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

(57) **Abstract:** The present invention provides antibodies and antigen-binding molecules thereof which specifically bind the α and/or β subunits of the non-active form of the GPIIb/IIIa receptor. The antibodies and antigen-binding molecules can be genetically fused and/or conjugated to heterologous moieties and used, for example, as targeting moieties. The invention also includes methods for screening for these antibodies, as well as methods of making and methods of using chimeric molecules derived from the antibodies.



ANTI-GPIIB/IIIA ANTIBODIES OR USES THEREOF

BACKGROUND OF THE INVENTION

- [0001] 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.
- [0002] Although such recombinant molecules are effective, there is a need for improved versions which localize the therapeutic to sites of coagulation, have improved pharmacokinetic properties, have improved manufacturability, have reduced thrombogenicity, or have enhanced activity, or more than one of these characteristics.
- [0003] 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;1 1:387-96), 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;1 14:2802-281 1) and conjugation with albumin (Schulte S., *Thromb. Res.* 2008;122 Suppl 4:S14-9).
- [0004] Recombinant FVIIa (rFVIIa; NOVOSEVEN®) 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. NOVOSEVEN® displays 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

option for hemophilia patients with inhibitors in which the activity of the FVIIa protein is increased.

BRIEF SUMMARY OF THE INVENTION

[0005] The present disclosure provides antibodies against GPIIb/IIIa that are capable of targeting the non-active form of the receptor (see FIG. 2E). The GPIIb/IIIa antibodies and antigen-binding molecules derived from these GPIIb/IIIa antibodies can be used, for example, to target therapeutic molecules (*e.g.*, clotting factors) to the platelet. In addition to their use as targeting moieties, GPIIb/IIIa antibodies and antigen-binding molecules thereof of the present invention can be used for diagnostics, for example, by conjugation to a detectable label.

[0006] The present invention also relates to chimeric molecules comprising the GPIIb/IIIa antibodies and antigen-binding molecules thereof disclosed herein as targeting moieties, and one or more heterologous moieties. For example, a chimeric molecule can comprise a heterologous moiety comprising a therapeutic molecule (for example, a procoagulant molecule such as the FVIIa clotting factor), and optionally a second heterologous moiety comprising, for example, a PK enhancing moiety (*i.e.*, a molecule which can improve various pharmacokinetic properties, *e.g.*, circulation half-life).

[0007] The present disclosure provides an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, wherein the antibody or antigen-binding molecule thereof specifically bind to the same GPIIb/IIIa epitope as an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4. The invention also includes an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, wherein the antibody or antigen-binding molecule thereof competitively inhibits GPIIb/IIIa binding by an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4. Also provided is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, wherein the antibody or antigen-binding molecule thereof comprises at least one, at least two, at least three, at least four, or at least five complementarity determining regions (CDR) or variants thereof of an antibody selected from the 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6,

13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4 antibodies. In some embodiments, the antibody or antigen-binding molecule thereof comprises six CDRs or variants thereof of an antibody selected from the 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4 antibodies.

[0008] The present disclosure also provides an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 25, 31, 37, 43 or 111; (ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 26, 32, 38, 44, or 112; (iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 27, 33, 39, 45, or 113; (iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 28, 34, 40, 117, or 114; (v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 29, 35, 41, 118, or 115; and (vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60, 70, 80, 90, or 95% identical to any one of SEQ ID NOS: 30, 36, 42, 119, or 116.

[0009] Also provided is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a VH-CDR1 comprising the consensus sequence XjYAMS wherein X₁ represents amino acid residues Thr (T), Ser (S), or Ala (A); (ii) a VH-CDR2 comprising the consensus sequence SIX2X3GX₄X₅TYX6X7DSVKX8 wherein X₂ represents amino acid residues Ser (S) or Asn (N), X₃ represents amino acid residues Ser (S) or Gly (G), X₄ represents amino acid residues Ser (S) or Gly (G), X₅ represents amino acid residues Ser (S), Asn (N), or Thr (T), X₆ represents amino acid residues Tyr (Y) or Phe (F), X₇ represents amino acid residues Leu (L) or Pro (P), and X₈ represents amino acids Gly (G) or Arg (R); (iii) a VH-CDR3 comprising the consensus sequence GGDYGYAX₉DY, wherein X₉ represents amino acid residues Leu (L) or Met (M); (iv) a VL-CDR1 comprising the sequence RASSSVNYMY (SEQ ID NO: 28); (v) a VL-CDR2 comprising the sequence YTSNLAP (SEQ ID NO: 29); and, (vi) a VL-CDR3 comprising the sequence QQFSSSPWT (SEQ ID NO: 30). In some embodiments, the antibody or antigen-binding molecule thereof

comprises (i) a VH-CDR1 sequence selected from SEQ ID NO: 25, 31, 37, 43, or 111; (ii) a VH-CDR2 sequence selected from SEQ ID NOS: 26, 32, 38, 44, or 112; (iii) a VH-CDR3 sequence selected from SEQ ID NOS: 27, 33, 39, 45, or 113; (iv) a VL-CDR1 sequence selected from of SEQ ID NOS: 28, 34, 40, 117, or 114; (v) a VL-CDR2 sequence selected from SEQ ID NOS: 29, 35, 41, 118, or 115; and (vi) a VL-CDR3 sequence selected from SEQ ID NOS: 30, 36, 42, 119, or 116.

[0010] The instant disclosure also provides an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 1, 3, 5, 7, or 97, and a VL comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 2, 4, 6, 99, or 98. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 1 and a VL comprising the amino acid sequence of SEQ ID NO: 2.

[0011] In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 3 and a VL comprising the amino acid sequence of SEQ ID NO: 4. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 5 and a VL comprising the amino acid sequence of SEQ ID NO: 6. In other embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 7 and a VL comprising the amino acid sequence of SEQ ID NO: 99. In other embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 97 and a VL comprising the amino acid sequence of SEQ ID NO: 98.

[0012] In some embodiments, the antibody or antigen-binding molecule thereof can bind to a GPIIb/IIIa epitope located in the extracellular domain of the alpha subunit of GPIIb/IIIa or in the extracellular domain of the GPIIb/IIIa complex. In some embodiments, the antibody or antigen-binding molecule thereof does not compete with fibrinogen for binding to GPIIb/IIIa.

[0013] The present disclosure also provides an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprising (i) a variable heavy

chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 46, 52, 120, or 126; (ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 47, 53, 121, or 127; (iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 48, 54, 122, or 128; (iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 49, 55, 123, or 129; (v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 50, 56, 124, or 130; and (vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NO: 51, 57, 125, or 131.

[0014] Also provided is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a VH-CDR1 comprising the sequence NYLIE (SEQ ID NO: 46); (ii) a VH-CDR2 comprising the sequence VINPGSGGTNYNEKFKG (SEQ ID NO: 47); (iii) a VH-CDR3 comprising the sequence GRYEWYFDV (SEQ ID NO: 48); (iv) a VL-CDR1 comprising the consensus sequence RASQDIX_iNYLN wherein X_i represents amino acid residues Ser (S) or Thr (T); (v) a VL-CDR2 comprising the sequence YTSRLHS (SEQ ID NO:50); and (vi) a VL-CDR3 comprising the sequence QQGYTLPYT (SEQ ID NO:51). In some embodiments, the antibody or antigen-binding molecule thereof comprises (i) a VH-CDR1 sequence selected from SEQ ID NO: 46, 52, 120, or 126; (ii) a VH-CDR2 sequence selected from SEQ ID NO: 47, 53, 121, or 127; (iii) a VH-CDR3 sequence selected from SEQ ID NO: 48, 54, 122, or 128; (iv) a VL-CDR1 sequence selected from SEQ ID NO: 49, 55, 123, or 129; (v) a VL-CDR2 sequence selected from SEQ ID NO: 50, 56, 124, or 130; and, (vi) a VL-CDR3 sequence selected from SEQ ID NO: 51, 57, 125, or 131.

[0015] Also provided is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 8, 10, 100, or 102, and a VL comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 9, 11, 101, or 103. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH

comprising the amino acid sequence of SEQ ID NO: 8, and a VL comprising the amino acid sequence of SEQ ID NO: 9. In other embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 10 and a VL comprising the amino acid sequence of SEQ ID NO: 11.

[0016] In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 100 and a VL comprising the amino acid sequence of SEQ ID NO: 101. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 102 and a VL comprising the amino acid sequence of SEQ ID NO: 103. In some embodiments, the antibody or antigen-binding molecule thereof binds to a GPIIb/IIIa epitope located in the extracellular domain of the alpha subunit of GPIIb/IIIa. In some embodiments, the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

[0017] The present disclosure also provides an antibody or antigen-binding molecule thereof which specifically binds to a gpIIb/IIIa epitope, comprising (i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 58; (ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 59; (iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 60; (iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 61; (v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 62; and, (vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 63.

[0018] Also provided is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a VH-CDR1 sequence comprising SEQ ID NO: 58; (ii) a VH-CDR2 sequence comprising SEQ ID NO: 59; (iii) a VH-CDR3 sequence comprising SEQ ID NO: 60; (iv) a VL-CDR1 sequence comprising SEQ ID NO: 61; (v) a VL-CDR2 sequence comprising SEQ ID NO: 62; and (vi) a VL-CDR3 sequence comprising SEQ ID NO: 63. The present disclosure also provides an antibody or antigen-binding molecule thereof which specifically binds to a

GPIIb/IIIa epitope comprising a VH comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 12 and a VL comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 13. In some embodiments, the antibody or antigen-binding molecule thereof can bind to a GPIIb/IIIa epitope located in the extracellular domain of the alpha subunit of GPIIb/IIIa. In some embodiments, the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

[0019] The instant disclosure also provides an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 64, 70, or 135; (ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 65, 71, or 136; (iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 66, 72, or 137; (iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 67, 132, or 138; (v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 68, 133, or 139; and (vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 69, 134, or 140.

[0020] The invention also includes an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a VH-CDR1 comprising the sequence SYWIE (SEQ ID NO: 64); (ii) a VH-CDR2 comprising the consensus sequence EILPGX₄GX₁₅TKYNX₆KFKG (SEQ ID NO: 187), wherein X₁₄ represents amino acid residues Ser (S) or Thr (T), X₁₅ represents amino acid residues Ile (I) or Tyr (Y), and X₁₆ represents amino acid residues Asp (D) or Glu (E); (iii) a VH-CDR3 comprising the sequence LISYYYAMDY (SEQ ID NO: 66); (iv) a VL-CDR1 comprising the sequence RASQDISNYLN (SEQ ID NO: 67); (v) a VL-CDR2 comprising the sequence YTSRLHS (SEQ ID NO: 68); and, (vi) a VL-CDR3 comprising the sequence QQGNTLPPT (SEQ ID NO: 69).

[0021] Also provided is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a VH-CDR1 sequence selected

from SEQ ID NO: 64, 70, or 135; (ii) a VH-CDR2 sequence selected from SEQ ID NO: 65, 71, or 136; (iii) a VH-CDR3 sequence selected from SEQ ID NO: 66, 72, or 137; (iv) a VL-CDR1 sequence selected from SEQ ID NO: 67, 132, or 138; (v) a VL-CDR2 sequence selected from SEQ ID NO: 68, 133, or 139; and, (vi) a VL-CDR3 sequence selected from SEQ ID NO: 69, 134, or 140.

[0022] Also provided herein is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 14, 16, or 105 and a VL comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 15, 104, or 106. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 14 and a VL comprising the amino acid sequence of SEQ ID NO: 15. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 16 and a VL comprising the amino acid sequence of SEQ ID NO: 104. In other embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 105 and a VL comprising the amino acid sequence of SEQ ID NO: 106. In some embodiments, the antibody or antigen-binding molecule thereof binds to a GPIIb/IIIa epitope located in the extracellular domain of the beta subunit of GPIIb/IIIa. In some embodiments, the antibody or antigen-binding molecule thereof does not compete with fibrinogen for binding to GPIIb/IIIa.

[0023] The present disclosure also provides an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 73, 76, 79, 85, or 147; (ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 74, 77, 80, 86, or 148; (iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 75, 78, 81, 87, or 149; (iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 141, 144, 82, 88, or 150; (v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100%

identical to any one of SEQ ID NOS: 142, 145, 83, 89, or 151; and, (vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NO: 143, 146, 84, 90, or 152. Also provided is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a VH-CDR1 comprising the consensus sequence TSGX_nGVG, wherein X₁₁ represents amino acid residues Met (M) or Leu (L); (ii) a VH-CDR2 comprising the consensus sequence HIWWDDDKRYNPX₁₂LKS, wherein X₁₂ represents amino acid residues Ala (A) or Thr (T); (iii) a VH-CDR3 comprising the consensus sequence SHYX₁₃GTFYFDXi₄, wherein X₁₃ represents amino acid residues Tyr (Y) or Asn (N), and X₁₄ represents amino acid residues Tyr (Y) or Phe (F); (iv) a VL-CDR1 comprising the sequence RASKSISKYLA (SEQ ID NO: 82); (v) a VL-CDR2 comprising the sequence SGSTLQS (SEQ ID NO: 83); and, (vi) a VL-CDR3 comprising the sequence QQHIEYPWT (SEQ ID NO: 84).

[0024] In some embodiments, the antibody or antigen-binding molecule thereof comprises (i) a VH-CDR1 sequence selected from SEQ ID NOS: 73, 76, 79, 85, or 147; (ii) a VH-CDR2 sequence selected from SEQ ID NOS: 74, 77, 80, 86, or 148; (iii) a VH-CDR3 sequence selected from SEQ ID NOS: 75, 78, 81, 87, or 149; (iv) a VL-CDR1 sequence selected from SEQ ID NOS: 141, 144, 82, 88, or 150; (v) a VL-CDR2 sequence selected from SEQ ID NOS: 142, 145, 83, 89, or 151; and (vi) a VL-CDR3 sequence selected from SEQ ID NOS: 143, 146, 84, 90, or 152. Also provided is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 17, 18, 19, 21, or 109 and a VL comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 107, 108, 20, 22, or 110.

[0025] In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 17 and a VL comprising the amino acid sequence of SEQ ID NO: 107. In other embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 18 and a VL comprising the amino acid sequence of SEQ ID NO: 108. In some embodiments, the antibody or antigen-binding molecule thereof

comprises a VH comprising the amino acid sequence of SEQ ID NO: 109 and a VL comprising the amino acid sequence of SEQ ID NO: 110.

[0026] In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 19 and a VL comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH comprising the amino acid sequence of SEQ ID NO: 21 and a VL comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen-binding molecule thereof binds to a GPIIb/IIIa epitope located in the extracellular domain of the beta subunit of GPIIb/IIIa. In some embodiments, the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

[0027] Also provided in the present disclosure is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 91; (ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 92; (iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 93; (iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 94; (v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 95; and (vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 96.

[0028] The present disclosure also provides an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a VH-CDR1 sequence comprising SEQ ID NO: 91; (ii) a VH-CDR2 sequence comprising SEQ ID NO: 92; (iii) a VH-CDR3 sequence comprising SEQ ID NO: 93; (iv) a VL-CDR1 sequence comprising SEQ ID NO: 94; (v) a VL-CDR2 sequence comprising SEQ ID NOS: 95; and (vi) a VL-CDR3 sequence comprising SEQ ID NOS: 96.

[0029] Also provided is an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 23 and a

VL comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100%, identical to SEQ ID NO: 24. In some embodiments, the antibody or antigen-binding molecule thereof binds to a GPIIb/IIIa epitope located in the extracellular domain of the beta subunit of GPIIb/IIIa. In some embodiments, the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

[0030] In some embodiments, the antibody or antigen-binding molecule thereof disclosed herein comprises or consists of (a) a single chain Fv ("scFv"); (b) a diabody; (c) a minibody; (d) a polypeptide chain of an antibody; (e) F(ab')₂; or (f) F(ab).

[0031] The present disclosure also provides a chimeric molecule comprising (i) a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein; (ii) a heterologous moiety; and, (iii) an optional linker. In some embodiments, the chimeric molecule has the formula (i) Tm-(L)-H; or (ii) H-(L)-Tm, wherein, H is a heterologous moiety; L is an optional linker; and, Tm is an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein. In some embodiments, the heterologous moiety comprises a clotting factor. In some embodiments, the clotting factor is independently selected from the group consisting of FVII, FIX, FX, and any combinations thereof. In some embodiments, the clotting factor is 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 chimeric molecule further comprises a second heterologous moiety.

[0032] In some embodiments, the chimeric molecule has a formula selected from (i) H1-(L1)-Tm-(L2)-H2; (ii) H2-(L2)-Tm-(L1)-H1; (iii) H1-(L1)-H2-(L2)-Tm; (iv) H2-(L2)-H1-(L1)-Tm; (v) Tm-(L1)-H1-(L2)-H2; or (vi) Tm-(L2)-H2-(L1)-H1; wherein, Tm 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, L2 is a second optional linker. In some embodiments, the first heterologous moiety and the second heterologous moiety are the same or different. In some embodiments, the second heterologous moiety comprises a half-life extending moiety.

[0033] In some embodiments, L1 and L2 are the same or different. In some embodiments, the first heterologous moiety comprises a clotting factor and the second heterologous moiety comprises a half-life extending moiety. In some embodiments, the second heterologous moiety comprises a low-complexity polypeptide. In some embodiments* the

second heterologous moiety comprises albumin, albumin binding polypeptide or fatty acid, Fc, 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, a clearance receptor or fragment thereof which blocks binding of the chimeric molecule to a clearance receptor or any combinations thereof. In some embodiments, the clotting factor comprises a single polypeptide chain or two polypeptide chains.

[0034] Also provided herein is 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 and a heterologous moiety, and the second polypeptide chain comprises a heavy chain of the clotting factor and a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein; (2) wherein the first polypeptide chain comprises a light chain of a clotting factor 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 and a heterologous moiety; (3) wherein the first polypeptide chain comprises a light chain of a clotting factor and the second polypeptide chain comprises a heavy chain of the clotting factor, a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, and a heterologous moiety; (4) wherein the first polypeptide chain comprises a light chain of a clotting factor and the second polypeptide chain comprises a heavy chain of the clotting factor, a heterologous moiety, and a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein; (5) wherein the first polypeptide chain comprises a light chain of a clotting factor, a heterologous 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; or (6) wherein the first polypeptide chain comprises a light chain of a clotting factor, a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, and a heterologous moiety and the second polypeptide chain comprises a heavy chain of the clotting factor.

[0035] 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 CFL-H or H-CFL and the second polypeptide chain comprises $\text{CF}_H\text{-Tm}$ or Tm-CFH ; (2) wherein the first polypeptide chain comprises CFL-

T_m or THI-CFL and the second polypeptide chain comprises CF_H-H or H-CF_H; (3) wherein the first polypeptide chain comprises CFL and the second polypeptide chain comprises CF_n-T_m-H or H-T_m-CF_H; (4) wherein the first polypeptide chain comprises CF_L and the second polypeptide chain comprises CF_n-H-T_m or T_m-H-CF_H; (5) wherein the first polypeptide chain comprises CF_L-H-T_m or T_m-H-CF_L and the second polypeptide chain comprises CF_H; or (6) wherein the first polypeptide chain comprises CFL-T_mH or H-T_m-CF_L and the second polypeptide chain comprises CF_H; wherein CFL is a light chain of a clotting factor; CF_H is a heavy chain of the clotting factor; T_m is an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein; or H is a heterologous moiety. In some embodiments, the clotting factor is independently selected from FVII, FIX, and FX.

[0036] In other embodiments, the chimeric molecule comprises one or more of the linkers, wherein one or more of the linkers comprise a peptide linker. In some embodiments, the peptide linker comprises at least two, 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 some embodiments, the peptide linker comprises at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, at least 1200, at least 1300, at least 1400, at least 1500, at least 1600, at least 1700, at least 1800, at least 1900, or at least 2000 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. In other embodiments, one or more of the linkers comprise a non-peptide linker.

[0037] In some embodiments, the heterologous moiety comprises a half-life extending moiety comprising a low-complexity polypeptide. In some embodiments, the chimeric molecule comprises a half-life extending moiety comprising albumin, albumin binding polypeptide or fatty acid, Fc, 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, a clearance receptor or fragment thereof which blocks binding of the chimeric molecule to a clearance receptor, or 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. In some embodiments, the chimeric molecule comprises a clotting

factor comprising FVII zymogen, activatable FVII, activated FVII, FIX zymogen, activatable FIX, activated FIX, FX zymogen, activatable FX, or activated FX.

[0038] The instant 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 and a targeting moiety, which binds to a platelet, and the second polypeptide chain comprises a heavy chain of the clotting factor and a heterologous moiety; (2) wherein the first polypeptide chain comprises a light chain of a clotting factor and a heterologous moiety and the second polypeptide chain comprises a heavy chain of the clotting factor and a targeting moiety, which binds to a platelet; (3) wherein the first polypeptide chain comprises a light chain of a clotting factor, a heterologous moiety, and a targeting moiety, which binds to a platelet, and the second polypeptide comprises a heavy chain of the clotting factor; or (4) wherein the first polypeptide chain comprises a light chain of a clotting factor, a targeting moiety, which binds to a platelet, and a heterologous moiety and the second polypeptide chain comprises a heavy chain of the clotting factor, and wherein the clotting factor is FVII, FIX, or FX.

[0039] 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 CFL-H or H-CFL and the second polypeptide chain comprises CF_H -Tm or Tm- CF_H ; (2) wherein the first polypeptide chain comprises CF_L -Tm or Tm-CFL and the second polypeptide chain comprises CFH-H or H-CFH; (3) wherein the first polypeptide chain comprises CFL-H-Tm or Tm-H-CFL and the second polypeptide chain comprises CF_H ; or (4) wherein the first polypeptide chain comprises CFL-Tm-H or H-Tm-CFL and the second polypeptide chain comprises CF_H ; wherein H is a heterologous moiety, CF_H is a heavy chain of a clotting factor, CF_L is a light chain of the clotting factor, Tm is a targeting moiety which binds to a platelet, and, L is an optional linker. In some embodiments, the chimeric molecule comprises a formula selected from (1) Tm- CF_H : CF_L -H; (2) H- CF_H : CF_L -Tm; (3) Tm-H- CF_L : CF_H ; or (4) H-Tm- CF_L :CFH; wherein, H is a heterologous moiety; CFH is a heavy chain of a clotting factor; CFL is a light chain of the clotting factor; Tm is a targeting moiety, which binds to a platelet; L is an optional linker; and : represents a covalent or non-covalent bond between CFH and CF_L .

[0040] 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. In other embodiment, 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. In some embodiments, the covalent bond is a disulfide bond.

[0041] The present disclosure also provides a chimeric molecule comprising a single polypeptide chain, which comprises, from N terminus to C terminus, (a) a light chain of a clotting factor, a heterologous moiety, a protease cleavage site, a heavy chain of the clotting factor, and a targeting moiety which binds to a platelet or (b) a light chain of a clotting factor, a targeting moiety which binds to a platelet, a protease cleavage site, a heavy chain of the clotting factor, and a heterologous moiety, wherein 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 PC5, PACE, PC7, and any combinations thereof.

[0042] In some embodiments, the targeting moiety in the chimeric molecule is selected from: an antibody or antigen binding molecule thereof, a receptor binding portion of a receptor, and a peptide. In some embodiments, the targeting moiety selectively binds to a resting platelet or an activated platelet. In other embodiments, the targeting moiety selectively binds to a target selected from the group consisting of: GPIIb, GPVI, GPIX, a nonactive form of glycoprotein IIb/IIIa ("GPIIb/IIIa"), an active form of GPIIb/IIIa, P selectin, GMP-33, LAMP-1, LAMP-2, CD40L, LOX-1, and any combinations thereof. In certain embodiments, the targeting moiety is an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof. In specific embodiments, the anti-GPIIb/IIIa antibody or antigen-binding molecule thereof is a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein. In some embodiments, a heterologous moiety in the chimeric molecule is a half-life extending moiety. In some embodiments, the half-life extending moiety is a low-complexity polypeptide. In some embodiments, the half-life extending moiety is selected from albumin, albumin binding polypeptide or fatty acid, Fc, 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, a clearance receptor or fragment thereof which blocks binding of the chimeric molecule to a clearance receptor, and any combinations thereof.

[0043] Also provided is a nucleic acid molecule or a set of nucleic acid molecules encoding an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or any of the chimeric molecules disclosed herein, or a complement thereof. 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. Also provided is a host cell comprising the vector.

[0044] The instant disclosure also provides a method for producing an anti-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. Also provided is a pharmaceutical composition comprising (i) an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein, or (ii) a chimeric molecule disclosed herein, or (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. Also provided is method of reducing a frequency or degree of a bleeding episode in a subject in need thereof comprising administering (i) a chimeric molecule disclosed herein, (ii) a nucleic acid molecule or set of nucleic acid molecules disclosed herein, (iii) a vector or set of vectors disclosed herein, or (iii) a pharmaceutical composition disclosed herein.

[0045] Also provided is a method of reducing or preventing an occurrence of a bleeding episode in a subject in need thereof comprising administering (i) a chimeric molecule disclosed herein, (ii) a nucleic acid molecule or set of nucleic acid molecules disclosed herein, (iii) a vector or the set of vectors disclosed herein, or (iv) a pharmaceutical composition disclosed 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. In some embodiments, the inhibitor against FVIII or FIX is a neutralizing antibody against FVIII, FIX, or both. In some embodiments, the bleeding episode is caused by a blood coagulation disorder. In some embodiments, the blood coagulation disorder is hemophilia A or hemophilia B. In some embodiments, the bleeding episode is derived from 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.

[0046] The instant disclosure also provides the chimeric molecule disclosed herein, the nucleic acid molecule or the set of nucleic acid molecules disclosed herein, the vector or the set of vectors disclosed herein, or the pharmaceutical composition disclosed herein for use in reducing a frequency or degree of a bleeding episode or reducing or preventing an occurrence of a bleeding episode in a subject in need thereof. Also provided is the use of the chimeric molecule disclosed herein, the nucleic acid molecule or the set of nucleic acid molecules disclosed herein, the vector or the set of vectors disclosed herein, or the pharmaceutical composition disclosed herein for the manufacture of a medicament for reducing a frequency or degree of a bleeding episode or reducing or preventing an occurrence of a bleeding episode in a subject in need thereof.

[0047] Also provided is a method to target a therapeutic or prophylactic agent to the surface of platelets comprising fusing the agent to one of the GPIIb/IIIa antibodies or antigen-binding molecules thereof disclosed herein. Also provided is a method to increase the activity of a therapeutic or prophylactic agent comprising fusing the agent to a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein. In some embodiments, the therapeutic or prophylactic agent is a clotting factor.

[0048] The present disclosure also 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, the method further comprises fusing or conjugating the clotting factor and/or the GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein to a half-life extending moiety.

[0049] 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.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

- [0050] FIG. 1 is a diagram summarizing the screening process used to identify the antibodies of the present disclosure.
- [0051] FIG 2A shows a diagram of an anti-GPIIb/IIIa antibody. FIGS. 2B, 2C, and 2D are schematic representations of the possible effects of the binding of antibodies to non-active GPIIb/IIIa. Antibodies can be inert (no activation) (FIG. 2B), activating (FIG. 2C), or capable of indirect activation via simultaneous interaction with GPIIb/IIIa and the FcγRIIA receptor (FIG. 2D). Also shown is a schematic representation of the nonactive (bent) and active (extended) conformations of GPIIb/IIIa (FIG. 2E). The cross over FIG. 2C indicates that platelet activating antibodies are not selected as targeting moieties for clotting factors.
- [0052] FIG. 3A shows the markers detected in the X- and y-axes in the flow cytometry profiles shown in FIGS. 3B, 3C, 3D, and 3E. FIG. 3B shows flow cytometry profiles of platelets exposed to culture medium only or with an agonist in the presence or absence of an FcγRIIA inhibitory antibody. FIG. 3C shows flow cytometry profiles of platelets incubated with hybridoma clone P24H2 supernatant in the presence or absence of an FcγRIIA inhibitory antibody. FIG. 3D shows flow cytometry profiles of platelets incubated with hybridoma clone 35E2 supernatant in the presence or absence of an FcγRIIA inhibitory antibody. FIG. 3E shows flow cytometry profiles of platelets incubated with supernatants from hybridoma clones P24A4, P31G2, and P35E9, respectively, in the presence or absence of an FcγRIIA inhibitory antibody.
- [0053] FIGS. 4A and 4B show identity matrices corresponding to heavy chain variable domain (VH) sequences (FIG. 4A) and light chain variable domain (VL) sequences (FIG. 4B) of antibodies against GPIIb/IIIa in which the shaded cells indicate which antibodies share identical VL or VH sequences.
- [0054] FIGS. 5A and 5B show ClustalX multiple sequence alignments of the VH sequences of monoclonal antibodies 1H6, 38A8, 12B2, 38F6, 2A2, 36A8, 4B1 1, 34D10, 28F4, 23C10, 28C2, 5C4, 9D6, and 18F7 (FIG. 5A) and of the VL sequences of monoclonal antibodies 28C2, 9D6, 1H6, 38A8, 12B2, 18F7, 28F4, 34D10, 36A8, and 2A2 (FIG. 5B). Degree of amino acid conservation is indicating above the alignment ("*" = identical; ":" = strongly conserved; "." = poorly conserved), as well as the bars below the alignment. The ClustalX highlighting scheme, which is dependent on amino acid

physicochemical and/or structural properties and conservation, was used to highlight the sequences.

[0055] FIG. 6 shows a ClustalX multiple sequence alignment of the VH sequences of the 1H6, 38A8, 12B2, 38F6, 2A2, 36A8, 4B1 1, 34D10, 28F4, 23C10, 28C2, 5C4, 9D6, and 18F7 antibodies, indicating the location of the complementarity determining regions (CDR). The location of each CDR (CDR1, CDR2, and CDR3) according to the EU index is indicated. The location of identical, conserved and partially conserved amino acid residues is indicated below the alignment.

[0056] FIG. 7 shows a ClustalX multiple sequence alignment of the VL sequences of the 28C2, 9D6, 1H6, 38A8, 12B2, 18F7, 28F4, 34D10, 36A8, and 2A2 antibodies, indicating the location of CDR1, CDR2, and CDR3 according to the EU index. The location of identical, conserved and partially conserved amino acid residues is indicated below the alignment.

[0057] FIG. 8 shows percentage identity matrices corresponding to the sequences included in the ClustalX multiple sequence alignments shown in FIG. 3 (top matrix) and FIG. 4 (bottom matrix).

[0058] FIG. 9 shows ClustalX multiple sequence alignments corresponding to the VH sequences in FIG. 2 clustered according to their specificity for the α or β subunit of GPIIb/IIIa.

[0059] FIG. 10 shows ClustalX multiple sequence alignments corresponding to the VL sequences in FIG. 3 clustered according to their specificity for the α or β subunit of GPIIb/IIIa.

[0060] FIG. 11 shows ClustalX multiple sequence alignments corresponding to the VH sequences in FIG. 2 clustered according to their ability to compete with fibrinogen for binding to GPIIb/IIIa.

[0061] FIG. 12 shows ClustalX multiple sequence alignments corresponding to the VL sequences in FIG. 2 clustered according to their ability to compete with fibrinogen for binding to GPIIb/IIIa.

[0062] FIGS. 13A and 13B are schematic representations of an anti-GPIIb/IIIa antibody in scFv format recombinantly fused to the FVIIa clotting factor heavy chain (FIG. 13A) or light chain (FIG. 13B) for platelet targeting.

- [0063] FIG. 14 is a diagram summarizing the experimental method used to study the binding kinetics and affinity of a platelet-targeted rFVIIa chimeric molecule for GPIIb/IIIa. GPIIb/IIIa constructs were doubly labeled with biotin and the HPC4 purification tag and incorporated into GPIIb/IIIa nanodiscs. Binding of the platelet-targeted rFVIIa to the nanodiscs was studied using Surface Plasmon Resonance (SPR).
- [0064] FIG. 15 shows single cycle steady-state binding of a platelet-targeted rFVIIa chimeric molecule to GPIIb/IIIa nanodiscs measured by SPR. FIG. 15A shows the binding as a function of time, and FIG. 15B shows the binding as a function of concentration.
- [0065] FIG. 16 shows ROTEM assay results corresponding to rFVIIa compared to FVII-189 (a platelet-targeted FVIIa chimeric molecule where the targeting moiety is an scFv derived from the 34D10 antibody) (FIG. 16A), and to rFVIIa compared to FVII-206 (a platelet-targeted FVIIa chimeric molecule where the targeting moiety is an scFv derived from the 12B2 antibody) (FIG. 16B).
- [0066] FIGS. 17 shows ROTEM assay results corresponding to rFVIIa compared to FVII-204 (a platelet-targeted FVIIa chimeric molecule where the targeting moiety is an scFv derived from the 38A8 antibody) (FIG. 17A), and to rFVIIa compared to FVII-163 (a platelet-targeted FVIIa chimeric molecule where the targeting moiety is an scFv derived from the PDG13 antibody) and FVII-189 (FIG. 17B)..
- [0067] FIG. 18 shows the result of flow cytometry based platelet-binding assays, in which the median fluorescence intensity (MFI) was plotted against the concentration of the chimeric molecules spiked in blood. MFI represented the relative concentration of the chimeric molecules that bound to human platelets.
- [0068] FIG. 19 shows flow cytometry profiles of the activation of gel-purified platelets. Platelets were non-activated, activated after incubation with an agonist, or incubated with chimeric molecules FVII-189, FVII-206, and FVII-130 (a platelet-targeted FVII comprising an scFv derived from the AP3 antibody and an Fc moiety).
- [0069] FIG. 20 shows ADP-induced aggregation of platelet-rich plasma in the presence of chimeric molecules FVII-189 (FIG. 20A) or FVII-206 (FIG. 20B). In both cases, ADP and REOPRO® (Abciximab) were used as controls.

- [0070] FIG. 21 shows human platelet clearance from NSG mice after administration of the chimeric molecules FVII-189, FVII-206, FVII204, or FVII-130. The NSG mice had been transfused with human platelets before treating with the chimeric molecules.
- [0071] FIG. 22 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.
- [0072] FIG. 23 shows possible configurations for chimeric molecules comprising the heavy and light chains of a clotting factor (*e.g.*, FVII), an 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.

DETAILED DESCRIPTION

- [0073] The clotting factor FVIIa has low affinity for platelets, the site of action for clot formation. Thus, a possible approach to increase activity of FVIIa is to target the clotting to platelet receptors via targeting moieties (*e.g.*, Fab or scFv), which can increase the affinity of FVIIa for platelets thereby boosting activity. Multiple receptors are expressed exclusively on platelet. For example, GPIIb/IIIa (α IIb β 3) is an integrin specific to platelets which expressed at high levels. Activated GPIIb/IIIa receptor binds fibrinogen and modulates platelet aggregation. Upon activation, the GPIIb/IIIa receptors change from a bent low ligand affinity conformation to an extended high ligand affinity conformation. Thus, targeting strategies can be directed either the active or the non-active conformations of the receptor.
- [0074] The present disclosure provides antibodies against GPIIb/IIIa that are capable of targeting the non-active form of the receptor. The GPIIb/IIIa antibodies and antigen-binding molecules derived from these antibodies can be non-activating, and be used, for example, to target therapeutic molecules (*e.g.*, clotting factors or other molecules capable of having a pharmacological effect in platelets) to the platelet surface. In addition to their use as targeting moieties, these antibodies and antigen-binding molecules thereof can be used for diagnostics, for example, by conjugation to a detectable label.
- [0075] In some embodiments, the GPIIb/IIIa antibodies and antigen-binding molecules thereof can be activating, for example, (i) by directly activating the GPIIb/IIIa receptors through binding to the α and/or β subunits of the receptor, (ii) by indirectly activating the

GPIIb/IIIa via simultaneous binding to the α and/or β subunits of the receptor and to an Fc receptor (*e.g.*, an Fc γ IIb receptor), or (iii) by interfering with the interaction between the GPIIb/IIIa receptor and fibrinogen.

[0076] In addition, the present invention relates to chimeric molecules comprising the GPIIb/IIIa antibodies and antigen-binding molecules thereof disclosed herein as targeting moieties, and one or more heterologous moieties. For example, a heterologous moiety comprising a therapeutic molecule (for example, a procoagulant molecule such as a clotting factor), and optionally a second heterologous moiety comprising, for example, a PK enhancing moiety (*i.e.*, a molecule which can improve various pharmacokinetic properties, *e.g.*, half-life).

[0077] Exemplary GPIIb/IIIa antibodies and antigen-binding molecules thereof, as well as exemplary constructs (chimeric molecules) comprising such antibodies and antigen-binding molecules thereof (*e.g.*, scFv or F(ab)) are illustrated in the instant description and figures. In some embodiments, the invention pertains to chimeric molecules having the structures set forth, for example, in FIGS. 22 and 23. In other embodiments, the invention pertains to polynucleotide encoding chimeric molecule constructs disclosed herein.

[0078] In order to provide a clear understanding of the specification and claims, the following definitions are provided below.

I. Definitions

[0079] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0080] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

- [0081] 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.
- [0082] The use of numerical values in the various ranges specified in this application, unless expressly indicated otherwise, are stated as approximations as though the minimum and maximum values within the stated ranges were both preceded by the word "about." In this manner, slight variations above and below the stated ranges can be used to achieve substantially the same results as values within the ranges. As used herein, the terms "about" and "approximately" when referring to a numerical value shall have their plain and ordinary meanings to one skilled in the art relevant to the range or element at issue.
- [0083] The amount of broadening from the strict numerical boundary depends upon many factors. For example, some of the factors to be considered can include the criticality of the element and/or the effect a given amount of variation will have on the performance of the claimed subject matter, as well as other considerations known to those of skill in the art. Thus, as a general matter, "about" or "approximately" broaden the numerical value. For example, in some cases, "about" or "approximately" can mean $\pm 5\%$, or $\pm 10\%$, depending on the relevant technology. Also, the disclosure of ranges is intended as a continuous range including every value between the minimum and maximum values recited.
- [0084] Unless defined otherwise, 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 disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.
- [0085] Units, prefixes, and symbols are denoted in their Systeme International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various embodiments of the disclosure, which can be by reference to the specification as a whole.

Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety. Amino acids are referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, are referred to by their commonly accepted single-letter codes.

[0086] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" can be used instead of, or interchangeably with any of these terms.

[0087] As used herein the term "protein" is intended to encompass a molecule comprised of one or more polypeptides, which can in some instances be associated by bonds other than amide bonds.

[0088] Polypeptides can be either monomers or multimers. For example, in one embodiment, an antibody, an antigen-binding molecule thereof, or a chimeric molecule of the invention can be a dimeric polypeptide. A dimeric antibody, an antigen-binding molecule thereof can comprise two polypeptide chains or can consist of one polypeptide chain (e.g., in the case of an scFc molecule). In one embodiment, the dimers can be a homodimer, comprising two identical monomeric subunits or polypeptides (e.g., two identical Fc moieties or two identical biologically active moieties). In another embodiment, the dimers are heterodimers, comprising two non-identical monomeric subunits or polypeptides (e.g., comprising two different clotting factors or portions thereof or one clotting factor only). See, e.g., U.S. patent 7,404,956, incorporated herein by reference.

[0089] The terms "polypeptide" and "protein" are also intended to refer to the products of post-expression modifications, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide or protein can be derived from a natural biological source or produced by

recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0090] A polypeptide which is "isolated" is a polypeptide which is in a form not found in nature. Isolated polypeptides include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a polypeptide which is isolated is substantially pure.

[0091] "Derivatives" of GPIIb/IIIa antibodies, antigen-binding molecules thereof, or chimeric molecules of the invention are polypeptides or proteins which have been altered so as to exhibit additional features not found on the native polypeptide or protein. Also included as "derivatives" are those peptides that contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. A polypeptide or amino acid sequence "derived from" a designated polypeptide or protein refers to the origin of the polypeptide. In one embodiment, the polypeptide or amino acid sequence which is derived from a particular sequence has an amino acid sequence that is essentially identical to that sequence or a portion thereof, wherein the portion consists of at least about 10 to about 20 amino acids, at least about 20 to about 30 amino acids, or at least about 30 to about 50 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the sequence.

[0092] Polypeptides that are "variants" of another polypeptide can have one or more mutations relative to the starting polypeptide, *e.g.*, one or more amino acid residues which have been substituted with another amino acid residue or which has one or more amino acid residue insertions or deletions. In one embodiment, the polypeptide comprises an amino acid sequence which is not naturally occurring. Such variants necessarily have less than 100% sequence identity or similarity with the starting polypeptide. In another embodiment, the variant will have an amino acid sequence from about 75% to less than 100% amino acid sequence identity or similarity with the amino acid sequence of the starting polypeptide, for example, from about 80% to less than 100%, from about 85% to less than 100%, from about 90% to less than 100% (*e.g.*, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) and from about 95% to less than 100%, *e.g.*, over the length of the variant molecule. In one embodiment, there is one amino acid difference between a starting polypeptide sequence and the sequence derived therefrom.

- [0093] The term "fragment" when referring to GPIIb/IIIa antibodies, antigen-binding molecules thereof, chimeric molecules of the invention, or clotting factors refers to any polypeptides or proteins which retain at least some of the properties of the reference polypeptide or protein. Fragments of polypeptides include proteolytic fragments, as well as deletion fragments.
- [0094] The term "sequence" as used to refer to a protein sequence, a peptide sequence, a polypeptide sequence, or an amino acid sequence means a linear representation of the amino acid constituents in the polypeptide in an amino-terminal to carboxyl-terminal direction in which residues that neighbor each other in the representation are contiguous in the primary structure of the polypeptide.
- [0095] The term "amino acid" includes alanine (Ala or A); arginine (Arg or R); asparagine (Asn or N); aspartic acid (Asp or D); cysteine (Cys or C); glutamine (Gln or Q); glutamic acid (Glu or E); glycine (Gly or G); histidine (His or H); isoleucine (Ile or I); leucine (Leu or L); lysine (Lys or K); methionine (Met or M); phenylalanine (Phe or F); proline (Pro or P); serine (Ser or S); threonine (Thr or T); tryptophan (Trp or W); tyrosine (Tyr or Y); and valine (Val or V).
- [0096] Non-traditional amino acids are also within the scope of the invention and include norleucine, ornithine, norvaline, homoserine, and other amino acid residue analogues such as those described in Ellman *et al.* Meth. Enzym. 202:301-336 (1991). To generate such non-naturally occurring amino acid residues, the procedures of Noren *et al.* Science 244:182 (1989) and Ellman *et al.*, *supra*, can be used. Briefly, these procedures involve chemically activating a suppressor tRNA with a non-naturally occurring amino acid residue followed by *in vitro* transcription and translation of the RNA. Introduction of the non-traditional amino acid can also be achieved using peptide chemistries known in the art. As used herein, the term "polar amino acid" includes amino acids that have net zero charge, but have non-zero partial charges in different portions of their side chains (*e.g.*, M, F, W, S, Y, N, Q, and C). These amino acids can participate in hydrophobic interactions and electrostatic interactions. As used herein, the term "charged amino acid" includes amino acids that can have non-zero net charge on their side chains (*e.g.* R, K, H, E, and D). These amino acids can participate in hydrophobic interactions and electrostatic interactions.

[0097] An "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a predetermined amino acid sequence (an amino acid sequence of a starting polypeptide) with a second, different "replacement" amino acid residue. An "amino acid insertion" refers to the incorporation of at least one additional amino acid into a predetermined amino acid sequence. While the insertion will usually consist of the insertion of one or two amino acid residues, the present larger "peptide insertions", can be made, e.g. insertion of about three to about five or even up to about ten, fifteen, or twenty amino acid residues. The inserted residue(s) can be naturally occurring or non-naturally occurring as disclosed above. An "amino acid deletion" refers to the removal of at least one amino acid residue from a predetermined amino acid sequence.

[0098] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., Lys, Arg, and His), acidic side chains (e.g., Asp and Glu), uncharged polar side chains (e.g., Gly, Asn, Gln, Ser, Thr, Tyr, and Cys), nonpolar side chains (e.g., Ala, Val, Leu, Ile, Pro, Phe, Met, and Trp), beta-branched side chains (e.g., Thr, Val, and Ile) and aromatic side chains (e.g., Tyr, Phe, Trp, and His). Thus, if an amino acid in a polypeptide is replaced with another amino acid from the same side chain family, the substitution is considered to be conservative. In another embodiment, a string of amino acids can be conservatively replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0099] Non-conservative substitutions include those in which (i) a residue having an electropositive side chain (e.g., Arg, His, or Lys) is substituted for, or by, an electronegative residue (e.g., Glu or Asp), (ii) a hydrophilic residue (e.g., Ser or Thr) is substituted for, or by, a hydrophobic residue (e.g., Ala, Leu, Ile, Phe, or Val), (iii) a cysteine or proline is substituted for, or by, any other residue, or (iv) a residue having a bulky hydrophobic or aromatic side chain (e.g., Val, Ile, Phe, or Trp) is substituted for, or by, one having a smaller side chain (e.g., Ala or Ser) or no side chain (e.g., Gly).

[0100] The term "percent sequence identity" between two polynucleotide or polypeptide 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.

[0101] 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.

[0102] 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). B12seq 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.

[0103] Different regions within a single polynucleotide or polypeptide target sequence that aligns with a polynucleotide or polypeptide reference sequence can each have their own percent sequence identity. It is noted that the percent sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

[0104] 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.

[0105] 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.

[0106] It will also be appreciated that sequence alignments can be generated by integrating sequence data with data from heterogeneous sources such as structural data (*e.g.*, crystallographic protein structures), functional data (*e.g.*, location of mutations), or phylogenetic data. A suitable program that integrates heterogeneous data to generate a multiple sequence alignment is T-Coffee, available at www.tcoffee.org, and alternatively available, *e.g.*, from the EBI. It will also be appreciated that the final alignment used to calculate percent sequence identity can be curated either automatically or manually.

(01071 In one embodiment, the antibodies and antigen-binding molecules thereof, as well as the chimeric molecules of the invention can comprise an amino acid sequence derived from a human protein sequence. However, the antibodies and antigen-binding molecules thereof, as well as the chimeric molecules of the invention can comprise one or more amino acids from another mammalian species. In a particular embodiment, the antibodies and antigen-binding molecules thereof, as well as the chimeric molecules of the invention are not immunogenic.

[0108] As used herein, the terms "linked," "fused", or "fusion" refer to linkage via a peptide bonds (*e.g.*, genetic fusion), chemical conjugation, or other means known in the art. For example, one way in which molecules or moieties can be linked employs peptide linkers which link the molecules or moieties via peptide bonds. The terms "genetically fused," "genetically linked," or "genetic fusion" are used interchangeably and refer to the co-linear, covalent linkage or attachment of two or more proteins, polypeptides, or

fragments thereof via their individual peptide backbones, through genetic expression of a single polynucleotide molecule encoding those proteins, polypeptides, or fragments. Such genetic fusion results in the expression of a single contiguous genetic sequence.

[0109] Preferred genetic fusions are in frame, *i.e.*, two or more open reading frames (ORFs) are fused to form a continuous longer ORF, in a manner that maintains the correct reading frame of the original ORFs. Thus, the resulting recombinant fusion protein is a single polypeptide containing two or more protein segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature). In this case, the single polypeptide is cleaved during processing to yield dimeric molecules comprising two polypeptide chains.

[0110] As used herein the term "associated with" refers to a covalent or non-covalent bond formed between a first amino acid chain and a second amino acid chain. In one embodiment, the term "associated with" means a covalent, non-peptide bond or a non-covalent bond. 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:CF_L" 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.

[0111] Examples of covalent bonds include, but are not limited to, a peptide bond, a metal bond, a hydrogen bond, a disulfide bond, a sigma bond, a pi bond, a delta bond, a glycosidic bond, an agnostic bond, a bent bond, a dipolar bond, a Pi backbond, a double bond, a triple bond, a quadruple bond, a quintuple bond, a sextuple bond, conjugation, hyperconjugation, aromaticity, hapticity, or antibonding. Non-limiting examples of non-covalent bond include an ionic bond (*e.g.*, cation-pi bond or salt bond), a metal bond, an hydrogen bond (*e.g.*, dihydrogen bond, dihydrogen complex, low-barrier hydrogen bond, or symmetric hydrogen bond), van der Waals force, London dispersion force, a mechanical bond, a halogen bond, aurophilicity, intercalation, stacking, entropic force, or chemical polarity.

[0112] As used herein, the terms "chemically crosslinked" and "conjugated" are used interchangeably and refer to chemically linking by covalent bonds between acid side

chains of amino acids, either directly or via a linker, *e.g.*, a peptide linker. Chemical crosslinking does not include intramolecular or intermolecular disulfide bonds between Fc moieties of a dimeric Fc region, or non-engineered disulfide bonds between an amino acid of the activatable clotting factor and an amino acid of the enhancer moiety. Chemical crosslinking generally takes place by addition of a cross-linking agent, *e.g.*, a heterobifunctional crosslinking agent. Examples of chemical crosslinking includes one or more photo-reactive bonds by chemically connecting photo-Ile, photo-Met, and photo-Leu (*see*, Suchanek *et al.*, (2005) *Nature Methods*, 2: 261-267).

[0113] 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.

[0114] 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, C1. 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 (FW).

[0115] Each VH and VL region is composed of three CDRs and four FWs, arranged from amino-terminus to carboxy-terminus in the following order: FW1, CDR1, FW2, CDR2, FW3, CDR3, FW4. 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, and Fv fragments), single chain Fv (scFv), minibodies, 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, etc.

[0116] There are at least two techniques for determining the location of CDRs: (1) an approach based on cross-species sequence variability (*i.e.*, Kabat *et al.* Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda Md.)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani *et al.* (1997) *J. Molec. Biol.* 273:927-948)). In addition, combinations of these two approaches are sometimes used in the art to determine CDRs.

[0117] The amino acid position numbering as in Kabat, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Using this numbering system, the actual linear amino acid sequence can contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FW or CDR of the variable domain. For example, a heavy chain variable domain can include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.*, residues 82a, 82b, and 82c, *etc.* according to Kabat) after heavy chain FW residue 82.

[0118] The Kabat numbering of residues can be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence. Chothia refers instead to the location of the structural loops (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software.

[0119] IMGT (ImMunoGeneTics) also provides a numbering system for the immunoglobulin variable regions, including the CDRs. See *e.g.*, Lefranc, M.P. *et al.*, *Dev. Comp. Immunol.* 27: 55-77(2003). The IMGT numbering system was based on an alignment of more than 5,000 sequences, structural data, and characterization of hypervariable loops and allows for easy comparison of the variable and CDR regions for all species. 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.

[0120] As used throughout the specification the VH CDR sequences described herein correspond to the classical Kabat numbering locations, namely Kabat VH-CDR1 is at positions 31-35, VH-CDR2 is a positions 50-65, and VH-CDR3 is at positions 95-102. VL-CDR1, VL-CDR2, and VL-CDR3 also correspond to classical Kabat numbering locations, namely positions 14-24, 50-56 and 89-97, respectively.

[0121] The term "consensus sequence," as used herein with respect to a CDR in the light chain (VL) or heavy chain (VH) variable regions, refers to a composite or genericized amino acid sequence defined based on information as to which amino acid residues are present at a given position based in multiple sequence alignments. Thus, in a "consensus sequence" for a VL or VH chain CDR1, CDR2, or CDR3, certain amino acid positions are occupied by one of multiple possible amino acid residues at that position. For example, if an arginine (R) or a serine (S) occur at a particular position X, then that particular position within the consensus sequence can be either arginine or serine (R or S). Such occurrence would be represented, for example, as $N-Z_1Z_2X_nZ_{t-1}Z_t-c$, where Z_i are invariant amino acids in the multiple sequence alignment, X represent a position occupied by variant amino acids (*e.g.*, R or S), and the subindex n is an ordinal. As used herein, referring to a polypeptide sequence as consisting of or comprising a consensus sequence means that the polypeptide sequence consists of or comprises one of the of multiple possible amino acid sequences represented by the consensus sequence.

[0122] 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 antibody fragments include, but are not limited to

Fab, Fab', F(ab')₂, and Fv fragments, linear antibodies, single chain antibodies, and multispecific antibodies formed from antibody fragments.

[0123] 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.

[0124] The term "Fab'" refers to an antibody fragment that is essentially equivalent to that obtained by reduction of the disulfide bridge or bridges joining the two heavy chain pieces in the F(ab')₂ fragment. 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.

[0125] 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.

[0126] The term "Fv" refers to an antibody fragment that consists of one NH and one N domain held together by noncovalent interactions.

[0127] The term "monoclonal antibody" refers to a homogeneous antibody population involved in the highly specific recognition and binding of a single antigenic determinant, or epitope. This is in contrast to polyclonal antibodies that typically include different antibodies directed against different antigenic determinants. The term "monoclonal antibody" encompasses both intact and full-length monoclonal antibodies as well as antibody fragments (such as Fab, Fab', F(ab')₂, or Fv), single chain (scFv) mutants, fusion proteins comprising an antibody portion, and any other modified immunoglobulin molecule comprising an antigen recognition site. Furthermore, "monoclonal antibody" refers to such antibodies made in any number of ways including, but not limited to, by hybridoma, phage selection, recombinant expression, and transgenic animals.

[0128] The term "human antibody" refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any technique known in the art. This definition of a human antibody includes intact or full-length antibodies, fragments thereof, and/or antibodies comprising at least one human heavy and/or light chain polypeptide such as, for example, an antibody comprising murine light chain and human heavy chain polypeptides. The term "humanized antibody" refers to an antibody derived from a non-human (*e.g.*, murine) immunoglobulin, which has been engineered to contain minimal non-human (*e.g.*, murine) sequences. The term "chimeric antibodies" refers to antibodies wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. Typically, the variable region of both light and heavy chains corresponds to the variable region of antibodies derived from one species of mammals (*e.g.*, mouse, rat, rabbit, etc.) with the desired specificity, affinity, and capability while the constant regions are homologous to the sequences in antibodies derived from another (usually human) to avoid eliciting an immune response in that species.

[0129] In one embodiment, an anti-GPIIa/IIIb antibody of the invention comprises an antibody variant. The term "antibody variant" or "modified antibody" includes an antibody which does not occur in nature and which has an amino acid sequence or amino acid side chain chemistry which differs from that of a naturally-derived antibody by at least one amino acid or amino acid modification as described herein. As used herein, the term "antibody variant" includes synthetic forms of antibodies which are altered such that they are not naturally occurring, *e.g.*, antibodies that comprise at least two heavy chain portions but not two complete heavy chains (such as, domain deleted antibodies or minibodies); multispecific forms of antibodies (*e.g.*, bispecific, trispecific, etc.) altered to bind to two or more different antigens or to different epitopes on a single antigen; heavy chain molecules joined to scFv molecules; single-chain antibodies; diabodies; triabodies; and antibodies with altered effector function and the like.

[0130] 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* 30:101 17 (1991); Milenic *et al*, *Cancer Research* 51:6363 (1991); Takkinen *et al*, *Protein Engineering* 4:837 (1991).

[0131] 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.

[0132] As used herein, the term "antigen-binding molecule" refers to a molecule comprising an anti-GPIIb/IIIa antibody fragment, variant, or derivative thereof, comprising at least one CDR from one or more of the anti-GPIIb/IIIa antibodies disclosed herein. In some embodiments, the antigen-binding molecule is a protein. In other embodiments, the antigen-binding molecule is a protein scaffold (*e.g.*, a fibronectin type III domain) or non-protein scaffold comprising at least one CDR from one of the anti-GPIIb/IIIa antibodies disclosed herein. In some embodiments, the antigen-binding molecule is an anti-GPb/IIIa antibody identified according to the methods disclosed herein, comprising at least one CDR identical to one of the CDR sequences disclosed herein. The term "antigen-binding molecule" also encompasses any molecule comprising a VH and/or VL region from one or more of the anti-GPIIb/IIIa antibodies disclosed herein.

[0133] The term "polynucleotide" or "nucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA) or plasmid DNA (pDNA). In certain embodiments, a polynucleotide comprises a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)).

[0134] The term "nucleic acid" refers to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. Examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially

or substantially) from other polynucleotides in a solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides of the present invention. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid can include regulatory elements such as promoters, enhancers, ribosome binding sites, or transcription termination signals.

[0135] As used herein, a "coding region" or "coding sequence" is a portion of polynucleotide which consists of codons translatable into amino acids. Although a "stop codon" (tag, tga, or taa) is typically not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl terminus of the resulting polypeptide.

[0136] Two or more coding regions of the present invention can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. It follows, then, that a single vector can contain just a single coding region, or comprise two or more coding regions, *e.g.*, a single vector can separately encode a binding domain-A and a binding domain-B as described below. In addition, a vector, polynucleotide, or nucleic acid of the invention can encode heterologous coding regions, either fused or unfused to a nucleic acid encoding a binding domain of the invention. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0137] The term "vector" or "expression vector" is used herein to mean vectors used in accordance with the present invention as a vehicle for introducing into and expressing a desired polynucleotide in a cell. As known to those skilled in the art, such vectors can easily be selected from plasmids, phages, viruses, or retroviruses. In general, vectors compatible with the instant invention will comprise a selection marker, appropriate restriction sites to facilitate cloning of the desired gene and the ability to enter and/or replicate in eukaryotic or prokaryotic cells.

[0138] Numerous expression vector systems can be employed to produce the antibody, antigen-binding molecule thereof, or a chimeric molecule of the invention. For example, one class of vector utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma vims, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV) or SV40 virus. Additionally, cells which have integrated the DNA into their chromosomes can be selected by introducing one or more markers which allow selection of transfected host ceils. 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 ceil by cotransformation. In one embodiment, an inducible expression system can be employed. Additional elements can also be needed for optimal synthesis of mRNA. These elements can include signal sequences, splice signals, as well as transcriptional promoters, enhancers, and termination signals. In one embodiment, a secretion signal, *e.g.*, any one of several well characterized bacterial leader peptides (*e.g.*, pe!B, phoA, or ompA), can be fused in-frame to the N terminus of a polypeptide of the invention to obtain optimal secretion of the polypeptide. (Lei *et al.* (1988), *Nature*, 331:543; Better *et al* (1988) *Science*, 240:1041; Mullinax *et al*, (1990). *PNAS*, 87:8095).

[0139] Certain proteins secreted by mammalian cells are associated with a secretory signal peptide which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that signal peptides are generally fused to the N-terminus of the polypeptide, and are cleaved from the complete or "full-length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, a native signal peptide, *e.g.*, an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, *e.g.*, a human tissue plasminogen activator (TPA) or mouse B-glucuronidase signal peptide, or a functional derivative thereof, can be used.

[0140] A "recombinant" polypeptide or protein refers to a polypeptide or protein produced via recombinant DNA technology, Recombinantly produced polypeptides and

proteins expressed in host cells are considered isolated for the purpose of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0141] The term "host cell" refers to a cell that has been transformed with a vector constructed using recombinant DNA techniques and encoding at least one heterologous gene. In descriptions of processes for isolation of proteins from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of protein unless it is clearly specified otherwise. In other words, recovery of protein from the "cells" can mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells. The host cell line used for protein expression is most preferably of mammalian origin; those skilled in the art are credited with ability to preferentially determine particular host cell lines which are best suited for the desired gene product to be expressed therein. Exemplary host cell lines include, but are not limited to, CHO cell line, BHK cell line, HEK cell line, DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative of CVI with SV40 T antigen), R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), PerC6 cells, HAK (hamster kidney line), SP2/0 (mouse myeloma), P3x63-Ag3.653 (mouse myeloma), BFA-lc1BPT (bovine endothelial cells), and RAJI (human lymphocyte). Host cell lines are typically available from commercial services, the American Tissue Culture Collection or from published literature.

II. Anti-GPIIb/IIIa Antibodies

[0142] The present invention provides antibodies and antigen-binding molecules thereof that specifically bind to GPIIb/IIIa receptors located on the surface of platelets. As used herein, 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 35,000 to 100,000 GPIIb/IIIa complexes; most are distributed on the platelet surface, with a smaller pool 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. See Uniprot entries P05106 (ITB3_HUMAN; GPIIIa; CD61; integrin beta-3; integrin β 3) and P08514 (ITA2B_HUMAN; GPIIb; CD41; integrin alpha-2b; integrin all) as published in Universal Protein Resource (Uniprot) database release 2011_05 (May 1, 2013), which are incorporated by reference in their entireties.

[0143] The GPIIb/IIIa receptor is a target of several GpIIb/IIIa inhibitor drugs including abciximab (REOPRO®), eptifibatid (INTEGRILIN®), and tirofiban (AGGRASTAT®). GPIIb/IIIa inhibitors can be used during percutaneous coronary intervention (angioplasty with or without intracoronary stent placement) to preventing platelet aggregation and thrombus formation by inhibiting the GPIIb/IIIa receptor on the surface of the platelets. They can also be used to treat acute coronary syndromes, without percutaneous coronary intervention, depending on TIMI risk.

[0144] 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 (K_D) of $\leq 1 \mu\text{M}$, $< 100 \text{ nM}$, $< 50 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $< 0.1 \text{ nM}$, $\leq 10 \text{ pM}$, $\leq 1 \text{ pM}$, or $< 0.1 \text{ pM}$.

[0145] In some embodiments, the antibody or antigen-binding molecules thereof specifically bind to a GPIIb/IIIa epitope, which comprises or overlaps with the GPIIb/IIIa binding epitope of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4 (see TABLE 1). In some embodiments, the antibody or antigen-binding molecules thereof specifically bind to a GPIIb/IIIa epitope, which is the same GPIIb/IIIa binding epitope of

an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4 (see TABLE 1). As used herein, the term "epitope" designates a specific amino acid sequence, modified amino acid sequence, or protein secondary or tertiary structure which is specifically recognized by an antibody. The terms "specifically recognizing," "specifically recognizes," and any grammatical variants mean that the antibody or antigen-binding molecule thereof is capable of specifically interacting with and/or binding to at least two, at least three, or at least four amino acids of an epitope, *e.g.*, a GPIIb/IIIa epitope. Such binding can be exemplified by the specificity of a "lock-and-key-principle." Thus, specific motifs in the amino acid sequence of the antigen-binding domain the GPIIb/IIIa antibody or antigen-binding molecule thereof and the epitope bind to each other as a result of their primary, secondary or tertiary structure as well as the result of secondary modifications of the structure.

[0146] In other embodiments, the antibody or antigen-binding molecule thereof of the present invention specifically binds to GPIIb/IIIa and competitively inhibits GPIIb/IIIa binding by an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4 (see TABLE 1). In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises at least one, at least two, at least three, at least four, or at least five complementarity determining regions (CDR) or variants thereof of an antibody selected from one or more of the 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4 antibodies disclosed in TABLE 1. In other embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises six CDRs or variants thereof of an antibody selected from one or more of the 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4 antibodies disclosed herein. In some embodiments, CDRs are independently selected from CDRs or variants thereof derived from the VH and/or VL region of one, two, three, four, or six antibodies selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4.

[0147] In certain embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR1 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR2 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR3 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VL-CDR1 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VL-CDR2 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4, and/or

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60, 70, 80, 90, or 95% identical to VL-CDR3 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4.

[0148] In certain embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR1 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR2 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4; and

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR3 of an antibody selected from 4D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4.

[0149] In other embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VL-CDR1 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(ii) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VL-CDR2 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4, and

(iii) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60, 70, 80, 90, or 95% identical to VL-CDR3 of an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4.

[0150] In certain embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR1 of an antibody selected from 34D10, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, or 18F7;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR2 of an antibody selected from 34D10, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, or 18F7;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR3 of an antibody selected from 34D10, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, or 18F7;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VL-CDR1 of an antibody selected from 34D10, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, or 18F7;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VL-CDR2 of an antibody selected from 34D10, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, or 18F7, and/or

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60, 70, 80, 90, or 95% identical to VL-CDR3 of an antibody selected from 34D10, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, or 18F7.

[0151] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR1 of an antibody selected from 12B2, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR2 of an antibody selected from 12B2, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VH-CDR3 of an antibody selected from 12B2, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VL-CDR1 of an antibody selected from 12B2, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to VL-CDR2 of an antibody selected from 12B2, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4, and/or

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60, 70, 80, 90, or 95% identical to VL-CDR3 of an antibody selected from 12B2, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4.

[0152J] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 25, 31, 37, 43, or 111;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 26, 32, 38, 44, or 112;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 27, 33, 39, 45, or 113;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 28, 34, 40, 117, or 114;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 29, 35, 41, 118, or 115; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60, 70, 80, 90, or 95% identical to any one of SEQ ID NOS: 30, 36, 42, 119, or 116.

[0153] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprises:

(i) a VH-CDR1 comprising the consensus sequence XiYAMS wherein Xi represents any amino acid residue, *e.g.*, an amino acid residue with uncharged polar side chain or nonpolar side chain, *e.g.*, Thr (T), Ser (S), or Ala (A);

(ii) a VH-CDR2 comprising the consensus sequence SIX₂X₃GX₄X₅TYX₆X₇DSVKX₈ wherein X₂ represents any amino acid residue, *e.g.*, an amino acid residue with uncharged polar side chain, *e.g.*, Ser (S) or Asn (N), X₃ represents any amino acid residue, *e.g.*, an amino acid residue with uncharged polar side chain, *e.g.*, Ser (S) or Gly (G), X₄ represents any amino acid residue, *e.g.*, an amino acid residue with uncharged polar side chain, *e.g.*, Ser (S) or Gly (G), X₅ represents any amino acid residue, *e.g.*, an amino acid residue with uncharged polar side chain, *e.g.*, Ser (S), Asn (N), or Thr (T), X₆ represents any amino acid residue, *e.g.*, an amino acid residue with aromatic side chain, *e.g.*, Tyr (Y) or Phe (F), X₇ represents any amino acid residue, *e.g.*, an amino acid residue with nonpolar side chains, *e.g.*, Leu (L) or Pro (P). and X₈ represents any amino

acid residue, *e.g.*, an amino acid residue with basic side chains or uncharged polar side chains, *e.g.*, Gly (G) or Arg (R);

(iii) a VH-CDR3 comprising the consensus sequence GGDYGYAX₉DY, wherein X₉ represents any amino acid residue, *e.g.*, an amino acid residue with nonpolar side chains, *e.g.*, Leu (L) or Met (M);

(iv) a VL-CDR1 comprising the sequence RASSSVNYMY (SEQ ID NO: 28);

(v) a VL-CDR2 comprising the sequence YTSNLAP (SEQ ID NO: 29); and,

(vi) a VL-CDR3 comprising the sequence QQFSSSPWT (SEQ ID NO: 30).

[0154] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a VH-CDR1 sequence selected from SEQ ID NOS: 25, 31, 37, 43, or 111;

(ii) a VH-CDR2 sequence selected from SEQ ID NOS: 26, 32, 38, 44, or 112;

(iii) a VH-CDR3 sequence selected from SEQ ID NOS: 27, 33, 39, 45, or 113;

(iv) a VL-CDR1 sequence selected from SEQ ID NOS: 28, 34, 40, 117, or 114;

(v) a VL-CDR2 sequence selected from SEQ ID NOS: 29, 35, 41, 118, or 115;

and,

(vi) a VL-CDR3 sequence selected from SEQ ID NOS: 30, 36, 42, 119, or 116.

[0155] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 1, 3, 5, 7, or 97 and a VL region comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 2, 4, 6, 99, or 98. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 1 and a VL region comprising the amino acid sequence of SEQ ID NO: 2. In other embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 3 and a VL region comprising the amino acid sequence of SEQ ID NO: 4. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 5 and a VL region comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 7 and a VL region comprising the amino acid sequence of SEQ ID NO: 99.

In some embodiments, the antibody or antigen binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 97 and a VL region comprising the amino acid sequence of SEQ ID NO: 98. In some embodiment, the anti-GPIIb/IIIa antibody or antigen-binding molecule thereof binds to a GPIIb/IIIa epitope located in the extracellular domain of the alpha subunit of GPIIb/IIIa or to a binding site formed by the extracellular domains of the GPIIb/IIIa complex. In some embodiments, the GPIIb/IIIa antibody or antigen-binding molecule thereof does not compete with fibrinogen for binding to GPIIb/IIIa.

[0156] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

- (i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 46, 52, 120, or 126;
- (ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 47, 53, 121, or 127;
- (iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 48, 54, 122, or 128;
- (iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 49, 55, 123, or 129;
- (v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 50, 56, 124, or 130; and,
- (vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NO: 51, 57, 125, or 131 .

[0157] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprises:

- (i) a VH-CDR1 comprising the sequence NYLIE (SEQ ID NO: 46);
- (ii) a VH-CDR2 comprising the sequence VINPGSGGTNYNEKFKG (SEQ ID NO: 47);
- (iii) a VH-CDR3 comprising the sequence GRYEWYFDV (SEQ ID NO: 48);
- (iv) a VL-CDR1 comprising the consensus sequence RASQDIXioNYLN wherein Xio represents any amino acid residue, *e.g.*, an amino acid residue with uncharged polar side chain, *e.g.*, Ser (S) or Thr (T);
- (v) a VL-CDR2 comprising the sequence YTSRLHS (SEQ ID NO: 50); and.

(vi) a VL-CDR3 comprising the sequence QGGYTLPYT (SEQ ID NO:51).

[0158] In some embodiments, the antibody or antigen-binding molecule thereof comprises:

- (i) a VH-CDR1 sequence selected from SEQ ID NOS: 46, 52, 120, or 126;
- (ii) a VH-CDR2 sequence selected from SEQ ID NOS: 47, 53, 121, or 127;
- (iii) a VH-CDR3 sequence selected from SEQ ID NOS: 48, 54, 122, or 128;
- (iv) a VL-CDR1 sequence selected from SEQ ID NOS: 49, 55, 123, or 129;
- (v) a VL-CDR2 sequence selected from SEQ ID NOS: 50, 56, 124, or 130; and,
- (vi) a VL-CDR3 sequence selected from SEQ ID NOS: 51, 57, 125, or 131.

[0159] In some embodiments, the antibody or antigen-binding molecule thereof, which specifically binds to a GPIIb/IIIa epitope, comprises a VH region comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 8, 10, 100, or 102 and a VL region comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 9, 11, 101, or 103. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 8 and a VL region comprising the amino acid sequence of SEQ ID NO: 9. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 10 and a VL region comprising the amino acid sequence of SEQ ID NO: 11. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 100 and a VL region comprising the amino acid sequence of SEQ ID NO: 101. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 102 and a VL region comprising the amino acid sequence of SEQ ID NO: 103. In some embodiments, the antibody or antigen-binding molecule thereof binds to a GPIIb/IIIa epitope located in the extracellular domain of the alpha subunit of GPIIb/IIIa or to a binding site formed by the extracellular domains of the GPIIb/IIIa complex. In some embodiments, the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

[0160] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 58;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 59;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 60;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 61;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 62; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 63.

[0161] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a VH-CDR1 sequence comprising SEQ ID NO: 58;

(ii) a VH-CDR2 sequence comprising SEQ ID NO: 59;

(iii) a VH-CDR3 sequence comprising SEQ ID NO: 60;

(iv) a VL-CDR1 sequence comprising SEQ ID NO: 61;

(v) a VL-CDR2 sequence comprising SEQ ID NO: 62; and,

(vi) a VL-CDR3 sequence comprising SEQ ID NO: 63.

[0162] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises a VH region comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 12, and a VL region comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 13. In some embodiments, the antibody or antigen-binding molecule thereof binds to a GPIIb/IIIa epitope is located in the extracellular domain of the alpha subunit of GPIIb/IIIa. In some embodiments, the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

[0163] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 64, 70, or 135;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 65, 71, or 136;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 66, 72, or 137;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 67, 132, or 138;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 68, 133, or 139; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 69, 134, or 140.

[0164] In other embodiments, the invention includes an antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising (i) a VH-CDR1 comprising the sequence SYWIE (SEQ ID NO: 64); (ii) a VH-CDR2 comprising the consensus sequence EILPGX₁₄GX₁₅TKYNX₁₆KFKG (SEQ ID NO: 187), wherein X₁₄ represents any amino acids, *e.g.*, an amino acid residue with uncharged polar side chain, *e.g.*, Ser (S) or Thr (T), X₁₅ represents any amino acids, *e.g.*, an amino acid residue with uncharged polar side chains or beta-branched side chains, *e.g.*, Ile (I) or Tyr (Y), and X₁₆ represents any amino acid, *e.g.*, an amino acid residue with acidic side chains, *e.g.*, Asp (D) or Glu (E); (iii) a VH-CDR3 comprising the sequence LISYYYAMDY (SEQ ID NO: 66); (iv) a VL-CDR1 comprising the sequence RASQDISNYLN (SEQ ID NO: 67); (v) a VL-CDR2 comprising the sequence YTSRLHS (SEQ ID NO: 68); and, (vi) a VL-CDR3 comprising the sequence QQGNTLPPT (SEQ ID NO: 69).

[0165] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a VH-CDR1 sequence selected from SEQ ID NOS: 64, 70, or 135;

(ii) a VH-CDR2 sequence selected from SEQ ID NOS: 65, 71, or 136;

(iii) a VH-CDR3 sequence selected from SEQ ID NOS: 66, 72, or 137;

(iv) a VL-CDR1 sequence selected from SEQ ID NOS: 67, 132, or 138;

(v) a VL-CDR2 sequence selected from SEQ ID NOS: 68, 133, or 139; and,

(vi) a VL-CDR3 sequence selected from SEQ ID NOS: 69, 134, or 140.

[0166] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises a VH region comprising an amino

acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 14, 16, or 105 and a VL region comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 15, 104, or 106. In some embodiments, the antibody or antigen-binding molecule comprises a VH region comprising the amino acid sequence of SEQ ID NO: 14 and a VL region comprising the amino acid sequence of SEQ ID NO: 15. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 16 and a VL region comprising the amino acid sequence of SEQ ID NO: 104. In some embodiments, the antibody or antigen-binding molecule comprises a VH region comprising the amino acid sequence of SEQ ID NO: 105 and a VL region comprising the amino acid sequence of SEQ ID NO: 106. In some embodiments, the antibody or antigen-binding molecule thereof binds to a GPIIb/IIIa epitope located in the extracellular domain of the beta subunit of GPIIb/IIIa. In some embodiments, the antibody or antigen-binding molecule thereof does not compete with fibrinogen for binding to GPIIb/IIIa.

[0167] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 73, 76, 79, 85, or 147;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 74, 77, 80, 86, or 148;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 75, 78, 81, 87, or 149;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 141, 144, 82, 88, or 150;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 142, 145, 83, 89, or 151; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NO: 143, 146, 84, 90, or 152.

[0168] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a VH-CDR1 comprising the consensus sequence TSGX_nGVG, wherein X₁₁ represents any amino acid residue, *e.g.*, an amino acid residue with nonpolar side chains, *e.g.*, Met (M) or Leu (L);

(ii) a VH-CDR2 comprising the consensus sequence HIWWDDDKRYNPX₁₂LKS, wherein X₁₂ represents any amino acid residue, *e.g.*, an amino acid residue with nonpolar side chains or beta-branched side chains, *e.g.*, Ala (A) or Thr (T);

(iii) a VH-CDR3 comprising the consensus sequence SHYX_oGTFYFDX_i₄, wherein X₁₃ represents any amino acid residue, *e.g.*, an amino acid residue with uncharged polar side chain, *e.g.*, Tyr (Y) or Asn (N), and X₁₄ represents any amino acid residue, *e.g.*, an amino acid residue with aromatic side chain, *e.g.*, Tyr (Y) or Phe (F);

(iv) a VL-CDR1 comprising the sequence RASKSISKYLA (SEQ ID NO: 82);

(v) a VL-CDR2 comprising the sequence SGSTLQS (SEQ ID NO: 83); and,

(vi) a VL-CDR3 comprising the sequence QQHIEYPWT (SEQ ID NO: 84).

[0169] In some embodiments, the antibody or antigen-binding molecule thereof according comprises:

(i) a VH-CDR1 sequence selected from SEQ ID NOS: 73, 76, 79, 85, or 147;

(ii) a VH-CDR2 sequence selected from SEQ ID NOS: 74, 77, 80, 86, or 148;

(iii) a VH-CDR3 sequence selected from SEQ ID NOS: 75, 78, 81, 87, or 149;

(iv) a VL-CDR1 sequence selected from SEQ ID NOS: 141, 144, 82, 88, or 150;

(v) a VL-CDR2 sequence selected from SEQ ID NOS: 142, 145, 83, 89, or 151;

and,

(vi) a VL-CDR3 sequence selected from SEQ ID NOS: 143, 146, 84, 90, or 152.

[0170] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises a VH region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 17, 18, 19, 21, or 109 and a VL region comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 107, 108, 20, 22, or 110.

[0171] In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 17 and a VL region comprising the amino acid sequence of SEQ ID NO: 107. In other embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 18 and a VL region comprising the amino acid sequence of SEQ ID NO: 108. In some embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 109 and a VL region comprising the amino acid sequence of SEQ ID NO: 110. In other embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 19 and a VL region comprising the amino acid sequence of SEQ ID NO: 20. In other embodiments, the antibody or antigen-binding molecule thereof comprises a VH region comprising the amino acid sequence of SEQ ID NO: 21 and a VL region comprising the amino acid sequence of SEQ ID NO: 22. In some embodiments, the antibody or antigen-binding molecule thereof binds to a GPIIb/IIIa epitope located in the extracellular domain of the beta subunit of GPIIb/IIIa. In other embodiments, the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

[0172] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 91;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 92;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 93;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 94;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 95; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 96.

[0173] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises:

- (i) a VH-CDR1 sequence comprising SEQ ID NO: 91;
- (ii) a VH-CDR2 sequence comprising SEQ ID NO: 92;
- (iii) a VH-CDR3 sequence comprising SEQ ID NO: 93;
- (iv) a VL-CDR1 sequence comprising SEQ ID NO: 94;
- (v) a VL-CDR2 sequence comprising SEQ ID NOS: 95; and,
- (vi) a VL-CDR3 sequence comprising SEQ ID NOS: 96.

[0174] In some embodiments, the antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope comprises a VH region comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 23 and a VL region comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 24. In some embodiments, the antibody or antigen-binding molecule thereof binds to a GPIIb/IIIa epitope located in the extracellular domain of the "molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

[0175] In some embodiments, the anti-GPIIb/IIIa antibody or antigen-binding molecule thereof comprises or consists of (a) a single chain Fv ("scFv"); (b) a diabody; (c) a minibody; (d) a polypeptide chain of an antibody; (e) F(ab')₂; or (f) F(ab).

[0176] In some embodiments, the anti-GPIIb/IIIa antibody or antigen-binding molecule thereof can be a targeting moiety. 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. 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.

III. Chimeric Molecules

[0177] The present disclosure also provides "chimeric molecules" comprising, for example, at least one of the GPIIb/IIIa antibodies or antigen-binding molecules thereof disclosed herein which is fused and/or conjugated and/or otherwise associated with at least one heterologous moiety. Thus, a chimeric molecule disclosed herein encompasses any molecule comprising (i) a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein (*e.g.*, a scFv derived, for example, from the 34D10 antibody or the 12B2 antibody), and (ii) at least one heterologous moiety (*e.g.*, a therapeutic moiety such as a clotting factors, and optionally a half-life extending moiety). 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.

[0178] 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 2 different biological sources. A biological source can include any non-synthetically produced nucleic acid or amino acid sequence (*e.g.*, a genomic or cDNA sequence, a plasmid or viral vector, a native virion or a mutant or analog, as further described herein, of any of the above). A synthetic source can include a protein or nucleic acid sequence produced chemically and not by a biological system (*e.g.*, solid phase synthesis of amino acid sequences). A chimeric 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.

[0179] As used herein the term "moiety" refers to a component part or constituent of a chimeric molecule of the present invention. As used herein, the term "heterologous moiety" refers to a moiety genetically fused, conjugated, and/or otherwise associated to a GPIIb/IIIa antibody or antigen-binding molecule thereof. In some embodiments, the chimeric molecule has, for example, a formula:

- (i) Tm-(L)-H or
- (ii) H-(L)-Tm,

wherein, H is a heterologous moiety; L is an optional linker; and, Tm is an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein.

[0180] 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)-Tm-(L2)-H2;
- (ii) H2-(L2)-Tm-(L1)-H1;
- (iii) H1-(L1)-H2-(L2)-Tm;
- (iv) H2-(L2)-H1-(L1)-Tm;
- (v) Tm-(L1)-H1-(L2)-H2; or,
- (vi) Tm-(L2)-H2-(L1)-H1;

wherein, Tm 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.

[0181] 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.

[0182] 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 GPIIb/IIIa antibodies or antigen-binding molecules 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. Exemplarily chimeric molecules are presented in FIG. 22.

[0183] 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).

[0184] In some embodiments, the chimeric molecule comprises a formula:

(1) wherein the first polypeptide chain comprises **CF_L-H** or **H-CFL** and the second polypeptide chain comprises **CF_H-Tm** or **Tm-CF_H**;

(2) wherein the first polypeptide chain comprises **CFL-Tm** or **Tm-CFL** and the second polypeptide chain comprises **CFH-H** or **H-CFH**;

(3) wherein the first polypeptide chain comprises **CFL** and the second polypeptide chain comprises **CF_H-Tm-H** or **H-Tm-CF_H**;

(4) wherein the first polypeptide chain comprises **CFL** and the second polypeptide chain comprises **CF_n-H-Tm** or **Tm-H-CF_n**;

(5) wherein the first polypeptide chain comprises **CF_L-H-Tm** or **Tm-H-CFL** and the second polypeptide chain comprises **CFH**; or

(6) wherein the first polypeptide chain comprises **CpL-Tm-H** or **H-Tm-CFL** and the second polypeptide chain comprises **CFH**;

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); Tm 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.

[0185] The instant 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.

[0186] 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 would be 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, for example, 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 a 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.

[0187] 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 CFL-H or H-CFL and the second polypeptide chain comprises CF_H-Tm or Tm-CF_H; (2) wherein the first polypeptide chain comprises CF_L-Tm or Tm-CFL and the second polypeptide chain comprises CF_H-H or H-CF_H; (3) wherein the first polypeptide chain comprises CFL-H-Tm or Tm-H-CF_L and the second polypeptide chain comprises CF_H; or (4) wherein the first polypeptide chain comprises CF_L-Tm-H or H-Tm-CFL 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), Tm is a targeting moiety which binds to a platelet, and L is an optional linker.

[0188] In some embodiments, the chimeric molecule comprises a formula selected from (1) Tm- CF_H:CF_L-H; (2) H- CF_H:CF_L -Tm; (3) Tm-H- CF_L:CF_H; or (4) H-Tm-CF_L: 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, FIX, or FX); CF_L is a light chain of a clotting factor (*e.g.*, FVII); T_m is a targeting moiety, which binds to a platelet; L is an optional linker; and : represents a covalent or non-covalent bond between CF_H and CF_L (*e.g.*, a disulfide bond).

[0189] 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.

[0190] 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.

[0191] In some embodiments, the targeting moiety in the chimeric molecule is selected from: an antibody or antigen binding molecule thereof, a receptor binding portion of a receptor, and a peptide. In some embodiments, the targeting moiety selectively binds to a resting platelet or an activated platelet. In other embodiments, the targeting moiety selectively binds to a target selected from the group consisting of: GPIIb (Uniprot: E7ES66; E7ES66HUMAN), GPVI (Uniprot: Q9HCN6; GPVI_HUMAN), GPIX (Uniprot: PI4770; GPIX_HUMAN), a nonactive form of glycoprotein IIb/IIIa

("GPIIb/IIIa"), an active form of GPIIb/IIIa, P-selectin (Uniprot: Q14242; SELPL_HUMAN), GMP-33 (*see, e.g., Damas et al, Thromb. Haemost. 86:887-93 (2001)*), LAMP-1 (Uniprot: PI1279; LAMP1_HUMAN), LAMP-2 (Uniprot: P13473; LAMP2_HUMAN), CD40L (Uniprot: P29965; CD40L_HUMAN), LOX-1 (Uniprot: P78380; OLR1_HUMAN), and any combinations thereof. The above referenced Uniprot identifiers correspond to the entries published in the Universal Protein Resource (Uniprot) database release 2013_05 (May 1, 2013), and are incorporated by reference in their entireties. In certain embodiments, the targeting moiety comprises a GPIIb/IIIa antibody or antigen-binding molecule thereof. In specific embodiments, the GPIIb/IIIa antibody or antigen-binding molecule thereof is a GPIIb/IIIa antibody or antigen-binding molecule thereof disclosed herein.

IIIA Heterologous moieties

[0192] The heterologous moiety or moieties of the chimeric molecules disclosed herein can comprise, consist of, or consist essentially of prophylactic and/or therapeutic agents (*e.g., clotting factors*), molecules capable of improving a pharmacokinetic (PK) property (*e.g., plasma half-life extending moieties*), detectable moieties (*e.g., fluorescent molecules or radionuclides*), etc.

[0193] As used herein, 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 chimeric molecule comprises a clotting factor.

[0194] In some embodiments, a heterologous moiety 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*).

[0195] 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.

[0196] In other embodiments, a heterologous moiety increases stability of the chimeric molecule of the invention or a fragment thereof. As used herein, 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.

IIIA.1 Clotting Factors

[0197] In some embodiments, chimeric molecules of the invention comprises at least one polypeptide heterologous moiety which is (i) a clotting factor, or (ii) a procoagulant peptide (*e.g.*, a synthetic procoagulant peptide). 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 (*e.g.*, the heavy chain and the light chain of FVII). 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.

[0198] As used herein, the term "clotting factor" refers to molecules, or analogs thereof, naturally occurring or recombinantly produced which prevent or decrease the duration of a bleeding episode in a subject. In other words, it means molecules having pro-clotting activity, *i.e.*, are responsible for the conversion of fibrinogen into a mesh of insoluble fibrin causing the blood to coagulate or clot. 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. 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.

[0199] In some embodiments, the chimeric molecule comprises a single clotting factor, which in the chimeric molecule is represented by a formula as H, HI 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.

III.A.1.a Factor VII

[0200] 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. FVII occurs as a single chain zymogen, an activated zymogen-like two-chain polypeptide and a fully activated two-chain form.

[0201] 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.

[0202] Exemplary FVII variants include those with increased specific activity, *e.g.*, mutations that increase the activity of FVII by increasing its enzymatic activity (K_{cat} or K_m). Such variants have been described in the art and include, *e.g.*, mutant forms of the molecule as described for example in Persson *et al*, *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).

[0203] 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 (LQQRKVGDSPN, corresponding to the 170-loop) from the FVII mature sequence with amino acids EASYPGK (SEQ ID NO: 188) 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 these references are incorporated herein by reference.

[0204] 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.

III.A.1.b Factor IX

[0205] 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 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)).

[0206] 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)).

III.A.1.C Factor X

[0207] 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 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 Asp 103 as well as N- and O-type glycosylation.

[0208] 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.

[0209] Exemplary modified polypeptides include, but are not limited to, tissue-specific isoforms and allelic variants thereof, synthetic molecules prepared by translation of nucleic acids, proteins generated by chemical synthesis, such as syntheses that include ligation of shorter polypeptides, through recombinant methods, proteins isolated from human and non-human tissue and cells, chimeric polypeptides and modified forms thereof. The instant clotting factors 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.

[0210] As used herein, the term "Gla domain" refers to the conserved membrane binding motif which is present in vitamin K-dependent proteins, such as as prothrombin, coagulation factors VII, IX and X, proteins C, S, and Z. These proteins require vitamin K for the posttranslational synthesis of 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.

[0211] 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.

[0212] 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).

[0213] 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.

III.A.2 Half-life Extending Moieties

[0214] In some embodiments, the chimeric molecule comprises at least one heterologous moiety that is a "half-life extending moiety." 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).

[0215] Half-life extending moieties, as discussed below in detail, can comprise, for example, (i) low complexity peptides, (ii) albumin, (iii) albumin binding polypeptide or fatty acid, (iv) Fc, (v) transferrin, (vi) PAS, (vii) the C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin, (viii) polyethylene glycol (PEG), (ix) hydroxyethyl starch (HES), (x) albumin-binding small molecules, (xi) vWF, (xii) a clearance receptor or fragment thereof which blocks binding of the chimeric molecule to a clearance receptor, or (xiii) 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.

[0216] In certain embodiments, a chimeric molecule of the invention comprises at least one half-life extending moiety which increases the *in vivo* half-life of the chimeric molecule with respect to 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.

[0217] 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.

[0218] 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.

III.A.2.a Fc region

[0219] In certain embodiments, the chimeric molecule comprises at least a 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 PCX Publication Nos. WO2011/069164, WO2012/006623, WO2012/006635, or WO 2012/006633, all of which are incorporated herein by reference in their entireties.

[0220] 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 an Fc region.

III.A.2.b scFc (single chain Fc) Region

[0221] 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). 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.*, Clq)) in order to improve half-life or trigger an immune effector function (*e.g.*, antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cytotoxicity (CDCC) and/or to improve manufacturability).

[0222] 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 an scFc region.

III.A.2.c Albumins

[0223] 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.

[0224] 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 an albumin.

III.A.2.d Albumin Binding Polypeptides and Lipids

[0225] 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, wherein Xaa₁ is Asp, Asn, Ser, Thr, or Trp; Xaa₂ is Asn, Gin, 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. Pat. No. US2003/0069395 or Dermis *et al.* (2002) *J Biol. Chem.* 277, 35035-35043.

[0226] 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: 162). *See, e.g.*, Dennis *et al.*, *J Biol. Chem.* 277: 35035-35043 (2002). Examples of albumin-binding antibody fragments are disclosed in Muller and Kontermann, *Curr. Opin. Mol. Ther.* 9:319-326 (2007); Rooverse *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 Trussed *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)-methyl)-7-sulfo-9H-fluoren-2-ylamino)-3-oxopropyl)-2,5-dioxopyrrolidin-3-ylthio) hexadecanoic acid (see, *e.g.*, WO 2010/140148).

[0227] 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 an albumin binding polypeptide or lipid.

III.A.2.e CTP

[0228] 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.

[0229] Exemplary CTP peptides include DPRFQDSSSSKAPPPSLPSPSRLPGPSDTPIL (SEQ ID NO: 153) or SSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 154). See, *e.g.*, U.S. Patent Appl. Publ. No. US 2009/008741 1, 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.

[0230] 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 CTP,

DI.A.2.f PAS

[0231] 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.

[0232] 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.

[0233] The amino acids different from alanine, serine and proline can be selected from Arg, Asn, Asp, Cys, Gin, Glu, Gly, His, He, Leu, Lys, Met, Phe, Thr, Trp, Tyr, or Val.

[0234] 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.

[0235] 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: 155), AAPASPAPAAPSAPAPAAPS (SEQ ID NO: 156), APSSSPSPAPSSSPSPASPSS (SEQ ID NO: 157), APSSSPSPAPSSSPSPASPS (SEQ ID NO: 158), SSPSPSPSPSPASPS (SEQ ID NO: 159), AASPAAPSAPPAAASPAAPSAPPA (SEQ ID NO: 160) and ASAAAPAAASAAASAPSAAA (SEQ ID NO: 161) 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.

[0236] 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 PAS.

III.A.2.g HAP

[0237] 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 $(\text{Gly})_n$, $(\text{Gly}_4\text{Ser})_n$ or $\text{S}(\text{Gly}_4\text{Ser})_n$, 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).

[0238] 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 HAP.

III.A.2.h Transferrin

[0239] 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 AAA61 140. 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.

[0240] 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 (201 1); 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 (201 1)).

[0241] 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 transferrin.

IIA.2.i PEG

[0242] 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.

[0243] 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.

[0244] 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.

[0245] 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.

[0246] 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).

[0247] 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).

[0248] 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. W092/16221 and W095/34326.

[0249] 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 PEG.

III.A.2.j HES

- [0250] 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)).
- [0251] 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.
- [0252] 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.
- [0253] 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.

[0254] 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.

[0255] 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 HES.

III.A.2.k PSA

[0256] 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. (Birkhauser Verlag, Basel, Switzerland), pp 335—348.. They can be produced in various degrees of polymerisation 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 alpha-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 alpha-2,8 alpha-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 foetal development (wherein the polymer has an anti-adhesive function) Cho and Troy, *P.N.A.S., USA*, 91 (1994) 11427-1 1431, although there are no known receptors for polysialic acids in mammals. The alpha-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 invention. 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-0 187922, and US 2007/0191597 A1, which are incorporated herein by reference in their entireties.

[0257] 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.

III.A.2.1 Clearance Receptors

[0258] 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 LRPI, or fragments thereof, can block binding of Factor X to clearance receptors and thereby extend its *in vivo* half-life.

[0259] LRPI 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). 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).

[0260] 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 clearance receptor, fragment, variant, or derivative thereof.

III.B Linkers

[0261] As used herein, the term "linker" or "linker moiety" (represented as L, LI, 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.

[0262] 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.

[0263] 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.

[0264] The peptide linker can comprise 1-5 amino acids, 1-10 amino acids, 1-20 amino acids, 10-50 amino acids, 50-100 amino acids, 100-200 amino acids, 200-300 amino acids, 300-400 amino acids, 400-500 amino acids, 500-600 amino acids, 600-700 amino acids, 700-800 amino acids, 800-900 amino acids, 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.

[0265] 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. In one embodiment, the peptide linker comprises the sequence G_n , where n can be an integer from 1 to 100. In a specific embodiment, the specific embodiment, the sequence of the peptide linker is GGGG. The peptide linker can comprise the sequence $(GA)_n$. The peptide linker can comprise the sequence $(GGS)_n$. In other embodiments, the

peptide linker comprises the sequence $(GGGS)_n$ (SEQ ID NO: 166). In still other embodiments, the peptide linker comprises the sequence $(GGS)_n(GGGGS)_n$ (SEQ ID NO: 189). 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: 190), GGSGGSGGSGGSGGG (SEQ ID NO: 191), GGSGGSGGGGSGGGGS (SEQ ID NO: 192), GGSGGSGGSGGSGGSGGS (SEQ ID NO: 193), or GGGGSGGGGSGGGGS (SEQ ID NO: 194). In other embodiments, the linker is a poly-G sequence $(GGGG)_n$, where n can be an integer from 1-100.

[0266] An exemplary Gly/Ser peptide linker comprises the amino acid sequence $(Gly_4Ser)_n$ (SEQ ID NO: 195), 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: 196). In one embodiment, $n=2$, *i.e.*, the linker is $(Gly_4Ser)_2$ (SEQ ID NO: 197). In another embodiment, $n=3$, *i.e.*, the linker is $(Gly_4Ser)_3$ (SEQ ID NO: 198). In another embodiment, $n=4$, *i.e.*, the linker is $(Gly_4Ser)_4$ (SEQ ID NO: 199). In another embodiment, $n=5$, *i.e.*, the linker is $(Gly_4Ser)_5$ (SEQ ID NO: 200). In yet another embodiment, $n=6$, *i.e.*, the linker is $(Gly_4Ser)_6$ (SEQ ID NO: 202). In another embodiment, $n=7$, *i.e.*, the linker is $(Gly_4Ser)_7$ (SEQ ID NO: 203). In yet another embodiment, $n=8$, *i.e.*, the linker is $(Gly_4Ser)_8$ (SEQ ID NO: 204). In another embodiment, $n=9$, *i.e.*, the linker is $(Gly_4Ser)_9$ (SEQ ID NO: 205). In yet another embodiment, $n=10$, *i.e.*, the linker is $(Gly_4Ser)_{10}$ (SEQ ID NO: 206).

[0267] Another exemplary Gly/Ser peptide linker comprises the amino acid sequence $Ser(Gly_4Ser)_n$ (SEQ ID NO: 201), 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 $Ser(Gly_4Ser)$ (SEQ ID NO: 207). In one embodiment, $n=2$, *i.e.*, the linker is $Ser(Gly_4Ser)_2$ (SEQ ID NO: 208). In another embodiment, $n=3$, *i.e.*, the linker is $Ser(Gly_4Ser)_3$ (SEQ ID NO: 209). In another embodiment, $n=4$, *i.e.*, the linker is $Ser(Gly_4Ser)_4$ (SEQ ID NO: 210). In another embodiment, $n=5$, *i.e.*, the linker is $Ser(Gly_4Ser)_5$ (SEQ ID NO: 211). In yet another embodiment, $n=6$, *i.e.*, the linker is $Ser(Gly_4Ser)_6$ (SEQ ID NO: 212). In yet another embodiment, $n=7$, *i.e.*, the linker is $Ser(Gly_4Ser)_7$ (SEQ ID NO: 213). In yet another embodiment, $n=8$, *i.e.*, the linker is $Ser(Gly_4Ser)_8$ (SEQ ID NO: 214). In yet another embodiment, $n=9$, *i.e.*, the linker is

Ser(Gly₄Ser)₉ (SEQ ID NO: 215). In yet another embodiment, n=10, *i.e.*, the linker is Ser(Gly₄Ser)₁₀ (SEQ ID NO: 216).

[0268] 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 (Gly₄Ser)_n) (SEQ ID NO: 195)).

[0269] 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 or 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.

[0270] 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. C Protease Cleavage Site

[0271] 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 XIIIa, 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: 217), DFTR (SEQ ID NO: 218), TQSFNDFTR (SEQ ID NO: 219) and SVSQTSLTR (SEQ ID NO: 220). Exemplary thrombin cleavage sites include, *e.g.*, DFLAEGGGVR (SEQ ID NO: 221), TTKIKPR (SEQ ID NO: 222), LVPRG (SEQ ID NO: 223) and ALRPR (SEQ ID NO: 224).

[0272] 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.

[0273] Examples of the intracellular processing enzymes include furin, a yeast Kex2, PCSK1 (also known as PCI/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.

IV. Methods of Preparation

[0274] 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.

[0275] 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).

[0276] 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).

[0277] 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.**

[0278] 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.

[0279] 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.

[0280] 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.

[0281] 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.

[0282] 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.

[0283] 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).

[0284] 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.

[0285] For the purposes of this invention, 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.

[0286] Commonly, expression vectors contain selection markers (*e.g.*, ampicillin-resistance, hygromycin-resistance, tetracycline resistance or neomycin resistance) to permit detection of those cells transformed with the desired DNA sequences (see, *e.g.*, Itakura *et al.*, 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.

[0287] 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.

[0288] 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.

[0289] 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.

[0290] 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.

[0291] 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.

[0292] 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.

[0293] 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.

[0294] 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.* 32:1 1586-90. 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.

[0295] Nucleotide and amino acid sequences of rodent PCS 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.

[0296] 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.

[0297] 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.

[0298] 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.

[0299] 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.

[0300] 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.

[0301] *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) can optionally be attached or included within the polypeptide sequence to facilitate downstream purification.

[0302] 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.

V. Methods of Use

[0303] The present disclosure also provides is a pharmaceutical composition comprising (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;
- (iv) a vector or set of vectors disclosed herein; or
- (v) any combinations thereof,

and a pharmaceutically acceptable carrier.

[0304] 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.

[0305] 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 derived from 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.

[0306] The instant disclosure also provides:

(a) a method to target 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 molecules thereof disclosed herein;

(b) 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; or.

(c) 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.

[0307] In some embodiments, these methods further comprise fusing or conjugating the 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 FVII, FIX, or FX.

[0308] 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.

[0309] The invention also relates to 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 invention 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. Compositions for administration to a subject include nucleic acid molecules which comprise a nucleotide sequence encoding a chimeric molecule the invention.

[0310] 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 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.

[0311] In one embodiment, the composition (*e.g.*, the polypeptide or nucleic acid molecule encoding the polypeptide) 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.

- [0312] The chimeric molecule of the invention can be administered intravenously, subcutaneously, intramuscularly, or via any mucosal surface, *e.g.*, orally, sublingually, buccally, sublingually, nasally, rectally, vaginally or via pulmonary route. The chimeric 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.
- [0313] 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 invention are conveniently delivered in the form of an aerosol spray from a pressurized pack or nebulizer (*e.g.*, in PBS), with a suitable propellant.
- [0314] 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.

[0315] 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.

[0316] 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.

[0317] 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.

[0318] 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.

[0319] 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.

[0320] Effective doses of the compositions of the present invention, for the treatment of conditions vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but non-human mammals including transgenic mammals can also be treated. Treatment dosages can be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

[0321] In one embodiment, the dose of a biologically active moiety (*e.g.*, comprising FVII), can range from about 90 to 270 $\mu\text{g}/\text{kg}$ 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 $1\mu\text{g}/\text{kg}$ to $400\text{mg}/\text{kg}$.

[0322] Dosages can range from 1000 $\mu\text{g}/\text{kg}$ to 0.1 ng/kg body weight. In one embodiment, the dosing range is $1\mu\text{g}/\text{kg}$ to $100\mu\text{g}/\text{kg}$. 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).

[0323] 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.

[0324] 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.

[0325] 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.

[0326] 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).

[0327] 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.

[0328] 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.

[0329] 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.

[0330] As previously discussed, the polypeptides of the present invention, 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 invention comprise a pharmaceutically acceptable, non-toxic, sterile carrier such as physiological saline, non-toxic buffers, preservatives and the like. Of course, the pharmaceutical compositions of the present invention can be administered in single or multiple doses to provide for a pharmaceutically effective amount of the polypeptide.

[0331] 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.

[0332] 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.

[0333] The chimeric molecules of the invention have many uses as will be recognized by one skilled in the art, including, but not limited to methods of treating a subject with a disease or condition. The disease or condition can include, but is not limited to, hemostatic disorders.

[0334] In one embodiment, the invention relates to a method of treating a subject having a hemostatic disorder comprising administering a therapeutically effective amount of at least one chimeric molecule of the invention.

[0335] 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.

[0336] 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 a GPIIb/IIIa antibody or antigen-binding molecule thereof 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.

[0337] Chimeric molecules of the invention comprising a clotting factor (*e.g.*, FVII) can be used to prophylactically treat a subject with a hemostatic 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.

[0338] 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).

[0339] The invention also relates to methods of treating a subject who does not have a hemostatic disorder or a secondary disease or condition resulting in acquisition of a hemostatic disorder. The invention thus relates to a method of treating a subject in need of a general hemostatic agent comprising administering a therapeutically effective amount of at least one chimeric 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.

[0340] Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention. All patents and publications referred to herein are expressly incorporated by reference in their entireties.

Examples

General Materials and Methods

[0341] In general, the practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, biophysics, molecular biology, recombinant DNA technology, immunology (especially, *e.g.*, antibody technology), and standard techniques in electrophoresis. See, *e.g.*, Sambrook, Fritsch and Maniatis, *Molecular Cloning*: Cold Spring Harbor Laboratory Press (1989); *Antibody Engineering Protocols* (Methods in Molecular Biology), 510, Paul, S., Humana Pr (1996); *Antibody Engineering: A Practical Approach* (Practical Approach Series, 169), McCafferty, Ed., Irl Pr (1996); *Antibodies: A Laboratory Manual*, Harlow et al., C.S.H.L. Press, Pub. (1999); and *Current Protocols in Molecular Biology*, eds. Ausubel et al., John Wiley & Sons (1992).

Example 1

Identification and Characterization of Platelet-Targeted Antibodies

[0342] A summary of the process used for production, identification, and characterization of antibodies against GP b/IIIa disclosed herein, and constructs derived from these antibodies is presented in FIG. 1.

[0343] Hybridomas were generated from BALB/C mice immunized with plasmids containing DNA sequences encoding GPIIb/IIIa (SEQ ID NOs: 183 and 184) 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. Following a 30 minute incubation, cells were fixed in 1% formaldehyde. Following fixation, cells were washed in Tyrode's buffer and a detection antibody was added (Jackson Immunoresearch goat anti-mouse IgG-PE conjugated). Antibody binding was detected by flow cytometry.

[§344] The binding of supernatants from hybridomas to human GPIIb/IIIa (allbp) was determined by using ELISA as follows., Costar plates (Cat. No. 3590) were coated with 100 μ l/well of 5 μ g/mL human GPIIb/IIIa (Calbiochem Cat No. 528240) in measuring buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 1mM $MgCl_2$, and 1mM $MnCl_2$) and incubated for 1 hour at 37°C with shaking. Wells were washed three times with TBST using a plate washer. Blocking was performed using 200 μ l of measuring buffer containing 5% BSA (Bovine Serum albumin, Jackson Cat No 001 000 173) per well, and incubating 1 hour at 37°C with shaking. 100 μ L of hybridoma supernatant were added assay wells, incubated for 1 hour at 37°C with shaking, and washed three times with TBST. A 1:10,000 dilution of goat anti mouse IgGHRP (Southern Biotech (Cat. No. 1010 05) in measuring buffer was added, incubated for 1 hour at 37°C with shaking, and washed three times with TBST. HRP presence was developed using TMB and O.D. read at 450 nm using a Molecular Devices plate reader.

[0345] The supernatants from hybridomas which tested positive in the ELISA assays were mixed with platelets and screened for platelet activation using flow cytometry as follows.

(a) Reagents : Citrated human whole blood; Sepharose 2B beads (GE Healthcare); Tyrode's buffer with 1mg/mL BSA (no calcium); Tyrode's buffer with 5mM $CaCl_2$ and 1mg/mL BSA; 32% paraformaldehyde (PFA) (EM Sciences); PAC1 FITC antibody (BD Cat. No. 340507); CD62 PE antibody (BD Cat. No. 555524); ADP; SFFLRN peptide (Anaspec, Cat. No. 24191); IV.3 Fabb anti CD32 (StemCell, Cat. No. 01470).

(b) Platelet purification: A 10 mL Sepharose 2B bead column was packed and equilibrated with 30 mL of Tyrode's buffer containing 1mg/mL BSA. A volume of 1 to 1.5 mL of platelet-rich plasma (PRP) was loaded onto the equilibrated Sepharose column

and allowed to enter the packed beads by gravity, followed with approximately 5mL of Tyrode's buffer. The turbid drops, which contained the platelets, were collected.

(c) Assay: First, 50 μ l aliquots of hybridoma supernatant were added to assay wells of a 96 well round bottom plate. 10 μ L of PAC1 FITC and 10 μ L of CD62PE were added to all control and assay wells. 10 μ L of ADP and 10 μ L of SFFLRN were added to all control wells (no hybridoma supernatant). 10 μ L of IV.3 inhibitor (antibody to Fc γ RIIA) were added wells to see if activation was Fc or antibody mediated. Next, a 50 μ L aliquot of concentrated resting platelets, which was purified as described above, was added to all wells. Plates were incubated for 30 minutes in the dark and at room temperature. Cells were fixed with 1% PFA (final concentration) for 10 minutes at room temperature (a volume of 2% PFA equal to the content of each well was added). After fixation, samples were analyzed by flow cytometry.

[0346] The antibodies that did not activate platelets upon binding to GPIIb/IIIa (see FIG. 2B) were selected as candidates for clotting factor targeting moieties. The antibodies that activate platelets upon binding to GPIIb/IIIa were excluded from selection (see FIG. 2C)

[0347] Antibodies can also activate platelets by binding to the Fc γ RIIA receptor via the Fc region (see FIG. 2D), which were not excluded from the selection because their antigen binding portion contain no Fc region and therefore not bind to the Fc γ RIIA receptor. These antibodies can be identified by blocking the Fc γ RIIA receptor with an inhibitor..

[0348] FIG. 3 shows flow cytometry profiles from platelet activation assays in which CD62p and PAC1 were detected (FIG. 3A). CD62p, also known as P-selectin, is expressed on the platelet surface upon platelet activation. PAC1 is a pentarneric IgM that binds to agonist-stimulated platelets, and PAC1 binding is therefore indicative of GPIIb/IIIa-mediated activation. Experiments were performed in the absence and presence of an Fc γ RIIA receptor inhibitor (IV.3 Fab; see Naik *et al*, *Biochem. J* 310:155-162 (1995)) in order to detect antibodies capable of activating platelets indirectly via simultaneous binding to GPIIb/IIIa and to the Fc γ RIIA receptor (see FIG. 3B, 3C, 3D, and 3E).

[0349] FIG. 3B shows flow cytometry profiles observed when only cell culture medium was present, and when ADP and a PARI platelet receptor agonist peptide with amino sequence SFLLRN (SEQ ID NO: 225) were present. FIG. 3C shows flow cytometry

profiles corresponding to an antibody capable of activating platelets both in the presence and absence of the FcγRIIA inhibitor monoclonal antibody. Thus, the antibody activates platelets by direct binding to GPIIb/IIIa. FIG. 3D shows flow cytometry profiles corresponding to an antibody not capable of activating platelets both in the presence and absence of the FcγRIIA inhibitor monoclonal antibody. FIG. 3E shows flow cytometry profiles corresponding to antibodies indirectly activating platelets, *i.e.*, the antibodies bind simultaneously to GPIIa/IIIb and to the FcγRIIA receptor.

[0350] 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.

[0351] Antibody binding to the α and/or β subunit of GPIIb/IIIa was assessed using ELISA, whereas antibody competition with fibrinogen was assessed using flow cytometry. Antibodies determined to be non-activating (*e.g.*, clones 34D10, 2A2, 35D1, 36A8, 4B11, 1H6, 38G8, 21F10, 38A8, 18F7, 12B2, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, 13A1) were clustered into 6 different groups according to the VH domain sequence similarity, α or β subunit specificity, ability to compete with fibrinogen, and relative strength of the signals measured via ELISA and flow cytometry (see TABLE 1).

TABLE 1: Platelet Specific Non-Activating Anti-GPIIb/IIIa Monoclonal Antibodies

Group / Antibody Characteristics	Antibody / Chain		SEQ ID	CDR SEQ IDs		
				CDR1	CDR2	CDR3
1 / α subunit specific No fibrinogen competition	2A2	HC	3	25	26	27
		LC	4	28	29	30
	34D10 ⁸	HC	1	31	32	33
		LC	2	34	35	36
	35D1	HC	97	111	112	113
		LC	98	114	115	116

	36A8	HC	5	37	38	39
		LC	6	40	41	42
	4B11	HC	7	43	44	45
		LC	99	117	118	119
2 / α subunit specific. Fibrinogen competition	1H6	HC	8	46	47	48
		LC	9	49	50	51
	38G8	HC	100	120	121	122
		LC	101	123	124	125
	21F10	HC	102	126	127	128
		LC	103	129	130	131
	38A8	HC	10	52	53	54
		LC	11	55	56	57
3 / α subunit specific. Fibrinogen competition. Low ELISA signal v flow cytometry	18F7	HC	12	58	59	60
		LC	13	61	62	63
4 / β subunit specific. No fibrinogen competition	12B2 ^s	HC	14	64	65	66
		LC	15	67	68	69
	38F6	HC	16	70	71	72
		LC*	104	132	133	134
	13C1	HC	105	135	136	137
		LC*	106	138	139	140
5 / β subunit specific. Fibrinogen competition	5C4	HC	17	73	74	75
		LC*	107	141	142	143
	23C10	HC	18	76	77	78
		LC*	108	144	145	146
	37C7	HC	109	147	148	149
		LC*	110	150	151	152
	28C2	HC	19	79	80	81
		LC	20	82	83	84
	9D6	HC	21	85	86	87
	9D6	LC	22	88	89	90
6 / β subunit specific. Fibrinogen	28F4	HC	23	91	92	93

competition. Low ELISA signal v	J	ILC	24	94	95	96
flow cytometry						

* = Not sequenced.

HC = Heavy Chain (*i.e.*, VH). LC = Light Chain (*i.e.*, VL)

: a subunit or complex specific

\$: β subunit specific

[0352] Several non-activating antibodies identified using the screening method described above shared the same VH or VL domains, as show in FIGS. 4. For example, 35D1 and 34D10 shared the same VH domain.

[0353] Unique VH and VL sequences were aligned using the ClustalX program using standard parameters (FIG. 5). Sequence designators in FIG. 5, which are also used in the sequence alignments of FIGS. 6 to 12, follow the schema "SEQ ID NO-Antibody-BindingDomain." Accordingly "SEQ22-9D6LC" corresponds to SEQ ID NO:22, which is the Light Chain variable domain (*i.e.*, VL) of the 9D6 antibody. Similarly, "SEQ08-1H6HC" corresponds to SEQ ID NO:8, which is the Heavy Chain variable domain (*i.e.*, VH) of the 1H6 antibody.

[0354] The character "*" in the multiple sequence alignments indicates positions which have a single, fully conserved amino acid residue. The character ":" indicates that one strong conservation group is fully conserved. Strong conservation amino acid groups are STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, and FYW. The character "." indicates that one weaker conservation group is fully conserved. Weaker conservation amino acid groups are CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, FVLIM, and HFY. Amino acids are shaded in FIG. 5 according to the rules set out below. Rules are specified according to the formula: (A,C, and D): {50%, P,Q,RSTV} {85%, W,Y}, where amino acid residue or residues in the alignment column are given first in the round brackets. More than one amino acid can be specified between the round brackets, in which case the rules apply to each of these amino acid residues. Next, the rule or rules to assign a given color are provided between curly braces. Only one rule has to be met for the color to be applied. The minimum percentage of occurrence in the alignment column is given first, followed by the amino acid residue or residues which must meet or exceed this percentage within the column. If a group of amino acid residues is concatenated together, such as "RSTV," then any combination of these residues in total must meet or exceed the given percentage for the color to be applied. For

amino acid residues or residue groups separated by commas, at least one of these must by itself exceed the percentage.

[0355] Highlighting (shading) rules in FIG. 5 are based on conservation and common physicochemical and/or structural properties. For example, tiny residues (G) and prolines (P) are always highlighted when present. The following highlighting rules were applied:

- * W, L, V, I, M, F, A, and C amino acid residues are highlighted according to the following set of rules: (W, L, V, I, M, F): {50%, P} {60%, WLVIAMFCYHP}; (A): {50%, P} {60%, WLVIAMFCYHP} {85%, T, S, G}; and (C): {50%, P} {60%, WLVIAMFCYHP} {85%, S}.
- * K and R amino acid residues are highlighted according to the following set of rules: (K, R): {60%, KR} {85%, Q}.
- * T, S, N, and Q amino acid residues are highlighted according to the following set of rules: (T): {50%, TS} {60%, WLVIAMFCYHP}; (S): {50%, TS} {80%, WLVIAMFCYHP}; (N): {50%, N} {85%, D}; and, (Q): {50%, QE} {60%, KR}.
- * C amino acid residues are highlighted according to the following rule: (C): {85%, C}.
- * D and E amino acid residues are highlighted according to the following set of rules: (D): {50%, DE, N}; and (E): {50%, DE, QE}.
- * G amino acid residues are highlighted in according to the following rule: (G): {Always}.
- * H and Y amino acid residues highlighted according to the following set of rules: (H,Y): {50%, P} {60%, WLVIAMFCYHP}.
- * P amino acid residues are highlighted according to the following rule: (P): {Always}.

[0356] Multiple sequence alignments corresponding to the VH and VL domains of the above identified antibodies are shown in FIG. 6 and FIG. 7, respectively. The multiple sequence alignments show the location of the complementarity determining regions CDR1, CDR2, and CDR3 in the VH and VL domain and their location according to the EU numbering system (Kabat, E.A., Wu, T.T., Perry, H., Gottesman, K., and Foeller, C. (1991) "Sequences of Proteins of Immunological Interest," 4th ed., U.S. Govt. Printing Off. No. 165-492, Bethesda, MD),

[0357] FIG. 8 shows percent identity matrices showing the percentage of sequence identity among each pair of the VH and VL sequences shown in the multiple sequence alignments of FIGS. 6 and 7. Column designations correspond to the designation applied

to each row in the matrix. For example, column 2 corresponds to "2: SEQ 22-9D6LC." The sequences aligned in FIG. 6 and FIG. 7 were clustered according to the specificity of the antibodies for the α or β subunits of GPIIb/IIIa (see FIG. 9 and FIG. 10), and according to their ability to compete with fibrinogen for binding to GPIIb/IIIa (see FIG. 11 and FIG. 12).

Example 2

Platelet-Targeted Chimeric Molecules Comprising Anti-GPIIb/IIIa Antibodies and FVIIa

[0358] The above described monoclonal antibodies against GPIIb/IIIa were used to target the FVIIa clotting factor to the surface of platelets. Accordingly, scFv's derived from the platelet-specific monoclonal antibodies identified according to the methods disclosed in Example 1 were recombinantly fused to FVIIa using molecular biology methods known in the art. In the resulting chimeric molecules, the C-terminus of the heavy chain of FVIIa was fused to the N-terminus of an scFv comprising a VH and a VL domain derived from non-activating platelet-targeting antibodies identified in Example 1 (see FIG. 13). A chimeric molecule comprising an scFv derived from the GPIIb/IIIa α subunit/complex-specific 34D10 antibody was designated "FVII-189". A second chimeric molecule comprising an scFv derived from the GPIIb/IIIa β subunit-specific 12B2 monoclonal antibody was designated "FVII-206". A chimeric molecule comprising an scFv from a known antibody (PDG13) was designated as "FVII-163". A chimeric molecule containing an scFv from 38A8 monoclonal antibody was designated as "FVII-204". These platelet-targeted FVIIa variants were characterized for their affinity to GPIIb/IIIa receptor, their ability to bind to platelets, their procoagulant activity, their effect on platelet activation and platelet function, and their effect on platelet clearance in animals.

[0359] The affinity of platelet-targeted FVII-189, FVII-206, FVII-163 chimeric molecules to GPIIb/IIIa receptor was measured using surface plasmon resonance (SPR). GPIIb/IIIa was modified to incorporate a tag for *in vivo* biotinylation, and an HPC4 tag for purification (see FIG. 14). The GPIIb/IIIa protein were transiently expressed in HEK293 cells and subsequently purified. GPIIb/IIIa nanodiscs were formed by first combining the GPIIb/IIIa protein with the scaffold protein, phospholipids, and detergent, followed with the removal of the detergent (FIG. 14). (*see, Zhu et al, Sci. Transl. Med. 4, 125ra32 (2012), which is hereby incorporated by reference in its entirety.*)

[0360] The nanodiscs, which contained the biotinylated GPIIb/IIIa receptor, were bound to BIACORE® streptavidin chips, and binding of the FVII-189, FVII-206, and FVII-163 chimeric molecules was measured using SPR (see FIG. 15 and TABLE 2).

TABLE 2: Affinity of FVII-189 and FVII-206 Chimeric Molecules to GPIIb/IIIa receptor as measured by SPR

FVIIa Variant	Antibody (scFv) Moiety	Affinity (nM)
FVII-189	34D10	5.2
FVII-206	12B2	5.3
FVII-163	PDG13	8.8
Recombinant FVIIa	None	No binding observed

[0361] SPR results indicate that both α -targeted and β -targeted chimeric molecules bound to GPIIb/IIIa with similar affinity. Compared to the known antibody (PDG13), the 34D10 and 12B2 antibodies provide stronger GPIIb/IIIa affinity to FVIIa.

[0362] The ability of the FVIIa chimeric molecules to bind to platelets was measured in a flow cytometry-based platelet-binding assay. Briefly, the FVIIa chimeric molecules were spiked into a citrated human whole blood and incubated for 30 mins. The blood was then stained with an APC-conjugated anti-human CD42b antibody and a FITC-conjugated anti-FVII polyclonal antibody to label the platelets and FVII protein, respectively. After fixing with paraformaldehyde, the stained blood was analyzed by flow cytometry. The platelets were identified by scatter gating followed by APC-fluorescence gating; and relative FVII concentration was represented by the median fluorescent value of FITC-fluorescence gating on gated platelets. As shown in FIG. 18, all platelet-targeted FVII chimeric molecules described above (FVII-163, FVII-189, FVII-204, and FVII-206) were capable of binding to human platelets. No binding was observed to human platelets for recombinant FVIIa alone (in the absence of a targeting moiety). See also Table 2.

[0363] The procoagulant activity of the platelet-targeted FVIIa chimeric molecules was measured using a FVIIa specific soluble tissue factor prothrombin time (sTF-PT) and rotational thromboelastometry (ROTEM) assays. The specific activity of the platelet-targeted rFVIIa variants FVII-189, FVII-206, and FVII-204, as measured by sTF-PT assays, was found to be comparable to that of rFVIIa, indicating that linking of the

platelet-targeting moiety does not affect rFVIIa's catalytic activity. In the presence of platelets, such as the ROTEM assays using the whole blood from human Hemophila A donors, the platelet-targeted rFVIIa variants showed improved clotting activities. Specifically, FVII-189 and FVII-206 displayed a 25-50 fold increase in activity compared to rFVIIa (FIGS. 16A,16B, 17B). Improvement in activity was also observed in FVII-204 (FIG 17A).

[0364] Platelet activation was measured using flow cytometry as described in Example 1. FIG. 19 shows that platelets were not activated by either the platelet-targeted FVII-189 chimeric molecule (targeting the α chain/complex) or the platelet-targeted FVII-206 chimeric molecule (targeting the β chain). FVII-130 is a platelet-targeted chimeric molecule known to activate platelets and was used as a positive control using the same experimental conditions (see FIG. 19).

[0365] FVII-189 (FIG. 20A) and FVII-206 (FIG. 20B) did not inhibit ADP-induced platelet aggregation in platelet-rich plasma, indicating that none of these chimeric molecules inhibited platelet function. REOPRO® (abciximab), a monoclonal antibody that blocks the pathway to platelet aggregation, was used as a control. Accordingly, platelet-targeted chimeric molecules comprising targeting moieties derived from the anti-GPIIb/IIIa antibodies disclosed herein did not activate or inhibit platelet function.

[0366] The effect of platelet-targeting rFVIIa chimeric molecules on platelet clearance *in vivo* was investigated in the NOD SCID gamma (NSG) mice with circulating human platelets since none of the targeting moieties is capable of binding mouse platelets. Human platelet-rich plasma (PRP) was prepared from the citrated whole blood. The PRP was concentrated and administrated via retro-orbital into NSG mice. After 30 minutes, the clotting factors were dosed at 5 nmol/kg by tail-vein injection, and the blood samples were collected via tail laceration at 5 minutes before, and at various times after clotting factor dosing. To quantify the human platelets, the blood was stained with a cocktail containing PE-conjugated anti-mouse CD61, APC-conjugated anti-human CD42b to visualize by flow cytometry the mouse platelets and human platelet, respectively. The relative human platelet counts was obtained by the scattering gating for platelets, and the PE, APC fluorescent gating for mouse and human platelets, respectively. As shown in FIG.21, a control protein FVII-130, which is known to promote platelet clearance and cause thrombocytopenia, led a rapid human platelet clearance in this model; more than

90% of injected human platelets were cleared within 1 hour following the administration of FVII-130. On the contrary, no effect on human platelet clearance can be detected when treated the mice with FVII-189, FVII-206, and FVII-204, indicating that these platelet-targeted rFVIIa chimeric molecules do not affect the human platelet clearance *in vivo*.

[0367] The present invention has been described above with the aid of functional building blocks illustrating the implementation of specified functions and relationships thereof. The boundaries of these functional building blocks have been arbitrarily defined herein for the convenience of the description. Alternative boundaries can be defined so long as the specified functions and relationships thereof are appropriately performed.

[0368] The present application claims benefit to U.S. Provisional Application No. 61/827,165, filed May 24, 2014, which is incorporated herein by reference in its entirety.

[0369] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0370] The breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein.

[0371] All patents and publications cited herein are incorporated by reference herein in their entirety.

SEQUENCE LISTING:

>SEQ_ID_NO:1 34D10 HC
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>SEQ_ID_No:2 34D10 LC
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 SLTISSMEGEDAATYYCQQFSSSPWTFGGGTKLEIKR

>SEQ_ID_NO:3 2A2 HC
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 RDNARNILYLQMSLRSEDTAMYCCARGGDYGYALDYWGQTSVTVSS

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 SLTISSLEGEDAGTYCQQFSSSPWTFGGGTKLEIKR

>SEQ_ID_NO:5 36A8 HC
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>SEQ_ID_NO:6 36A8 LC
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 SLTISSMEGEDAATYYCQQFS SSPWTFGGGS KLEIKR

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>SEQ_ID_NO:8 1H6 HC
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>SEQ_ID_NO: 10 38A8 HC
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>SEQ_ID_NO:14 12B2 HC
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 TYAMS

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 YTSNLAP

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sequenc ing_pending

>SEQ_ID_NO :107 5C4 LC*
sequenc ing_pending

>SEQ_ID_NO: 108 23C10 LC*
sequenc ing_pending

>SEQ_ID_NO: 109 37C7 HC
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sequenc ing_pending

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>SEQ_ID_NO: 113 35D1 HC CDR3
GGDYGALDY

>SEQ_ID_NO: 114 35D2 LC CDR1
RASSSVNYMY

>SEQ_ID_NO: 115 35D2 LC CDR2
YTSNLAP

>SEQ_ID_NO: 116 35D2 LC CDR3
QQFSSSPWT

>SEQ_ID_NO: 117 4B11 LC CDR1
RASSSVNYMY

>SEQ_ID_NO: 118 4B11 LC CDR2
YTSNLAP

>SEQ_ID_NO: 119 4B11 LC CDR3
QQFSSSPWT

>SEQ_ID_NO: 120 38G8 HC CDR1
NYLIE

>SEQ_ID_NO: 121 38G8 HC CDR2
VINPGSGGTNYNEKFKG

>SEQ_ID_NO: 122 38G8 HC CDR3
GRYEWYFDV

>SEQ_ID_NO: 123 38G8 LC CDR1
RASQDITNYLN

>SEQ_ID_NO: 124 38G8 LC CDR2
YTSSLHS

>SEQ_ID_NO: 125 38G8 LC CDR3
QQGYTLPYT

>SEQ_ID_NO: 126 21F10 HC CDR1
NYLIE

>SEQ_ID_NO: 127 21F10 HC CDR2
VINPGSGGTNYNEKFKG

>SEQ_ID_NO: 128 21F10 HC CDR3
GRYEWYFDV

>SEQ_ID_NO: 129 21F10 LC CDR1
RASQDITNYLN

>SEQ_ID_NO: 130 21F10 LC CDR2
YTSRLHS

>SEQ_ID_NO: 131 21F10 LC CDR3
QQGYTLPYT

>SEQ_ID_NO: 132 38F6 LC* CDR1
sequencing_pending

>SEQ_ID_NO: 133 38F6 LC* CDR2
sequencing_pending

>SEQ_ID_NO: 134 38F6 LC* CDR3
sequencing_pending

>SEQ_ID_NO: 135 13C1 HC CDR1
SYWIE

>SEQ_ID_NO: 136 13C1 HC CDR2
EILPGSGITKYNDKFKG

>SEQ_ID_NO: 137 13C1 HC CDR3
LISYYYAMDY

>SEQ_ID_NO: 138 13C1 LC* CDR1
sequencing_pending

>SEQ_ID_NO: 139 13C1 LC* CDR2
sequencing_pending

>SEQ_ID_NO: 140 13C1 LC* CDR3
sequencing_pending

>SEQ_ID_NO: 141 5C4 LC* CDR1
sequencing_pending

>SEQ_ID_NO: 142 5C4 LC* CDR2
sequencing_pending

>SEQ_ID_NO: 143 5C4 LC* CDR3
sequencing_pending

>SEQ_ID_NO: 144 23C10 LC* CDR1
sequencing_pending

>SEQ_ID_NO: 145 23C10 LC* CDR2
sequencing_pending

>SEQ_ID_NO: 146 23C10 LC* CDR3
sequencing_pending

>SEQ_ID_NO: 147 37C7 HC CDR1
TSGMGVG

>SEQ_ID_NO: 148 37C7 HC CDR2
HIWDDDKRYNPALKS

>SEQ_ID_NO: 149 37C7 HC CDR3
SHYGTIFYFDY

>SEQ_ID_NO: 150 37C7 LC* CDR1
sequencing_pending

>SEQ_ID_NO: 151 37C7 LC* CDR2
sequencing_pending

>SEQ_ID_NO: 152 37C7 LC* CDR3
sequencing_pending

SEQ ID NO: 153
>CTP peptide 1
DPRFQDSSSSKAPPSLPSRLPGPSDTPIL

SEQ ID NO: 154
>CTP peptide 2
SSSSKAPPSLPSRLPGPSDTPILPQ

SEQ ID NO: 155
>PAS peptide 1
ASPAAPAPAS PAAPAPSAPA

SEQ ID NO: 156
>PAS peptide 2
AAPAS PAPAAPSAPAPAAPS

SEQ ID NO: 157
>PAS peptide 3
APSSSPSAPSSSPASPSS

SEQ ID NO: 158
>PAS peptide 4
APSSSPSAPSSSPASPSS

SEQ ID NO: 159
>PAS peptide 5
SSPSAPSPSPASPSPSSPA

SEQ ID NO: 160
>PAS peptide 6
AASPAAPSAPPAAASPAAPSAPPA

SEQ ID NO: 161
>PAS peptide 7
ASAAAPAAASAAASAPS;»Aiv

SEQ ID NO: 162
 >Albumin Binding Peptide Core Sequence
 DICLPRWGCLW

SEQ ID NO: 163
 >GFP protein sequence (Genbank ID AAG34521.1)
 MSKGEELFTGWPIILVELDGDVNGHKFSVSGEGEGDATYGLKTLKFICTTGKLPVPWPPTLVTTFGYGVQCFARYPDHMKQHDFFKS
 AMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGLDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIE
 DGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDMVLLFVTAAGITHGMDELYKSRTSGSPGLQEFDIKLIIDTV
 DLESCN

SEQ ID NO: 164
 >Example: Single-chain Human IgG1 Fc. (Fc sequences with Gly/Ser linker underlined.)
 DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRWSVL
 TVLHQDWLNGKEYCKVSNKALPAPIEKTI S KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK GGGSGGGSGGGSGGGGS DKTHTCPPCPA
 PELLGGPSVFLFPPKPKDTLMI SRTPEVTCWVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRWSVLTVLHQDWLNGK
 EYCKVSNKALPAPIEKTI S KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGS
 FFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

SEQ ID NO: 165
 >Mature human albumin protein sequence (derived from NCBI Ref. Sequence NP_000468) :
 RGVFRRDAHKSEVAHRFKDLGEEFKALVLI AFAQYLQCPFDHVKLVNEVTEFAKTCVADESAENC DKSLHTLFGDKLCTVATL
 RETY GEMADCCAKQEPERNECF LQHKDDNP LRLV RPEVDMCTAFHDNEETF LKKYLYE IARRHPYFYAP ELLFFAKRYKAAFT
 ECCQAADKAACLLPKLDEL RDEGKASSAKQRLK CASLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGD LLE
 CADDRADLAKYICENQDSISSK LKECCEKPLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRH
 PDYSWLLLR LAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC E LFEQLGEYKFNALLVRYTKKVPQVSTPTLV
 EVSRNLGKVGSKCKKHPEAKRMPCAEDYLSWLNQLCVLHEKTPVSDRVTKCTESLraRRPCFSALEVD ETYVPKEFNAETFTFH
 ADICTLSEKERQIKKQ TALVELVKHKPKATKEQLKAVMDDFAAFVEKCKADDKETCF AE EGKLVAA SQAALGL

SEQ ID NO: 166
 >Linker, n = 0, 1, 2, 3, 4 or more
 (GGGS)_n

SEQ ID NO: 167
 >Albumin binding peptide 1
 RLIEDICLPRWGCLWEDD

SEQ ID NO: 168
 >Albumin binding peptide 2
 QRLMEDICLPRWGCLWEDDF

SEQ ID NO: 169
 >Albumin binding peptide 3
 QGLIGD ICLPRWGCLWGDS VK

SEQ ID NO: 170
 >Albumin binding peptide 4
 GEWWEDICLPRWGCLWEEED

SEQ ID NO: 171
 >Cysteine-containing peptide
 GGGSGCGGGS

SEQ ID NO: 172
 >Human LRPL sequence (signal peptide and transmembrane segment underlined; NCBI Reference Sequence: CAA32112)
MLTPPLLLLLPLLSALVAA AIDAPKTCSPKQFACRDQITCISKGWRC DGERDCPDGSDEAPEIC POSKAORCQ PNEHNCLGTEL CV
 PMSRLCNGVQDCMDGSD EGP HCRELQGNCSRLGCQHHCVPTLDGPTCYCNSS FQLQADGKTCKDFDECSVYGTCSQLCTNTDGSFI
 CGCVEGYLLQPDNRSCAKNEPVD RPPVLLIANSQNILATYLSGAQVSTITPTSTRQTTAMD FSYANETVCWVHVGD SAAQTQLKC
 ARMPGLKGFVDEHTINISLSLHHVEQMAIDWLTGNFYFVDDIDDRIFVCN RRGDTCVTL LLDLELYNPKGIALDPAMGKVFFTDYGO
 IPKVERCDMDGQNRKTLVDSKIVFPHGITLDLVSRLVYWADAYLDYIEWDYEGKGRQTI IQGILIEHLYGLTVFENYLYATNSDN
 ANAQKQTSVIRVNRFNST EYQWTRVDKGGALHIYHQRQPRVRS HACENDQYKPGGCS DICLLANS HKARTCRCSGFS LGSDG
 KSCKKPEHELFLVY GKRPGIIRGMDMGAKVPDEHMIP IENLMNPRALDFHAETGF IYFADTTSYLIGRQKIDGTERETILKDG I H
 NVEGVAVDWMGDNL YWTD DGP KKTISVARLEKAAQTRKTLIEGKMTHPRAIWDPLNGWMYWTDWEEDPKDSRRGR LERAWMDGSH:

RDIFVTSKTVLWPNGLSLDIPAGRLYVWDAFYDRIETILLNGTDRKIVYEGPELNHAFGLCHHGNYLFWTEYRSGSVYRLERGVGG
 APPTVTLRLSERPPIFEIRMYDAQQQVGTNKRNVNNGGCSLCLATPGSRQCACAEDQVLDADGVTCLANPSYVPPQCQPGFEFA
 CANSRCIQERWKCDGDNDCDLSNDEAPALCHQHTCPSDRFKCENNRCPINRWLDCGDNDGCGNSEDESATCSARTCPPNQFSCASG
 RCIPISWTCDLDDCGDRSDESA SCAYPTCFPLTQFTCNNGR IN INWRCDNDNDGCGNSDEAGCSHSCSSTQFKCNSGRCIPEHW
 TCDGDNDGCGDYSDETHANCTNQAATRPGGCHTDEFQCRDLGLCIPLRWRCGDGTDGMDSSDEKSCGEGVTHVCDPSVKFGCKDSARC
 ISKAWVCDGDNDCEDNSDEENCESLACRPPSHPCANNTSVCLPPDKLDCGNDGCGDGSDEGELCDQCSSLNNGGCSHNCSVAPGEGI
 VCSCPLGMELGPDNHTCQIQSYCAKHLKCSQKCDQNKFSVKCSCSYEGWVLEPDGEGESRSLDPFKPFIIFSNRHEIRRIDLHKGDYS
 VLVPGLRNTIALDFHLSALYWDWEDKIRYKGLLDNGALTSFEWIIQYGLATPEGLAVDWDIAGNIYWVESNLDQIEVAKLDGT
 LRTTLLAGDIEHPRAIALDPRDGLFWDWASLPRIEAASMSGAGRRTVHRETGSGGWPNGLTVDYLEKRIILWIDARSDAIYSAR
 YDGSGHMEVLRGHEFLSHFAVTVLYGGEVYWTWRTNTLAKANKWTGHNVTWQRTNTQPFDLQVYHPSRQPMAPNPCEANGGQGP
 CSHLCLINYNRTVSCACPHMLKHKDNTTCYEFKKFLLYARQMEIRGVDLDAPYYNYIISFTVPDIDNVTVDYDAREQRVYWSDV
 RTQAIKRAFINGTGVETWSADLPNAHGLAVDWWVRNLFWTSYDITNKKQINVARLDGSPKNAWQGLEQPHGLWHPLRGKLYWTD
 GDNISMANMDGSRNTLLFSGQKGPVGLAIDFPESKLYWISSGNHTINRNLGSGLEVIDAMRSQLGKATALAIMGDKLWWADQVS
 EKMGTCSKADGSGSWLRNSTTLVMHKVYDESIQLDHKGTNPCSVNNGDCSQCCLPTSETTRSCMCTAGYSLRSGQQACEGVSF
 LLYSVHEGIRGILPDPNDKSDALVPVSGTSLAVGIDFHAENDTIYVWDMGLSTISRAKRDQWTREDWTNGIRVEGIAVDWIAGN
 IYWTDQGFVIEVARLNGSFRYWISQGLDKPRAITVHPEKGYLFWTEWQYPIERSRLDGTERTWLVNVSISWPNGISVDYQDG
 KLYWCDARTDKIERIDLETGENREWLSSNNMDFSVSVFEDFIYWSDRTHANGSIKRGSKDNATDSVPLRTGIGVQLDKIKVFN
 DRQKGTNVCAVANGGCQQLCLYRGRQRACACAHGMLAEDGASCREYAGYLLYSERTILKSIHLSDERNLNAPVQPFEDPEHMKNV
 IALAFDYRAGTSPGTPNRIFFSDIHFNGIQQINDGSRRTITIVENVGSVEGLAYHRGWDTLTYWTSYTTSTITRHTVDQTRPGAFER
 ETHVITMSGDDHPRAFLVLEDCQNLMTNWNNEQHPSIMRAALSGANVLTLEKDIRTPNGLAIDHRAEKLYFSDATLKERCEYD
 SHYVILKSEPVHPFGLAVYGEHIFWTDWVRAVQRANKHVGSNMKLLRVDIPQQPMGIIAVANDTNSCELSPCRINNGGCQDLCL
 LTHQGHVNCSCRRGRIQLQDDLTCLRAWSSCRAQDEFECANGECNFSLTCDGVPHCKDKSDEKPSYCNRRCKKTFRQCSNRCV
 NMLWCNGADDCGDSDEIPCNKTACGVGEFRCDGTICGNSSRCNQFVDCEDASDEMNCSATDCSSYFRLGVKGVLFQPCERTSLC
 YAPSWVCDGANDCGDYSDERDCPGVKRPRCPNLYFACPSGRCPMSWTCDEKEDDCEHGEDETHCNKFCSEAQFECQNHRCISKQWL
 CDGSDDCGDSDEAAHC EGKTCGPFSCPGTHVCPERWLCGDKDCADGADESIAAGCLYNSTCDDREFMQNRQCIPKHFVCD
 HDRCADGSDSPECEYPTCGPSEFRANCGRCLSSRQWECGDENDCHDQSDPEAPKNPHCTSPHEKCNASSQFLCSSGRCVAAELLC
 NGQDDCGDSSDERGCHI NECLSRKLSGCSQDCEDLIGFKCRCPGRFRLKDDGRTCADVDECESTTFPCSQRINTHGSYKCLCQVEG
 YAPRGGDPHSCAKAVTDEPFLLIFANRYLRLKLNLDGSNYTLKQGLNNAVALDFDYREQMIYWTDVTTQSMIRRMHLNNGSNVQVL
 HRTGLSNPDGLAVDWWGNYLWCDKGRDITIEVSKLNGAYRTVLVSSGLREPRALWDVQNGYLYWTDWGDHSLIGRIGMDGSSRSV
 XVDTKITWPNGLTLDYVTERIYWADAREDI EFASLDGSRNHWLSQDIPHIFALTLFEDYVYWTDWETKINRAHKTGTNKTLL
 ISTLHRPMDLHVHFALRQFDVPHPGKVNNGGCSNLCLLSPGGGHCACPTNFYLGSDGRTCVSNCTASQFVCKNDKCI PFWWKCD
 TEDDCGDHSDPEPPDCPEFKRPGQFQCSTGICTNPAFICDGDNDGQDNDSEANCDIHVCLPSQFKCTNTNRCIPGIFRCNGQDNCG
 DGEDERDCPEVTCAPNQFQCSITKRCIPRVVWCDRDNDGVDGSDPEANCTQMTCGVDEFRCRDSGRCPARWKCDEDDCGDGSDE
 PKECEDERTCEPYQFRCKNNRCPVGRWQCDYDNDGDNSEESCTPRPCESEFSCANGRCIAGRWKCDGDHDCADGSDKDCCTPR
 CDMDQFQCKSGHC1PLRWRCADADACMDGSDDEACGTGVRTPLDEFQCNNTLCKPLAWKCDGEDDCGDNSENDEEPCARFVCPN
 RPFRCNDRVCLWIGRQCDGTDNCGDGTDEEDCEPPTAHTTHCKDKKEFLCRNQRCLESSLRCNMFDCCGDSDEEDCSIDPKLTS
 CATNASICGDEARCVRTEKAAYCACRSGFHTVPGQPGQDINECLRFGTCSQCLCNNTKGGHLCSCARNFMKTHNTCKAEGSEYQVL
 YIADDNEIRSLFPGHPHSAQEAFQGDSEVRIDAMDVHVKAGRVYWTNWHGTISYRSLPPAAPPTTSNRHRRQIDRGVTHLNSG
 LKMPRGIAIDWVAGNVYWTDSGRDVIEVQMKGENRKTLSGMIDEPHAIWDLRGTMYWSDWGNHPKIEAAMDGTLRETLVQD
 NIQWPTGLAVDYHNERLYWADAKLSVIGSIRLNGTDPXVAADSKRGLSHPPSIDVFEDYIYGVTYINNRFVKIHKHFGHSPLVNLTG
 GLSHASDWLYHQKQPEVTNPNCDRKKCEWLCLLSPSGVMCTCPNGKRLDNGTCVVPSPPTPPDAPRPGTCNLQCFNGGSCFLNA
 RRQPKRCQPRYTGDKCELDQCWEHCRNGGTCAASPSGMPTCRCPRTGFTGPKCTQVQCAGYCANNSTCTVNQGNQPCRCCLPGFLG
 DRQCQRQCSGYCENFGTCQMAADGSRQCRCTAYFEGSRCEVKNKCSRCLLEGACWNKQSGDVTNCNCTDGRVAPSCLTCVGHCSNNGS
 CTMNSKMMPEQCQPPHMTGPRCEEHVFSQQQPGHIASILIPLLLLLLLLLVAG VVFWYKRRVQGAQGFQHQRMNTGAMNVEIGNPT
 YKMYEGGEPDDVGLLDADFALDPDKPTNFTNPVYATLYMGHGSRHSLASTDEKRELLGRGPEDEIGDPLA

- SEQ ID NO: 173
 >Biotin Acceptor Peptide (BAP)
 LNDIFEAQKXEW
- SEQ ID NO: 174
 >Lipoate Acceptor Peptide 2 (LAP2)
 GFEIDKVVYDLDA
- SEQ ID NO: 175
 >HAPylation motif, n= 1 to 400
 (Gly4Ser) n
- SEQ ID NO: 176
 >Alternative linker
 PEAPTDPEAPTD
- SEQ ID NO: 177
 >CTP
 DSSSSKAPPPSLPSPSRLPGPSDTPILPp

SEQ ID NO: 178
>FVII-HC

IVGGKVCV KGECPWQVLL LVNGAQLCGG TLINTIWVVS AAHCFDKIKN WRNLI AVLGE HDLSEHDGDE QSRRVAQVII
PSTYVPGTTN HDIALLRHLQ PVVLT DHVVP LCLPERTFSE RTLAFVRFSL VSGWGQLLDR GATALELMVL
NVPRMTQDC LQQSRKVGDS PNITEYMFCA GYSDGSKDSC KGDSGGPHAT HYRGTWYLTG
IVSWGQGCAT VGHFGVYTRV SQYIEWLQKL MRSEPRPGVL LRAPFP

SEQ ID NO:179
>FVII-LC

ANAFLEELRP GSLERECKEE QCSFEEAREI FKDAERTKLF WISYSDGDQC ASSPCQNGGS
CKDQLQSYIC FCLPAFEGRN CETHKDDQLI CVNENGGCEQ YCSDHTGTRK SCRCHEGYSL
LADGVSTPT VEYPCGKIPI LEKRNASKPQ GR

SEQ ID NO: 180
>FVII zymogen.

MVSQALRLLC LLLGLQGCLA AGGVAKASGG ETRDMPWPKG PHRVFVTQEE AHGVLHRRRR
ANAFLEELRP GSLERECKEE QCSFEEAREI FKDAERTKLF WISYSDGDQC ASSPCQNGGS
CKDQLQSYIC FCLPAFEGRN CETHKDDQLI CVNENGGCEQ YCSDHTGTRK SCRCHEGYSL
LADGVSTPT VEYPCGKIPI LEKRNASKPQ GRIVGGKVCV KGECPWQVLL LVNGAQLCGG
TLINTIWVVS AAHCFDKIKN WRNLI AVLGE HDLSEHDGDE QSRRVAQVII PSTYVPGTTN
HDIALLRHLQ PVVLT DHVVP LCLPERTFSE RTLAFVRFSL VSGWGQLLDR GATALELMVL
NVPRMTQDC LQQSRKVGDS PNITEYMFCA GYSDGSKDSC KGDSGGPHAT HYRGTWYLTG
IVSWGQGCAT VGHFGVYTRV SQYIEWLQKL MRSEPRPGVL LRAPFP

SEQ ID NO: 181

>FIX zymogen. Signal sequence (1-28) , Propeptide (29-46)

MQRVNMIMAESPLITICLLGYLLSAECTVFLDHENANKILNRPKRYNSGKLEEFVQGNLERECMEEKCSFEEAREVFENTERTTE
FWKQYVDGDCESNPCLNGGCKDDINSYECWCPFGFEGKNCELDVTNCIKNGRCEQFCKNADNKWCSCTEGYRLAENQKSCPE
AVPFFCGRVSVSQTSLTRAETVFPDVDYVNSTEAETILDNITQSTQSFNDFTRWGGEDAKPGQFPWQLNGKVDFAFCGGS IW
EKWIVTAAHCVETGVKITWAGEHNIETEHETEQRNVIRIIPHNNYNAAINKYNHDIALLELDEPLVLNSYVTPICIAKEYTNI
FLKFGSGYVSGWRVFKGRSALVLQYLRVPLVDRATCLRSTKFTIYNMFCAGFHEGGRDSCQGDSSGGPHVTEVEGTSFLTGIIS
WGEECAMKGYGIYTKVSRVYVNIKEKTKLT

SEQ ID NO: 182

>FX zymogen. Signal sequence (1-23) , Propeptide (24-40)

MGRPLHLVLLSASLAGLLLLGESLFXRREQANNILARVTRANSFLEEMKGLHRECEMETCSYEEAREVFEDES DKTNEFWNKYKD
GDQCETSPCQNGKCKDGLGEYTCLEGFEGKNCLEFTRKLCSLDNGDCDQFCHEEQNSWCSCARGYTLADNGKACIPTGPYPC
GKQTLERRKRSVAQATSSSGEAPDSITWKPYDAADLDPTENPFLLDFNQTPERGDNNLTRIVGGQECKDGCEPQWALLINEENE
GFCGGTILSEFYILTAACHLYQAKRFKVRVGDNRTEQEEGGEAVHEVEWIKHNRFTKETYDFDIAVLRLLKTPITFRMNVAPACLP
ERDWAESTLMTQKTGIVSGFGRTHEKGRQSTRKMLEVYVDRNSCKLSSSFIIITQNMFCAGYD-TKQEDACQGDSSGGPHVTRFKDT
YFVTGIVSWGEGCARKGKYGIYTKVTAFLKWIDRSMKTRGLPKAKSHAPEVITSSPLK

SEQ ID NO: 183

>Human GPIIb. Signal sequence (1-31) . Transmembrane (981-1019)... Cytoplasmic (1020-1039)

MARALCPLQALWLLWVLLLLGPCAAPPAAWALNLDPVQLTFYAGPNGSQFGFSLDFHKDSHGRVAIWGAPRTLGPQSQEETGGVFL
CPWRAEGGQCPSSLFDLRDETRNVGSQTLQTFKARQGLGASWSWSDVIVACAPWQHWNVLEKTEEAETKTPVGSFLAQPEGARRA
EYSPCRGNTLSRIYVENDFSWDKRYCEAGFSSWTQAGELVLGAPGGYFLGLLAQAPVADIFSSYRPGILLWHVSSQSLSFSSN
PEYFDGYWGYSAVGEFDDGLNTEYWGAPTWSWTLGAVEILDSYYQRLHRLRGEQMASYFGHSVAVTDVNGDGRHLLVGLY
MESRADRKLAEVGRVYFLQPRGPHALGAPSLLLTGTQLYGRFGSAIAPLGLDRDGYNDIAVAAPYGGPSGRGQVLVFLGQSEGL
RSRPSQVLDSFPPTGSFAFGSLRGAVIDDNGYPDLIVGAYGANQVAVYRAQPWKASVQLLVQDSLNPVKS CVLPQTKTPVSCF
NIQMCV GATGHNIPQKLSLNAELQLDRQKPRQRRVLLLSGQQAGTTLNLDLGGKHSPICHTTMAFLRDEADFRDKLSPIVLSLNV
SLPTEAGMAPAVVLHGDTHVQEQTRIVLDCGEDDVCVPQLQLTASVTGSPLLVGDNVLELQMDAANEAGEGAYEALAVHLPQGA
HYMRLSNVEGFERLICNKKENETRWLCELGNPMKKNQIGIAMLVSVGNLEEAGESVSFQLQIRSKNSQNPNSKIVLLDVPVR
AEAQVELRGNSFPASLWAAEEGEREQNSLDSWGPKEVHTYELHNNPGTVNGLHLSIHLPGQSQPSDLLYILDIPQGGGLQCFPQ

PPVNP LKVDWGLPI PIPSPSP IHPAHHKRDRRQIFLPEPEQPSRLQDPVLVSCDSA PCTVVQCDLQEMARGQRAMVTVIA FLWLPSLY
RPLD₀FVL₀SHAWFNVSSLPYAVPPLSLPRGEA₀VWT₀LLRALEERAIP IWWVLVGVLGLLLLLITILVLAMWKVGFVKRNRPPLE
EDDEEGE

SEQ ID NO: 184

>Human GPIIIa. Signal sequence (1-26), Transmembrane (719-747), Cytoplasmic (748-788)

MRARPRRPLWATV LALGALAGVGVGPNICTTRGVSSCQQLAVSPMCAWCSDEALPLGSPRCDLKENLLKDNCAPE SIEFPVSE
ARVLEDRPLSDKSGSDSS₀VT₀VSP₀RIALRLRPDDSKNF SIQRVQVEDY PVDIYYLMDLSYSMKDDLWSIQNLGTKLATQMRKLT
SNLRIGFGAFVDPKVPSPYMYISPPEALENPCYDMKTTCLPMFGYKHVLTLDQVTRFN EEVKKQSVSRNRDAPEGGFDAMQATVC
DEKIGWRNDASHLLVFTTDAKTHIALDGRLAGIVQPNDGQCHVGSNDHY SASTTMDYPSLGLMTEKLSQKNINLIFAVTENWNL Y
QNYSELIPGTTVGVLSMDSNVLQLIVDAYGKXRSKVELEVRDLPEELSLSFNATCLNNEVI PGLKSCMGLKIGDTSVFSIEAKVR
GCPQEKEKSFTIKPVGFKDSLIVQVTFDCDCACQQAEPNSHRCNNNGN GTFECGVCRCGPGWLGSQCECSEEDYRPSQQDECSPRE
GQPVC SQRGECLCGQCVCHSSDFGKITGKYCEDDFSCVR YK GEMCSHGQCSCGDCLCSDSWTGYCNC TTRTDTCMSNGLLCS
GRGKCECGSCVCIQPGSYGDTCEKCP TPCDACTFKKECVECKKFD RGLHDENTCNRYCRDEIESVKELKDTGKDAVNCTYKNEDD
CVVRFQYYEDSSGKSILYVVEEPECPKGPDILVLLSVMGAILLIGLAALLIWKLLITIHDRKEFAKFEEERARAKWDTANNPLYK
EATSTFTNITYRGT

SEQ ID NO: 185

>Human GPIIb DNA.

ATGGCCAGAGCTTTGTGTCCACTGCAAGCCCTCTGGCTTCTGGAGTGGGTGCTGCTGCTCTACCGGTCTCGAAACACAGGTGACGT
TCGGGAGACCGAAGACCTCACCCACGACGACGAGTTGGGACCTTGTGCTGCCCTCCAGCCTGGGCCTTGAACCTGGACCCAGTGC
AGCTACCAACCCCTGGAACACGACGGGGAGGTCGGAGCCGGAACCTGGACCTGGGTACGTCGAGTGGTTCTATGGAGGCCCAAT
GGCAGCCAGTTTGGATTTTCACTGGACTTCCACAAGGACAGCAAGATACGTCGGGGTTACCGTCCGTCAAACCTAAAAGTGACCT
GAAGGTGTTCCCTGTCGCATGGGAGAGTGGCCATCGTGGTGGGCGCCCCGCGGACCTGGGCCCCAGCCAGGAGGAGGTACCCCTCTC
ACCGGTAGCACCACCCGCGGGGCGCCTGGGACCCGGGGTGGTCCCTCACGGGCGGCGTGTCTCTGTGCCCTGGAGGGCCGAG
GGCGGCCAGTGCCTCGCTGCTGCCCCGCCACAAGGACACGGGGACCTCCCGGCTCCCGCGGTACGGGGAGCGACGAGTT
TGACCTCCGTGATGAGACCGAAATGTAGGCTCCCAAACCTTACAACCTCAAGGCCAAACTGGAGCCACTACTCTGGGCTTTAC
ATCCGAGGGTTTGAATGTTTGAAGTTCGGCGCCAAGGACTGGGGGCGTGGTCTGTCAGTGGAGCGACGTCATTGTGGCCTGC
GCCCCGCGGTTCTGACCCCCGACGACGAGTGCACCTCGTGCAGTAACACCGGACGCGGGGTGGCAGCACTGGAACGTCTT
AGAAAAGACTGAGGAGGTGAGAAGACGCCGTAGGTAGCACCGTCTGACCTTGCAAGGATCTTTTCTGACTGCTCCGACTCTTCT
GCGGGCATCCATCGTGTCTTTTGGCTCAGCCAGAGAGCGCCGCGCGCCGAGTACTCCCCCTGTGCGGGGAACACGAAAAACCGA
GTCGGTCTCTGCGCGGCCGCGGGCTCATGAGGGGACAGCGCCCTTGACCCTGAGCCGATTTACGTGGAAAAATGATTTTAGCTG
GGACAAGCGTTACTGTGAAGCTGGGACTCGGCGTAAATGCACCTTTTACTAAAATCGACCCTGTTGCAATGACACTTCGCGGCT
TCAGTCCGTTGGTCACTCAGGCCGAGAGCTGGTGTCTTGGGGCTCTGGCGGCTATCCGAAGTCGAGGCACTACTGACCCGCT
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SEQ ID NO: 186

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AATT

WHAT IS CLAIMED IS:

1. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope,

(a) wherein the antibody or antigen-binding molecule thereof specifically bind to the same GPIIb/IIIa epitope as an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4;

(b) wherein the antibody or antigen-binding molecule thereof competitively inhibits GPIIb/IIIa binding by an antibody selected from 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4; or

(c) wherein the antibody or antigen-binding molecule thereof comprises at least one, at least two, at least three, at least four, or at least five complementarity determining regions (CDR) or variants thereof of an antibody selected from the 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4 antibodies.

2. The antibody or antigen-binding molecule thereof of claim 1, which comprises six CDRs or variants thereof of an antibody selected from the 34D10, 12B2, 2A2, 35D1, 36A8, 4B1 1, 1H6, 38G8, 21F10, 38A8, 18F7, 38F6, 13C1, 5C4, 23C10, 37C7, 28C2, 9D6, or 28F4 antibodies.

3. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 25, 31, 37, 43 or 111;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 26, 32, 38, 44, or 112;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 27, 33, 39, 45, or 113;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 28, 34, 40, 117, or 114;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 29, 35, 41, 118, or 115; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60, 70, 80, 90, or 95% identical to any one of SEQ ID NOS: 30, 36, 42, 119, or 116.

4. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a VH-CDR1 comprising the consensus sequence XiYAMS wherein Xi represents amino acid residues Thr (T), Ser (S), or Ala (A);

(ii) a VH-CDR2 comprising the consensus sequence SIX₂X₃GX₄X₅TYX₆X₇DSVKX₈ wherein X₂ represents amino acid residues Ser (S) or Asn (N), X₃ represents amino acid residues Ser (S) or Gly (G), X₄ represents amino acid residues Ser (S) or Gly (G), X₅ represents amino acid residues Ser (S), Asn (N), or Thr (T), X₆ represents amino acid residues Tyr (Y) or Phe (F), X₇ represents amino acid residues Leu (L) or Pro (P), and X₈ represents amino acids Gly (G) or Arg (R);

(iii) a VH-CDR3 comprising the consensus sequence GGDYGYAX₉DY, wherein X₉ represents amino acid residues Leu (L) or Met (M);

(iv) a VL-CDR1 comprising the sequence RASSSVNYMY (SEQ ID NO: 28);

(v) a VL-CDR2 comprising the sequence YTSNLAP (SEQ ID NO: 29); and,

(vi) a VL-CDR3 comprising the sequence QQFSSSPWT (SEQ ID NO: 30).

5. The antibody or antigen-binding molecule thereof according to claim 4, comprising

(i) a VH-CDR1 sequence selected from SEQ ID NOS: 25, 31, 37, 43, or 111;

(ii) a VH-CDR2 sequence selected from SEQ ID NOS: 26, 32, 38, 44, or 112;

(iii) a VH-CDR3 sequence selected from SEQ ID NOS: 27, 33, 39, 45, or 113;

(iv) a VL-CDR1 sequence selected from SEQ ID NOS: 28, 34, 40, 117, or 114;

(v) a VL-CDR2 sequence selected from SEQ ID NOS: 29, 35, 41, 118, or 115; and,

(vi) a VL-CDR3 sequence selected from SEQ ID NOS: 30, 36, 42, 119, or 116.

6. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 1, 3, 5, 7, or 97 and a VL

comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 2, 4, 6, 99, or 98.

7. The antibody or antigen-binding molecule thereof of any one of claims 1 to 6, wherein the VH comprises the amino acid sequence of SEQ ID NO: 1 and the VL comprises the amino acid sequence of SEQ ID NO: 2 (34D10 antibody).

8. The antibody or antigen-binding molecule thereof of any one of claims 1 to 6, wherein the VH comprises the amino acid sequence of SEQ ID NO: 3 and the VL comprises the amino acid sequence of SEQ ID NO: 4 (2A2 antibody).

9. The antibody or antigen-binding molecule thereof of any one of claims 1 to 6, wherein the VH comprises the amino acid sequence of SEQ ID NO: 5 and the VL comprises the amino acid sequence of SEQ ID NO: 6 (36A8 antibody).

10. The antibody or antigen-binding molecule thereof of any one of claims 1 to 6, wherein the VH comprises the amino acid sequence of SEQ ID NO: 7 and the VL comprises the amino acid sequence of SEQ ID NO: 99 (4B1 1 antibody).

11. The antibody or antigen-binding molecule thereof of any one of claims 1 to 6, wherein the VH comprises the amino acid sequence of SEQ ID NO: 97 and the VL comprises the amino acid sequence of SEQ ID NO: 98 (35D1 antibody).

12. The antibody or antigen-binding molecule thereof of any one of claims 1 to 11, wherein the GPIIb/IIIa epitope is located in the extracellular domain of the alpha subunit of GPIIb/IIIa or the extracellular domain of the GPIIb/IIIa complex.

13. The antibody or antigen-binding molecule thereof of any one of claims 1 to 12, wherein the antibody or antigen-binding molecule thereof does not compete with fibrinogen for **binding to GPIIb/IIIa**.

14. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 46, 52, 120, or 126;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 47, 53, 121, or 127;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 48, 54, 122, or 128;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 49, 55, 123, or 129;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 50, 56, 124, or 130; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NO: 51, 57, 125, or 131.

15. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a VH-CDR1 comprising the sequence NYLIE (SEQ ID NO: 46);

(ii) a VH-CDR2 comprising the sequence VINPGSGGTNYNEKFKG (SEQ ID NO: 47);

(iii) a VH-CDR3 comprising the sequence GRYEWYFDV (SEQ ID NO: 48);

(iv) a VL-CDR1 comprising the consensus sequence RASQDIX_iNYLN wherein X_i represents amino acid residues Ser (S) or Thr (T);

(v) a VL-CDR2 comprising the sequence YTSRLHS (SEQ ID NO:50); and,

(vi) a VL-CDR3 comprising the sequence QQGYTLPYT (SEQ ID NO:51).

16. The antibody or antigen-binding molecule thereof according to claim 15, comprising

(i) a VH-CDR1 sequence selected from SEQ ID NO: 46, 52, 120, or 126;

(ii) a VH-CDR2 sequence selected from SEQ ID NO: 47, 53, 121, or 127;

(iii) a VH-CDR3 sequence selected from SEQ ID NO: 48, 54, 122, or 128;

(iv) a VL-CDR1 sequence selected from SEQ ID NO: 49, 55, 123, or 129;

(v) a VL-CDR2 sequence selected from SEQ ID NO: 50, 56, 124, or 130; and,

(vi) a VL-CDR3 sequence selected from SEQ ID NO: 51, 57, 125, or 131.

17. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 8, 10, 100, or 102, and a VL comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 9, 11, 101, or 103.

18. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 14 to 17, wherein the VH comprises the amino acid sequence of SEQ ID NO: 8 and the VL comprises the amino acid sequence of SEQ ID NO: 9 (VH of 1H6 antibody).

19. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 14 to 17, wherein the VH comprises the amino acid sequence of SEQ ID NO: 10 and the VL comprises the amino acid sequence of SEQ ID NO: 11 (38A8 antibody).

20. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 14 to 17, wherein the VH comprises the amino acid sequence of SEQ ID NO: 100 and the VL comprises the amino acid sequence of SEQ ID NO: 101 (38G8 antibody).

21. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 14 to 17, wherein the VH comprises the amino acid sequence of SEQ ID NO: 102 and the VL comprises the amino acid sequence of SEQ ID NO: 103 (21F10 antibody).

22. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 14 to 21, wherein the GPIIb/IIIa epitope is located in the extracellular domain of the alpha subunit of GPIIb/IIIa.

23. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 14 to 22, wherein the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

24. An antibody or antigen-binding molecule thereof which specifically binds to a gpIIb/IIIa epitope, comprising

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 58;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 59;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 60;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 61;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 62; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 63.

25. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a VH-CDR1 sequence comprising SEQ ID NO: 58;

(ii) a VH-CDR2 sequence comprising SEQ ID NO: 59;

(iii) a VH-CDR3 sequence comprising SEQ ID NO: 60;

(iv) a VL-CDR1 sequence comprising SEQ ID NO: 61;

(v) a VL-CDR2 sequence comprising SEQ ID NO: 62; and,

(vi) a VL-CDR3 sequence comprising SEQ ID NO: 63.

26. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 12 and a VL comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 13.

27. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 24 to 26, wherein the GPIIb/IIIa epitope is located in the extracellular domain of the alpha subunit of GPIIb/IIIa.

28. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 24 to 27, wherein the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

29. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 64, 70, or 135;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 65, 71, or 136;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 66, 72, or 137;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 67, 132, or 138;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 68, 133, or 139; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 69, 134, or 140.

30. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a VH-CDR1 comprising the sequence SYWIE (SEQ ID NO: 64);

(ii) a VH-CDR2 comprising the consensus sequence EILPGX₁₄GX₁₅TKYNX₁₆KFKG (SEQ ID NO: 187) wherein X₁₄ represents amino acid residues Ser (S) or Thr (T), X₁₅ represents amino acid residues Ile (I) or Tyr (Y), and X₁₆ represents amino acid residues Asp (D) or Glu (E);

(iii) a VH-CDR3 comprising the sequence LISYYYAMDY (SEQ ID NO: 66);

(iv) a VL-CDR1 comprising the sequence RASQDISNYLN (SEQ ID NO: 67);

(v) a VL-CDR2 comprising the sequence YTSRLHS (SEQ ID NO: 68); and,

(vi) a VL-CDR3 comprising the sequence QQGNTLPPT (SEQ ID NO: 69).

31. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

- (i) a VH-CDR1 sequence selected from SEQ ID NO: 64, 70, or 135;
- (ii) a VH-CDR2 sequence selected from SEQ ID NO: 65, 71, or 136;
- (iii) a VH-CDR3 sequence selected from SEQ ID NO: 66, 72, or 137;
- (iv) a VL-CDR1 sequence selected from SEQ ID NO: 67, 132, or 138;
- (v) a VL-CDR2 sequence selected from SEQ ID NO: 68, 133, or 139; and,
- (vi) a VL-CDR3 sequence selected from SEQ ID NO: 69, 134, or 140.

32. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 14, 16, or 105 and a VL comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 15, 104, or 106.

33. The antibody or antigen-binding molecule of any one of claims 1, 2, and 29 to 32, wherein the VH comprises the amino acid sequence of SEQ ID NO: 14 and the VL comprises the amino acid sequence of SEQ ID NO: 15 (12B2 antibody).

34. The antibody or antigen-binding molecule of any one of claims 1, 2, and 29 to 32, wherein the VH comprises the amino acid sequence of SEQ ID NO: 16 and the VL comprises the amino acid sequence of SEQ ID NO: 104 (38F6 antibody).

35. The antibody or antigen-binding molecule of any one of claims 1, 2, and 29 to 32, wherein the VH comprises the amino acid sequence of SEQ ID NO: 105 and the VL comprises the amino acid sequence of SEQ ID NO: 106 (13C1 antibody).

36. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 29 to 35, wherein the GPIIb/IIIa epitope is located in the extracellular domain of the beta subunit of GPIIb/IIIa,

37. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 29 to 36, wherein the antibody or antigen-binding molecule thereof does not compete with fibrinogen for binding to GPIIb/IIIa.

38. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 73, 76, 79, 85, or 147;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 74, 77, 80, 86, or 148;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 75, 78, 81, 87, or 149;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 141, 144, 82, 88, or 150;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 142, 145, 83, 89, or 151; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to any one of SEQ ID NO: 143, 146, 84, 90, or 152.

39. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a VH-CDR1 comprising the consensus sequence TSGX_nGVG, wherein X₁₁ represents amino acid residues Met (M) or Leu (L);

(ii) a VH-CDR2 comprising the consensus sequence HIWWDDDKRYNPX₁₂LKS, wherein X₁₂ represents amino acid residues Ala (A) or Thr (T);

(iii) a VH-CDR3 comprising the consensus sequence SHYX₁₃GTFYFDX₁₄, wherein X₁₃ represents amino acid residues Tyr (Y) or Asn (N), and X₁₄ represents amino acid residues Tyr (Y) or Phe (F);

(iv) a VL-CDR1 comprising the sequence RASKSISKYLA (SEQ ID NO: 82);

(v) a VL-CDR2 comprising the sequence SGSTLQS (SEQ ID NO: 83); and,

(vi) a VL-CDR3 comprising the sequence QQHIEYPWT (SEQ ID NO: 84).

40. The antibody or antigen-binding molecule thereof according to claim 39, comprising

- (i) a VH-CDR1 sequence selected from SEQ ID NOS: 73, 76, 79, 85, or 147;
- (ii) a VH-CDR2 sequence selected from SEQ ID NOS: 74, 77, 80, 86, or 148;
- (iii) a VH-CDR3 sequence selected from SEQ ID NOS: 75, 78, 81, 87, or 149;
- (iv) a VL-CDR1 sequence selected from SEQ ID NOS: 141, 144, 82, 88, or 150;
- (v) a VL-CDR2 sequence selected from SEQ ID NOS: 142, 145, 83, 89, or 151; and,
- (vi) a VL-CDR3 sequence selected from SEQ ID NOS: 143, 146, 84, 90, or 152.

41. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 17, 18, 19, 21, or 109 and a VL comprising an amino acid sequence at least 80%, 85%, 90%, 95%, or 100% identical to any one of SEQ ID NOS: 107, 108, 20, 22, or 110.

42. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 38 to 41, wherein the VH comprises the amino acid sequence of SEQ ID NO: 17 and the VL comprising the amino acid sequence of SEQ ID NO: 107 (5C4 antibody).

43. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 38 to 41, wherein the VH comprises the amino acid sequence of SEQ ID NO: 18 and the VL comprising the amino acid sequence of SEQ ID NO: 108 (23C10 antibody).

44. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 38 to 41, wherein the VH comprises the amino acid sequence of SEQ ID NO: 109 and the VL comprising the amino acid sequence of SEQ ID NO: 110 (37C7 antibody).

45. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 38 to 41, wherein the VH comprises the amino acid sequence of SEQ ID NO: 19 and the VL comprising the amino acid sequence of SEQ ID NO: 20 (28C2 antibody).

46. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 38 to 41, wherein the VH comprises the amino acid sequence of SEQ ID NO: 21 and the VL comprising the amino acid sequence of SEQ ID NO: 22 (9D6 antibody).

47. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 38 to 46, wherein the GPIIb/IIIa epitope is located in the extracellular domain of the beta subunit of GPIIb/IIIa.

48. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 38 to 47, wherein the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

49. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a variable heavy chain CDR-1 (VH-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 91;

(ii) a variable heavy chain CDR-2 (VH-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 92;

(iii) a variable heavy chain CDR-3 (VH-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 93;

(iv) a variable light chain CDR-1 (VL-CDR1) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 94;

(v) a variable light chain CDR-2 (VL-CDR2) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 95; and,

(vi) a variable light chain CDR-3 (VL-CDR3) sequence at least about 60%, 70%, 80%, 90%, 95%, or 100% identical to SEQ ID NO: 96.

50. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising

(i) a VH-CDR1 sequence comprising SEQ ID NO: 91;

(ii) a VH-CDR2 sequence comprising SEQ ID NO: 92;

(iii) a VH-CDR3 sequence comprising SEQ ID NO: 93;

- (iv) a VL-CDR1 sequence comprising SEQ ID NO: 94;
- (v) a VL-CDR2 sequence comprising SEQ ID NO: 95; and,
- (vi) a VL-CDR3 sequence comprising SEQ ID NO: 96.

51. An antibody or antigen-binding molecule thereof which specifically binds to a GPIIb/IIIa epitope, comprising a VH comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 23 and a VL comprising an amino acid sequence at least about 80%, 85%, 90%, 95%, or 100% identical to SEQ ID NO: 24 (28F4 antibody).

52. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 49 to 51, wherein the GPIIb/IIIa epitope is located in the extracellular domain of the beta subunit of GPIIb/IIIa.

53. The antibody or antigen-binding molecule thereof of any one of claims 1, 2, and 49 to 52, wherein the antibody or antigen-binding molecule thereof competes with fibrinogen for binding to GPIIb/IIIa.

54. The antibody or antigen-binding molecule thereof of any one of claims 1 to 53, wherein the antibody or antigen-binding molecule thereof comprises or consists of:

- (a) a single chain Fv ("scFv");
- (b) a diabody;
- (c) a minibody;
- (d) a polypeptide chain of an antibody;
- (e) F(ab')₂; or,
- (f) F(ab).

55. A chimeric molecule comprising,

- (i) the antibody or antigen-binding molecule thereof of any one of claims 1 to 54;
- (ii) a heterologous moiety; and,
- (iii) an optional linker.

56. The chimeric molecule of claim 55, which comprises a formula:

- (i) Tm-(L1)-HI; or
- (ii) HI-(L1)-Tm,

wherein,

HI is the heterologous moiety;

L1 is the optional linker; and,

Tm is the antibody or antigen-binding molecule thereof of any one of claims 1 to 53.

57. The chimeric molecule according to claim 56, wherein the heterologous moiety comprises a clotting factor.

58. The chimeric molecule according to claim 57, wherein the clotting factor is independently selected from the group consisting of FVII, FIX, FX, and any combinations thereof.

59. The chimeric molecule according to claim 58, wherein the clotting factor is FVII zymogen, activatable FVII, activated FVII (FVIIa), FIX zymogen, activatable FIX, activated FIX (FIXa), FX zymogen, activatable FX, or activated FX (FXa).

60. The chimeric molecule according to any one of claims 55 to 59, further comprising a second heterologous moiety.

61. The chimeric molecule according to claim 60, which comprises a formula selected from:

- (i) HI-(L1)-Tm-(L2)-H2;
- (ii) H2-(L2)-Tm-(L1)-HI;
- (iii) HI-(L1)-H2-(L2)-Tm;
- (iv) H2-(L2)-HI-(L1)-Tm;
- (v) Tm-(L1)-HI-(L2)-H2; or,
- (vi) Tm-(L2)-H2-(L1)-HI

wherein,

Tm is the antibody or antigen-binding molecule thereof of any one of claims 1 to 54;

H1 is the first heterologous moiety,
H2 is the second heterologous moiety,
L1 is the first optional linker,
L2 is the second optional linker.

62. The chimeric molecule according to claim 60 or 61, wherein the first heterologous moiety and the second heterologous moiety are the same or different.

63. The chimeric molecule according to any one of claims 60 to 62, wherein the second heterologous moiety comprise a half-life extending moiety.

64. The chimeric molecule according to any one of claims 61 to 63, wherein L1 and L2 are the same or different.

65. The chimeric molecule according to claim 61, wherein the first heterologous moiety comprises a clotting factor and the second heterologous moiety comprises a half-life extending moiety.

66. The chimeric molecule according to any one of claims 60 to 65, wherein the second heterologous moiety comprises a low-complexity polypeptide.

67. The chimeric molecule according to any one of claims 60 to 65, wherein the second heterologous moiety comprises albumin, albumin binding polypeptide or fatty acid, Fc, 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, a clearance receptor or fragment thereof which blocks binding of the chimeric molecule to a clearance receptor or any combinations thereof.

68. The chimeric molecule according to any one of claims 60 to 67, wherein the clotting factor comprises a single polypeptide chain or two polypeptide chains.

69. A chimeric protein 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 and a heterologous moiety, and the second polypeptide chain comprises a heavy chain of the clotting factor and the antibody or antigen-binding molecule thereof of any one of claims 1 to 54;

(2) wherein the first polypeptide chain comprises a light chain of a clotting factor and the antibody or antigen-binding molecule thereof of any one of claims 1 to 54 and the second polypeptide chain comprises a heavy chain of the clotting factor and a heterologous moiety;

(3) wherein the first polypeptide chain comprises a light chain of a clotting factor and the second polypeptide chain comprises a heavy chain of the clotting factor, the antibody or antigen-binding molecule thereof of any one of claims 1 to 54, and a heterologous moiety;

(4) wherein the first polypeptide chain comprises a light chain of a clotting factor and the second polypeptide chain comprises a heavy chain of the clotting factor, a heterologous moiety, and the antibody or antigen-binding molecule thereof of any one of claims 1 to 54;

(5) wherein the first polypeptide chain comprises a light chain of a clotting factor, a heterologous moiety, and the antibody or antigen-binding molecule thereof of any one of claims 1 to 54 and the second polypeptide chain comprises a heavy chain of the clotting factor; or

(6) wherein the first polypeptide chain comprises a light chain of a clotting factor, the antibody or antigen-binding molecule thereof of any one of claims 1 to 54, and a heterologous moiety and the second polypeptide chain comprises a heavy chain of the clotting factor.

70. The chimeric molecule of claim 69,

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

(2) wherein the first polypeptide chain comprises CF_L -Tm or Tm- CFL and the second polypeptide chain comprises CFH -H or H- CFH;

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

(4) wherein the first polypeptide chain comprises CFL and the second polypeptide chain comprises CFH -H-Tm or Tm-H- CFH;

(5) wherein the first polypeptide chain comprises CF_L -H-Tm or Tm-H- CFL and the second polypeptide chain comprises CFH; or

(6) wherein the first polypeptide chain comprises CF_L -Tm-H or H-Tm- CFL and the second polypeptide chain comprises CFH,

wherein CFL is the light chain of the clotting factor;

CFH is the heavy chain of the clotting factor;

Tm is the antibody or antigen-binding molecule thereof; or

H is the heterologous moiety.

71. The chimeric molecule according to claim 69 or 70, wherein the clotting factor is independently selected from FVII, FIX, or FX.

72. The chimeric molecule according to any one of claims 55 to 71, wherein one or more of the linkers comprise a peptide linker.

73. The chimeric molecule according to claim 72, wherein the peptide linker comprises at least two, 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.

74. The chimeric molecule according to claims 73, wherein the peptide linker comprises at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1100, at least 1200, at least 1300, at least 1400, at least 1500, at least 1600, at least 1700, at least 1800, at least 1900, or at least 2000 amino acids.

75. The chimeric molecule according to any one of claims 72 to 74, wherein 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.

76. The chimeric molecule according to any one of claims 55 to 75, wherein one or more of the linkers comprise a non-peptide linker.

77. The chimeric molecule according to claim 69 to 76, wherein the heterologous moiety comprise a half-life extending moiety comprising a low-complexity polypeptide.

78. The chimeric molecule according to claim 77, wherein the half-life extending moiety comprising albumin, albumin binding polypeptide or fatty acid, Fc, 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, a clearance receptor or fragment thereof which blocks binding of the chimeric molecule to a clearance receptor, or any combinations thereof.

79. The chimeric molecule according to claim 78, wherein the half-life extending moiety comprises an Fc region.

80. The chimeric molecule according to claim 79, wherein the half-life extending moiety comprises two Fc regions fused by a linker.

81. The chimeric molecule according to any one of claims 69 to 80, wherein the clotting factor comprises FVII zymogen, activatable FVII, activated FVII, FIX zymogen, activatable FIX, activated FIX, FX zymogen, activatable FX, or activated FX.

82. 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 and a targeting moiety, which binds to a platelet, and the second polypeptide chain comprises a heavy chain of the clotting factor and a heterologous moiety;

(2) wherein the first polypeptide chain comprises a light chain of a clotting factor and a heterologous moiety and the second polypeptide chain comprises a heavy chain of the clotting factor and a targeting moiety, which binds to a platelet;

(3) wherein the first polypeptide chain comprises a light chain of a clotting factor, a heterologous moiety, and a targeting moiety, which binds to a platelet, and the second polypeptide comprises a heavy chain of the clotting factor; or

(4) wherein the first polypeptide chain comprises a light chain of a clotting factor, a targeting moiety, which binds to a platelet, and a heterologous moiety and the second polypeptide chain comprises a heavy chain of the clotting factor, and

wherein the clotting factor is FVII, FIX, or FX.

83. The chimeric molecule of claim 82,

(1) wherein the first polypeptide chain comprises CF_L-L1-H or $H-L1-CF_L$ and the second polypeptide chain comprises $CFH-L2-Tm$ or $Tm-L2-CFH$;

(2) wherein the first polypeptide chain comprises $CF_L-L1-Tm$ or $Tm-L1-CF_L$ and the second polypeptide chain comprises $CFH-L2-H$ or $H-L2-CFH$;

(3) wherein the first polypeptide chain comprises $CF_L-L1-L2-Tm$ or $Tm-L1-L2-CF_L$ and the second polypeptide chain comprises CF_H ; or

(4) wherein the first polypeptide chain comprises $CF_L-L1-Tm-L2-H$ or $H-L1-Tm-L2-CF_L$ and the second polypeptide chain comprises CF_H ;

wherein

H is the heterologous moiety;

CF_H is the heavy chain of the clotting factor;

CF_L is the light chain of the clotting factor;

Tm is the targeting moiety, which binds to a platelet;

L1 is a first optional linker; and

L2 is a second optional linker.

84. The chimeric molecule of claim 82 or 83, comprising a formula

(1) $Tm-L1-CF_H:CF_L-L2-H$;

(2) $H-L1-CF_L:CF_H-L2-Tm$;

(3) $Tm-L1-H-L2-CF_L:CF_H$; or

(4) $H-L1-Tm-L2-CF_L:CF_H$,

wherein,

H is the heterologous moiety;

CFH is the heavy chain of the clotting factor;

CF_L is the light chain of the clotting factor;

Tm is the targeting moiety, which binds to a platelet,

L1 is a first optional linker;

L2 is a second optional linker; and

: represents a covalent or non-covalent bond between CFH and CF_L .

85. The chimeric molecule according to claim 82 or 83, wherein the association between the first polypeptide chain and the second polypeptide chain is a covalent bond or a non-covalent bond.

86. The chimeric molecule according to claim 85, wherein the association between the first polypeptide chain and the second polypeptide chain is a covalent bond between the heavy chain and the light chain of the clotting factor.

87. The chimeric molecule of claim 86, wherein the covalent bond is a disulfide bond.

88. A chimeric molecule comprising a single polypeptide chain, which comprises, from N terminus to C terminus, (a) a light chain of a clotting factor, a heterologous moiety, a protease cleavage site, a heavy chain of the clotting factor, and a targeting moiety which binds to a platelet or (b) a light chain of a clotting factor, a targeting moiety which binds to a platelet, a protease cleavage site, a heavy chain of the clotting factor, and a heterologous moiety, wherein the clotting factor is FVII, FIX, or FX.

89. The chimeric molecule of claim 88, wherein the protease cleavage site is an intracellular processing site.

90. The chimeric molecule of claim 89, wherein the intracellular processing site is processed by a proprotein convertase.

91. The chimeric molecule of claim 90, wherein the proprotein convertase is selected from the group consisting of PC5, PACE, PC7, and any combinations thereof.

92. The chimeric molecule of any one of claims 82 to 91, wherein the targeting moiety is selected from: an antibody or antigen binding molecule thereof, a receptor binding portion of a receptor, and a peptide.

93. The chimeric molecule of claim 92, wherein the targeting moiety selectively binds to a resting platelet or an activated platelet.

94. The chimeric molecule of any one of claims 82 to 93, wherein the targeting moiety selectively binds to a target selected from the group consisting of: GPIIb, GPVI, GPIX, a nonactive form of glycoprotein IIb/IIIa ("GPIIb/IIIa"), an active form of GPIIb/IIIa, P selectin, GMP-33, LAMP-1, LAMP-2, CD40L, LOX-1, and any combinations thereof.

95. The chimeric molecule of claim 94, wherein the targeting moiety is an anti-GPIIb/IIIa antibody or antigen-binding molecule thereof.

96. The chimeric molecule of claim 95, wherein the anti-GPIIb/IIIa antibody or antigen-binding molecule thereof is the antibody or antigen-binding molecule thereof of any one of claims 1 to 53.

97. The chimeric molecule of any one of claims 82 to 96, wherein the heterologous moiety is a half-life extending moiety.

98. The chimeric molecule of claim 97, wherein the half-life extending moiety is a low-complexity polypeptide.

99. The chimeric molecule of claim 97, wherein the half-life extending moiety is selected from the group consisting of albumin, albumin binding polypeptide or fatty acid, Fc, 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, a clearance receptor or fragment thereof which blocks binding of the chimeric molecule to a clearance receptor, and any combinations thereof.

100. A nucleic acid molecule or a set of nucleic acid molecules encoding an antibody or antigen-binding molecule thereof of any one of claims 1 to 54 or the chimeric molecule according to any one of claims 55 to 99 or a complement thereof.

101. A vector or a set of vectors comprising the nucleic acid molecule or the set of the nucleic acid molecules of claim 100 or a complement thereof.

102. A host cell comprising the vector according to claim 101.

103. A method for producing a chimeric molecule comprising culturing the host cell according to claim 102 and recovering the antibody or antigen-binding molecule thereof or the chimeric molecule from the culture medium.

104. A pharmaceutical composition comprising the antibody or antigen-binding molecule thereof of any one of claims 1 to 54 or the chimeric molecule of any one of claims 55 to 99, the nucleic acid molecule or the set of nucleic acid molecules according to claim 100, the vector or the set of vectors according to claim 101 and a pharmaceutically acceptable carrier.

105. A method of reducing a frequency or degree of a bleeding episode in a subject in need thereof comprising administering the chimeric molecule of any one of claims 55 to 99, the nucleic acid molecule or the set of nucleic acid molecules according to claim 100, the vector or the set of vectors according to claim 101, or the pharmaceutical composition according to claim 104.

106. A method of reducing or preventing an occurrence of a bleeding episode in a subject in need thereof comprising administering the chimeric molecule of any one of claims 55 to 99, the nucleic acid molecule or the set of nucleic acid molecules according to claim 100, the vector or the set of vectors according to claim 101, or the pharmaceutical composition according to claim 104.

107. The method of any one of claim 105 or 106, wherein the subject has developed or has a tendency to develop an inhibitor against Factor VIII ("FVIII"), Factor IX ("FIX"), or both.

108. The method of claim 107, wherein the inhibitor against FVIII or FIX is a neutralizing antibody against FVIII, FIX, or both.

109. The method according to any one of claims 105 to 108, wherein the bleeding episode is caused by a blood coagulation disorder.

110. The method according to claim 109, wherein the blood coagulation disorder is hemophilia A or hemophilia B.

111. The method according to any one of claims 105 to 110, wherein the bleeding episode is derived from 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.

112. The method according to any one of claims 105 to 111, wherein the subject is a human subject.

113. The chimeric molecule of any one of claims 55 to 99, the nucleic acid molecule or the set of nucleic acid molecules according to claim 100, the vector or the set of vectors according to claim 101, or the pharmaceutical composition according to claim 104 for use in reducing a frequency or degree of a bleeding episode or reducing or preventing an occurrence of a bleeding episode in a subject in need thereof.

114. Use of the chimeric molecule of any one of claims 55 to 99, the nucleic acid molecule or the set of nucleic acid molecules according to claim 100, the vector or the set of vectors according to claim 101, or the pharmaceutical composition according to claim 104 for the manufacture of a medicament for reducing a frequency or degree of a bleeding episode or reducing or preventing an occurrence of a bleeding episode in a subject in need thereof.

115. A method to target a therapeutic or prophylactic agent to the surface of platelets comprising fusing the agent to the antibody or antigen-binding molecule thereof according to any one of claims 1 to 54.

116. A method to increase the activity of a therapeutic or prophylactic agent comprising fusing the agent to the antibody or antigen-binding molecule thereof according to any of claims 1 to 54.

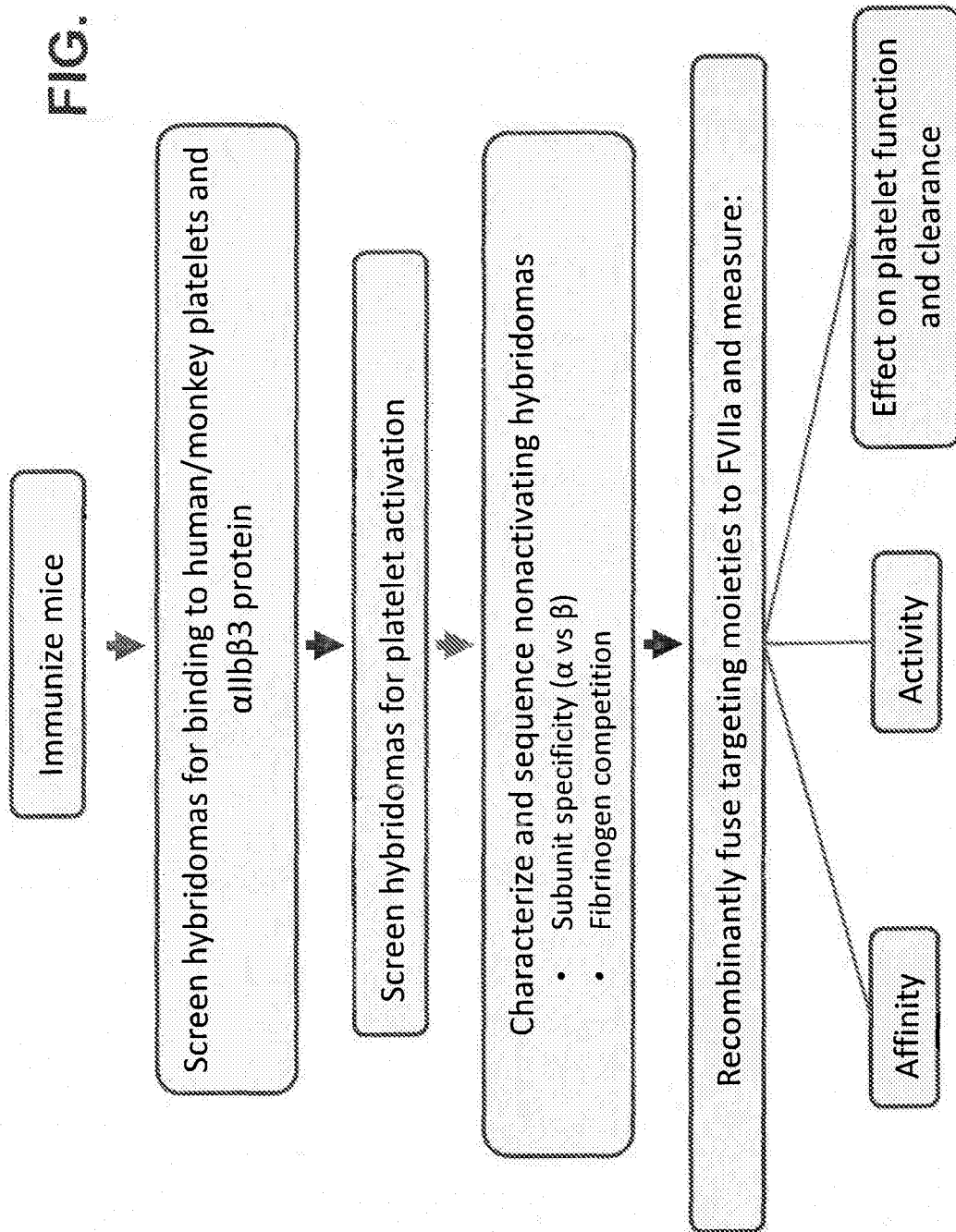
117. The method according to any one of claim 115 or 116, wherein therapeutic or prophylactic agent is a clotting factor.

118. A method to improve the pharmacokinetic properties of a clotting factor comprising fusing the clotting factor to the antibody or antigen-binding molecule thereof according to any of claims 1 to 54.

119. The method according to claim 118, further comprising fusing the clotting factor and/or the antibody or antigen-binding molecule thereof according to any of claims 1 to 54 to a half-life extending moiety.

120. A method of measuring the level of platelets in plasma of a subject in need thereof comprising contacting the antibody or antigen binding molecule thereof of any one of claims 1 to 54 with the plasma from the subject and measuring the plasma level.

FIG. 1



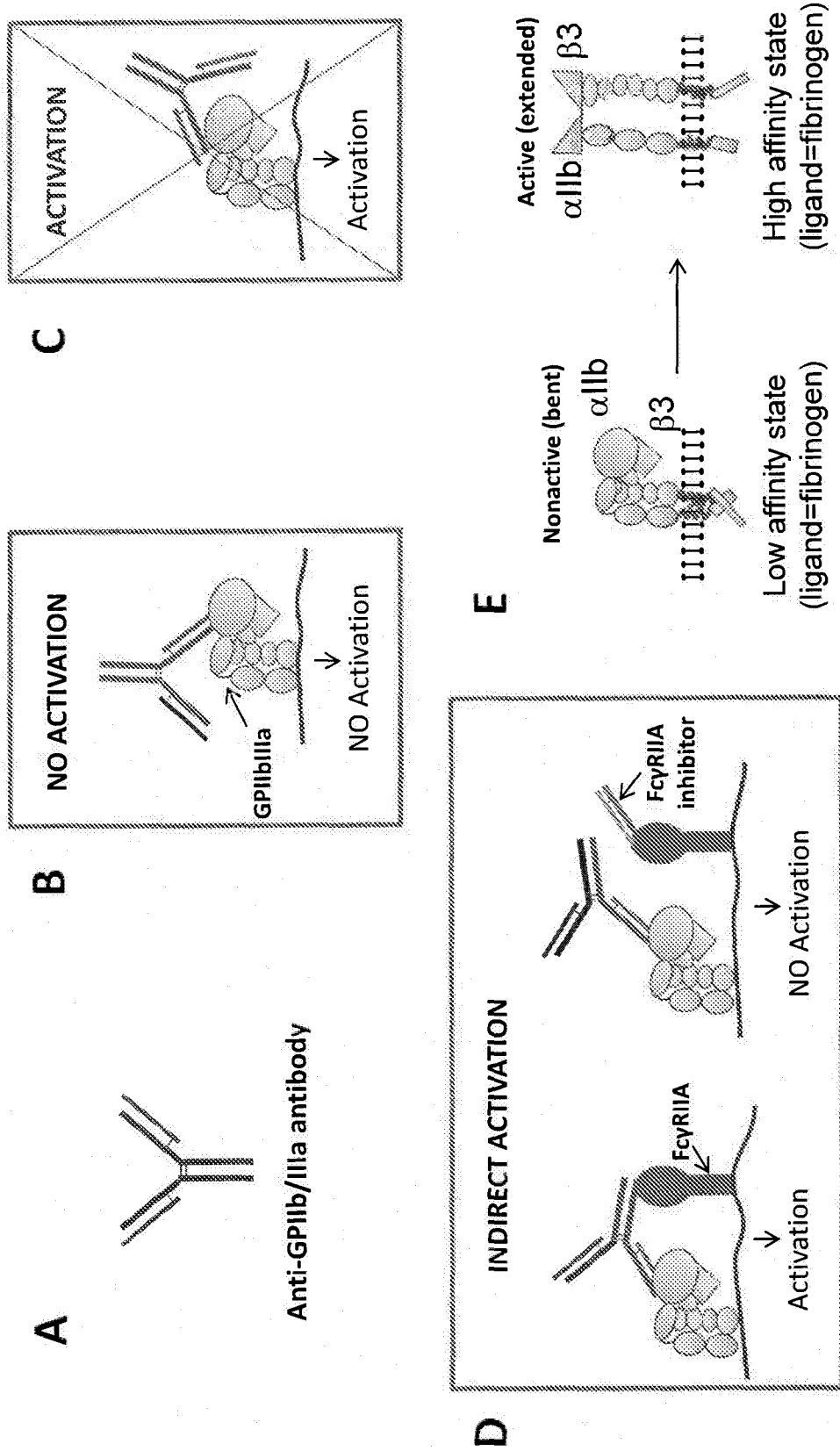
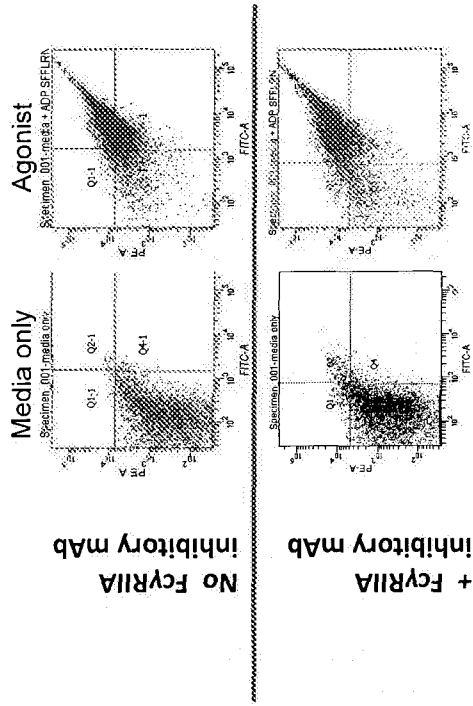
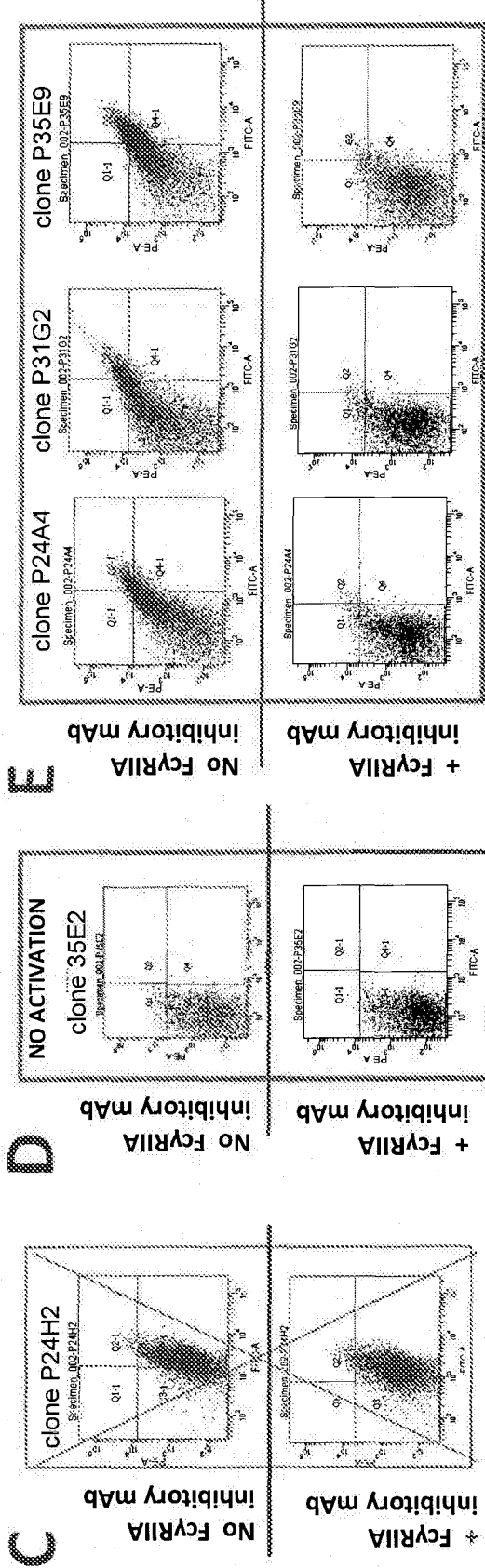
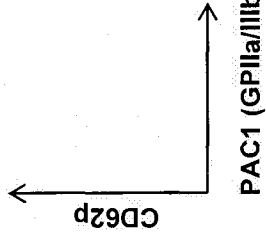


FIG. 2



B

A Platelet activation assay
 (flow cytometry)



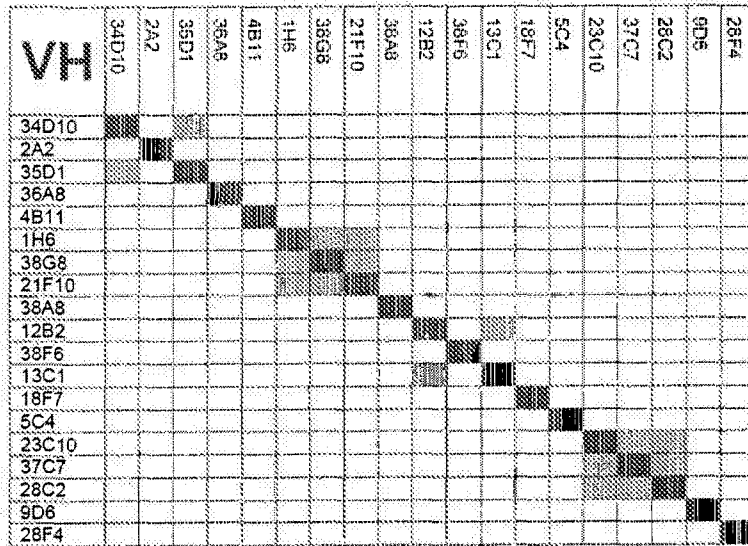
E

D

C

FIG. 3

A



B

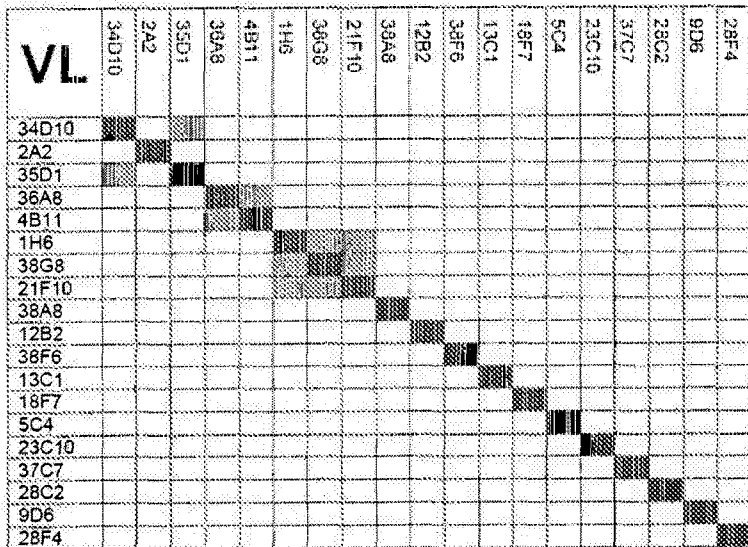
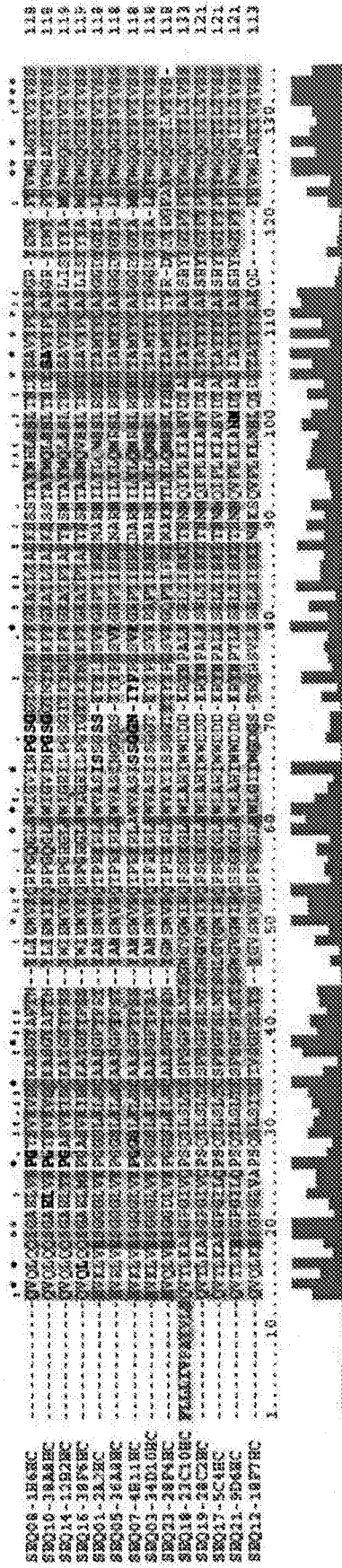


FIG. 4

A



B

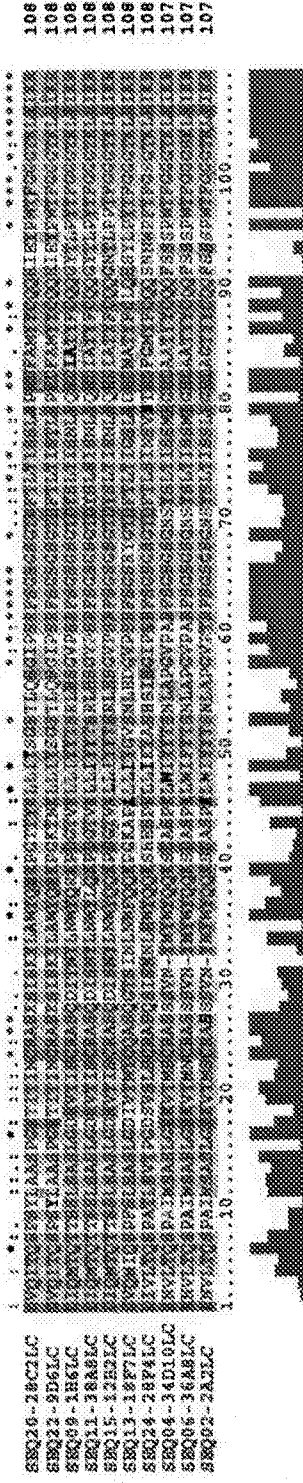


FIG. 5

VH SEQUENCES – Full Alignment (All Sequenced VH and VL Included)

CLUSTAL 2.1 multiple sequence alignment

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                                Kabat 31-35
                                CDR1
SEQ08-1H6HC      -----QVQLQQSGAELVRPGTSVKVSCKASGYAFN--YLIENWVNRPGGGL
SEQ10-38A8HC     -----QVQLQQSGAELVRPGTSVKVSCKASGYAFN--YLIENWVNRPGGGL
SEQ14-12B2HC     -----QVQLQQSGAELTKPGASVKISCKATGYTFSS--YWIENWVNRPGHGLE
SEQ16-38F6HC     -----QVQLQQSGAELMRPGASVKISCKATGYTFSS--YWIENWVNRPGHGLE
SEQ03-2A2HC      -----EVRLVESGGGLVKPGGSLKLSCAASGFTFST--YAMSVVWVQTPEKRL
SEQ05-36A8HC     -----EVRLVESGGGLVKPGGSLKLSCAASGFTFST--YAMSVVWVQTPEKRL
SEQ07-4B11HC     -----EVRLVESGGGLVKPGGSLKLSCAASGFTFSS--YAMSVVWVQTPEKRL
SEQ01-34D10HC    -----EVRLVESGGGLVKPGGSLKLSCAASGFTFSS--YAMSVVWVQTPEKRL
SEQ23-28F4HC     -----EVQLVESGGDLVKPGGSLKLSCAASGFTFSS--YGMSSVWVQTPEKRL
SEQ18-23C10HC    FLLLVIPAYVLSQVTLKASGPGIVQPSQTLSSLTCSFSGFSLNLSGGMGVGNIRQPSGKGL
SEQ19-28C2HC     -----QVTLKASGPGIVQPSQTLSSLTCSFSGFSLNLSGGMGVGNIRQPSGKGL
SEQ17-5C4HC      -----QVTLKASGPGIQLQPSQTLSSLTCSFSGFSLNLSGGLGVGNIRQPSGKGL
SEQ21-9D6HC      -----QVTLKESGPGIQLQPSQTLSSLTCSFSGFSLNLSGGMGVGNIRQPSGKGL
SEQ12-18F7HC     -----QVQLKESGPGLVAPSSQLSITCTVSGFSLNLS--YGVSSVWVROPKGL
                                : * * * * : * . : : : * : * : : : ( : * * * . : *

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                                Kabat 50-65          Kabat 95-102
                                CDR2              CDR3
SEQ08-1H6HC      WIRVINGPSSGDTNYNEKFKKSAITLADKSSSTAYMHLSSLTSDSVAVYFCARER-YEWY
SEQ10-38A8HC     WIRVINGPSSGDTNYNEKFKKSAITLADKSSSTAYMQLSSLTSDSVAVYFCARER-YEWY
SEQ14-12B2HC     WIRVILPSSGDTKYNDKFKKSAITLADTSSNTAYMQLSSLTSEDSVAVYFCARERLSYYYA
SEQ16-38F6HC     WIRVILPSSGDTKYNEKFKKSAITLADTSSNTAYMQLSSLTSEDSVAVYFCARERLSYYYA
SEQ03-2A2HC      WYVSISSGGS-TYYPLDSVKRFTISRDNARNILYLQMSLRSEDTAMYYCARGDYGYA
SEQ05-36A8HC     WYVSISSGGS-TYYPLDSVKRFTISRDNARNILYLQMSLRSEDTAMYYCARGDYGYA
SEQ07-4B11HC     WYVSISSGGS-TYYPLDSVKRFTISRDNARNILYLQMSLRSEDTAMYYCARGDYGYA
SEQ01-34D10HC    WYVSISSGGS-TYYPLDSVKRFTISRDNARNILYLQMSLRSEDTAMYYCARGDYGYA
SEQ23-28F4HC     WYVSISSGGS-TYYPLDSVKRFTISRDNARNILYLQMSLRSEDTAMYYCARGDYGYA
SEQ18-23C10HC    WLSHIWDDDD-KRYNPAKLSRLTISKDTSNNQIFLKIASVDTADTATYYCASHYNGTF
SEQ19-28C2HC     WLSHIWDDDD-KRYNPAKLSRLTISKDTSNNQIFLKIASVDTADTATYYCASHYNGTF
SEQ17-5C4HC      WLSHIWDDDD-KRYNPAKLSRLTISKDTSNNQIFLKIASVDTADTATYYCASHYNGTF
SEQ21-9D6HC      WLSHIWDDDD-KRYNPAKLSRLTISKDTSNNQIFLKIASVDTADTATYYCASHYNGTF
SEQ12-18F7HC     WLSHIWDDGS-TNYHSLKLSRLSISKDTSKSVFLKLSLQTDADTATYYCASHYNGTF
                                * : * . : * . : : : * : : : * : : : * * * * :

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```

SEQ08-1H6HC      FDVNGAGTTVTVSS
SEQ10-38A8HC     FDVNGAGTTVTVSS
SEQ14-12B2HC     MDVNGGQTSVTVSS
SEQ16-38F6HC     MDVNGGQTSVTVSS
SEQ03-2A2HC      LDVNGGQTSVTVSS
SEQ05-36A8HC     LDVNGGQTSVTVSS
SEQ07-4B11HC     MDVNGGQTSVTVSS
SEQ01-34D10HC    LDVNGGQTSVTVSS
SEQ23-28F4HC     FAVNGGQTLVTVSS
SEQ18-23C10HC    FDVNGGQTTLVVSS
SEQ19-28C2HC     FDVNGGQTTLVVSS
SEQ17-5C4HC      FDVNGGQTTLVVSS
SEQ21-9D6HC      FDVNGGQTTLVVSS
SEQ12-18F7HC     FDVNGAGTTVTVSS
                                ( * * * ; * *

```

* - Identical
: - Conserved
. - Partially conserved

FIG. 6

VL SEQUENCES - Percent Identity Matrix

	1	2	3	4	5	6	7	8	9	10
1: SEQ20-28C2IC	100	97	66	65	67	62	60	59	58	59
2: SEQ22-9D6IC	97	100	66	65	67	62	59	58	57	58
3: SEQ09-1H6IC	66	66	100	96	96	66	58	63	60	63
4: SEQ11-38A8IC	65	65	96	100	96	66	60	62	59	62
5: SEQ15-12B2IC	67	67	96	96	100	67	61	64	61	64
6: SEQ13-18F7IC	62	62	66	66	67	100	56	57	55	57
7: SEQ24-28F4IC	60	59	58	60	61	56	100	56	54	57
8: SEQ02-34D10IC	59	58	63	62	64	57	56	100	97	97
9: SEQ06-36A8IC	58	57	60	59	61	55	54	97	100	94
10: SEQ04-2A2IC	59	58	63	62	64	57	57	97	94	100

VH SEQUENCES - Percent Identity Matrix

Percent Identity Matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1: SEQ08-1H6HC	100	98	75	74	48	47	44	45	47	37	37	36	33	50
2: SEQ10-38A8HC	98	100	75	74	48	47	44	45	47	38	38	37	34	49
3: SEQ14-12B2HC	75	75	100	92	54	53	53	53	52	36	36	36	34	42
4: SEQ16-38F6HC	74	74	92	100	50	50	49	49	50	36	36	36	33	41
5: SEQ03-2A2HC	48	48	54	50	100	94	91	94	91	46	46	45	42	50
6: SEQ05-36A8HC	47	47	53	50	94	100	92	93	81	46	46	45	42	51
7: SEQ07-4B11HC	44	44	53	49	91	92	100	92	79	43	43	42	40	47
8: SEQ01-34D10HC	45	45	53	49	94	93	92	100	84	44	44	43	41	49
9: SEQ23-28F4HC	47	47	52	50	81	81	79	84	100	41	41	40	38	49
10: SEQ18-23C10HC	37	38	36	36	46	46	43	44	41	100	100	98	90	65
11: SEQ19-28C2HC	37	38	36	36	46	46	43	44	41	100	100	98	90	65
12: SEQ17-5C4HC	36	37	36	36	45	45	42	43	40	98	100	98	90	64
13: SEQ21-9D6HC	33	34	34	33	42	42	40	41	38	90	90	90	100	62
14: SEQ12-18F7HC	50	49	42	41	50	51	47	49	49	65	65	64	62	100

FIG. 8

VH Sequences Clustered According to Alpha/Beta Specificity

Only sequences with both VH and VL sequenced included

CLUSTAL 2.1 multiple sequence alignment

ALPHA SUBUNIT SPECIFIC

	Kabat 31-35 CDR1
SEQ08-1H6HC	-----QVQLQQSGAELVLRPGTSVKVSKKASGYAFTN-- YLI EVVKQRPGQGLE
SEQ10-38A8HC	-----QVQLQQSGAELVLRPGTSVKVSKKASGYAFTN-- YLI EVVKQRPGQGLE
SEQ03-2A2HC	-----EVKLVEGGGLVLRPGGSLKLSKAASGFTFST-- YAMS WVRQTPEKRL
SEQ05-36A8HC	-----EVLVEGGGLVLRPGGSLKLSKAASGFTFST-- YAMS WVRQTPEKRL
SEQ01-34D10HC	-----EVKLVEGGGLVLRPGGSLKLSKAASGFTFSA-- YAMS WVRQTPEKRL
SEQ12-18F7HC	-----QVQLKESGPGLVAPSQSLTITCTVSGFSLTIS-- YGV SWVRQPPGKGL

	Kabat 50-65 CDR2	Kabat 95-102 CDR3
SEQ08-1H6HC	WIG EL EPSSGTTNRRRRTT KAT LTADKSSSTAYMHLSSLTSDDSAVYFCAR ER TEWY	
SEQ10-38A8HC	WISVINPGSGGTYNYNEKFK KAT LTADKSSSTAYMQLSSLTSDDSAVYFCAR ER YENY	
SEQ03-2A2HC	WV S ISSGSS-TYYLDSVK GRFTI SRDNARNILYLMSSLRSEDTAMYCAR GGD YGYA	
SEQ05-36A8HC	WV S INGGGS-TYYLDSVK GRFTI SRDNARNILYLMSSLRSEDTAMYCAR GGD YGYA	
SEQ01-34D10HC	WV S ISSGGT-TYYLDSVK GRFTI SRDNARNILYLMSSLRSEDTAMYCT GGD YGYA	
SEQ12-18F7HC	WLS HI WDDDD-KRYNE ELK SRLTISKDTSNNQVFLKIANMDTADTATYYCAR SHY NGTFY	

SEQ08-1H6HC	FDV WGAGTIVTVSS
SEQ10-38A8HC	FDV WGAGTIVTVSS
SEQ03-2A2HC	LDY WGQGTIVTVSS
SEQ05-36A8HC	LDY WGQGTIVTVSS
SEQ01-34D10HC	LDY WGQGTIVTVSS
SEQ12-18F7HC	FDV WGAGTIVTVSS

BETA SUBUNIT SPECIFIC

	Kabat 31-35 CDR1
SEQ14-12B2HC	-----QVQLQQSGAELTKPGASVKISCKATGYTFSN-- YWI EVVKQRPGHGLE
SEQ23-28F4HC	-----EVQLVESGGDLVLRPGGSLKLSKAASGFTFSA-- YGM SWVRQTPDKRLE
SEQ19-28C2HC	-----QVTLKASGPGIVQPSQTLTLTCSFSGFSLT ISGM GVGNIROPSGKGLE
SEQ21-9D6HC	-----QVTLKESGPGILQPSQTLTLTCSFSGFSLT ISGM GVGNIROSSGKGLE

	Kabat 50-65 CDR2	Kabat 95-102 CDR3
SEQ14-12B3HC	WIG EL EPSSGTTNRRRRTT KAT FTADTSSNTAYMQLSSLTSEDSAVYSCAR LI SYIYA	
SEQ23-28F4HC	WV S ISSGGTYTYLDSVK GRFTI FRDNAKNTLYLMSSLKSEDTAMYCT ER DYDEYEG	
SEQ19-28C2HC	W L SIHWDDDD-KRYNE ELK SRLTISKDTSNNQVFLKIANMDTADTATYYCAR SHY NGTFY	
SEQ21-9D6HC	W L SIHWDDDD-KRYNE ELK SRLTISKDTSNNQVFLKIANMDTADTATYYCAR SHY NGTFY	

SEQ23-28F4HC	FAY WGQGTILTVSS
SEQ19-28C2HC	FDE WGQGTILTVSS
SEQ21-9D6HC	FDE WGQGTILTVSS
SEQ14-12B2HC	MDY WGQGTIVTVSS

FIG. 9

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VL Sequences Clustered According to Alpha/Beta Specificity

Only sequences with both VH and VL sequenced included

CLUSTAL 2.1 multiple sequence alignment

ALPHA SUBUNIT SPECIFIC

	Kabat 24-34 CDR1	Kabat 50-56 CDR2
SEQ09-1H6LC	DIQMTQTTSSLSASLGDRVTISCRASQSIINWYLNWYQKPKDGTVKLLI	YTSRLES
SEQ11-38A8LC	DIQMTQTTSSLSASLGDRVTISCRASQSIINWYLNWYQKPKDGTVKLLI	YTSRLES
SEQ13-18F7LC	DVQMIQSPFSLASLGDIVTMTCSASQGTISINLNWFOOKPGKAPKLLI	YVSNLES
SEQ02-34D10LC	ENVLTQSPAIMASLGEKVTMSCRASSSVN-YMYWYQOKSDASPRLWI	YTSNLA
SEQ06-36A8LC	ENVLTQSPAIMASLGEKVTMNCRASSSVN-YMYWYQOKSDASPRLWI	YTSNLA
SEQ04-2A2LC	ENVLTQSPAIMASLGEKVTMSCRASSSVN-YMYWYQOKSDASPRLWI	YTSNLA

	Kabat 89-97 CDR3
SEQ09-1H6LC	RFSGSGSGTDYSLTISNLEQEDIATYFCQQGKTLFPTFGGGTKLEIKR
SEQ11-38A8LC	RFSGSGSGTDYSLTISNLEQEDIATYFCQQGKTLFPTFGGGTKLEIKR
SEQ13-18F7LC	RFSGSRYGTDFTLTIGSLEDEDMATYFCQQHNYLPTFGGGTKLEIKR
SEQ02-34D10LC	RFSGSGSGNSYSLTISMEGEDAATYFCQQFSSSPWTFGGGTKLEIKR
SEQ06-36A8LC	RFSGSGSGNSYSLTISMEGEDAATYFCQQFSSSPWTFGGGTKLEIKR
SEQ04-2A2LC	RFSGSGSGNSYSLTISMEGEDAGTYFCQQFSSSPWTFGGGTKLEIKR

BETA SUBUNIT SPECIFIC

	Kabat 24-34 CDR1	Kabat 50-56 CDR2
SEQ20-28C2LC	DVQITQSPSYLAASPGETITINCRASKSISKYLAWYQKPKGTTKLLI	YSGSTLQSGIPS
SEQ22-9D6LC	DVQITQSPSYLAASPGETITINCRASKSISKYLAWYQKPKGTTKLLI	YSGSTLQSGIPS
SEQ15-12B2LC	DIQMTQTTSSLSASLGDRVTISCRASQSIINWYLNWYQKPKDGTVKLLI	YTSRLES
SEQ24-28F4LC	DIVLTQSPATLSVTPGDSVSLSCRASQSIINWYLNWYQKSHESPRLLI	YASHSISGIPS

	Kabat 89-97 CDR3
SEQ20-28C2LC	RFSGSGSGTDFLTISSLEPEDFAMYFCQQHIEYFPTFGGGTKLEIKR
SEQ22-9D6LC	RFSGSGSGTDFLTISTLEPEDFAMYFCQQHIEYFPTFGGGTKLEIKR
SEQ15-12B2LC	RFSGSGSGTDYSLTISNLEQEDIATYFCQQGKTLFPTFGGGTKLEIKR
SEQ24-28F4LC	RFSGSGSGTDFLTISNSVETEDFGMYFCQQSNWPFPTFGSGTKLEIKR

FIG. 10

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VH Sequence Clustered According to Competition with Fibrinogen

Only sequence with both VH and VL included

CLUSTAL O(1.1.0) multiple sequence alignment

No Fibrinogen Competition

	Kabat 31-35 CDR1	Kabat 50-65 CDR2
SEQ04-2A2HC	EVKLVESGGGLVKPGGSLKLSCAASGFTFR	YVNRQTPEKRLEWVA
SEQ01-34D10HC	EVKLVESGGGLVKPGGSLKLSCAASGFTFR	YVNRQTPEKRLEWVA
SEQ05-36A8HC	EVRLVESGGGLVKPGGSLKLSCAASGFTFR	YVNRQTPEKRLEWVA
SEQ14-12B2HC	QVQLQQSGAELTKPGASVKISCKATGYTFR	YVNRQTPDKRLEWVA

	Kabat 95-102 CDR3
SEQ04-2A2HC	LDSVKGKRTTISRDNARNILYLQMSLRSEDTAMYYCAR
SEQ01-34D10HC	PDSVKGKRTTISRDNARNILYLQMSLRSEDTAMYYCTR
SEQ05-36A8HC	PDSVKGKRTTISRDNARNILYLQMSLRSEDTAMYYCAR
SEQ14-12B2HC	NSKFKGKATFTADTSSNTAYMQLSLSLTSSEDSAVYSCAR

Fibrinogen Competition

	Kabat 31-35 CDR1	Kabat 50-65 CDR2
SEQ08-1H6HC	QVQLQQSGAELVLRPGTSVKVSKKASGYAFIN	YVNRQTPDKRLEWVA
SEQ10-38A8HC	QVQLQQSGAELVLRPGTSVKVSKKASGYAFIN	YVNRQTPDKRLEWVA
SEQ12-18F7HC	QVQLKESGPGLVAPQSLSITCTVSGFSLTS	YVNRQTPDKRLEWVA
SEQ23-28F4HC	EVLQVESGGDLVKPGGSLKLSCAASGFTFR	YVNRQTPDKRLEWVA
SEQ19-28C2HC	QVTLKASGPGIVQPSQTLTSLTCSFSGFSLT	YVNRQTPDKRLEWVA
SEQ21-9D6HC	QVTLKESGPGILQPSQTLTSLTCSFSGFSLT	YVNRQTPDKRLEWVA

	Kabat 95-102 CDR3
SEQ08-1H6HC	NYNEKFKGKATLTADKSSSTAYMHLSSLTSDDSAVYFCAR
SEQ10-38A8HC	NYNEKFKGKATLTADKSSSTAYMQLSLSLTSSEDSAVYFCAR
SEQ12-18F7HC	NYHSVLKSRLSISKDNSKQVFLKLNLSLQTDITATYYCAK
SEQ23-28F4HC	YYPDSVKGKQFTIFRDNAKNTLYLQMSLRSEDTAMYYCTR
SEQ19-28C2HC	RYNPALKSRILTISKDTSNNOIFLKIASVDTADTATYYCAR
SEQ21-9D6HC	RYNPILKSRILTISKDTSNNOVFLKIANMDTADTATYYCAR

SEQ08-1H6HC SS
 SEQ10-38A8HC SS
 SEQ12-18F7HC SS
 SEQ23-28F4HC S-
 SEQ19-28C2HC SS
 SEQ21-9D6HC SS
 *

FIG. 11

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VL Sequence Clustered According to Competition with Fibrinogen

Only sequence with both VH and VL included

CLUSTAL O (1.1.0) multiple sequence alignment

No Fibrinogen Competition

	Kabat 24-34 CDR1	Kabat 50-56 CDR2
SEQ04-2A2LC	ENVLTQSPAIMSASLGEKVTMS	ENVLTQSPAIMSASLGEKVTMS
SEQ02-34D10LC	ENVLTQSPAIMSASLGEKVTMS	ENVLTQSPAIMSASLGEKVTMS
SEQ06-36A8LC	ENVLTQSPAIMSASLGEKVTMS	ENVLTQSPAIMSASLGEKVTMS
SEQ15-12B2LC	DIQMTQTTSSLSASLGDRVTIS	DIQMTQTTSSLSASLGDRVTIS

	Kabat 89-97 CDR3
SEQ04-2A2LC	RFSGSGSGNSYSLTISSELEGEDAGTYFC
SEQ02-34D10LC	RFSGSGSGNSYSLTISSELEGEDAGTYFC
SEQ06-36A8LC	RFSGSGSGNSYSLTISSELEGEDAGTYFC
SEQ15-12B2LC	RFSGSGSGTDYSLTISNLEQEDIAIFYFC

Fibrinogen Competition

	Kabat 24-34 CDR1	Kabat 50-56 CDR2
SEQ09-1H6LC	DIQMTQTTSSLSASLGDRVTIS	DIQMTQTTSSLSASLGDRVTIS
SEQ13-18F7LC	DVQMIQSPFSLASLGDIVITMC	DVQMIQSPFSLASLGDIVITMC
SEQ11-38A8LC	DIQMTQTTSSLSASLGDRVTIS	DIQMTQTTSSLSASLGDRVTIS
SEQ20-28C2LC	DVQITQSPSYLAASPGETITINC	DVQITQSPSYLAASPGETITINC
SEQ22-9D6LC	DVQITQSPSYLAASPGETITINC	DVQITQSPSYLAASPGETITINC
SEQ24-28F4LC	DIVLTQSPATLSVTPGDSVLSLC	DIVLTQSPATLSVTPGDSVLSLC

	Kabat 89-97 CDR3
SEQ09-1H6LC	RFSGSGSGTDYSLTISNLEQEDIAIFYFC
SEQ13-18F7LC	RFSGSRYGDFILTIIGSLEEDMATIFYFC
SEQ11-38A8LC	RFSGSGSGTDYSLTISNLEQEDIAIFYFC
SEQ20-28C2LC	RFSGSGSGTDFILTISSLEPEDFAMYFC
SEQ22-9D6LC	RFSGSGSGTDFILTISSLEPEDFAMYFC
SEQ24-28F4LC	RFSGSGSGTDFILSINSVETEDFGMYFC

FIG. 12

Anti-GPIIb/IIIa Antibody in scFv format
Recombinantly fused to FVIIa

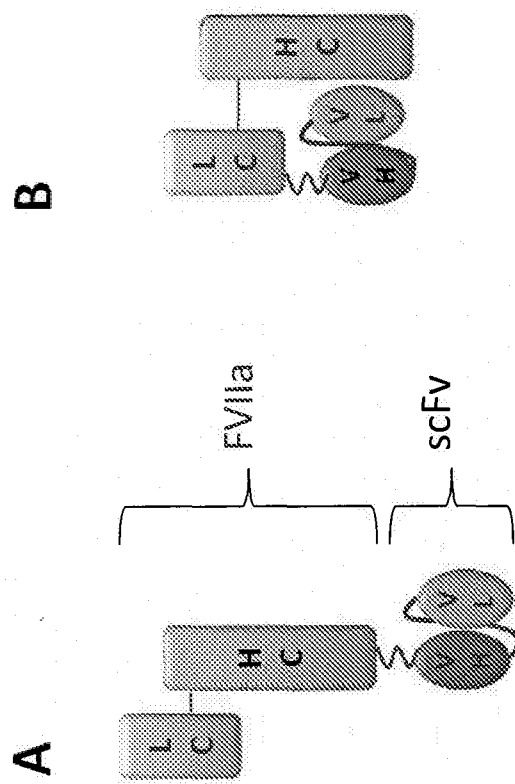


FIG. 13

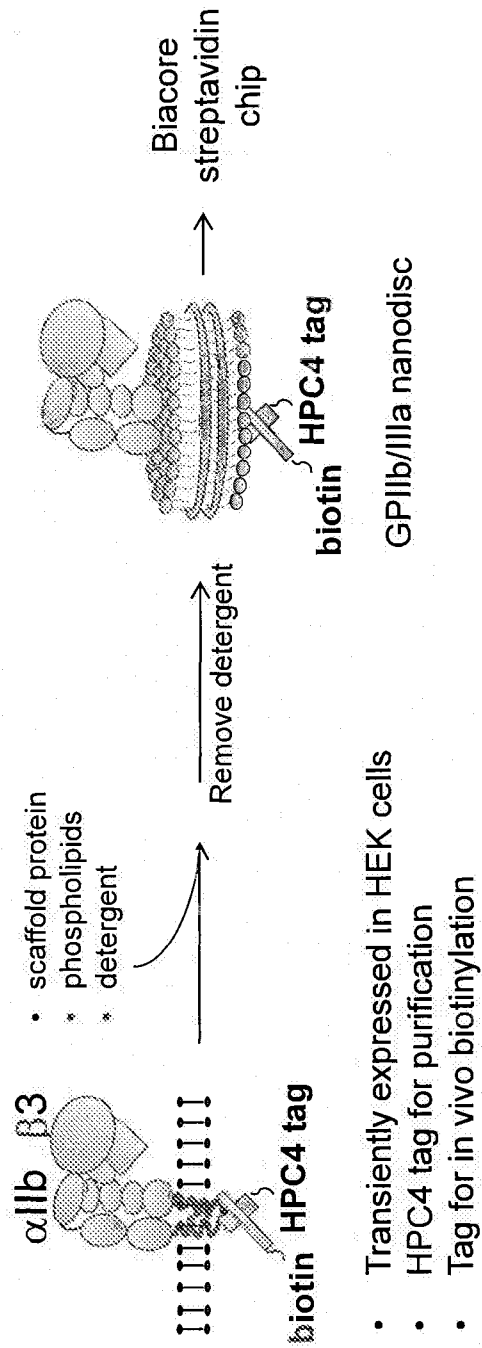


FIG. 14

Single cycle steady-state binding of platelet-targeted rFVIIa to
GP1Ib/IIIa nanodiscs measured by SPR

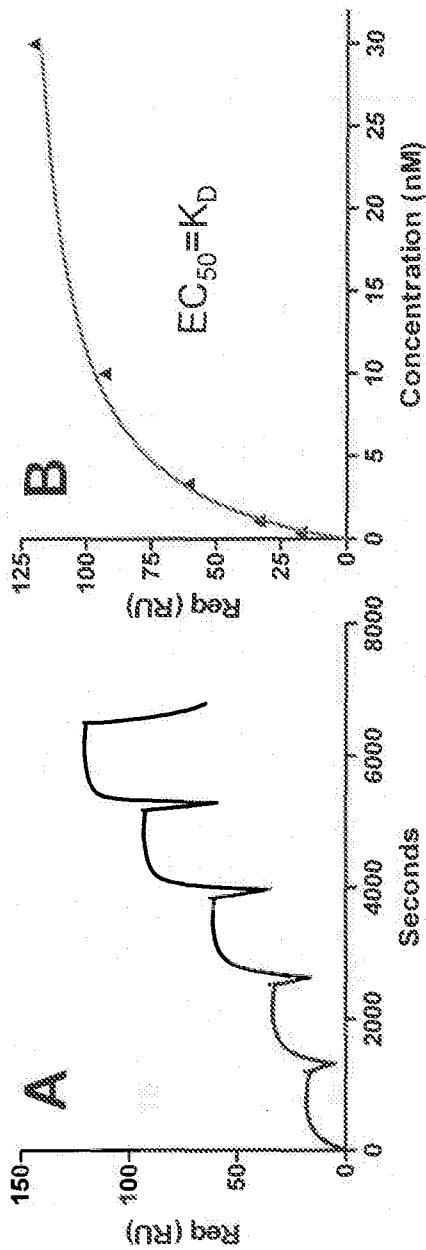


FIG. 15

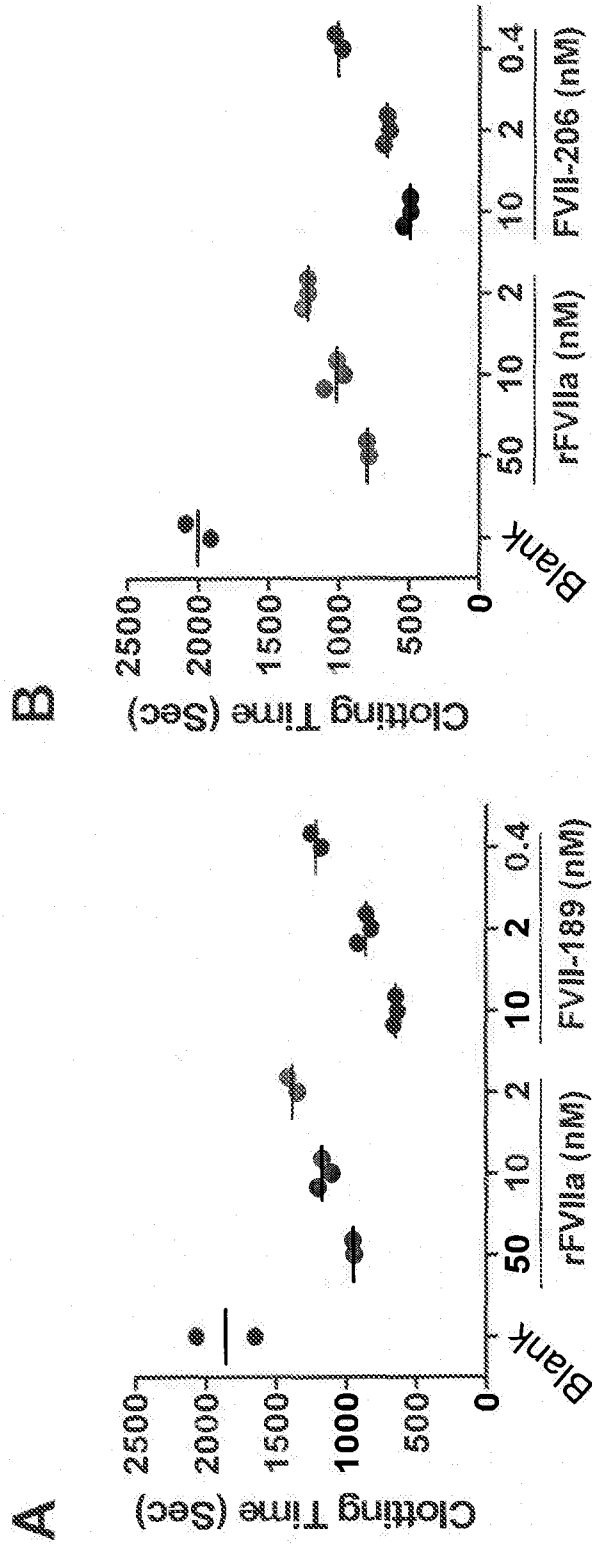


FIG. 16

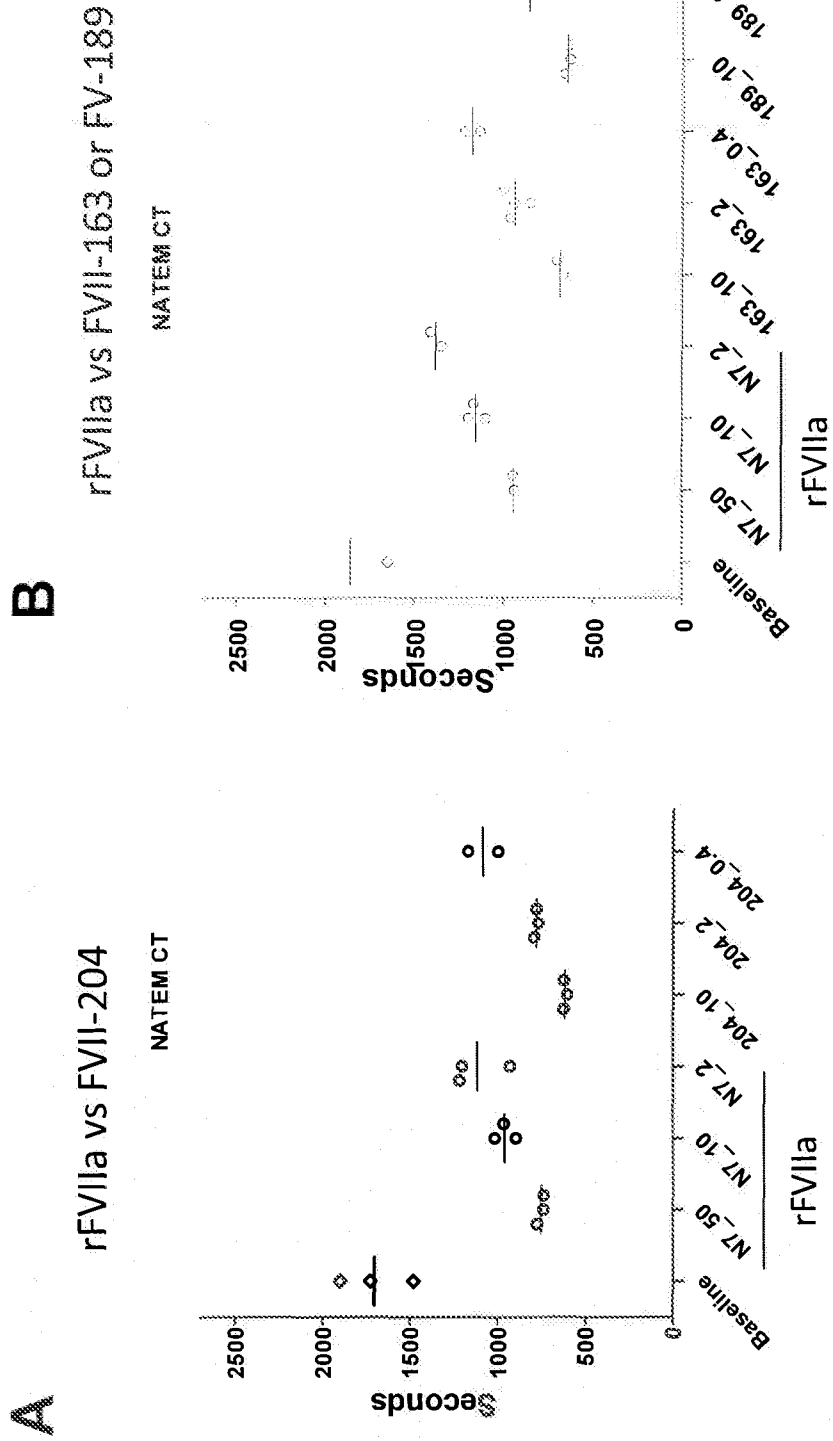


FIG. 17

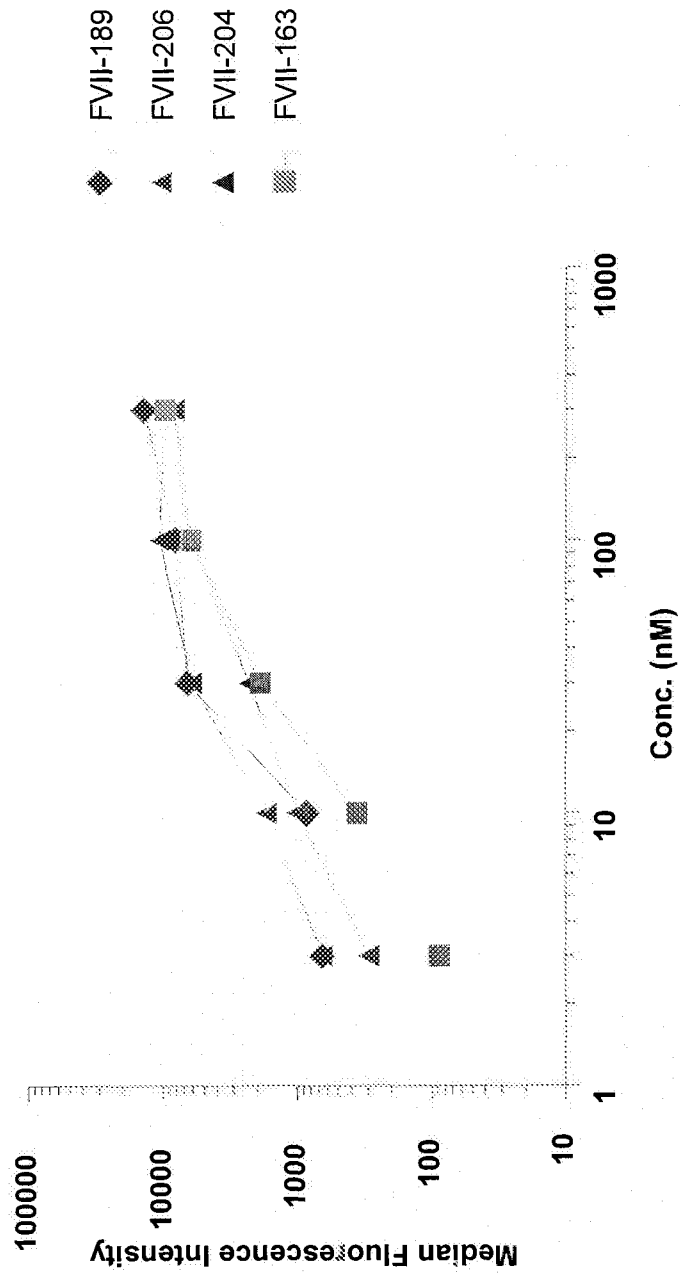
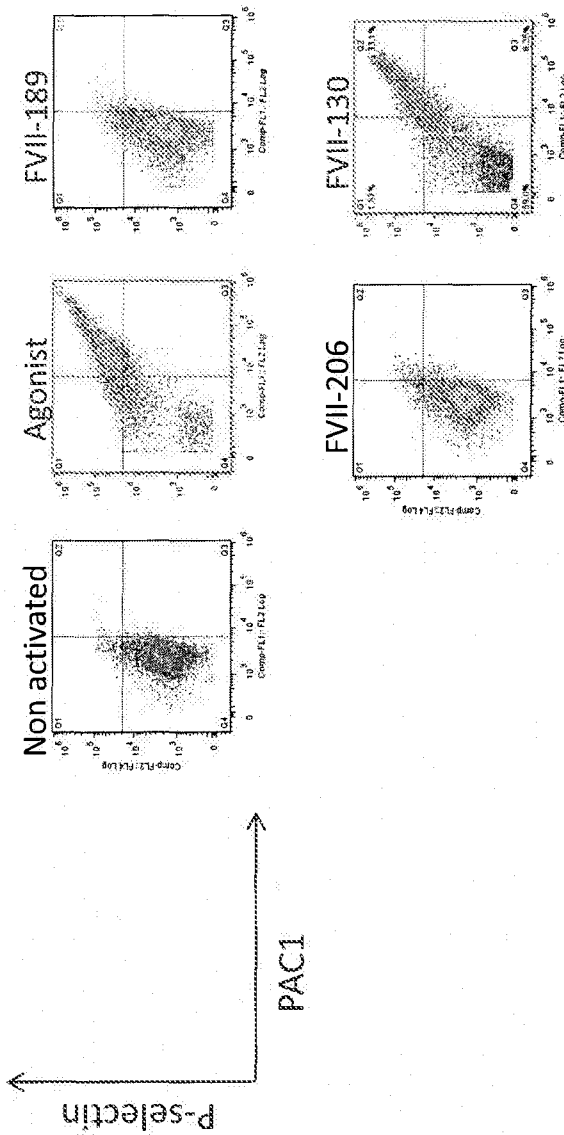


FIG. 18

Activation of gel-purified platelets determined by flow cytometry



Platelet-targeted variant known to activate platelets

FIG. 19

ADP-induced aggregation of platelet-rich plasma

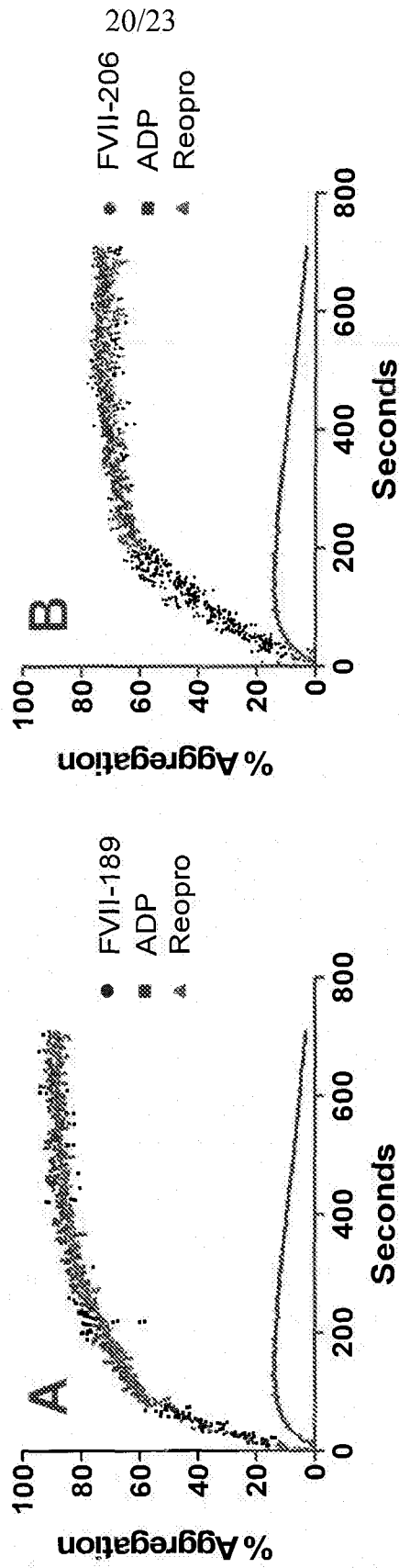


FIG. 20

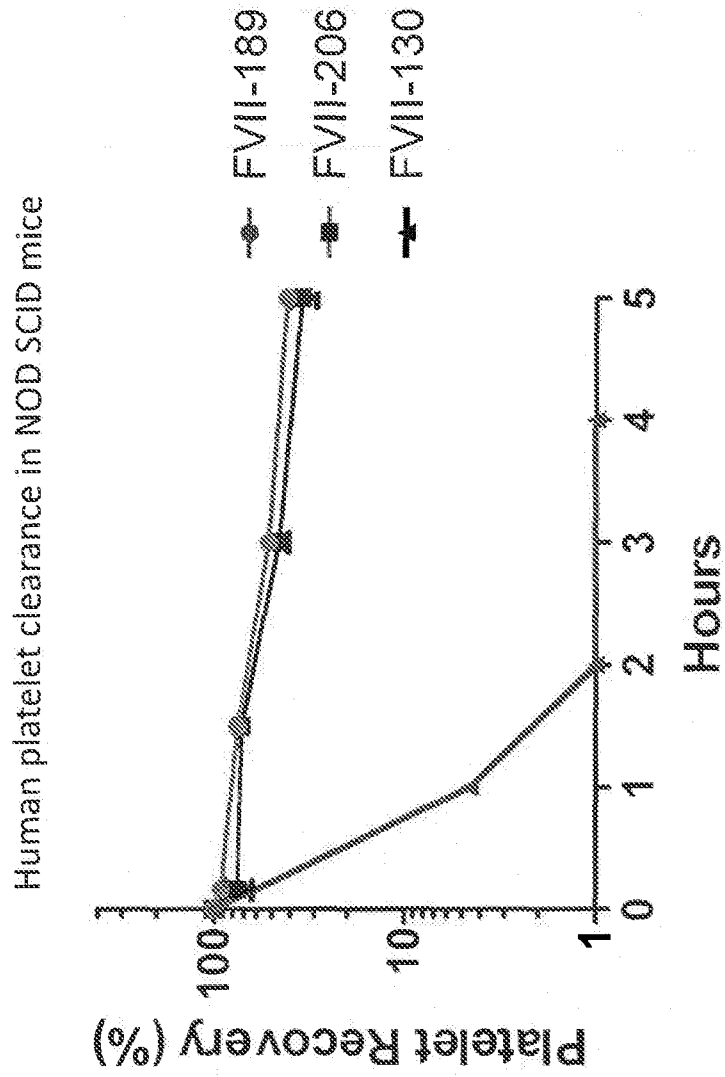
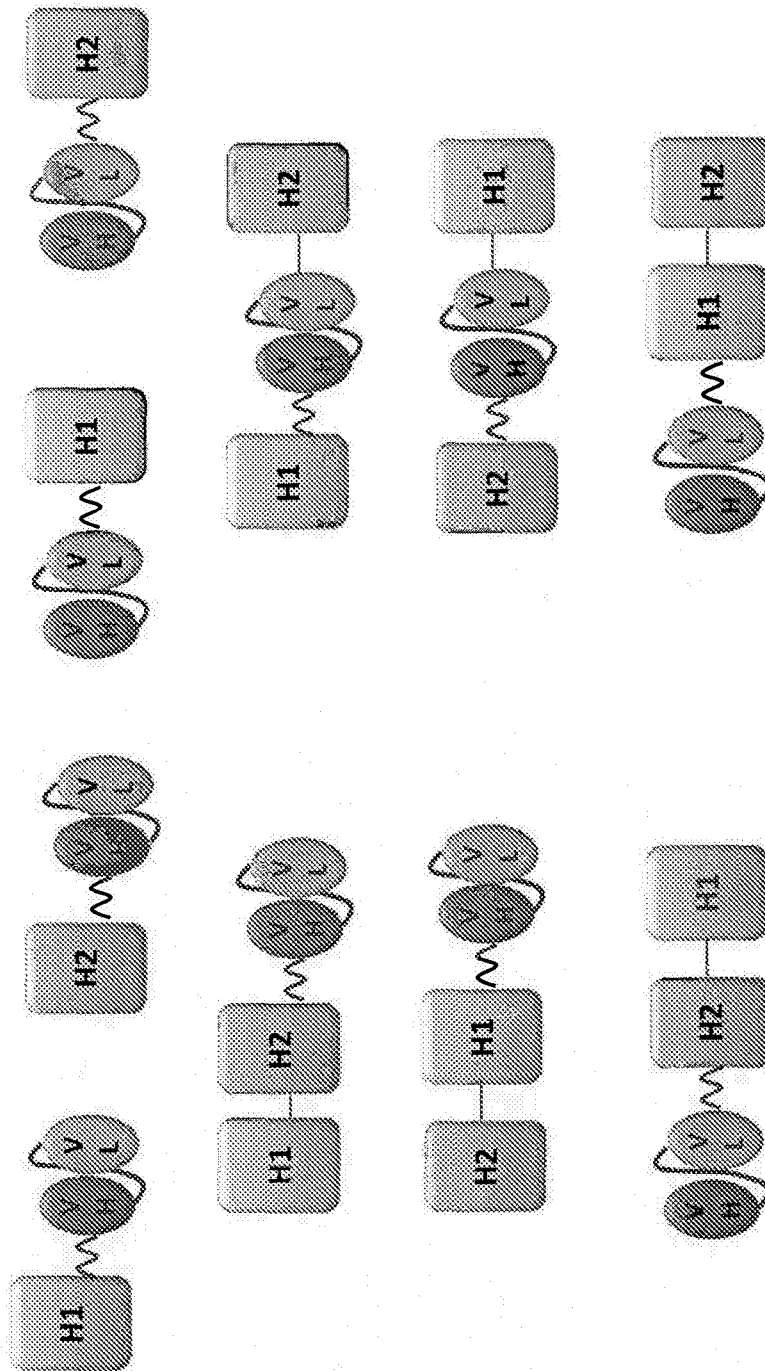


FIG. 21



VH-VL: scFv derived, e.g., from 34D10
 H1: E.g., half-life extending peptide
 H2: E.g., clotting factor (e.g., FVIIa)

FIG. 22

Schematic Diagram of Chimeric Molecules

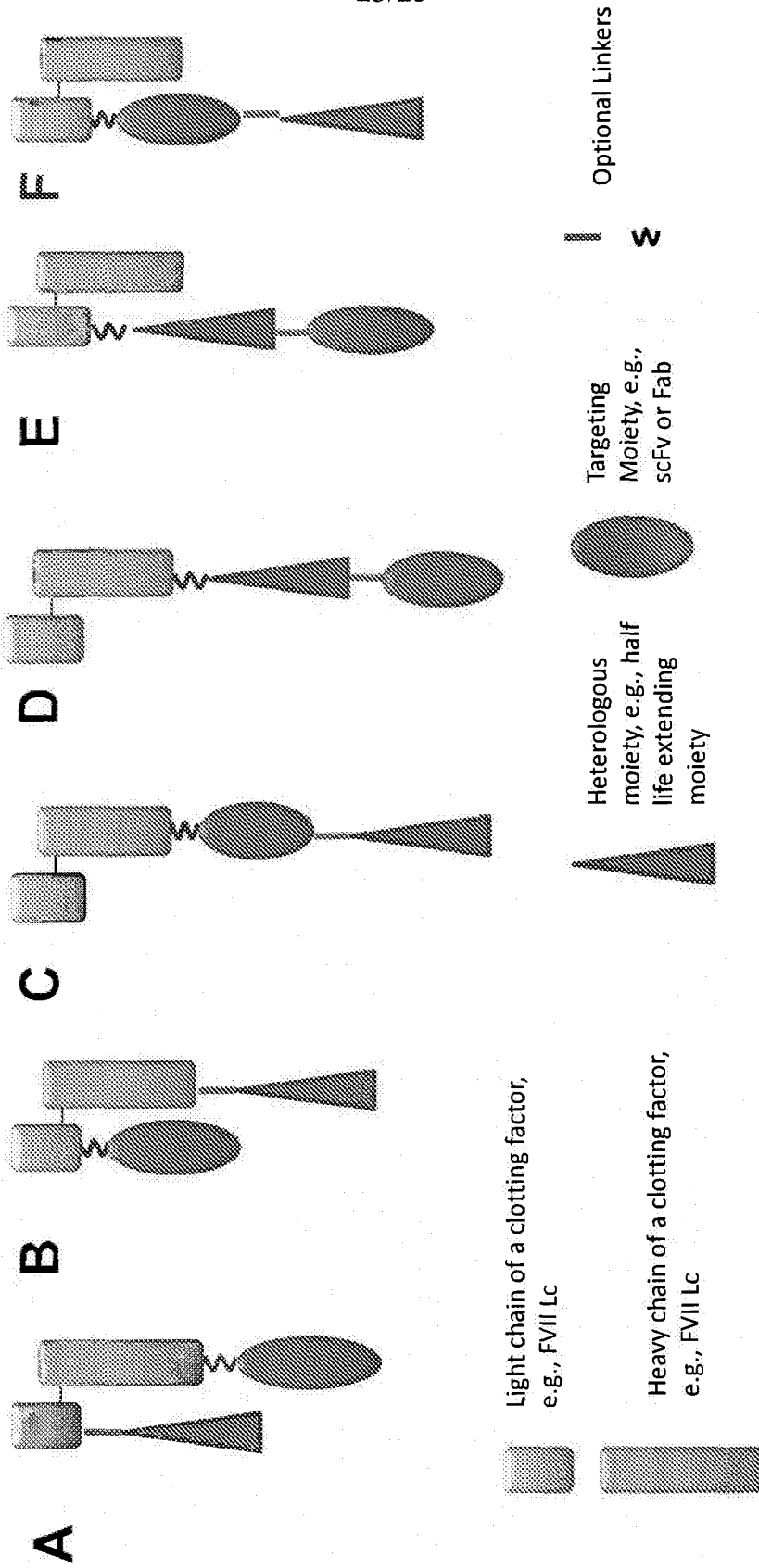


FIG. 23

专利名称(译)	抗gpiib / iiiia抗体或其用途		
公开(公告)号	EP3003368A4	公开(公告)日	2017-05-03
申请号	EP2014801129	申请日	2014-05-23
[标]申请(专利权)人(译)	生物遗传MA		
申请(专利权)人(译)	生物遗传MA INC.		
当前申请(专利权)人(译)	生物遗传MA公司		
[标]发明人	SALAS JOE TAN SIYUAN PETERS ROBERT		
发明人	SALAS, JOE TAN, SIYUAN PETERS, ROBERT		
IPC分类号	A61K39/395 G01N33/53 C07H21/00		
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优先权	61/827165 2013-05-24 US		
其他公开文献	EP3003368A2		
外部链接	Espacenet		

摘要(译)

本发明提供了抗体及其抗原结合分子，其特异性结合GPIIb / IIIa受体的非活性形式的 α 和/或 β 亚基。抗体和抗原结合分子可以与异源部分遗传融合和/或缀合，并用作例如靶向部分。本发明还包括筛选这些抗体的方法，以及制备方法和使用衍生自抗体的嵌合分子的方法。