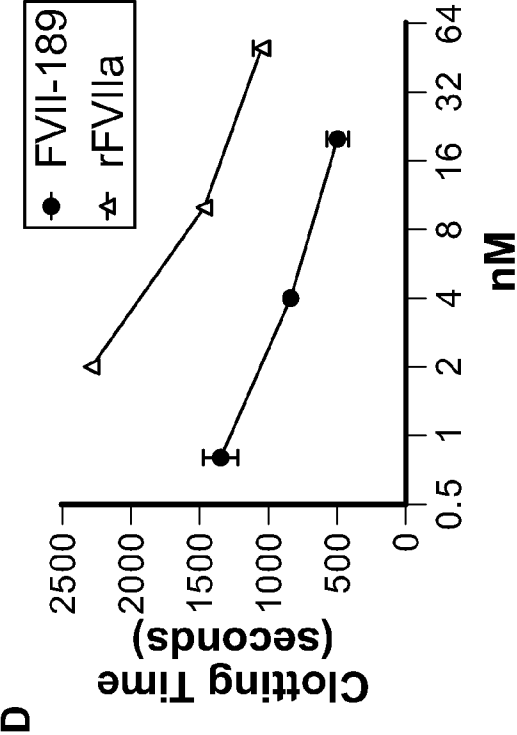
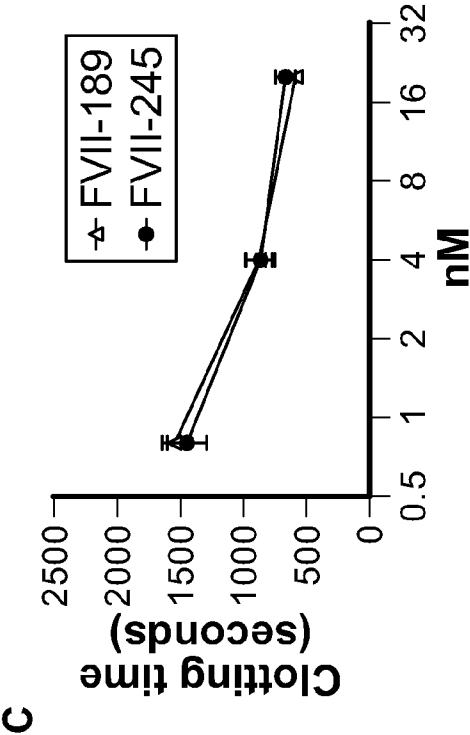
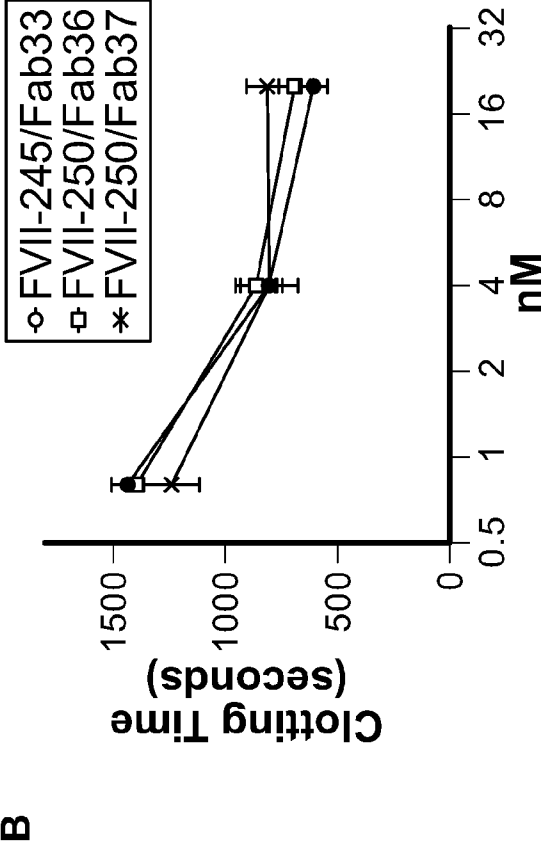
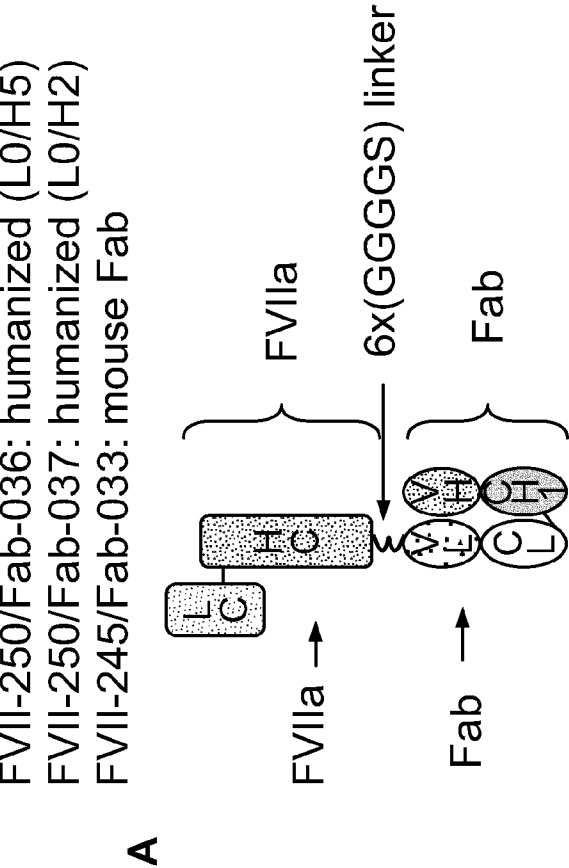


FIG. 4

FVII-251/Fab-036: humanized (L0/H5)
FVII-251/Fab-037: humanized (L0/H2)
FVII-200

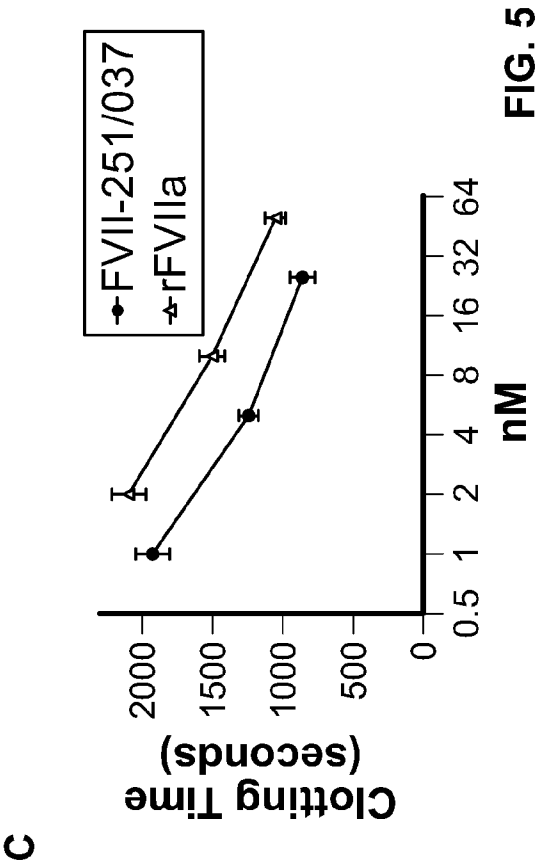
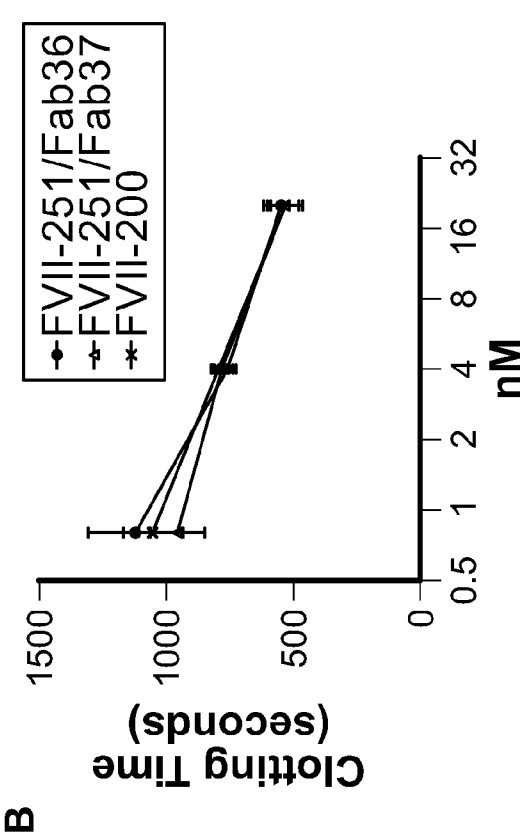
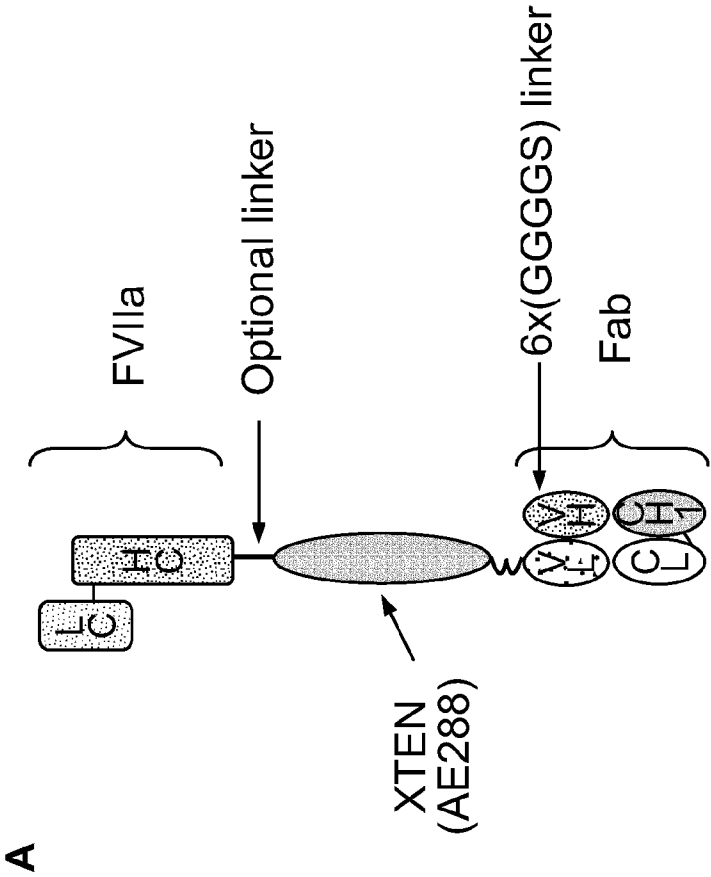


FIG. 5

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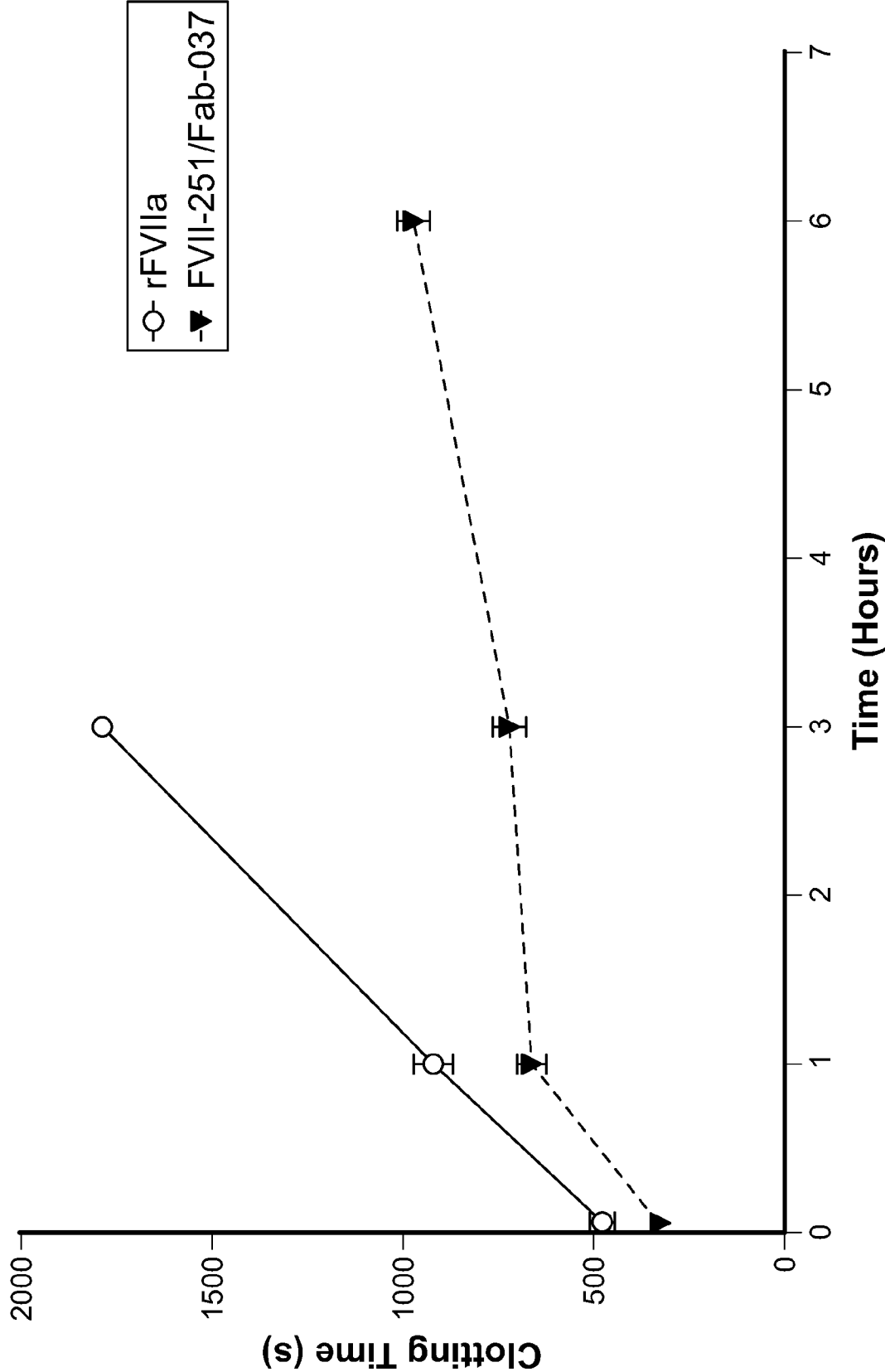


FIG. 6

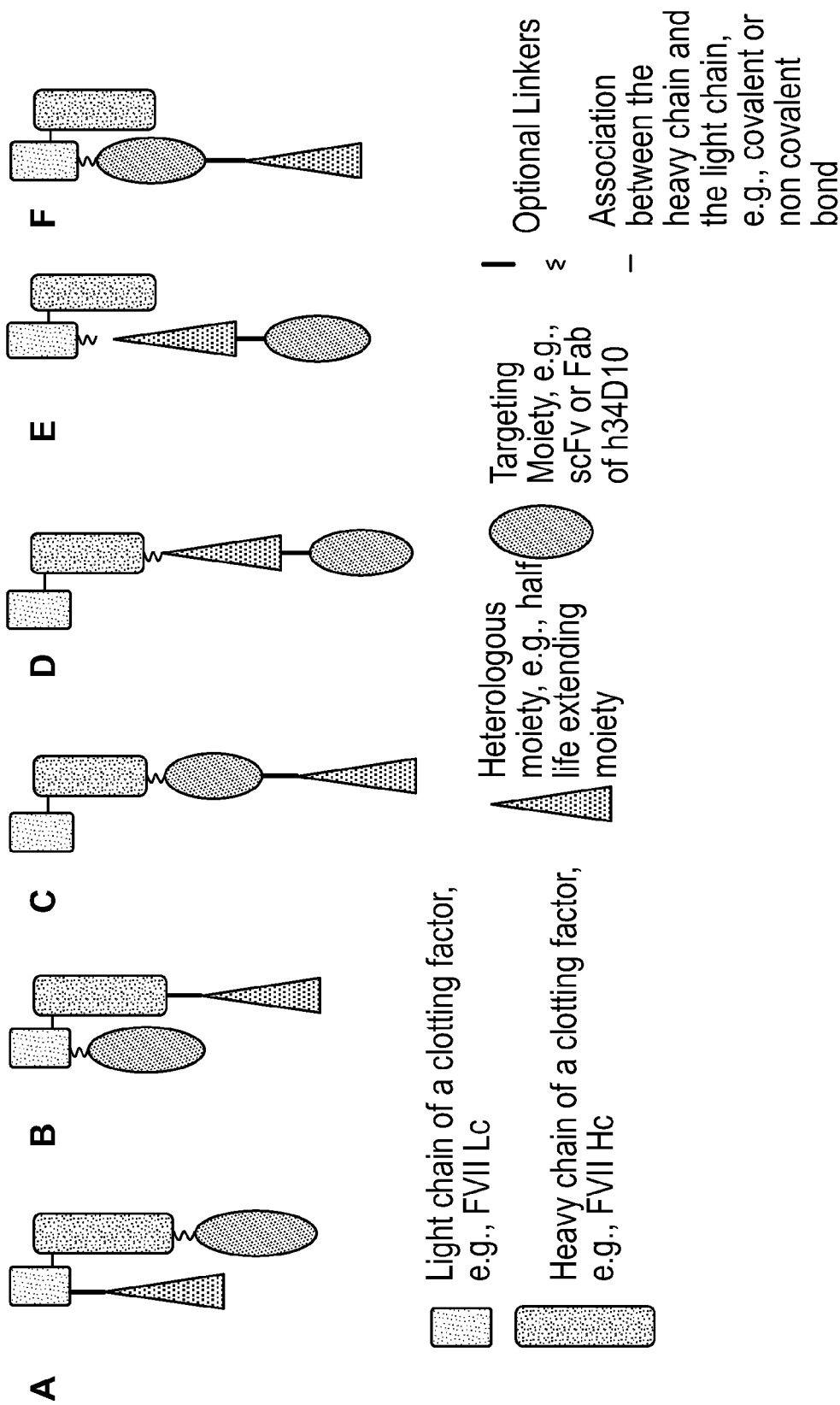
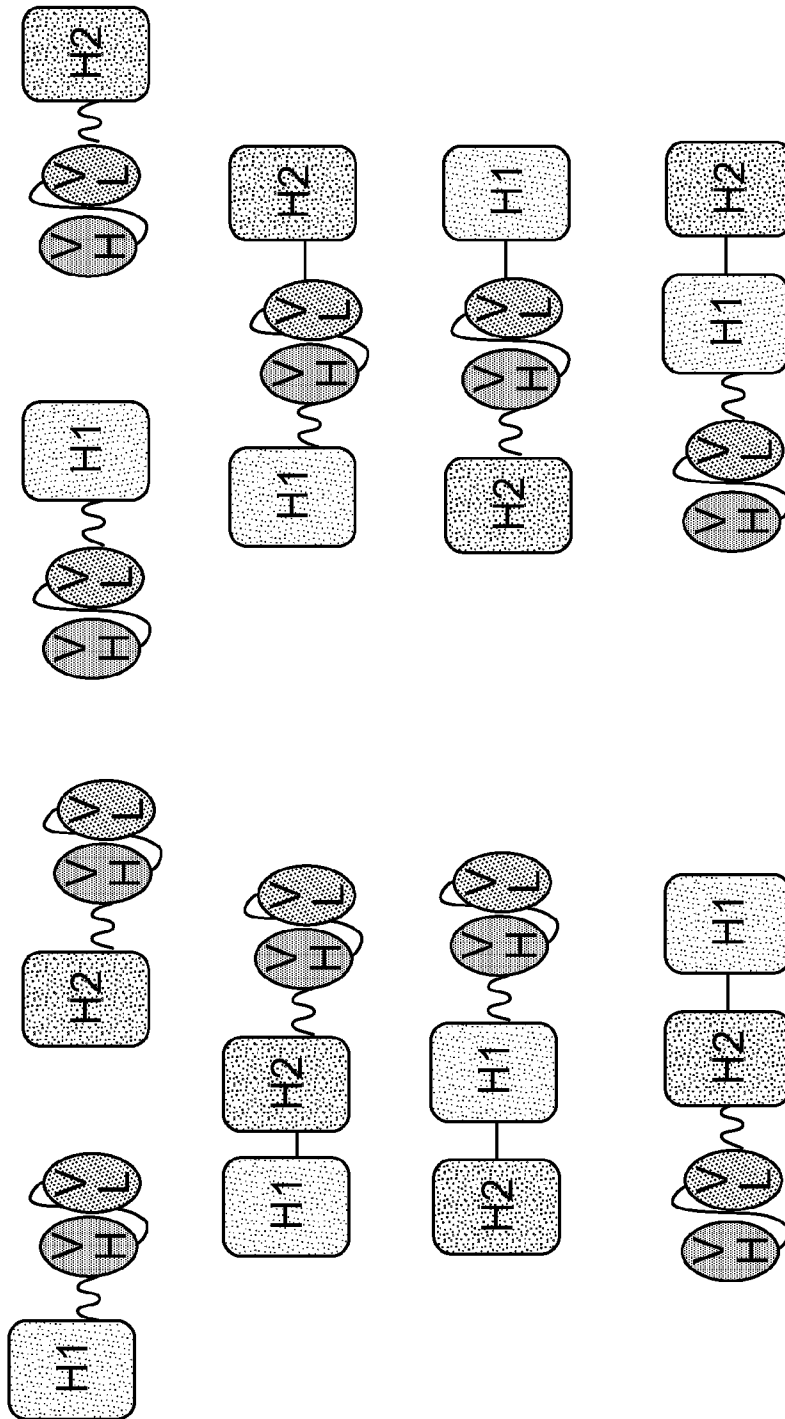


FIG. 7

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VH-VL: scFv derived, e.g., from h34D10 (note that an Fab of h34D10 can be used instead of an scFv)

H1: E.g., half-life extending peptide

H2: E.g., clotting factor (e.g., FVIIa)

~ An optional linker

FIG. 8

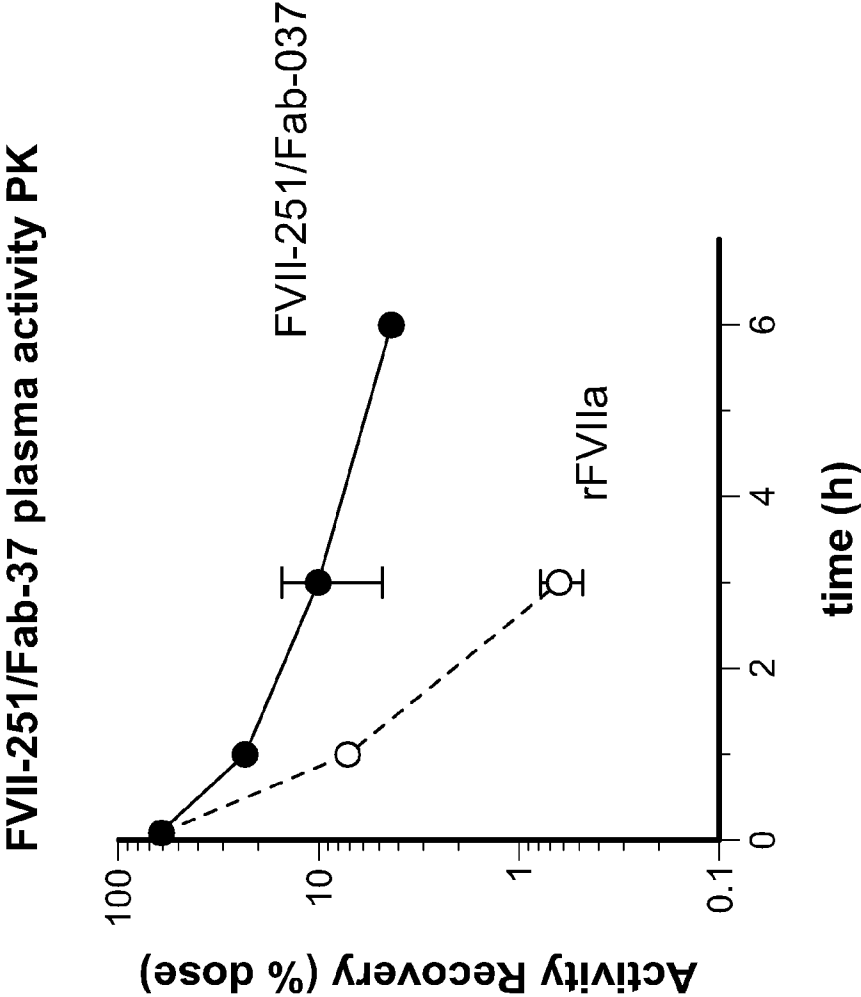


FIG. 9

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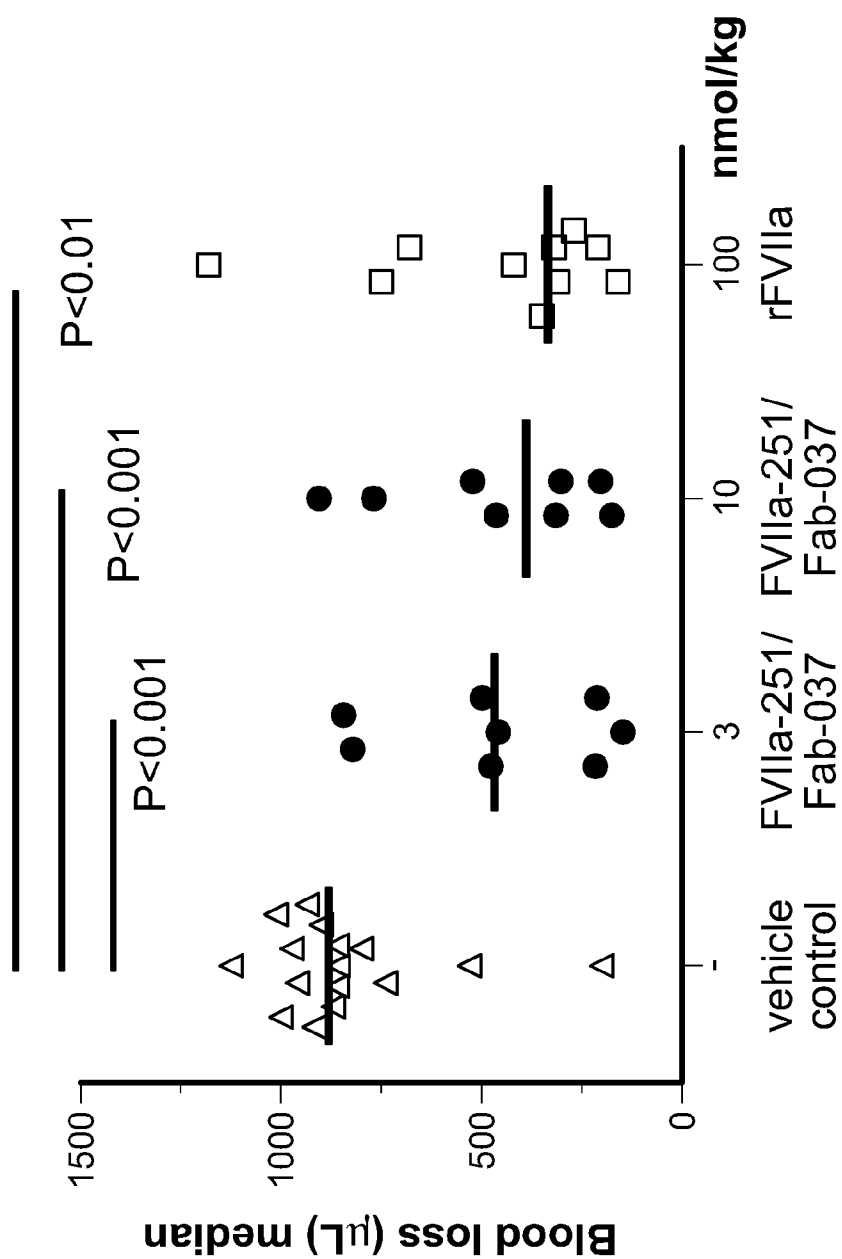


FIG. 10

h34D10

VH2: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 7)

VH100: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYSVALDYWGQGTLVTVSS (SEQ ID NO: 197)

VH101: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYSVALDYWGQGTLVTVSS (SEQ ID NO: 198)

VH102: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYSVALDYWGQGTLVTVSS (SEQ ID NO: 199)

VH103: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYSVALDYWGQGTLVTVSS (SEQ ID NO: 200)

VH104: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYSVALDYWGQGTLVTVSS (SEQ ID NO: 201)

VH105: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYSVALDYWGQGTLVTVSS (SEQ ID NO: 202)

VH106: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYSVALDYWGQGTLVTVSS (SEQ ID NO: 203)

VH107: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYSVALDYWGQGTLVTVSS (SEQ ID NO: 204)

VH108: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYSVALDYWGQGTLVTVSS (SEQ ID NO: 205)

VH109: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 206)

VH110: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 207)

VH111: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 208)

VH112: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 209)

VH113: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 210)

VH114: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 211)

VH115: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 212)

VH116: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 213)

VH117: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 214)

VH118: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 215)

VH119: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 216)

VH120: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 217)

VH121: EVQLVESGGGLVKPGGSLRLSCAASGFTFSAYAMSWRQAPGKGLVWVASISSGGITTPDPSVKRQFTISRDNAKNTLYLQMNSLRAEDTAVYYCTRGDDYGVALDYWGQGTLVTVSS (SEQ ID NO: 218)

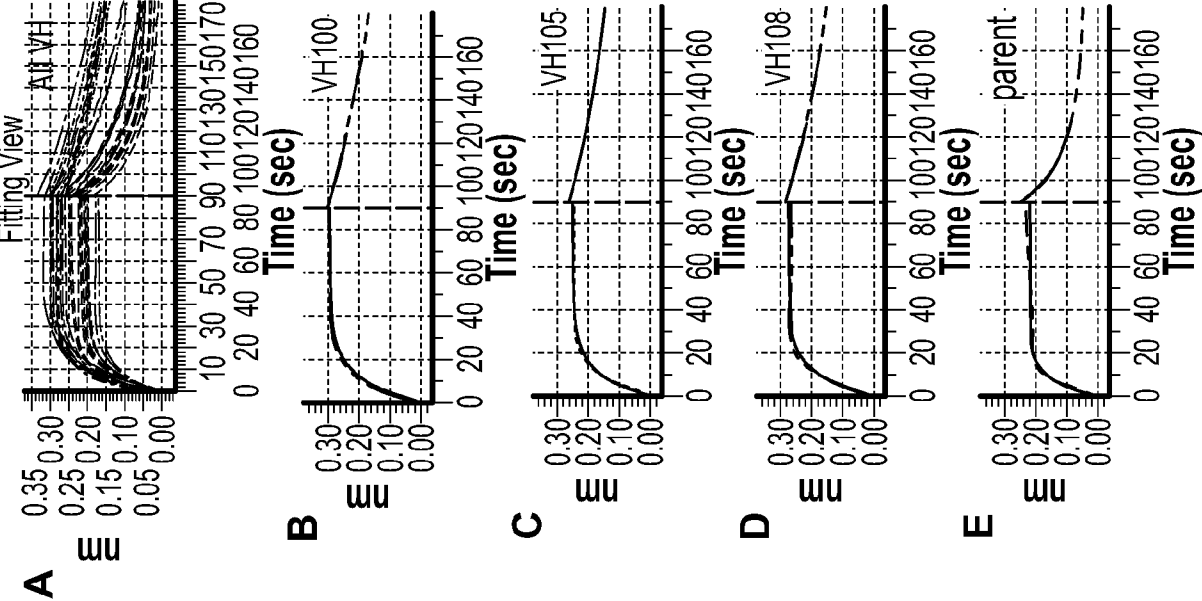
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FIG. 11

F

VH	KD (M)	K _{dis} (1/s)
VH100	2.90E-08	8.22E-03
VH101	2.50E-08	8.36E-03
VH102	3.60E-08	9.77E-03
VH103	3.70E-08	1.03E-02
VH104	3.10E-08	1.13E-02
VH105	4.20E-08	1.14E-02
VH106	4.40E-08	1.27E-02
VH107	4.80E-08	1.42E-02
VH108	4.90E-08	1.93E-02
VH109	1.20E-07	3.10E-02
VH110	1.30E-07	3.37E-02
VH111	1.50E-07	3.52E-02
VH112	1.60E-07	3.52E-02
VH113	1.60E-07	3.72E-02
VH114	1.30E-07	3.93E-02
VH115	1.20E-07	4.29E-02
VH116	1.30E-07	4.32E-02
VH117	1.60E-07	4.35E-02
VH118	2.00E-07	4.78E-02
VH119	1.40E-07	4.82E-02
VH120	2.40E-07	4.92E-02
VH121	2.30E-07	4.94E-02
Parent	1.55E-07	5.63E-02

FIG. 12



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VH	DSFT_m(°C)
VH100	78
VH101	78
VH102	79
VH103	76
VH104	79
VH105	79
VH106	79
VH107	78
VH108	79
VH109	78
VH110	80
VH111	79
VH112	76
VH113	78
VH114	79
VH115	80
VH116	80
VH117	78
VH118	80
VH119	81
VH120	80
VH121	80
Parent	81

FIG. 13

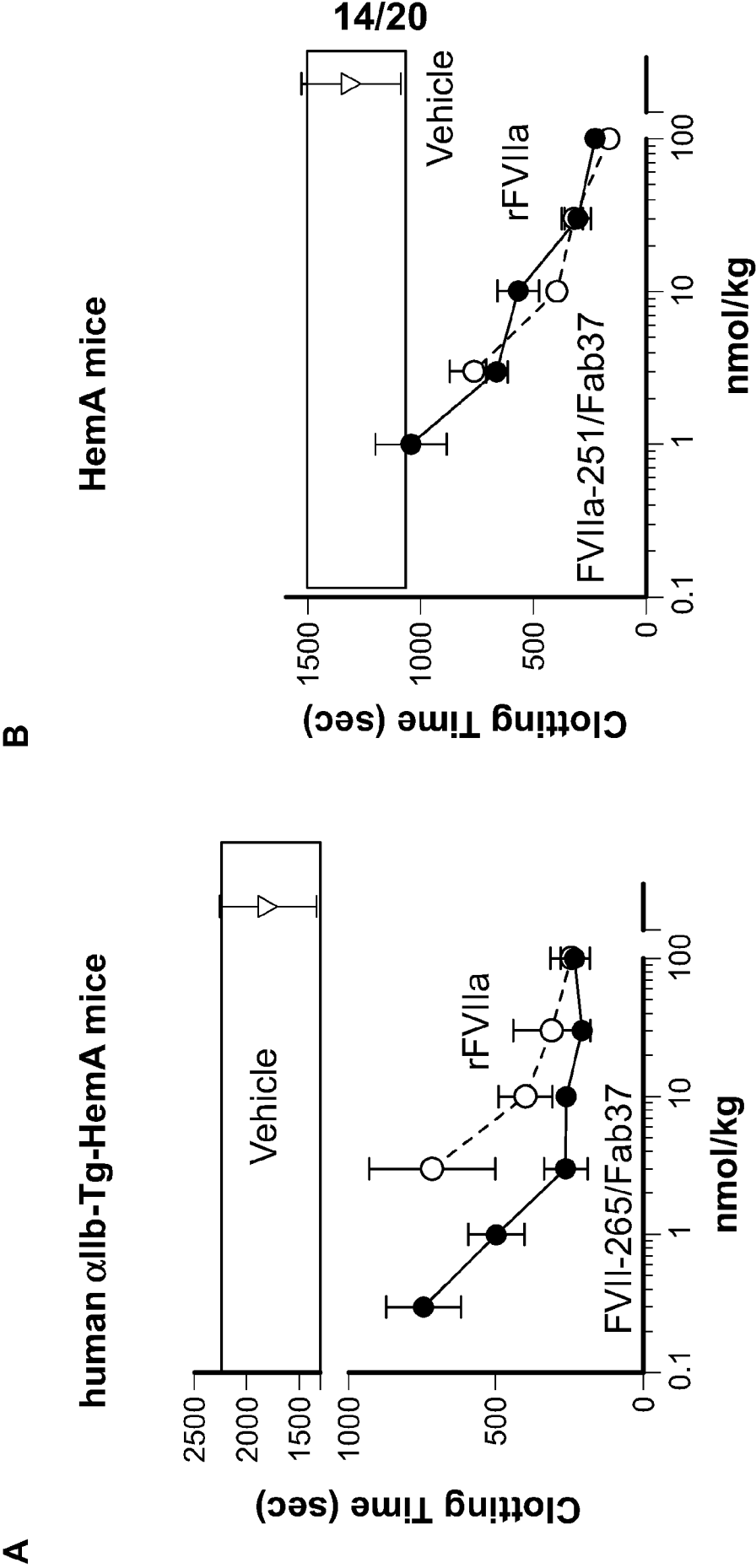


FIG. 14

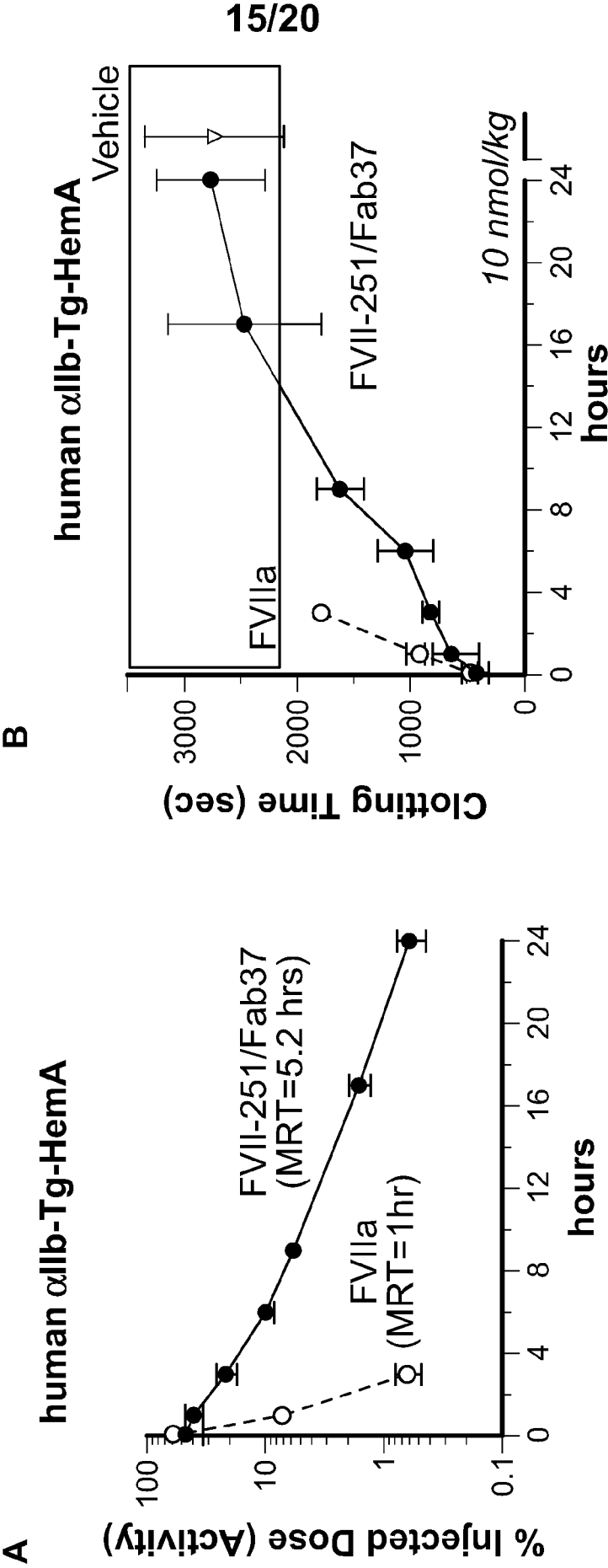


FIG. 15

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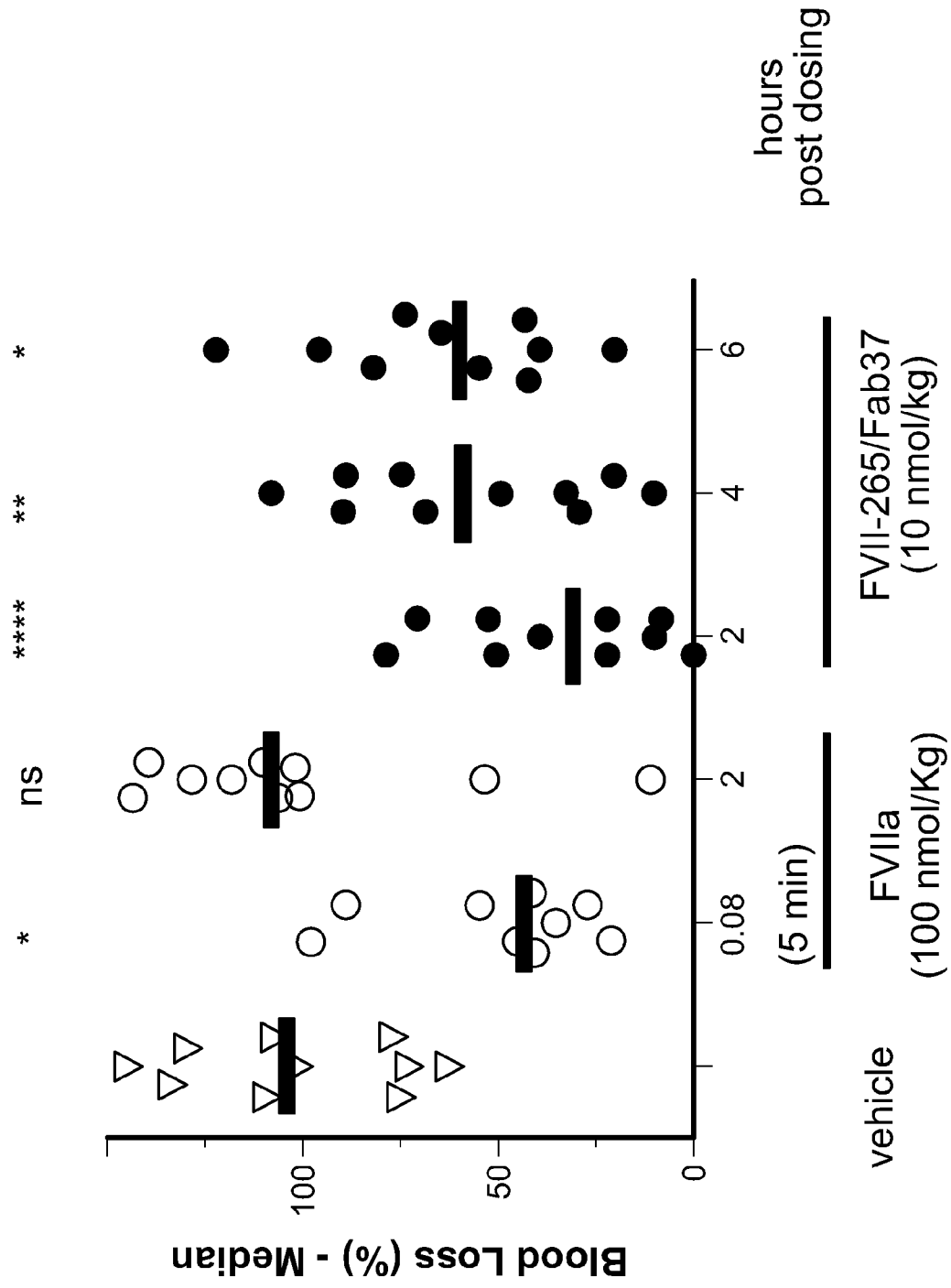


FIG. 16

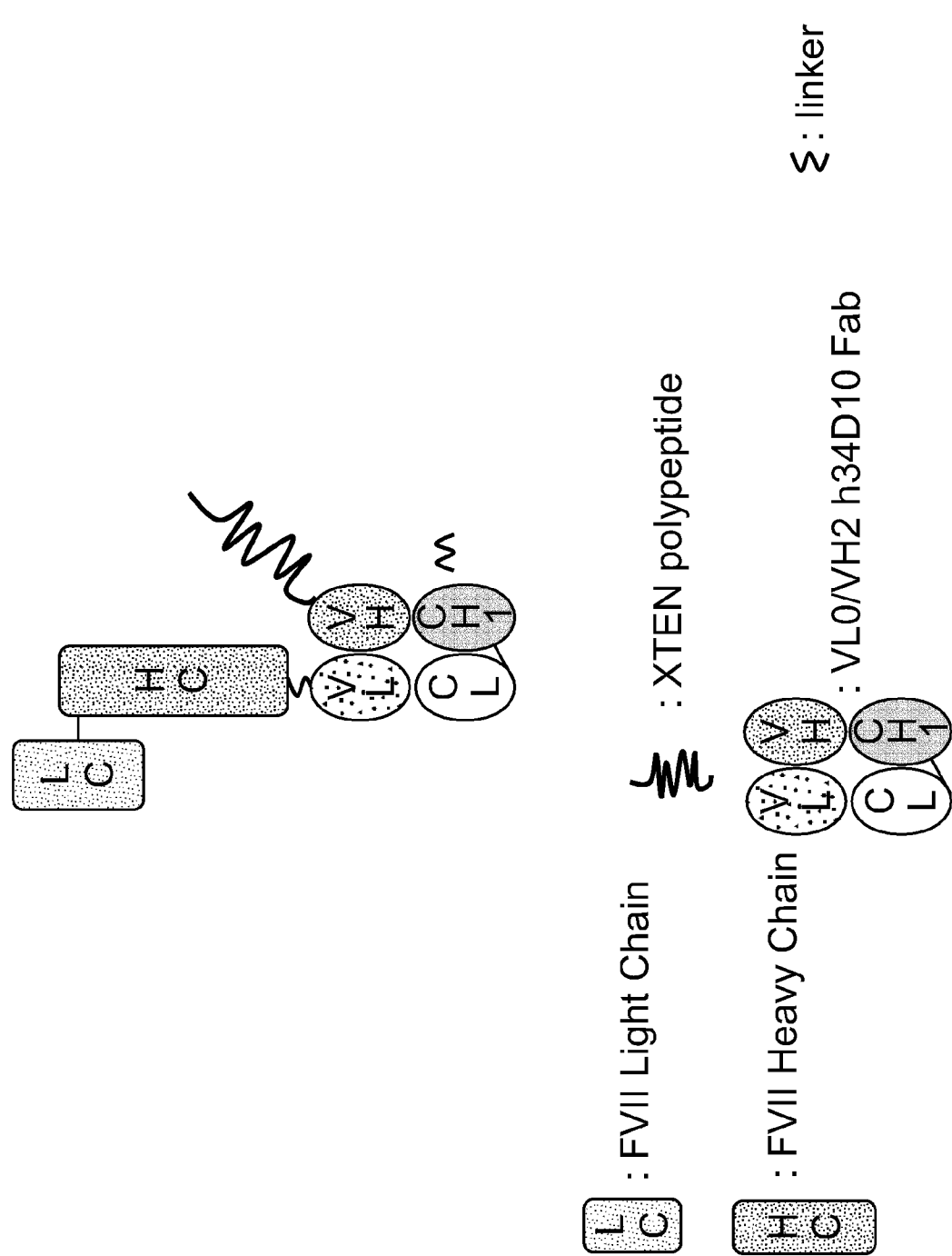


FIG. 17

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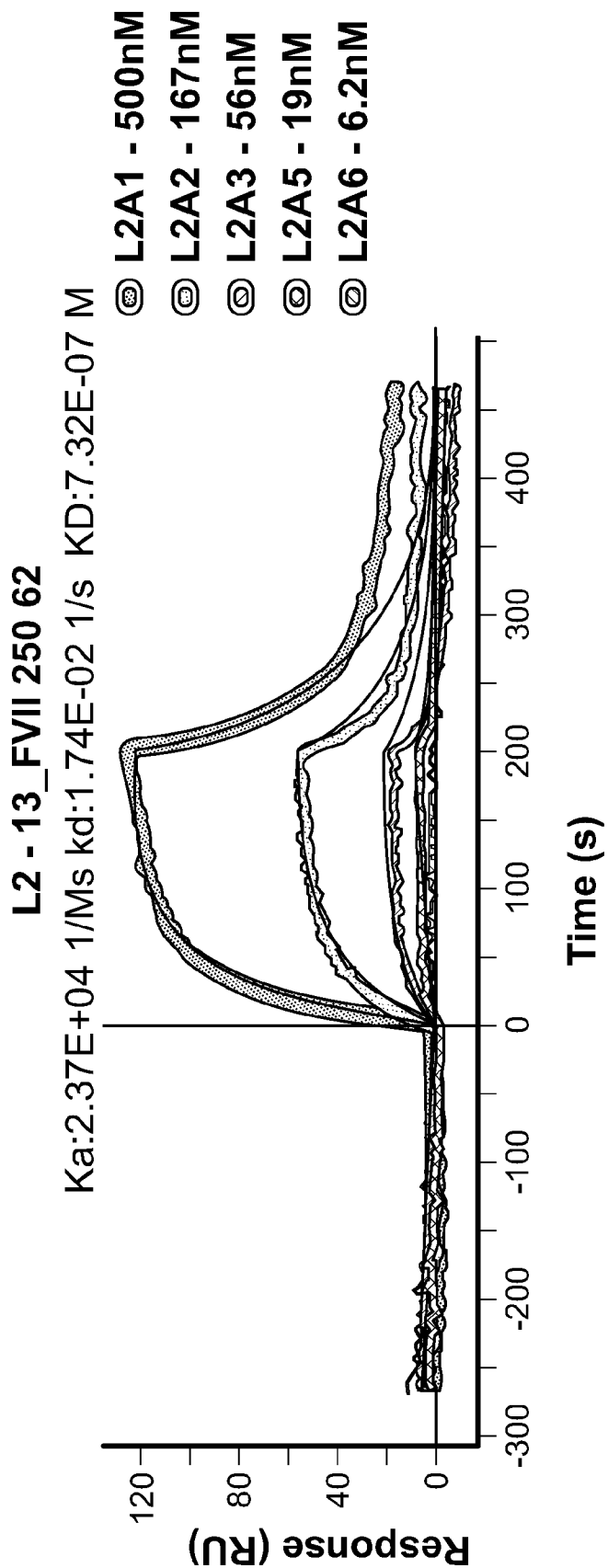


FIG. 18

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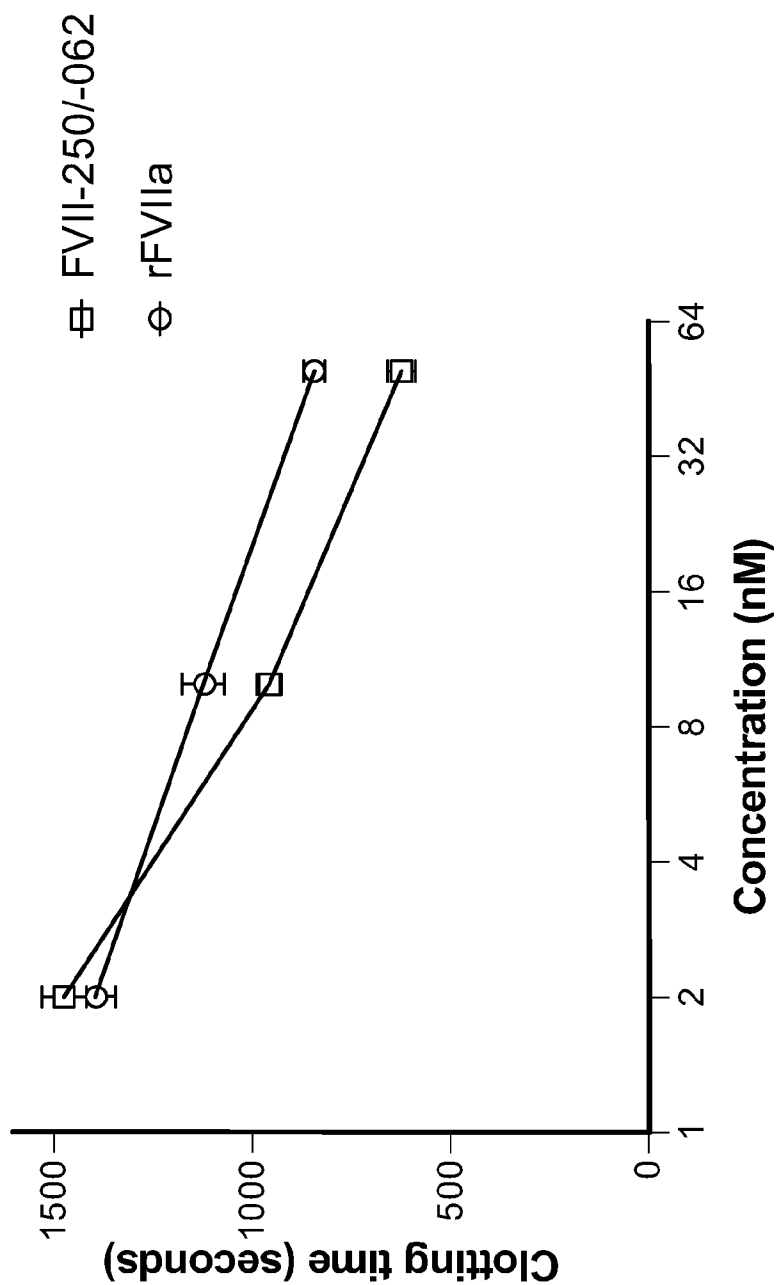
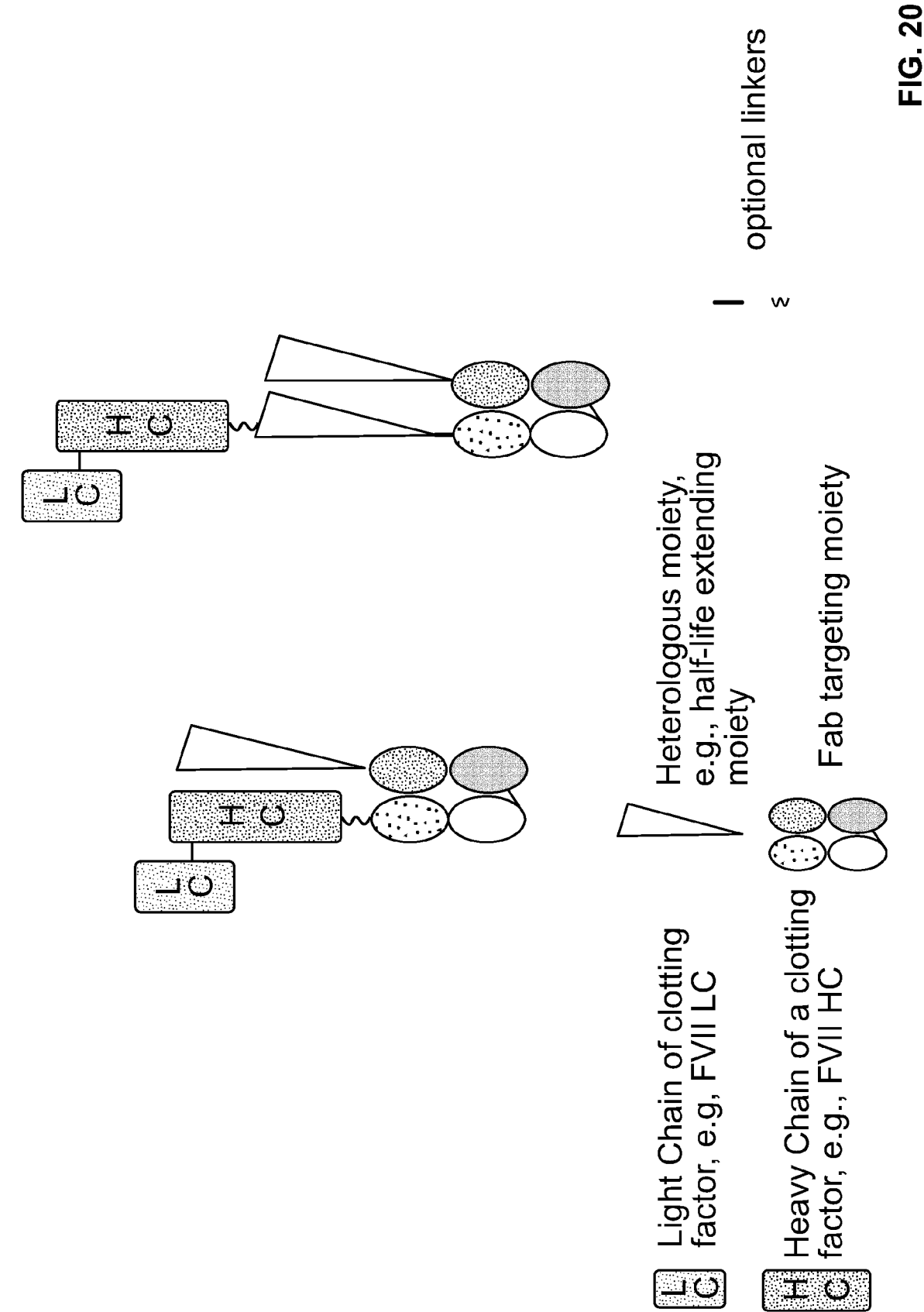


FIG. 19



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/057187

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/28 A61K38/00 A61K39/40 C07K14/00 A61K39/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/006633 A1 (BIOGEN IDEC HEMOPHILIA INC [US]; SALAS JOE [US]; PETERS ROBERT [US]; B) 12 January 2012 (2012-01-12) page all, paragraph all -----	1-81
A	A. M. SHIBEKO ET AL: "Predicting dosing advantages of factor VIIa variants with altered tissue factor-dependent and lipid-dependent activities", JOURNAL OF THROMBOSIS AND HAEMOSTASIS, vol. 12, no. 8, 1 August 2014 (2014-08-01), pages 1302-1312, XP55244624, GB ISSN: 1538-7933, DOI: 10.1111/jth.12628 table 1 -----	1-81



Further documents are listed in the continuation of Box C.



See patent family annex.

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"E" earlier application or patent but published on or after the international filing date

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Date of the actual completion of the international search

16 February 2016

Date of mailing of the international search report

23/02/2016

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2015/057187

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