

FACTOR VIII CHIMERIC PROTEINS AND USES THEREOF

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0001] The content of the electronically submitted sequence listing in ASCII text file (Name: 2159_441PC02_SequenceListing_ST25.txt; Size: 823,500 bytes; and Date of Creation: January 9, 2015) is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Haemophilia A is a bleeding disorder caused by defects in the gene encoding coagulation factor VIII (FVIII) and affects 1-2 in 10,000 male births. Graw *et al.*, *Nat. Rev. Genet.* 6(6): 488-501 (2005). Patients affected with hemophilia A can be treated with infusion of purified or recombinantly produced FVIII. All commercially available FVIII products, however, are known to have a half-life of about 8-12 hours, requiring frequent intravenous administration to the patients. See Weiner M.A. and Cairo, M.S., *Pediatric Hematology Secrets*, Lee, M.T., 12. Disorders of Coagulation, Elsevier Health Sciences, 2001; Lillicrap, D. *Thromb. Res.* 122 Suppl 4:S2-8 (2008). In addition, a number of approaches have been tried in order to extend the FVIII half-life. For example, the approaches in development to extend the half-life of clotting factors include pegylation, glycopegylation, and conjugation with albumin. See Dumont *et al.*, *Blood.* 119(13): 3024-3030 (Published online Jan. 13, 2012). Regardless of the protein engineering used, however, the long acting FVIII products currently under development are reported to have limited half-lives – only to about 1.5 to 2 hours in preclinical animal models. See *id.* Consistent results have been demonstrated in humans, for example, rFVIIIIFc was reported to improve half-life up to ~ 1.7 fold compared with ADVATE® in hemophilia A patients. See *Id.* Therefore, the half-life increases, despite minor improvements, may indicate the presence of other T_{1/2} limiting factors. See Liu, T. *et al.*, 2007 ISTH meeting, abstract #P-M-035; Henrik, A. *et al.*, 2011 ISTH meeting, abstract #P=MO-181; Liu, T. *et al.*, 2011 ISTH meeting abstract #P-WE-131.

[0003] Plasma von Willebrand Factor (VWF) has a half-life of approximately 16 hours (ranging from 13 to 18 hours). Goudemand J, *et al. J Thromb Haemost* 2005;3:2219–27. The VWF half-life may be affected by a number of factors: glycosylation pattern, ADAMTS-13 (a disintegrin and metalloprotease with thrombospondin motif-13), and various mutations in VWF.

[0004] In plasma, 95-98% of FVIII circulates in a tight non-covalent complex with full-length VWF. The formation of this complex is important for the maintenance of appropriate plasma levels of FVIII in vivo. Lenting *et al.*, *Blood.* 92(11): 3983-96 (1998); Lenting *et al.*, *J. Thromb.*

Haemost. 5(7): 1353-60 (2007). The full-length wild-type FVIII is mostly present as a heterodimer having a heavy chain (MW 200kD) and a light chain (MW 73kD). When FVIII is activated due to proteolysis at positions 372 and 740 in the heavy chain and at position 1689 in the light chain, the VWF bound to FVIII is removed from the activated FVIII. The activated FVIII, together with activated factor IX, calcium, and phospholipid ("tenase complex"), induces the activation of factor X, generating large amounts of thrombin. Thrombin, in turn, then cleaves fibrinogen to form soluble fibrin monomers, which then spontaneously polymerize to form the soluble fibrin polymer. Thrombin also activates factor XIII, which, together with calcium, serves to crosslink and stabilize the soluble fibrin polymer, forming crosslinked (insoluble) fibrin. The activated FVIII is cleared fast from the circulation by proteolysis.

[0005] Due to the frequent dosing and inconvenience caused by the dosing schedule, there is still a need to develop FVIII products requiring less frequent administration, *i.e.*, a FVIII product that has a half-life longer than the 1.5 to 2 fold half-life limitation.

BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides a chimeric protein comprising (i) a first polypeptide which comprises a Factor VIII ("FVIII") protein fused to a first immunoglobulin ("Ig") constant region or a portion thereof and (ii) a second polypeptide which comprises a von Willebrand Factor ("VWF") protein comprising a D' domain and a D3 domain of VWF fused to a second Ig constant region or a portion thereof by an XTEN sequence in-between, wherein the XTEN sequence contains less than 288 amino acid residues and wherein the first polypeptide is linked to or associated with the second polypeptide. Certain embodiments include the chimeric protein as described herein, wherein the XTEN sequence in the second polypeptide consists of an amino acid sequence having a length of between 12 amino acids and 287 amino acids.

[0007] Also disclosed is the chimeric protein as described herein, wherein the chimeric protein exhibits a longer half-life compared to a corresponding fusion protein comprising the first polypeptide and the second polypeptide wherein the second polypeptide of the fusion protein comprises an XTEN sequence containing at least 288 amino acids. Some embodiments include the XTEN sequence AE288, containing at least 288 amino acids. In some embodiments AE288 is SEQ ID NO: 8.

[0008] Also disclosed is the chimeric protein as described herein, wherein the XTEN sequence of the second polypeptide contains about 36, about 42, about 72, or about 144 amino acids. In some embodiments the XTEN sequence of the second polypeptide is selected from AE42, AE72, AE144, AG42, AG72, or AG144.

- [0009] Some embodiments include the chimeric protein as described herein, wherein the XTEN sequence of the second polypeptide is selected from SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63.
- [0010] In certain embodiments the first polypeptide further comprises a second XTEN sequence which links the FVIII protein with the first Ig constant region or a portion thereof. Also disclosed is the chimeric protein as described herein, wherein the first polypeptide comprises a third XTEN sequence which is inserted at one or more insertion sites within the FVIII protein. In some embodiments the first polypeptide further comprises a second XTEN sequence which is inserted at one or more insertion sites within the FVIII protein. In certain embodiments, the first polypeptide comprises a third XTEN sequence which links the FVIII protein with the first Ig constant region or a portion thereof.
- [0011] Also disclosed is the chimeric protein as described herein, wherein the second XTEN sequence, the third XTEN sequence, or the second and third XTEN sequences are each independently selected from AE42, AE72, AE864, AE576, AE288, AE144, AG864, AG576, AG288, and AG144. In some embodiments the second XTEN sequence, the third XTEN sequence, or the second and third XTEN sequences are each independently selected from SEQ ID NO: 8; SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 17; SEQ ID NO: 54; SEQ ID NO: 19; SEQ ID NO: 16; SEQ ID NO: 18; SEQ ID NO: 15; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63. In certain embodiments the second XTEN sequence, the third XTEN sequence, or both the second and third XTEN sequences are each independently AE288 or AG288. In some embodiments the XTEN sequence in the second polypeptide is fused to the second Ig constant region or a portion thereof by a linker. In certain embodiments the linker is a cleavable linker.
- [0012] Some embodiments include the chimeric protein as described herein, wherein the linker is cleavable by a protease selected from factor XIa, factor XIIa, kallikrein, factor VIIa, factor IXa, factor Xa, factor IIa (thrombin), Elastase-2, Granzyme-B, TEV, Enterokinase, Protease 3C, Sortase A, MMP-12, MMP-13, MMP-17, and MMP-20. In some embodiments the linker is cleavable by factor IIa (thrombin).
- [0013] Also disclosed is the chimeric protein as described herein, wherein the linker comprises one or more cleavage sites comprising an amino acid sequence selected from RRRR (SEQ ID NO: 102), RKRRKR (SEQ ID NO: 103), RRRRS (SEQ ID NO: 104), TQSFNDFTR (SEQ ID NO: 1), SVSQTSLTR (SEQ ID NO: 3), DFLAEGGGVR (SEQ ID NO: 4), TTKIKPR (SEQ ID

NO: 5), LVPRG (SEQ ID NO: 6), ALRPR (SEQ ID NO: 7), KLTRAET (SEQ ID NO: 121), DFTRVVG (SEQ ID NO: 122), TMTRIVGG (SEQ ID NO: 123), SPFRSTGG (SEQ ID NO: 124), LQVRIVGG (SEQ ID NO: 125), PLGRIVGG (SEQ ID NO: 126), IEGRTVGG (SEQ ID NO: 127), LTPRSLLV (SEQ ID NO: 128), LGPVSGVP (SEQ ID NO: 129), VAGDSLEE (SEQ ID NO: 130), GPAGLGGA (SEQ ID NO: 131), GPAGLRGA (SEQ ID NO: 132), APLGLRLR (SEQ ID NO: 133), PALPLVAQ (SEQ ID NO: 134), ENLYFQG (SEQ ID NO: 135), DDDKIVGG (SEQ ID NO: 136), LEVLFQGP (SEQ ID NO: 137), LPKTGSES (SEQ ID NO: 138), DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), and IEPRSFS (SEQ ID NO: 194). In some embodiments the linker comprises TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 146). In certain embodiments the cleavage sites comprise an amino acid sequence of LVPRG (SEQ ID NO: 6). In other embodiments the cleavage site comprises an amino acid sequence of IEPRSFS (SEQ ID NO: 194). In still other embodiments the cleavage site comprises an amino acid sequence of IEPRSFS (SEQ ID NO: 194), wherein the cleavage site is not the full length a2 region of FVIII. In some embodiments, the cleavage site comprises a fragment of an a2 region of FVIII comprising at least the sequence IEPR (SEQ ID NO: 200). In other embodiments, the cleavage site comprises a fragment of an a2 region of FVIII comprising at least the sequence IEPR (SEQ ID NO: 200), wherein the cleavage site is not the full length a2 region. In certain embodiments, the cleavage site is cleavable in a thrombin cleavage assay as provided herein or as known in the art.

[0014] Some embodiments include the chimeric protein as described herein, wherein the first Ig constant region or a portion thereof comprises a first Fc region and/or the second Ig constant region or a portion thereof comprises a second Fc region. In some embodiments the first Ig constant region or a portion thereof and the second Ig constant region or a portion thereof extend the half-life of the chimeric protein. In some embodiments the first polypeptide and the second polypeptide is fused by a linker. In certain embodiments the first polypeptide and the second polypeptide is fused by a processable linker. In some embodiments the first Ig constant region or a portion thereof is associated with the second Ig constant region or a portion thereof. In certain embodiments the first Ig constant region or a portion thereof is associated with the second Ig constant region or a portion thereof by a covalent bond. In some embodiments the covalent bond is a disulfide bond.

[0015] Also disclosed is the chimeric protein comprising each of the following formulae (a)-(hh):

- (a) FVIII-F1:F2-L2-X-L1-V;
- (b) FVIII-F1:V-L1-X-L2-F2;
- (c) F1-FVIII:F2-L2-X-L1-V;

- (d) F1-FVIII:V-L1-X-L2-F2;
- (e) FVIII-X2-F1:F2-L2-X1-L1-V;
- (f) FVIII-X2-F1:V-L1-X1-L2-F2;
- (g) FVIII(X2)-F1:F2-L2-X1-L1-V;
- (h) FVIII(X2)-F1:V-L1-X1-L2-F2;
- (i) F1-X2-F1:F2-L2-X1-L1-V;
- (j) F1-X2-F1:V-L1-X1-L2-F2;
- (k) V-L1-X-L2-F2-L3-FVIII-L4-F1;
- (l) V-L1-X-L2-F2-L3-F1-L4-FVIII;
- (m) F1-L4-FVIII-L3-F2-L2-X-L1-V;
- (n) FVIII-L4-F1-L3-F2-L2-X-L1-V;
- (o) FVIII-L4-F1-L3-V-L1-X-L2-F2;
- (p) FVIII-L4-F1-L3-F2-L2-X-L1-V;
- (q) F2-L2-X-L1-V-L3-F1-L4-FVIII;
- (r) F2-L2-X-L1-V-L3-FVIII-L4-F1;
- (s) V-L1-X1-L2-F2-L3-FVIII(X2)-L4-F1;
- (t) V-L1-X1-L2-F2-L3-F1-L4-FVIII(X2);
- (u) F1-L4-FVIII(X2)-L3-F2-L2-X1-L1-V;
- (v) F-L4-FVIII(X2)-L3-V-L1-X1-L2-F2;
- (w) FVIII(X2)-L4-F1-L3-V-L1-X1-L2-F2;
- (x) FVIII(X2)-L4-F1-L3-F2-L2-X1-L1-V;
- (y) F2-L2-X1-L1-V-L3-F1-L4-FVIII(X2);

- (z) F2-L2-X1-L1-V-L3-FVIII(X2)-L4-F1;
- (aa) V-L1-X2-L2-F2-L3-FVIII-L4-X2-L5-F1;
- (bb) V-L1-X2-L2-F2-L3-F1-L5-X2-L4-FVIII;
- (cc) F1-L5-X2-L4-FVIII-L3-F2-L2-X2-L1-V;
- (dd) F1-L5-X2-L4-FVIII-L3-V-L1-X2-L2-F2;
- (ee) FVIII-L5-X2-L4-F2-L3-V-L1-X1-L2-F1;
- (ff) FVIII-L5-X2-L4-F2-L3-F1-L2-X1-L1-V;
- (gg) F1-L2-X1-L1-V-L3-F2-L4-X2-L5-FVIII; or
- (hh) F1-L2-X1-L1-V-L3-FVIII-L5-X2-L4-F2;

wherein V is a VWF protein, which comprises a D' domain and a D3 domain, X or X1 is a first XTEN sequence that contains less than 288 amino acids, X2 is a second XTEN sequence, FVIII comprises a FVIII protein, FVIII(X2) comprises a FVIII protein having a second XTEN sequence inserted in one or more insertion sites within the FVIII protein, F1 is a first Ig constant region or a portion thereof, F2 is a second Ig constant region or a portion thereof, L1, L2, L3, L4, or L5 is an optional linker, (-) is a peptide bond; and (:) is a covalent bond or a non-covalent bond.

[0016] Some embodiments include the chimeric protein as described herein, wherein the X or X1 consists of an amino acid sequence in length between 12 amino acids and 287 amino acids.

[0017] In certain embodiments the chimeric protein as described herein exhibits a longer half-life compared to a corresponding chimeric protein comprising the formula except that the X or X1 is AE288. In some embodiments AE288 is SEQ ID NO:8.

[0018] Some embodiments include the chimeric protein as described herein, wherein the X or X1 in the formula contains about 36, about 42, about 72, or about 144 amino acids. In certain embodiments the X or X1 in the formula is selected from AE42, AE72, AE144, AG42, AG72, or AG144. In some embodiments the X or X1 in the formula is selected from SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63. In certain embodiments the X2 comprises an amino acid sequence having a length of at least about 36 amino acids, at least about 42 amino acids, at least about 144 amino acids, at least about 288 amino acids, at least about 576 amino acids, at least about 864 amino acids. In certain embodiments the X2 is selected from AE42, AE72, AE864, AE576, AE288, AE144, AG864,

AG576, AG288, and AG144. In some embodiments the X2 is selected from SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 17; SEQ ID NO: 54; SEQ ID NO: 19; SEQ ID NO: 16; SEQ ID NO: 18; SEQ ID NO: 15; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63. In certain embodiments the X2 is AE288 or AG288.

[0019] Also disclosed is the chimeric protein as described herein, comprising X or X1 and/or X2 that exhibits a longer half-life compared to the chimeric protein not comprising X or X1 and/or X2. In some embodiments, the L1 and/or L2 is a cleavable linker. In certain embodiments the L4 and/or L5 is a cleavable linker. In certain embodiments the linker is cleavable by a protease selected from factor XIa, factor XIIa, kallikrein, factor VIIa, factor IXa, factor Xa, factor IIa (thrombin), Elastase-2, Granzyme-B, TEV, Enterokinase, Protease 3C, Sortase A, MMP-12, MMP-13, MMP-17, and MMP-20. In some embodiments the linker is cleavable by factor IIa (thrombin).

[0020] Some embodiments include the chimeric protein as described herein, wherein the linker comprises one or more cleavage sites comprising an amino acid sequence selected from RRRR (SEQ ID NO: 102), RKRRKR (SEQ ID NO: 103), RRRRS (SEQ ID NO: 104), TQSFNDFTR (SEQ ID NO: 2), SVSQTSKLTR (SEQ ID NO: 3), DFLAEGGGVR (SEQ ID NO: 4), TTKIKPR (SEQ ID NO: 5), LVPRG (SEQ ID NO: 6), ALRPR (SEQ ID NO: 7), KLTRAET (SEQ ID NO: 121), DFTRVVG (SEQ ID NO: 122), TMTRIVGG (SEQ ID NO: 123), SPFRSTGG (SEQ ID NO: 124), LQVRIVGG (SEQ ID NO: 125), PLGRIVGG (SEQ ID NO: 126), IEGRTVGG (SEQ ID NO: 127), LTPRSLLV (SEQ ID NO: 128), LGPVSGVP (SEQ ID NO: 129), VAGDSLEE (SEQ ID NO: 130), GPAGLGGA (SEQ ID NO: 131), GPAGLRGA (SEQ ID NO: 132), APLGLRLR (SEQ ID NO: 133), PALPLVAQ (SEQ ID NO: 134), ENLYFQG (SEQ ID NO: 135), DDDKIVGG (SEQ ID NO: 136), LEVLFGQP (SEQ ID NO: 137), and LPKTGSES (SEQ ID NO: 138). In some embodiments the linker comprises TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 146). In certain embodiments the linker comprises an amino acid sequence of LVPRG (SEQ ID NO: 6). In some embodiments the linker comprises an a1 region of FVIII, an a2 region of FVIII, an a3 region of FVIII, or any combination thereof. In certain embodiments the linker comprises a fragment of the a2 region of FVIII. The fragment of the a2 region can in some cases comprise the sequence DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88). In still other embodiments a smaller fragment of the a2 region of FVIII can be used, including a fragment having the sequence of IEPRSFS (SEQ ID NO: 194). In one particular embodiment, the linker comprises the amino acid sequence of IEPRSFS (SEQ ID NO: 194). In another embodiment, the linker comprises the amino acid sequence of IEPRSFS (SEQ ID NO: 194), wherein the linker is not the full-length a2 region of FVIII.

- [0021] Also disclosed is the chimeric protein as described herein, wherein the a2 region of FVIII comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to either ISDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 106) or DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88). In some embodiments the a1 region comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to ISMKNNEEAEDYDDDLTDSEMDVVRFDDDNSPSFIQIRSV (SEQ ID NO: 107). In certain embodiments the a3 region comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to ISEITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQ (SEQ ID NO: 108). In some embodiments the F1 comprises a first Fc region and/or the F2 comprises a second Fc region.
- [0022] Some embodiments include the chimeric protein as described herein, wherein the chimeric protein comprising the F1 and the F2 exhibits a longer half-life compared to the chimeric protein not comprising the F1 and the F2. In certain embodiments the L3 is a processable linker. In some embodiments the VWF protein is associated with the FVIII protein by a non-covalent bond. In some embodiments the half-life of the chimeric protein is extended compared to a FVIII protein without the VWF protein and/or the XTEN sequence or compared to wild type FVIII. In certain embodiments the half-life of the chimeric protein is extended 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 a FVIII protein without the VWF protein or the XTEN sequence or than wild type FVIII.
- [0023] Also disclosed is the chimeric protein as described herein, wherein the half-life of the chimeric protein is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours. In some embodiments the half-life of the chimeric protein is about 40 hours in HemA mice. In certain embodiments the VWF protein does not bind substantially to a VWF clearance receptor. In some embodiments the VWF protein is capable of protecting the FVIII protein from one or more protease cleavages, protecting the FVIII protein from activation, stabilizing the heavy chain and/or the light chain of the FVIII protein, or preventing clearance of the FVIII protein by one or more scavenger receptors.

[0024] Some embodiments include the chimeric protein as described herein, wherein the VWF protein inhibits or prevents endogenous VWF from binding to the FVIII protein by shielding or blocking a VWF binding site on the FVIII protein. In certain embodiments the VWF binding site is located in the A3 domain or the C2 domain of the FVIII protein or both the A3 domain and the C2 domain. In some embodiments the VWF binding site comprises the amino acid sequence corresponding to amino acids 1669 to 1689 and 2303 to 2332 of SEQ ID NO: 65. In some embodiments the first Ig constant region or a portion thereof and the second Ig constant region or a portion thereof are identical or different. In certain embodiments the FVIII protein is linked to and/or inserted with at least two XTEN sequences, at least three XTEN sequences, at least four XTEN sequences, at least five XTEN sequences, or at least six XTEN sequences.

[0025] Also disclosed is the chimeric protein as described herein, wherein the FVIII protein comprises one or more domains of FVIII selected from an A1 domain, a1 acidic region, an A2 domain, a2 acidic region, a B domain, an A3 domain, a3 acidic region, a C1 domain, a C2 domain, one or more fragments thereof, and any combinations thereof.

[0026] Also disclosed is the chimeric protein as described herein, wherein the one or more insertion sites in the FVIII protein is located within one or more domains of the FVIII protein selected from the A1 domain, the a1 acidic region, the A2 domain, the a2 acidic region, the A3 domain, the B domain, the C1 domain, the C2 domain, and any combinations thereof or between one or more domains of the FVIII protein selected from the group consisting of the A1 domain and a1 acidic region, the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, the A3 domain and C1 domain, the C1 domain and C2 domain, and any combinations thereof or between two domains of the FVIII protein selected from the A1 domain and a1 acidic region, the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, the A3 domain and C1 domain, the C1 domain and C2 domain, and any combinations thereof. In some embodiments the one or more insertion sites in the FVIII protein are one or more amino acids selected from the group consisting of the amino acid residues in Table 7, Table 8, Table 9 and Table 10. In certain embodiments the insertion sites in the FVIII protein are located immediately downstream of amino acid 745 corresponding to the mature FVIII protein (SEQ ID NO: 65). In some embodiments the insertion sites in the FVIII protein are located immediately downstream of residue 1656 and residue 1900 corresponding to the mature FVIII protein (SEQ ID NO: 65). In some embodiments the insertion sites in the FVIII protein are immediately downstream of residues 26, 1656, and 1900 corresponding to the mature FVIII protein (SEQ ID NO: 65). In certain embodiments the insertion sites in the FVIII protein are immediately downstream of residues 403 and 745 corresponding to the mature FVIII protein (SEQ ID NO: 65).

In some embodiments the insertion sites in the FVIII protein are immediately downstream of residues 745 and 1900 corresponding to the mature FVIII protein (SEQ ID NO: 65). In certain embodiments the insertion sites in the FVIII protein are immediately downstream of residues 18 and 745 corresponding to the mature FVIII protein (SEQ ID NO: 65). In some embodiments the FVIII protein is a dual chain FVIII isoform. In some embodiments the FVIII protein is a single chain FVIII isoform. In certain embodiments the FVIII protein comprises B domain or a portion thereof. In some embodiments the FVIII protein is SQ B domain deleted FVIII.

[0027] Some embodiments include the chimeric protein as described herein, wherein the single chain FVIII isoform contains at least one amino acid substitution at a residue corresponding to residue 1648, residue 1645, or both residues corresponding to the full-length mature Factor VIII polypeptide (SEQ ID NO: 65) or residue 754, residue 751, or both residues of SQ BDD Factor VIII (SEQ ID NO: 67). In certain embodiments the amino acid substitution is an amino acid other than arginine. In some embodiments the dual chain FVIII isoform comprises a first chain comprising a heavy chain of FVIII and a second chain comprising a light chain of FVIII, wherein the heavy chain and the light chain are associated with each other by a metal bond. In certain embodiments the D' domain comprises an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 866 of SEQ ID NO: 21. In some embodiments the D3 domain comprises an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 867 to 1240 of SEQ ID NO: 21. In certain embodiments the VWF protein is a monomer.

[0028] Also disclosed is the chimeric protein as described herein, which comprises at least two VWF proteins, at least three VWF proteins, at least four VWF proteins, at least five VWF proteins, or at least six VWF proteins. In certain embodiments the VWF protein comprises an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 1240 of SEQ ID NO: 21. In some embodiments the VWF protein consists essentially of or consists of amino acids 764 to 1240 of SEQ ID NO: 21. In certain embodiments the VWF protein contains at least one amino acid substitution at a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 of SEQ ID NO: 21. In some embodiments the VWF protein contains an amino acid other than cysteine substituted for a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 of SEQ ID NO: 21. In certain embodiments the VWF protein further comprises the D1 domain, the D2 domain, or the D1 and D2 domains of VWF.

[0029] Some embodiments include the chimeric protein as described herein, wherein the VWF protein further comprises a VWF domain selected from the A1 domain, the A2 domain, the A3

domain, the D4 domain, the B1 domain, the B2 domain, the B3 domain, the C1 domain, the C2 domain, the CK domain, one or more fragments thereof, and any combinations thereof.

[0030] Also disclosed is the chimeric protein as described herein, wherein the VWF protein consists essentially of or consists of: (1) the D' and D3 domains of VWF or fragments thereof; (2) the D1, D', and D3 domains of VWF or fragments thereof; (3) the D2, D', and D3 domains of VWF or fragments thereof; (4) the D1, D2, D', and D3 domains of VWF or fragments thereof; or (5) the D1, D2, D', D3, and A1 domains of VWF or fragments thereof.

[0031] Some embodiments include the chimeric protein as described herein, wherein the VWF protein further comprises a signal peptide of VWF or FVIII which is operably linked to the VWF protein.

[0032] Also disclosed is the chimeric protein as described herein, wherein one or more of the linkers have a length of at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1200, 1400, 1600, 1800, or 2000 amino acid residues. In some embodiments one or more of the linkers have a length of about 1 to about 2000 amino acid residues. In certain embodiments one or more of the linkers comprise a gly/ser peptide. In some embodiments the gly/ser peptide has a formula of $(\text{Gly}_4\text{Ser})_n$ (SEQ ID NO: 94) or $\text{S}(\text{Gly}_4\text{Ser})_n$ (SEQ ID NO: 164), wherein n is a positive integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In certain embodiments the $(\text{Gly}_4\text{Ser})_n$ linker is $(\text{Gly}_4\text{Ser})_3$ (SEQ ID NO: 100) or $(\text{Gly}_4\text{Ser})_4$ (SEQ ID NO: 165). In some embodiments the linker comprises 20 amino acids, 35 amino acids, 48 amino acids, 73 amino acids, or 95 amino acids. In certain embodiments the cleavable linker is SGGGSGGGGSGGGGSGGGGSGGGGSLVPRGSGG (SEQ ID NO: 166).

[0033] In some embodiments, the chimeric protein as described herein is polysialylated, pegylated, or hesylated.

[0034] Also disclosed is the chimeric protein as described herein, wherein the first polypeptide comprises at least about 80%, 90%, 95%, 99%, or 100% identical to FVIII161 (SEQ ID NO: 69), FVIII169 (SEQ ID NO: 70), FVIII173 (SEQ ID NO: 72), FVIII195 (SEQ ID NO: 73), FVIII196 (SEQ ID NO: 74), FVIII199 (SEQ ID NO: 75), FVIII201 (SEQ ID NO: 76), FVIII203 (SEQ ID NO: 77), FVIII204 (SEQ ID NO: 78), FVIII205 (SEQ ID NO: 79), FVIII266 (SEQ ID NO: 80), FVIII267 (SEQ ID NO: 81), FVIII268 (SEQ ID NO: 82), FVIII269 (SEQ ID NO: 83), FVIII271 (SEQ ID NO: 84), FVIII272 (SEQ ID NO: 85), or FVIII282 (SEQ ID NO: 159), and the second polypeptide comprises at least about 80%, 90%, 95%, 99%, or 100% identical to either VWF057 (SEQ ID NO: 152) or VWF059 (SEQ ID NO: 197). In some embodiments, the first polypeptide comprises FVIII169 (SEQ ID NO: 70) and the second polypeptide comprises VWF057 (SEQ ID NO: 152). In other embodiments, the first polypeptide comprises FVIII169 (SEQ ID NO: 70) and

the second polypeptide comprises VWF059 (SEQ ID NO: 197). In yet another embodiment, the first polypeptide comprises FVIII169 (SEQ ID NO: 70) and the second polypeptide comprises VWF062 (SEQ ID NO: 199). In some embodiments, the chimeric protein is efficacious in preventing and/or stopping bleeding from a subject in need thereof.

- [0035] Also disclosed is a polynucleotide or a set of polynucleotides encoding the chimeric protein as described herein. In some embodiments, the polynucleotide as described herein, further comprises a polynucleotide chain, which encodes PC5 or PC7.
- [0036] Some embodiments include a vector comprising the polynucleotide as described herein and one or more promoter operably linked to the polynucleotide or the set of polynucleotides.
- [0037] In some embodiments the vector as described herein, further comprises an additional vector, which comprises a polynucleotide chain encoding PC5 or PC7.
- [0038] Also disclosed is a host cell comprising the polynucleotide or the vector as described herein. In some embodiments the host cell is a mammalian cell. In certain embodiments the mammalian cell is selected from HEK293 cell, CHO cell, and BHK cell.
- [0039] Also disclosed is a pharmaceutical composition comprising the chimeric protein, the polynucleotide, the vector, or the host cell as described herein, and a pharmaceutically acceptable carrier. In some embodiments the chimeric protein has extended half-life compared to wild type FVIII protein. In certain embodiments, the half-life of the chimeric protein is extended 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 wild type FVIII.
- [0040] Some embodiments include the composition as described herein, wherein the half-life of the chimeric protein is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours. In certain embodiments the half-life of the chimeric protein is about 40 hours in HemA mice. In some embodiments the composition as described herein is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration. In certain embodiments the parenteral administration is intravenous or subcutaneous administration.

- [0041] In some embodiments the composition as described herein is used to treat a bleeding disease or condition in a subject in need thereof. In certain embodiments the bleeding disease or condition is selected from the group consisting of a bleeding coagulation disorder, 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 and any combinations thereof. In some embodiments the subject is scheduled to undergo a surgery. In certain embodiments the treatment is prophylactic or on-demand.
- [0042] Also disclosed is a method of extending or increasing half-life of the chimeric protein, wherein the method comprises adding an effective amount of the chimeric protein, the polynucleotide, the vector, the host cell, or the composition as described herein to a subject in need thereof, wherein the VWF protein, the XTEN sequence, the first Ig constant region or a portion thereof, and the second Ig constant region or a portion thereof increase the half-life of the chimeric protein.
- [0043] Some embodiments include a method of treating a bleeding disease or disorder in a subject in need thereof comprising administering an effective amount of the chimeric protein, the polynucleotide, the vector, the host cell, or the composition as described herein, wherein the bleeding disease or disorder is selected from the group consisting of a bleeding coagulation disorder, 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, and bleeding in the iliopsoas sheath. In some embodiments the subject is an animal. In certain embodiments the animal is a human. In some embodiments the subject is suffering from hemophilia A. In certain embodiments the treatment is prophylactic or on-demand. In some embodiments the effective amount is 0.1 µg/kg to 500 mg/kg.
- [0044] Also disclosed is a method as described herein, wherein the chimeric protein, the polynucleotide, the vector, the host cell, or the composition as described herein is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration. In certain embodiments the parenteral administration is selected from the group consisting of intravenous administration, subcutaneous administration, intramuscular administration, and intradermal administration.

[0045] Some embodiments include a method of making a chimeric protein, comprising transfecting one or more host cell with the polynucleotide or the vector as described herein and expressing the chimeric protein in the host cell. In some embodiments, the method as described herein further comprises isolating the chimeric protein. In certain embodiments the chimeric protein is efficacious in stopping and/or preventing bleeding in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0046] Figure 1 shows a schematic diagram of a chimeric protein comprising a first polypeptide which comprises a FVIII protein (A1-A2-partial or full B-A3-C1-C2) fused to an Fc region, wherein an XTEN is inserted at an insertion site within the FVIII protein and a second polypeptide which comprises a VWF protein comprising D'D3 domains, an XTEN having less than 288 amino acids, a thrombin cleavable linker, and a second Fc region. XTEN insertions in the FVIII protein and/or fusions to the VWF protein extend a half-life of the chimeric protein by increasing the hydrodynamic radius and by blocking receptor-mediated clearance. The D'D3 domains of VWF block FVIII interaction with endogenous VWF, stabilize the FVIII protein, and extend a half-life of the chimeric protein. The Fc domains can covalently link the D'D3 domains with the FVIII protein and extend a half-life of the chimeric protein through FcRn-mediated recycling pathway. The thrombin-cleavable linker enables a release of the D'D3 domains upon FVIII activation and ensures the correct alignment between FVIII and the D'D3 domains of VWF.

[0047] Figure 2 shows three plasmid expression system for FVIII-XTEN-Fc:D'D3-XTEN-Fc heterodimers: a first plasmid comprising a nucleotide sequence encoding single chain FVIII-XTEN-Fc in which an XTEN is inserted in the B domain; a second plasmid comprising a nucleotide sequence encoding D1D2D'D3-XTEN-Fc, in which the XTEN sequence comprises less than 288 amino acids; and a third plasmid comprising a nucleotide sequence encoding PACE, a propeptide processing enzyme. When the three polypeptides are expressed from the three plasmids, the D1D2 propeptide domains of VWF can be processed from the D'D3 domains by intracellular processing. The resulting complex contains three products, the first molecule being FVIII-XTEN/D'D3 heterodimers, the second molecule being a by-product, homodimer of D'D3-XTEN-Fc, and the third molecule being another by-product, i.e., FVIII(XTEN)-Fc.

[0048] Figure 3 shows additive effects of XTEN insertions on the half-life extension of the heterodimers. FVIII169 comprises a B domain deleted FVIII protein fused to an Fc region, wherein an XTEN sequence (e.g., AE288) is inserted at amino acid 745 corresponding to mature full length FVIII. FVIII205 comprises a B domain deleted FVIII protein fused to an Fc region, wherein an XTEN sequence (e.g., AE144) is inserted at amino acid 18 corresponding to mature

full length FVIII and another XTEN sequence (e.g., AE288) is inserted at amino acid 745 corresponding to mature full length FVIII. VWF031 comprises a D' domain and a D3 domain of VWF fused to an Fc region by a thrombin cleavable linker (no XTEN). VWF034 comprises a D' domain and a D3 domain of VWF fused to AE288 and an Fc region. The half-life of FVIII169/VWF031 (inverted triangle) is 16.7 hours in HemA mice; the half-life of FVIII205/VWF031 (circle) is 29.4 hours in HemA mice; and the half-life of FVIII169/VWF034 (square) is 31.1 hours in HemA mice.

[0049] Figure 4 shows that AE144 XTEN confers better half-life extension than AE288 XTEN when inserted between the D'D3 domains of VWF and Fc domains. For example, while the half-life of VWF169/VWF034 (square) is 31.1 hours in HemA mice, the half-life of FVIII169/VWF057 (circle) is 42 hours in HemA mice. VWF057 comprises D'D3 domains of VWF fused to AE144 and an Fc region.

[0050] Figure 5 shows that Fc domains are needed for half-life extension of the chimeric protein heterodimers. When the half-life of FVIII205/VWF031(circle) was compared in HemA mice with that of FVIII263/VWF050 (square), which contains mutations at the FcRn binding sites (IHH triple mutation Fc) and thus cannot be recycled through FcRn pathway, the half-life of FVIII263/VWF050 (23 hours) is shorter than that of VWF205/VWF031 (29.4hours). This indicates that the Fc regions are necessary for half-life extension.

[0051] Figure 6A shows similar acute efficacy of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers compared to B domain deleted FVIII (SQ BDD FVIII) in HemA mice tail clip model. Mice were dosed at 75 IU/kg, and the activity was measured by aPTT assay. SQ BDD FVIII is shown as circle while FVIII169/VWF034 is shown as square, FVIII169/VWF057 is shown as diamond, and vehicle is shown as inverted triangle. The construct details of FVIII169, VWF034, and VWF057 are shown elsewhere herein. Figure 6B shows a comparison of the acute efficacy of FVIII169/VWF034 with B domain deleted FVIII (SQ BDD FVIII) in HemA mice at 37.5 IU/kg dose, and the activity was measured by aPTT assay. The median blood loss (uL) of mice in each treatment groups are indicated by the horizontal lines, blood loss (uL) in C57/BL6 mice is shown as hollow triangle; the blood loss (uL) after dosing of 37.5 IU/kg of rBDD-FVIII is shown as hollow circle; the blood loss (uL) after dosing of 37.5 IU/kg FVIII169/VWF034 is shown as hollow square and the blood loss (uL) after dosing of vehicle is shown as inverted triangle.

[0052] Figures 7A-B show that rFVIII169/VWF057 heterodimer provides longer protection to HemA mice in Tail Vein Transection Bleeding Model. Figure 7A shows the rebleeding data in mice that received rFVIII169/VWF057 at 72 hours before tail injury (square), SQ BDD-FVIII at 48 hours before tail injury (diamond), SQ BDD FVIII at 24 hours before tail injury (inverted triangle), and vehicle (circle). The activity was measured by aPTT assay. X-axis shows time in

hours, and the Y axis shows percent of Non-Bleeders. Figure 7B shows the corresponding survival data in the four categories of the mice shown in Figure 7A. The mice received 12 IU/kg of FVIII169/VWF057 72 hours prior to tail injury showed similar protection on re-bleeding and survival compared to the mice received SQ BDD FVIII treatment 24 hour before the tail injury.

[0053] Figure 8A shows the comparable rebleeding data in mice that received rFVIII-XTEN-Fc/D'D3-XTEN-Fc Heterodimers at 96 hours versus rBDD-FVIII at 24 hours before the injury. Filled squares show the rebleeding data in mice received FVIII169/VWF034 at 24 hours before the injury; hollow squares show the rebleeding data in mice received FVIII169/VWF034 at 96 hours before the injury; filled diamond show the rebleeding data in mice received FVIII169/VWF057 at 24 hours before the injury; hollow diamond show the rebleeding data in mice received FVIII169/VWF057 at 96 hours before the injury; filled circles show the rebleeding data in mice received rBDD-FVIII at 24 hours before the injury; hollow circles show the rebleeding data in mice received rBDD-FVIII at 48 hours before the injury; and filled triangle show the rebleeding data in mice received vehicle. X axis shows time in hours, and y axis shows percent of Non- Bleeders

[0054] Figure 8B shows the survival curve in mice that received rFVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers at 96 hours versus rBDD-FVIII at 24 hours before the injury. X axis shows time in hours, and y axis shows percent of survival. The symbols are the same as Figure 8A.

[0055] Figure 9 shows a diagram of representative FVIII-VWF heterodimers and FVIII169, FVIII286, VWF057, VWF059, and VWF062 constructs. For example, FVIII169 construct comprises a B domain deleted FVIII protein with R1648A substitution fused to an Fc region, wherein an XTEN sequence (*e.g.*, AE288) is inserted at amino acid 745 corresponding to mature full length FVIII (A1-a1-A2-a2-288XTEN-a3-A3-C1-C2-Fc). FVIII286 construct comprises a B domain deleted FVIII protein with R1648 substitution fused to an Fc region, wherein an XTEN sequence (*e.g.*, AE288) is inserted at amino acid 745 corresponding to mature full length FVIII, with additional a2 region in between FVIII and Fc (A1-a1-A2-a2-288XTEN-a3-A3-C1-C2-a2-Fc). VWF057 is a VWF-Fc fusion construct that comprises D'D3 domain of the VWF protein (with two amino acid substitutions in D'D3 domain, *i.e.*, C336A and C379A) linked to the Fc region via a VWF linker, which comprises LVPRG thrombin site ("LVPRG"; SEQ ID NO: 6) and GS linker ("GS"), wherein an XTEN sequence (*i.e.*, AE144) is inserted between D'D3 domain and the VWF linker (D'D3-144XTEN-GS+LVPRG-Fc). VWF059 is a VWF-Fc fusion construct that comprises D'D3 domain of the VWF protein (with two amino acid substitutions in D'D3 domain, *i.e.*, C336A and C379A) linked to the Fc region via an acidic region 2 (a2) of FVIII as a VWF linker, wherein an XTEN sequence (*i.e.*, AE144) is inserted between D'D3 domain and the VWF linker. VWF062 is a VWF-Fc fusion construct that comprises D'D3 domain of the VWF protein

(with two amino acid substitutions in D'D3 domain, *i.e.*, C336A and C379A) linked to the Fc region, wherein an XTEN sequence (*i.e.*, AE144) is inserted between D'D3 domain and the Fc region (D'D3-144XTEN-Fc).

[0056] Figure 10 shows a schematic diagram representing FVIII/VWF heterodimer constructs, for example, FVIII169/VWF057, FVIII169/VWF059, FVIII169/VWF059A, and FVIII169/VWF073. The arrow shows the site where an optional linker is added to introduce a thrombin cleavage site. FVIII169/VWF057 has a linker comprising LVPRG (SEQ ID NO: 6). FVIII169/VWF059 has a linker comprising the FVIII a2 region (*i.e.*, ISDKNTGDDYYEDSYEDISAYLLSKNNNAIEPRSFSDKTH (SEQ ID NO: 106)). FVIII169/VWF059A has a linker comprising a truncated FVIII a2 region (*i.e.*, DKNTGDDYYEDSYEDISAYLLSKNNNAIEPRSFSDKTH (SEQ ID NO: 88)). FVIII169/VWF073 has a linker within the VWF073 construct (SEQ ID NO: 175) comprising a fragment of the FVIII a2 region consisting of IEPRSF (SEQ ID NO: 194).

[0057] Figures 11A-C show SDS-PAGE images following thrombin digestion of FVIII169/VWF057 and a FVIII-Fc control. Figure 11A shows staining of the SDS-PAGE gel with an anti-D3 antibody (AB 96340). Arrows highlight "LCFc:D'D3-XTEN-Fc," which is the un-cleaved, full-length FVIII169/VWF057; and "D'D3-144 XTEN," which is the resulting fragment following cleavage by thrombin. Figure 11B shows staining of the SDS-PAGE gel with an anti-HC antibody (GMA012). Arrows highlight the FVIII heavy chain ("HC") and FVIII A2 domain. Figure 11C shows the overlay of panels A and B. Samples were collected at the time points indicated at the top of each panel. Arrows point to the relevant proteins.

[0058] Figures 12A-C shows SDS-PAGE images following thrombin digestion of FVIII169/VWF059. Figure 12A shows staining of the SDS-PAGE gel with an anti-D3 antibody (AB 96340). Arrows highlight "LCFc:D'D3-XTEN-Fc," which is the un-cleaved, full-length FVIII169/VWF059; and "D'D3-144 XTEN," which is the resulting fragment following cleavage by thrombin. Figure 12B shows staining of the SDS-PAGE gel with an anti-HC antibody (GMA012). Arrows highlight the un-cleaved, full length FVIII169/VWF059; D'D3-144 XTEN-a3, which is the resulting fragment following cleavage by thrombin; and "A2," which is the A2 domain of FVIII. Figure 12C shows the overlay of panels A and B. Samples were collected at the time points indicated at the top of each panel

[0059] Figure 13 shows acute efficacy data of HemA mice treated with FVIII169/VWF059 (circle) as compared to HemA mice treated with a BDD-FVIII control (Square). Blood loss value was measured following tail clip. $p = 0.9883$.

DETAILED DESCRIPTION OF THE INVENTION

[0060] The present invention is directed to a chimeric protein comprising two polypeptides, a first polypeptide comprising a FVIII protein fused to a first Ig constant region and a second polypeptide comprising a VWF protein fused to a second Ig constant region or a portion thereof by an XTEN sequence, wherein the XTEN sequence contains less than 288 amino acids.

I. Definitions

[0061] 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.

[0062] 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 aspects: 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).

[0063] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0064] 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.

[0065] Units, prefixes, and symbols are denoted in their Système 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 aspects of the disclosure, which can be had 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.

[0066] The term "about" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by

extending the boundaries above and below the numerical values set forth. In general, the term "about" can modify a numerical value above and below the stated value by a variance of, *e.g.*, 10 percent, up or down (higher or lower).

[0067] 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)). 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. For example, a recombinant polynucleotide encoding a Factor VIII polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further 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.

[0068] 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 may 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. 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.

- [0069] 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 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 β -glucuronidase signal peptide, or a functional derivative thereof, can be used.
- [0070] The term "downstream," when refers to a nucleotide sequence, means that a nucleic acid or a nucleotide sequence is located 3' to a reference nucleotide sequence. In certain embodiments, downstream nucleotide sequences relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription. The term "downstream," when refers to a polypeptide sequence, means that the amino acid or an amino acid insertion site is located at the C-terminus of the reference amino acids. For example, an insertion site immediately downstream of amino acid 745 corresponding to the mature wild type FVIII protein means that the insertion site is between amino acid 745 and amino acid 746 corresponding to the mature wild type FVIII protein.
- [0071] The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence. In certain embodiments, upstream nucleotide sequences relate to sequences that are located on the 5' side of a coding region or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.
- [0072] As used herein, the term "regulatory region" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding region, and which influence the transcription, RNA processing, stability, or translation of the associated coding region. Regulatory regions may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites and stem-loop structures. If a coding region is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.
- [0073] A polynucleotide which encodes a gene product, *e.g.*, a polypeptide, can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. In an operable association a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory regions in such a way as to place

expression of the gene product under the influence or control of the regulatory region(s). For example, a coding region and a promoter are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the gene product encoded by the coding region, and if the nature of the linkage between the promoter and the coding region does not interfere with the ability of the promoter to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can also be operably associated with a coding region to direct gene product expression.

[0074] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit β -globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

[0075] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0076] The term "expression" as used herein refers to a process by which a polynucleotide produces a gene product, for example, an RNA or a polypeptide. It includes without limitation transcription of the polynucleotide into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product, and the translation of an mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation or splicing, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

[0077] A "vector" refers to any vehicle for the cloning of and/or transfer of a nucleic acid into a host cell. A vector may be a replicon to which another nucleic acid segment may be attached so

as to bring about the replication of the attached segment. A "replicon" refers to any genetic element (*e.g.*, plasmid, phage, cosmid, chromosome, virus) that functions as an autonomous unit of replication *in vivo*, *i.e.*, capable of replication under its own control. The term "vector" includes both viral and nonviral vehicles for introducing the nucleic acid into a cell *in vitro*, *ex vivo* or *in vivo*. A large number of vectors are known and used in the art including, for example, plasmids, modified eukaryotic viruses, or modified bacterial viruses. Insertion of a polynucleotide into a suitable vector can be accomplished by ligating the appropriate polynucleotide fragments into a chosen vector that has complementary cohesive termini.

[0078] Vectors may be engineered to encode selectable markers or reporters that provide for the selection or identification of cells that have incorporated the vector. Expression of selectable markers or reporters allows identification and/or selection of host cells that incorporate and express other coding regions contained on the vector. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, kanamycin, hygromycin, bialaphos herbicide, sulfonamide, and the like; and genes that are used as phenotypic markers, *i.e.*, anthocyanin regulatory genes, isopentanyl transferase gene, and the like. Examples of reporters known and used in the art include: luciferase (Luc), green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), -galactosidase (LacZ), -glucuronidase (Gus), and the like. Selectable markers may also be considered to be reporters.

[0079] The term "plasmid" refers to an extra-chromosomal element often carrying a gene that is not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear, circular, or supercoiled, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

[0080] Eukaryotic viral vectors that can be used include, but are not limited to, adenovirus vectors, retrovirus vectors, adeno-associated virus vectors, and poxvirus, *e.g.*, vaccinia virus vectors, baculovirus vectors, or herpesvirus vectors. Non-viral vectors include plasmids, liposomes, electrically charged lipids (cytofectins), DNA-protein complexes, and biopolymers.

[0081] A "cloning vector" refers to a "replicon," which is a unit length of a nucleic acid that replicates sequentially and which comprises an origin of replication, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. Certain cloning vectors are capable of replication in one cell type, *e.g.*, bacteria and expression in another, *e.g.*, eukaryotic cells. Cloning vectors typically

comprise one or more sequences that can be used for selection of cells comprising the vector and/or one or more multiple cloning sites for insertion of nucleic acid sequences of interest.

[0082] The term "expression vector" refers to a vehicle designed to enable the expression of an inserted nucleic acid sequence following insertion into a host cell. The inserted nucleic acid sequence is placed in operable association with regulatory regions as described above.

[0083] Vectors are introduced into host cells by methods well known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter.

[0084] "Culture," "to culture" and "culturing," as used herein, means to incubate cells under *in vitro* conditions that allow for cell growth or division or to maintain cells in a living state. "Cultured cells," as used herein, means cells that are propagated *in vitro*.

[0085] 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, "protein," "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. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, 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 can be derived from a natural biological source or produced recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0086] An "isolated" polypeptide or a fragment, variant, or derivative thereof refers to a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can simply be removed from its native or natural environment. 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.

[0087] Also included in the present invention are fragments or variants of polypeptides, and any combination thereof. The term "fragment" or "variant" when referring to polypeptide binding domains or binding molecules of the present invention include any polypeptides which retain at least some of the properties (*e.g.*, FcRn binding affinity for an FcRn binding domain or Fc variant,

coagulation activity for an FVIII variant, or FVIII binding activity for the VWF fragment) of the reference polypeptide. Fragments of polypeptides include proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein, but do not include the naturally occurring full-length polypeptide (or mature polypeptide). Variants of polypeptide binding domains or binding molecules of the present invention include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants can be naturally or non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions.

[0088] The term "VWF protein" or "VWF proteins" used herein means any VWF fragments that interact with FVIII and retain at least one or more properties that are normally provided to FVIII by full-length VWF, *e.g.*, preventing premature activation to FVIIIa, preventing premature proteolysis, preventing association with phospholipid membranes that could lead to premature clearance, preventing binding to FVIII clearance receptors that can bind naked FVIII but not VWF-bound FVIII, and/or stabilizing the FVIII heavy chain and light chain interactions.

[0089] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, 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.

[0090] As known in the art, "sequence identity" between two polypeptides is determined by comparing the amino acid sequence of one polypeptide to the sequence of a second polypeptide. When discussed herein, whether any particular polypeptide is at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). BESTFIT

uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full-length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

[0091] As used herein, an "amino acid corresponding to" or an "equivalent amino acid" in a VWF sequence or a FVIII protein sequence is identified by alignment to maximize the identity or similarity between a first VWF or FVIII sequence and a second VWF or FVIII sequence. The number used to identify an equivalent amino acid in a second VWF or FVIII sequence is based on the number used to identify the corresponding amino acid in the first VWF or FVIII sequence.

[0092] As used herein, the term "insertion site" refers to a position in a FVIII polypeptide, or fragment, variant, or derivative thereof, which is immediately upstream of the position at which a heterologous moiety can be inserted. An "insertion site" is specified as a number, the number being the number of the amino acid in mature native FVIII (SEQ ID NO: 65) to which the insertion site corresponds, which is immediately N-terminal to the position of the insertion. For example, the phrase "a3 comprises an XTEN at an insertion site which corresponds to amino acid 1656 of SEQ ID NO: 65" indicates that the heterologous moiety is located between two amino acids corresponding to amino acid 1656 and amino acid 1657 of SEQ ID NO: 65.

[0093] The phrase "immediately downstream of an amino acid" as used herein refers to position right next to the terminal carboxyl group of the amino acid. Similarly, the phrase "immediately upstream of an amino acid" refers to the position right next to the terminal amine group of the amino acid. Therefore, the phrase "between two amino acids of an insertion site" as used herein refers to a position in which an XTEN or any other polypeptide is inserted between two adjacent amino acids. Thus, the phrases "inserted immediately downstream of an amino acid" and "inserted between two amino acids of an insertion site" are used synonymously with "inserted at an insertion site."

[0094] The terms "inserted," "is inserted," "inserted into" or grammatically related terms, as used herein refers to the position of an XTEN in a chimeric polypeptide relative to the analogous position in native mature human FVIII. As used herein the terms refer to the characteristics of the recombinant FVIII polypeptide relative to native mature human FVIII, and do not indicate, imply or infer any methods or process by which the chimeric polypeptide was made. For example, in reference to a chimeric polypeptide provided herein, the phrase "an XTEN is inserted into immediately downstream of residue 745 of the FVIII polypeptide" means that the chimeric

polypeptide comprises an XTEN immediately downstream of an amino acid which corresponds to amino acid 745 in native mature human FVIII, *e.g.*, bounded by amino acids corresponding to amino acids 745 and 746 of native mature human FVIII.

[0095] A "fusion" or "chimeric" protein comprises a first amino acid sequence linked to a second amino acid sequence with which it is not naturally linked in nature. The amino acid sequences which normally exist in separate proteins can be brought together in the fusion polypeptide, or the amino acid sequences which normally exist in the same protein can be placed in a new arrangement in the fusion polypeptide, *e.g.*, fusion of a Factor VIII domain of the invention with an Ig Fc domain. A fusion protein is created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship. A chimeric protein can further comprises a second amino acid sequence associated with the first amino acid sequence by a covalent, non-peptide bond or a non-covalent bond.

[0096] As used herein, the term "half-life" refers to a biological half-life of a particular polypeptide *in vivo*. Half-life may be represented by the time required for half the quantity administered to a subject to be cleared from the circulation and/or other tissues in the animal. When a clearance curve of a given polypeptide is constructed as a function of time, the curve is usually biphasic with a rapid α -phase and longer β -phase. The α -phase typically represents an equilibration of the administered Fc polypeptide between the intra- and extra-vascular space and is, in part, determined by the size of the polypeptide. The β -phase typically represents the catabolism of the polypeptide in the intravascular space. In some embodiments, FVIII and chimeric proteins comprising FVIII are monophasic, and thus do not have an alpha phase, but just the single beta phase. Therefore, in certain embodiments, the term half-life as used herein refers to the half-life of the polypeptide in the β -phase. The typical β phase half-life of a human antibody in humans is 21 days.

[0097] The term "linked" as used herein refers to a first amino acid sequence or nucleotide sequence covalently or non-covalently joined to a second amino acid sequence or nucleotide sequence, respectively. The first amino acid or nucleotide sequence can be directly joined or juxtaposed to the second amino acid or nucleotide sequence or alternatively an intervening sequence can covalently join the first sequence to the second sequence. The term "linked" means not only a fusion of a first amino acid sequence to a second amino acid sequence at the C-terminus or the N-terminus, but also includes insertion of the whole first amino acid sequence (or the second amino acid sequence) into any two amino acids in the second amino acid sequence (or the first amino acid sequence, respectively). In one embodiment, the first amino acid sequence can be linked to a second amino acid sequence by a peptide bond or a linker. The first nucleotide sequence can be linked to a second nucleotide sequence by a phosphodiester bond or a linker.

The linker can be a peptide or a polypeptide (for polypeptide chains) or a nucleotide or a nucleotide chain (for nucleotide chains) or any chemical moiety (for both polypeptide and polynucleotide chains). The term "linked" is also indicated by a hyphen (-).

[0098] 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. This association can be indicated by a colon, *i.e.*, (:). In another embodiment, it means a covalent bond except a peptide bond. For example, the amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a thiol group on a second cysteine residue. In most naturally occurring IgG molecules, the CH1 and CL regions are associated by a disulfide bond and the two heavy chains are associated by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system). 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.

[0099] The term "monomer-dimer hybrid" used herein refers to a chimeric protein comprising a first polypeptide chain and a second polypeptide chain, which are associated with each other by a disulfide bond, wherein the first chain comprises a clotting factor, *e.g.*, Factor VIII, and a first Fc region and the second chain comprises, consists essentially of, or consists of a second Fc region without the clotting factor. The monomer-dimer hybrid construct thus is a hybrid comprising a monomer aspect having only one clotting factor and a dimer aspect having two Fc regions.

[0100] As used herein, the term "cleavage site" or "enzymatic cleavage site" refers to a site recognized by an enzyme. Certain enzymatic cleavage sites comprise an intracellular processing site. In one embodiment, a polypeptide has an enzymatic cleavage site cleaved by an enzyme that is activated during the clotting cascade, such that cleavage of such sites occurs at the site of clot formation. Exemplary such sites include, *e.g.*, those recognized by thrombin, Factor XIa or Factor Xa. Exemplary FXIa cleavage sites include, *e.g.*, TQSFNDFTR (SEQ ID NO: 1) and SVSQTSKLTR (SEQ ID NO: 3). Exemplary thrombin cleavage sites include, *e.g.*, DFLAEGGGVR (SEQ ID NO: 4), TTKIKPR (SEQ ID NO: 5), LVPRG (SEQ ID NO: 6),

ALRPR (SEQ ID NO: 7), ISDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 106), DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), and IEPRSFS (SEQ ID NO: 194). Other enzymatic cleavage sites are known in the art and described in elsewhere herein.

[0101] As used herein, the term "processing site" or "intracellular processing site" refers to a type of enzymatic cleavage site in a polypeptide which is a target for enzymes that function after translation of the polypeptide. In one embodiment, such enzymes function during transport from the Golgi lumen to the trans-Golgi compartment. Intracellular processing enzymes cleave polypeptides prior to secretion of the protein from the cell. Examples of such processing sites include, *e.g.*, those targeted by the PACE/furin (where PACE is an acronym for Paired basic Amino acid Cleaving Enzyme) family of endopeptidases. These enzymes are localized to the Golgi membrane and cleave proteins on the carboxyterminal side of the sequence motif Arg-[any residue]-(Lys or Arg)-Arg. As used herein the "furin" family of enzymes includes, *e.g.*, PCSK1 (also known as PC1/Pc3), PCSK2 (also known as PC2), PCSK3 (also known as furin or PACE), PCSK4 (also known as PC4), PCSK5 (also known as PC5 or PC6), PCSK6 (also known as PACE4), or PCSK7 (also known as PC7/LPC, PC8, or SPC7). Other processing sites are known in the art.

[0102] In constructs that include more than one processing or cleavage site, it will be understood that such sites may be the same or different.

[0103] The term "Furin" refers to the enzymes corresponding to EC No. 3.4.21.75. Furin is subtilisin-like proprotein convertase, which is also known as PACE (Paired basic Amino acid Cleaving Enzyme). Furin deletes sections of inactive precursor proteins to convert them into biologically active proteins. During its intracellular transport, pro-peptide of VWF can be cleaved from mature VWF molecule by a Furin enzyme. In some embodiments, Furin cleaves the D1D2 from the D'D3 of VWF. In other embodiments, a nucleotide sequence encoding Furin can be expressed together with the nucleotide sequence encoding a VWF fragment so that D1D2 domains can be cleaved off intracellularly by Furin.

[0104] In constructs that include more than one processing or cleavage site, it will be understood that such sites may be the same or different.

[0105] A "processable linker" as used herein refers to a linker comprising at least one intracellular processing site, which are described elsewhere herein.

[0106] Hemostatic disorder, as used herein, means a genetically inherited or acquired condition characterized by a tendency to hemorrhage, either spontaneously or as a result of trauma, due to an impaired ability or inability to form a fibrin clot. Examples of such disorders include the hemophilias. The three main forms are hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency or "Christmas disease") and hemophilia C (factor XI deficiency, mild

bleeding tendency). Other hemostatic disorders include, *e.g.*, Von Willebrand disease, Factor XI deficiency (PTA deficiency), Factor XII deficiency, deficiencies or structural abnormalities in fibrinogen, prothrombin, Factor V, Factor VII, Factor X or factor XIII, Bernard-Soulier syndrome, which is a defect or deficiency in GPIb. GPIb, the receptor for VWF, can be defective and lead to lack of primary clot formation (primary hemostasis) and increased bleeding tendency), and thrombasthenia of Glanzman and Naegeli (Glanzmann thrombasthenia). In liver failure (acute and chronic forms), there is insufficient production of coagulation factors by the liver; this may increase bleeding risk.

[0107] The chimeric molecules of the invention can be used prophylactically. As used herein the term "prophylactic treatment" refers to the administration of a molecule prior to a bleeding episode. In one embodiment, the subject in need of a general hemostatic agent is undergoing, or is about to undergo, surgery. The chimeric protein of the invention can be administered prior to or after surgery as a prophylactic. The chimeric protein of the invention can be administered during or after surgery to control an acute bleeding episode. The surgery can include, but is not limited to, liver transplantation, liver resection, dental procedures, or stem cell transplantation.

[0108] The chimeric protein of the invention is also used for on-demand treatment. The term "on-demand treatment" refers to the administration of a chimeric molecule in response to symptoms of a bleeding episode or before an activity that may cause bleeding. In one aspect, the on-demand treatment can be given to a subject when bleeding starts, such as after an injury, or when bleeding is expected, such as before surgery. In another aspect, the on-demand treatment can be given prior to activities that increase the risk of bleeding, such as contact sports.

[0109] As used herein the term "acute bleeding" refers to a bleeding episode regardless of the underlying cause. For example, a subject may have trauma, uremia, a hereditary bleeding disorder (*e.g.*, factor VII deficiency) a platelet disorder, or resistance owing to the development of antibodies to clotting factors.

[0110] Treat, treatment, treating, as used herein refers to, *e.g.*, the reduction in severity of a disease or condition; the reduction in the duration of a disease course; the amelioration of one or more symptoms associated with a disease or condition; the provision of beneficial effects to a subject with a disease or condition, without necessarily curing the disease or condition, or the prophylaxis of one or more symptoms associated with a disease or condition. In one embodiment, the term "treating" or "treatment" means maintaining a FVIII trough level at least about 1 IU/dL, 2 IU/dL, 3 IU/dL, 4 IU/dL, 5 IU/dL, 6 IU/dL, 7 IU/dL, 8 IU/dL, 9 IU/dL, 10 IU/dL, 11 IU/dL, 12 IU/dL, 13 IU/dL, 14 IU/dL, 15 IU/dL, 16 IU/dL, 17 IU/dL, 18 IU/dL, 19 IU/dL, or 20 IU/dL in a subject by administering a chimeric protein or a VWF fragment of the invention. In another embodiment, treating or treatment means maintaining a FVIII trough level between about 1 and

about 20 IU/dL, about 2 and about 20 IU/dL, about 3 and about 20 IU/dL, about 4 and about 20 IU/dL, about 5 and about 20 IU/dL, about 6 and about 20 IU/dL, about 7 and about 20 IU/dL, about 8 and about 20 IU/dL, about 9 and about 20 IU/dL, or about 10 and about 20 IU/dL. Treatment or treating of a disease or condition can also include maintaining FVIII activity in a subject at a level comparable to at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or 20% of the FVIII activity in a non-hemophiliac subject. The minimum trough level required for treatment can be measured by one or more known methods and can be adjusted (increased or decreased) for each person.

II. Chimeric Proteins

[0111] The present invention is directed to extending a half-life of a chimeric protein using a VWF protein fused to an XTEN sequence by preventing or inhibiting a FVIII half-life limiting factor, *i.e.*, endogenous VWF, from associating with the FVIII protein. Endogenous VWF associates with about 95% to about 98% of FVIII in non-covalent complexes. While endogenous VWF is a FVIII half-life limiting factor, endogenous VWF bound to a FVIII protein is also known to protect FVIII in various ways. For example, full length VWF (as a multimer having about 250 kDa) can protect FVIII from protease cleavage and FVIII activation, stabilize the FVIII heavy chain and/or light chain, and prevent clearance of FVIII by scavenger receptors. But, at the same time, endogenous VWF limits the FVIII half-life by preventing pinocytosis and by clearing FVIII-VWF complex from the system through the VWF clearance pathway. It is believed, while not bound by a theory, that endogenous VWF is a half-life limiting factor that prevents the half-life of a chimeric protein fused to a half-life extender from being longer than about two-fold that of wild-type FVIII. Therefore, the present invention is directed to preventing or inhibiting interaction between endogenous VWF and a FVIII protein using a VWF protein comprising a D' domain and a D3 domain (e.g., a VWF fragment) and at the same time to increasing a half-life of resulting FVIII protein(s) by using an XTEN sequence in combination with an Ig constant region or a portion thereof. In particular, the present invention shows that a shorter XTEN sequence (*i.e.*, XTEN that contains less than 288 amino acids in length, *i.e.*, XTEN that is shorter than 288 amino acids) is better in extending a half-life of the chimeric protein.

[0112] In one embodiment, the invention is directed to a chimeric protein comprising (i) a first polypeptide which comprises a FVIII protein fused to a first Ig constant region or a portion thereof and (ii) a second polypeptide which comprises a VWF protein comprising a D' domain and a D3 domain of VWF fused to a second Ig constant region or a portion thereof by an XTEN sequence in-between, wherein the XTEN sequence contains less than 288 amino acid residues and wherein the first polypeptide is linked to or associated with the second polypeptide. In another embodiment, the XTEN sequence in the second polypeptide consists of an amino acid sequence

having a length of between 12 amino acids and 287 amino acids. In other embodiments, the chimeric protein exhibits a longer half-life compared to a corresponding fusion protein comprising the first polypeptide and the second polypeptide, wherein the second polypeptide comprises an XTEN sequence containing at least 288 amino acids, *e.g.*, AE288, *e.g.*, SEQ ID NO: 8. In still other embodiments, the XTEN sequence in the second polypeptide contains at least about 36, at least about 42, at least about 72, or at least about 144 amino acids, but less than 288 amino acids, *e.g.*, AE42, AE72, AE144 (AE144, AE144_2A, AE144_3B, AE144_4A, AE144_5A, AE144_6B), AG42, AG72, or AG144 (AG144, AG144_A, AG144_B, AG144_C, AG144_F), *e.g.*, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63.

[0113] The chimeric protein of the invention can further comprise a second XTEN sequence which links the FVIII protein with the first Ig constant region or a portion thereof.

[0114] In certain embodiments, the invention is directed to a chimeric protein comprising (i) a first polypeptide which comprises a FVIII protein fused to a first Ig constant region or a portion thereof and (ii) a second polypeptide which comprises a VWF protein comprising a D' domain and a D3 domain of VWF fused to a second Ig constant region or a portion thereof by a first XTEN sequence in-between, wherein the XTEN sequence contains less than 288 amino acid residues and wherein the first polypeptide are linked to or associated with the second polypeptide, and wherein the first polypeptide further comprises a second XTEN sequence which is inserted at one or more insertion sites within the FVIII protein or which is fused to the FVIII protein and/or the first Ig constant region or a portion thereof. Therefore, in one embodiment, a second XTEN sequence is inserted at one or more insertion sites within the FVIII protein. In another embodiment, a second XTEN sequence is fused to the FVIII protein and/or the first Ig constant region or a portion thereof. In other embodiments, a second XTEN sequence is inserted at one or more insertion sites within the FVIII protein and a third XTEN sequence is fused to the FVIII protein and/or the first Ig constant region or a portion thereof.

[0115] The second and/or third XTEN sequences can be any length of XTEN amino acids. For example, the second and/or third XTEN sequences are disclosed elsewhere herein, *e.g.*, AE42, AE72, AE864, AE576, AE288, AE144, AG864, AG576, AG288, and AG144, *e.g.*, SEQ ID NO: 8; SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 17; SEQ ID NO: 54; SEQ ID NO: 19; SEQ ID NO: 16; SEQ ID NO: 18; SEQ ID NO: 15; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63. In a particular embodiment, the second and/or third XTEN sequence is AE288 or AG288, *e.g.*, SEQ ID NO: 8 or 19.

[0116] In certain embodiments, the invention is directed to a chimeric protein comprising (i) a first polypeptide which comprises a FVIII protein fused to a first Ig constant region or a portion thereof by an optional linker, wherein an optional XTEN sequence (X2) is inserted at one or more insertion sites within the FVIII protein or is fused to the FVIII protein or to the first Ig constant region or a portion thereof, and (ii) a second polypeptide which comprises a VWF protein comprising a D' domain and a D3 domain of VWF fused to a second Ig constant region or a portion thereof by an XTEN sequence (X1) between the VWF protein and the second Ig constant region or a portion thereof, wherein the XTEN sequence (X1) contains less than 288 amino acid residues and is fused to the VWF protein by a linker and wherein the first polypeptide and the second polypeptide are associated. In some embodiments, the invention is directed to a chimeric protein comprising (i) a first polypeptide which comprises a FVIII protein fused to a first Ig constant region or a portion thereof by an optional linker, wherein an optional XTEN sequence (X2) is inserted at one or more insertion sites within the FVIII protein or is fused to the FVIII protein or to the first Ig constant region or a portion thereof, and (ii) a second polypeptide which comprises a VWF protein comprising a D' domain and a D3 domain of VWF fused to a second Ig constant region or a portion thereof by an XTEN sequence (X1) between the VWF protein and the second Ig constant region or a portion thereof, wherein the XTEN sequence (X1) contains less than 288 amino acid residues and is fused to the second Ig constant region or a portion thereof by a linker and wherein the first polypeptide and the second polypeptide are associated. In other embodiments, the linker fusing the XTEN sequence (X1) with the VWF protein or the second Ig constant region or a portion thereof is a cleavable linker. Non-limiting examples of the cleavable linkers are shown elsewhere herein. In a particular embodiment, the linker is a thrombin cleavable linker.

[0117] In some embodiments, the chimeric protein is two polypeptide chains, the first chain comprising the first polypeptide described above and the second chain comprising the second polypeptide described above. For example, the two polypeptide chains comprise (i) a first chain comprising a single chain FVIII protein, a first Ig constant region or a portion thereof, and an optional XTEN sequence which is inserted at one or more insertion sites within the FVIII protein or is fused to the FVIII protein or to the first Ig constant region or a portion thereof, and (ii) a second chain comprising a VWF protein fused to a second Ig constant region or a portion thereof by an XTEN sequence (X1) in-between, wherein the XTEN sequence (X1) contains less than 288 amino acids.

[0118] In certain embodiments, the chimeric protein is two polypeptide chains, a first chain comprising a heavy chain of a FVIII protein and a second chain comprising, from N-terminus to C-terminus, a light chain of a FVIII protein, an optional XTEN sequence which is inserted at one

or more insertion sites within the FVIII protein or is fused to the FVIII protein or to the first Ig constant region or a portion thereof, and a first Ig constant region or a portion thereof, an optional linker (e.g., a processable linker), a VWF protein, an XTEN sequence (X1), a second optional linker (e.g., a cleavable linker), and a second Ig constant region or a portion thereof.

[0119] In other embodiments, the chimeric protein is three polypeptide chains, (i) a first chain comprising a heavy chain of a FVIII protein, (ii) a second chain comprising a light chain of a FVIII protein, a first Ig constant region or a portion thereof, and an optional XTEN sequence which is inserted at one or more insertion sites within the heavy chain or the light chain of the FVIII protein or is fused to the FVIII protein or to the first Ig constant region or a portion thereof, and (iii) a third chain comprising a VWF protein fused to a second Ig constant region or a portion thereof by an XTEN sequence (X1) in-between, wherein the first chain and the second chain are associated by a non-covalent bond, e.g., a metal bond, and the second chain and the third chain are associated by a covalent bond, e.g., a disulfide bond.

[0120] In still other embodiments, the chimeric protein is a single chain comprising, from N terminus to C terminus, a single chain FVIII protein, an optional XTEN sequence which is inserted at one or more insertion sites within the FVIII protein or is fused to the FVIII protein or to the first Ig constant region or a portion thereof, and a first Ig constant region or a portion thereof, an optional linker (e.g., a processable linker), a VWF protein, an XTEN sequence (X1), a second optional linker (e.g., a cleavable linker), and a second Ig constant region or a portion thereof.

[0121] In certain embodiments, a chimeric protein comprises one of the following formulae (a)-(hh):

- (a) FVIII-F1:F2-L2-X-L1-V;
- (b) FVIII-F1:V-L1-X-L2-F2;
- (c) F1-FVIII:F2-L2-X-L1-V;
- (d) F1-FVIII:V-L1-X-L2-F2;
- (e) FVIII-X2-F1:F2-L2-X1-L1-V;
- (f) FVIII-X2-F1:V-L1-X1-L2-F2;
- (g) FVIII(X2)-F1:F2-L2-X1-L1-V;
- (h) FVIII(X2)-F1:V-L1-X1-L2-F2;
- (i) F1-X2-F1:F2-L2-X1-L1-V;
- (j) F1-X2-F1:V-L1-X1-L2-F2;

- (k) V-L1-X-L2-F2-L3-FVIII-L4-F1;
- (l) V-L1-X-L2-F2-L3-F1-L4-FVIII;
- (m) F1-L4-FVIII-L3-F2-L2-X-L1-V;
- (n) FVIII-L4-F1-L3-F2-L2-X-L1-V;
- (o) FVIII-L4-F1-L3-V-L1-X-L2-F2;
- (p) FVIII-L4-F1-L3-F2-L2-X-L1-V;
- (q) F2-L2-X-L1-V-L3-F1-L4-FVIII;
- (r) F2-L2-X-L1-V-L3-FVIII-L4-F1;
- (s) V-L1-X1-L2-F2-L3-FVIII(X2)-L4-F1;
- (t) V-L1-X1-L2-F2-L3-F1-L4-FVIII(X2);
- (u) F1-L4-FVIII(X2)-L3-F2-L2-X1-L1-V;
- (v) F-L4-FVIII(X2)-L3-V-L1-X1-L2-F2;
- (w) FVIII(X2)-L4-F1-L3-V-L1-X1-L2-F2;
- (x) FVIII(X2)-L4-F1-L3-F2-L2-X1-L1-V;
- (y) F2-L2-X1-L1-V-L3-F1-L4-FVIII(X2);
- (z) F2-L2-X1-L1-V-L3-FVIII(X2)-L4-F1;
- (aa) V-L1-X2-L2-F2-L3-FVIII-L4-X2-L5-F1;
- (bb) V-L1-X2-L2-F2-L3-F1-L5-X2-L4-FVIII;
- (cc) F1-L5-X2-L4-FVIII-L3-F2-L2-X2-L1-V;
- (dd) F1-L5-X2-L4-FVIII-L3-V-L1-X2-L2-F2;
- (ee) FVIII-L5-X2-L4-F2-L3-V-L1-X1-L2-F1;
- (ff) FVIII-L5-X2-L4-F2-L3-F1-L2-X1-L1-V;
- (gg) F1-L2-X1-L1-V-L3-F2-L4-X2-L5-FVIII; or
- (hh) F1-L2-X1-L1-V-L3-FVIII-L5-X2-L4-F2;

wherein V is a VWF protein, which comprises a D' domain and a D3 domain,

X or X1 is a first XTEN sequence that contains less than 288 amino acids,

X2 is a second XTEN sequence,

FVIII comprises a FVIII protein,

FVIII(X2) comprises a FVIII protein having a second XTEN sequence inserted in one or more insertion sites within the FVIII protein,

F1 is a first Ig constant region or a portion thereof,

F2 is a second Ig constant region or a portion thereof,

L1, L2, L3, L4, or L5 is an optional linker,

(-) is a peptide bond; and

(:) is a covalent bond or a non-covalent bond.

[0122] In one embodiment, the X or X1 consists of an amino acid sequence having a length of between 12 amino acids and 287 amino acids. In another embodiment, the chimeric protein exhibits a longer half-life compared to a corresponding fusion protein comprising a formula wherein the X or X1 is AE288, *e.g.*, SEQ ID NO: 8.

[0123] In other embodiments, the X or X1 in the formula contains at least about 36, at least about 42, at least about 72, or at least about 144 amino acids, but less than 288 amino acids, *e.g.*, AE42, AE72, AE144 (AE144, AE144_2A, AE144_3B, AE144_4A, , AE144_5A, AE144_6B) , AG42, AG72, or AG144 (AG144, AG144_A, AG144_B, AG144_C, AG144_F), *e.g.*, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63.

[0124] In yet other embodiments, the X2 comprises an amino acid sequence having a length of at least about 36 amino acids, at least 42 amino acids, at least 144 amino acids, at least 288 amino acids, at least 576 amino acids, or at least 864 amino acids, *e.g.*, AE42, AE72, AE864, AE576, AE288, AE144, AG864, AG576, AG288, or AG144, *e.g.*, SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 15; SEQ ID NO: 16; SEQ ID NO: 8; SEQ ID NO: 11; SEQ ID NO: 17; SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 14. In a particular embodiment, the X2 is AE288 or AG288, *e.g.*, SEQ ID NO: 8 or 19.

[0125] In certain embodiments, the chimeric protein comprising the X or X1 and/or X2 has an extended half-life compared to a chimeric protein without the X or X1 and/or X2. In other embodiments, the L1 and/or L2 is a cleavable linker. In still other embodiments, the L4 and/or L5 is a cleavable linker.

II.A. Von Willebrand Factor (VWF) Proteins

[0126] VWF (also known as F8VWF) is a large multimeric glycoprotein present in blood plasma and produced constitutively in endothelium (in the Weibel-Palade bodies), megakaryocytes (α -granules of platelets), and subendothelial connective tissue. The basic VWF monomer is a 2813 amino acid protein. Every monomer contains a number of specific domains with a specific function, the D'/D3 domain (which binds to Factor VIII), the A1 domain (which binds to platelet

GPIIb-receptor, heparin, and/or possibly collagen), the A3 domain (which binds to collagen), the C1 domain (in which the RGD domain binds to platelet integrin α IIb β 3 when this is activated), and the "cysteine knot" domain at the C-terminal end of the protein (which VWF shares with platelet-derived growth factor (PDGF), transforming growth factor- β (TGF β) and β -human chorionic gonadotropin (β HCG)).

[0127] In one embodiment, the VWF protein is a VWF fragment. The term "a VWF fragment" as used herein includes, but is not limited to, functional VWF fragments comprising a D' domain and a D3 domain, which are capable of inhibiting binding of endogenous VWF to FVIII. In one embodiment, the VWF fragment binds to the FVIII protein. In another embodiment, the VWF fragment blocks the VWF binding site on the FVIII protein, thereby inhibiting interaction of the FVIII protein with endogenous VWF. The VWF fragments include derivatives, variants, mutants, or analogues that retain these activities of VWF.

[0128] The 2813 monomer amino acid sequence for human VWF is reported as Accession Number _NP_000543.2_ in Genbank. The nucleotide sequence encoding the human VWF is reported as Accession Number _NM_000552.3_ in Genbank. A nucleotide sequence of human VWF is designated as SEQ ID NO: 20. SEQ ID NO: 21 is the amino acid sequence of full-length VWF. Each domain of VWF is listed in Table 1.

TABLE 1. VWF Sequences

VWF domains	Amino acid Sequence			
VWF Signal Peptide (Amino acids 1 to 22 of SEQ ID NO: 21)	1	<u>MIPARFAGVL LALALILPGT LC</u>		22
VWF D1D2 region (Amino acids 23 to 763 of SEQ ID NO: 21)	23	AEGTRGRS STARCSLFGS		
	51	DFVNTFDGSM YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLEFVNG		
	101	TVTQGDQRVSPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL		
	151	SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC		
	201	ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC		
	251	EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME		
	301	YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC		
	351	VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD		
	401	NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG		
	451	LHNSLVKLKH GAGVAMDGQD IQLP LLKGD L RIQHTVTASV RLSYGEDLQM		
	501	DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG		

	551	LAEPRVEDFG NAWKLHGDCQ	DLQKQHSDDPC	ALNPRMTRFS	EEACAVLTSP	
	601	TFEACHRAVS PLPYLRNCRY	DVCSGSDGRE	CLCGALASYA	AACAGRGVRV	
	651	AWREPGRCEL NCPKGQVYLQ	CGTPCNLTCT	SLSYPDEECN	EACLEGCFCP	
	701	PGLYMDERGD CVPKAQCPCY	YDGEIFQPED	IFSDHHTMCY	CEDGFMHCTM	
	751	SGVPGSLLPD AVLSSPLSHR	SKR			763
VWF D' Domain	764	<u>TCQNYDLECM</u>	<u>SLSCRPP</u>	<u>MVKLVCPADN</u>	<u>LRAEGLECTK</u>	
	801	<u>SMGCVSGCLC</u>	<u>PPGMVRHENR</u>	<u>CVALERCPCF</u>	<u>HOGKEYAPGE</u>	
	851	<u>TVKIGCNTCV</u> <u>CRDRKWNCTD</u>	<u>HVCDAT</u>			866
VWF D3 Domain	867	<u>YVLVQDYCGS</u>	<u>CSTI</u>	<u>GMAHYLTFDG</u>	<u>LKYLFPGEQ</u>	
	901	<u>NPGTFRILVG</u>	<u>NKGCSPSVK</u>	<u>CKKRVTLVE</u>	<u>GGEIELFDGE</u>	
	951	<u>VNVKRPMDKE</u> <u>THFEVVEGR</u>	<u>YIILLGKAL</u>	<u>SVVWDRHLSI</u>	<u>SVVLKQTYQE</u>	
	1001	<u>KVCGLCGNFD</u> <u>GIONNDLTSS</u>	<u>NLQVEEDPVD</u>	<u>FGNSWKVSSQ</u>	<u>CADTRKVPLD</u>	
	1051	<u>SSPATCHNNI</u> <u>MKQTMVDSSC</u>	<u>RILTSDVFQD</u>	<u>CNKLVDPPEY</u>	<u>LDVCIYDTCS</u>	
	1101	<u>CESIGDCACF</u> <u>CDTIAAYAHV</u>	<u>CAQHKGKVTW</u>	<u>RTATLCPQSC</u>	<u>EERNLRENGY</u>	
	1151	<u>ECEWRYNSCA</u> <u>PACQVTCQHP</u>	<u>EPLACPVCV</u>	<u>EGCHAHCPPG</u>	<u>KILDELLQTC</u>	
	1201	<u>VDPEDCPVCE</u> <u>VAGRRFASGK</u>	<u>KVTLNPSDPE</u>	<u>HCQICHCDVV</u>	<u>NLTCEACQEP</u>	
	1240					
VWF A1 Domain	1241	GGLVVPPTDA				
	1251	PVSPPTLYVE	DISEPPLHDF	YCSRLDLVF	LLDGSSRLSE	
	1301	AEFEVLKAFV VDMMERLRIS	QKWVRVAVVE	YHDGSHAYIG	LKDRKRPSEL	
	1351	RRIASQVKYA GSQVASTSEV	LKYTLFQIFS	KIDRPEASRI	ALLLMASQEP	
	1401	QRMSRNFVRY VQGLKKKKVI	VIPVGIGPHA	NLKQIRLIEK	QAPENKAFVL	
	1451	SSVDELEQQR DEIVSYLCDL	APEAPPPTLP	PDMAQVTVG		1479
	1480			P	GLLGVSTLGP	KRNSMVLDVA
	1501	FVLEGSDKIG	EADFNRSKEF	MEEVIQRMDV	GQDSIHVTVL	
	1551	QYSYMTVEY PFSEAQSKGD	ILQRVREIRY	QGGNRTNTGL	ALRYLSDHSF	
	1601	LVSQGDREQA PNLVYMTGN	PASDEIKRLP	GDIQVVPIGV	GPNAVQELE	1600
	1651	RIGWPNAPIL IQDFETLPRE	APDLVLQRCC	SGEGLQIPTL	SPAPDCSQPL	
	1701	DVILLLDGSS SFPASYFDEM	KSFAKAFISK	ANIGPRLTQV	SVLQYGSITT	
	1751	IDVPWNVPE KAHLLSLVDV	MQREGGPSQI	GDALGFAVRY	LTSEMHGARP	
	1801	GASKAVVILV TDVSVDSVDA	AADAARSNRV	TVFPIGIGDR	YDAAQLRILA	
		GPAGDSNVVK				

	1851	LQRIEDLPTM VTLGNSFLHK LCSGFVRICM DEDGNEKRP DVWTLDPDQCH
	1901	TVTCQPDGQT LLKSHRVNCD RGLRPSCPNS QSPVKVEETC GCRWTCPCVC
	1951	TGSSTRHIVT FDGQNFKL TG SCSYVLFQNK EQDLEVILHN GACSPGARQG
	2001	CMKSIEVKHS ALSVEXHSDM EVTVNGRLVS VPYVGGNMEV NVYGAIMHEV
	2051	RFNHLGHIFT FTPQNNEFQL QLSPKTFASK TYGLCGICDE NGANDFMLRD
	2101	GTVTTDWKTL VQEWTVQRPQ QTCQPILEEQ CLVPDSSHQC VLLLPLFAEC
	2151	HKVLAPATFY AICQQDSCHQ EQVCEVIASY AHLCRTNGVC VDWRTPDFCA
	2201	MSCPPSLVYN HCEHGCPRHC DGNVSSCGDH PSEGCFCPPD KVMLEGSCVP
	2251	EEACTQCIGE DGVQHQFLEA WVPDHQPCQI CTCLSGRKVN CTTQPCPTAK
	2301	APTCGLCEVA RLRQNADQCC PEYECVCDPV SCDLPPVPHC ERGLQPTLTN
	2351	PGECPNFTC ACRKEECKRV SPPSCPPHRL PTLRKTQCCD EYECACNCVN
	2401	STVSCPLGYL ASTATNDCGC TTTTCLPDKV CVHRSTIYPV GQFWEEGCDV
	2451	CTCTDMEDAV MGLRVAQCSQ KPCEDSCRSG FTYVLHEGEC CGRCLPSACE
	2501	VVTGSPRGDS QSSWKS VGSQ WASPENPCLI NECVRVKEEV FIQQRNVSCP
	2551	QLEVPVCPSP FQLSCKTSAC CPSCRCERME ACMLNGTVIG PGKTVMIDVC
	2601	TTCRCMVQVG VISGFKLECR KTTCNPCPLG YKEENNTGEC CGRCLPTACT
	2651	IQLRGGQIMT LKRDETLQDG CDTHFCKVNE RGEYFWEKRV TGCPPFDEHK
	2701	CLAEGGKIMK IPGTCCDTCE EPECNDITAR LQYVKVGSK SEVEVDIHYC
	2751	QGKCASKAMY SIDINDVQDQ CSCCSPTRTE PMQVALHCTN GSVVYHEVLN
	2801	AMECKCSPRK CSK
	Nucleotide Sequence (SEQ ID NO: 20)	
Full-length VWF	1	ATGATTCTCG CCAGATTTGC CGGGGTGCTG CTTGCTCTGG CCCTCATTTT 51 GCCAGGGACC CTTTGTGCAG AAGGAAC TCG CGGCAGGTCA TCCACGGCCC 101 GATGCAGCCT TTTCGGAAGT GACTTCGTCA ACACCTTTGA TGGGAGCATG 151 TACAGCTTTG CGGGATACTG CAGTTACCTC CTGGCAGGGG GCTGCCAGAA 201 ACGCTCCTTC TCGATTATTG GGGACTTCCA GAATGGCAAG AGAGTGAGCC 251 TCTCCGTGTA TCTTGGGGAA TTTTTTGACA TCCATTTGTT TGTC AATGGT 301 ACCGTGACAC AGGGGGACCA AAGAGTCTCC ATGCCCTATG CCTCCAAAGG 351 GCTGTATCTA GAAACTGAGG CTGGGTACTA CAAGCTGTCC GGTGAGGCCT 401 ATGGCTTTGT GGCCAGGATC GATGGCAGCG GCAACTTTCA AGTCCTGCTG 451 TCAGACAGAT ACTTCAACAA GACCTGCGGG CTGTGTGGCA ACTTTAACAT

501	CTTTGCTGAA	GATGACTTTA	TGACCCAAGA	AGGGACCTTG
	ACCTCGGACC			
551	CTTATGACTT	TGCCAACTCA	TGGGCTCTGA	GCAGTGGAGA
	ACAGTGGTGT			
601	GAACGGGCAT	CTCCTCCCAG	CAGCTCATGC	AACATCTCCT
	CTGGGGAAAT			
651	GCAGAAGGGC	CTGTGGGAGC	AGTGCCAGCT	TCTGAAGAGC
	ACCTCGGTGT			
701	TTGCCCCTG	CCACCCTCTG	GTGGACCCCG	AGCCTTTTGT
	GGCCCTGTGT			
751	GAGAAGACTT	TGTGTGAGTG	TGCTGGGGGG	CTGGAGTGCG
	CCTGCCCTGC			
801	CCTCCTGGAG	TACGCCCGGA	CCTGTGCCCA	GGAGGGAATG
	GTGCTGTACG			
851	GCTGGACCGA	CCACAGCGCG	TGCAGCCCAG	TGTGCCCTGC
	TGGTATGGAG			
901	TATAGGCAGT	GTGTGTCCCC	TTGCGCCAGG	ACCTGCCAGA
	GCCTGCACAT			
951	CAATGAAATG	TGTCAGGAGC	GATGCGTGGA	TGGCTGCAGC
	TGCCCTGAGG			
1001	GACAGCTCCT	GGATGAAGGC	CTCTGCGTGG	AGAGCACCAG
	GTGTCCCTGC			
1051	GTGCATTCCG	GAAAGCGCTA	CCCTCCCGGC	ACCTCCCTCT
	CTCGAGACTG			
1101	CAACACCTGC	ATTTGCCGAA	ACAGCCAGTG	GATCTGCAGC
	AATGAAGAAT			
1151	GTCCAGGGGA	GTGCCTTGTC	ACTGGTCAAT	CCCACTTCAA
	GAGCTTTGAC			
1201	AACAGATACT	TCACCTTCAG	TGGGATCTGC	CAGTACCTGC
	TGGCCCGGGA			
1251	TTGCCAGGAC	CACTCCTTCT	CCATTGTTCAT	TGAGACTGTC
	CAGTGTGCTG			
1301	ATGACCGCGA	CGCTGTGTGC	ACCCGCTCCG	TCACCGTCCG
	GCTGCCTGGC			
1351	CTGCACAACA	GCCTTGTGAA	ACTGAAGCAT	GGGGCAGGAG
	TTGCCATGGA			
1401	TGGCCAGGAC	ATCCAGCTCC	CCCTCCTGAA	AGGTGACCTC
	CGCATCCAGC			
1451	ATACAGTGAC	GGCCTCCGTG	CGCCTCAGCT	ACGGGGAGGA
	CCTGCAGATG			
1501	GACTGGGATG	GCCGCGGGAG	GCTGCTGGTG	AAGCTGTCCC
	CCGTCTATGC			
1551	CGGGAAGACC	TGCGGCCTGT	GTGGGAATTA	CAATGGCAAC
	CAGGGCGACG			
1601	ACTTCCTTAC	CCCCTCTGGG	CTGGCRGAGC	CCCGGGTGGA
	GGACTTCGGG			
1651	AACGCCTGGA	AGCTGCACGG	GGACTGCCAG	GACCTGCAGA
	AGCAGCACAG			
1701	CGATCCCTGC	GCCCTCAACC	CGCGCATGAC	CAGGTTCTCC
	GAGGAGGCGT			
1751	GCGCGGTCCT	GACGTCCCCC	ACATTCGAGG	CCTGCCATCG
	TGCCGTCAGC			
1801	CCGCTGCCCT	ACCTGCGGAA	CTGCCGCTAC	GACGTGTGCT
	CCTGCTCGGA			
1851	CGGCCGCGAG	TGCCTGTGCG	GCGCCCTGGC	CAGCTATGCC
	GCGGCCTGCG			
1901	CGGGGAGAGG	CGTGCGCGTC	GCGTGGCGCG	AGCCAGGCCG
	CTGTGAGCTG			
1951	AACTGCCCGA	AAGGCCAGGT	GTACCTGCAG	TGCGGGACCC
	CCTGCAACCT			
2001	GACCTGCCGC	TCTCTCTCTT	ACCCGGATGA	GGAATGCAAT
	GAGGCCTGCC			
2051	TGGAGGGCTG	CTTCTGCCCC	CCAGGGCTCT	ACATGGATGA
	GAGGGGGGAC			
2101	TGCGTGCCCA	AGGCCAGTG	CCCCTGTTAC	TATGACGGTG
	AGATCTTCCA			

2151	GCCAGAAGAC	ATCTTCTCAG	ACCATCACAC	CATGTGCTAC
	TGTGAGGATG			
2201	GCTTCATGCA	CTGTACCATG	AGTGGAGTCC	CCGGAAGCTT
	GCTGCCTGAC			
2251	GCTGTCCTCA	GCAGTCCCCT	GTCTCATCGC	AGCAAAAGGA
	GCCTATCCTG			
2301	TCGGCCCCCC	ATGGTCAAGC	TGGTGTGTCC	CGCTGACAAC
	CTGCGGGCTG			
2351	AAGGGCTCGA	GTGTACCAAA	ACGTGCCAGA	ACTATGACCT
	GGAGTGCATG			
2401	AGCATGGGCT	GTGTCTCTGG	CTGCCTCTGC	CCCCCGGGCA
	TGGTCCGGCA			
2451	TGAGAACAGA	TGTGTGGCCC	TGGAAAGGTG	TCCCTGCTTC
	CATCAGGGCA			
2501	AGGAGTATGC	CCCTGGAGAA	ACAGTGAAGA	TTGGCTGCAA
	CACTTGTGTC			
2551	TGTCGGGACC	GGAAGTGGAA	CTGCACAGAC	CATGTGTGTG
	ATGCCACGTG			
2601	CTCCACGATC	GGCATGGCCC	ACTACCTCAC	CTTCGACGGG
	CTCAAATACC			
2651	TGTTCCCCGG	GGAGTGCCAG	TACGTTCTGG	TGCAGGATTA
	CTGCGGCAGT			
2701	AACCTTGGGA	CCTTTCGGAT	CCTAGTGGGG	AATAAGGGAT
	GCAGCCACCC			
2751	CTCAGTGAAA	TGCAAGAAAC	GGGTCACCAT	CCTGGTGGAG
	GGAGGAGAGA			
2801	TTGAGCTGTT	TGACGGGGAG	GTGAATGTGA	AGAGGCCCAT
	GAAGGATGAG			
2851	ACTCACTTTG	AGGTGGTGGG	GTCTGGCCGG	TACATCATTC
	TGCTGCTGGG			
2901	CAAAGCCCTC	TCCGTGGTCT	GGGACCGCCA	CCTGAGCATC
	TCCGTGGTCC			
2951	TGAAGCAGAC	ATACCAGGAG	AAAGTGTGTG	GCCTGTGTGG
	GAATTTTGAT			
3001	GGCATCCAGA	ACAATGACCT	CACCAGCAGC	AACCTCCAAG
	TGGAGGAAGA			
3051	CCCTGTGGAC	TTTGGAAGT	CCTGGAAAGT	GAGCTCGCAG
	TGTGCTGACA			
3101	CCAGAAAAGT	GCCTCTGGAC	TCATCCCCTG	CCACCTGCCA
	TAACAACATC			
3151	ATGAAGCAGA	CGATGGTGGG	TTCCTCCTGT	AGAATCCTTA
	CCAGTGACGT			
3201	CTTCCAGGAC	TGCAACAAGC	TGGTGGACCC	CGAGCCATAT
	CTGGATGTCT			
3251	GCATTTACGA	CACCTGCTCC	TGTGAGTCCA	TTGGGGACTG
	CGCCTGCTTC			
3301	TGCGACACCA	TTGCTGCCTA	TGCCCACGTG	TGTGCCCAGC
	ATGGCAAGGT			
3351	GGTGACCTGG	AGGACGGCCA	CATTGTGCCC	CCAGAGCTGC
	GAGGAGAGGA			
3401	ATCTCCGGGA	GAACGGGTAT	GAGTGTGAGT	GGCGCTATAA
	CAGCTGTGCA			
3451	CCTGCCTGTC	AAGTCACGTG	TCAGCACCCCT	GAGCCACTGG
	CCTGCCCTGT			
3501	GCAGTGTGTG	GAGGGCTGCC	ATGCCCCTG	CCCTCCAGGG
	AAAATCCTGG			
3551	ATGAGCTTTT	GCAGACCTGC	GTTGACCCTG	AAGACTGTCC
	AGTGTGTGAG			
3601	GTGGCTGGCC	GGCGTTTTGC	CTCAGGAAAG	AAAGTCACCT
	TGAATCCCAG			
3651	TGACCCTGAG	CACTGCCAGA	TTTGCCACTG	TGATGTTGTG
	AACCTCACCT			
3701	GTGAAGCCTG	CCAGGAGCCG	GGAGGCCTGG	TGGTGCCTCC
	CACAGATGCC			
3751	CCGGTGAGCC	CCACCACTCT	GTATGTGGAG	GACATCTCGG
	AACCGCCGTT			

3801	GCACGATTC	TACTGCAGCA	GGCTACTGGA	CCTGGTCTTC
	CTGCTGGATG			
3851	GCTCCTCCAG	GCTGTCCGAG	GCTGAGTTTG	AAGTGCTGAA
	GGCCTTTGTG			
3901	GTGGACATGA	TGGAGCGGCT	GCGCATCTCC	CAGAAGTGGG
	TCCGCGTGGC			
3951	CGTGGTGGAG	TACCACGACG	GCTCCCACGC	CTACATCGGG
	CTCAAGGACC			
4001	GGAAGCGACC	GTCAGAGCTG	CGGCGCATTG	CCAGCCAGGT
	GAAGTATGCG			
4051	GGCAGCCAGG	TGGCCTCCAC	CAGCGAGGTC	TTGAAATACA
	CACTGTTCCA			
4101	AATCTTCAGC	AAGATCGACC	GCCCTGAAGC	CTCCCGCATC
	GCCCTGCTCC			
4151	TGATGGCCAG	CCAGGAGCCC	CAACGGATGT	CCCGBAACTT
	TGTCCGCTAC			
4201	GTCCAGGGCC	TGAAGAAGAA	GAAGGTCATT	GTGATCCCGG
	TGGGCATTGG			
4251	GCCCCATGCC	AACCTCAAGC	AGATCCGCCT	CATCGAGAAG
	CAGGCCCTG			
4301	AGAACAAGGC	CTTCGTGCTG	AGCAGTGTGG	ATGAGCTGGA
	GCAGCAAAGG			
4351	GACGAGATCG	TTAGCTACCT	CTGTGACCTT	GCCCCGAAG
	CCCCTCCTCC			
4401	TACTCTGCCC	CCCGACATGG	CACAAGTCAC	TGTGGGCCCCG
	GGGCTCTTGG			
4451	GGGTTTCGAC	CCTGGGGCCC	AAGAGGAACT	CCATGGTTCT
	GGATGTGGCG			
4501	TTCGTCTGG	AAGGATCGGA	CAAAATTGGT	GAAGCCGACT
	TCAACAGGAG			
4551	CAAGGAGTTC	ATGGAGGAGG	TGATTCAGCG	GATGGATGTG
	GGCCAGGACA			
4601	GCATCCACGT	CACGGTGCTG	CAGTACTCCT	ACATGGTGAC
	CGTGGAGTAC			
4651	CCCTTCAGCG	AGGCACAGTC	CAAAGGGGAC	ATCCTGCAGC
	GGGTGCGAGA			
4701	GATCCGCTAC	CAGGGCGGCA	ACAGGACCAA	CACTGGGCTG
	GCCCTGCGGT			
4751	ACCTCTCTGA	CCACAGCTTC	TTGGTCAGCC	AGGGTGACCG
	GGAGCAGGCG			
4801	CCCAACCTGG	TCTACATGGT	CACCGGAAAT	CCTGCCTCTG
	ATGAGATCAA			
4851	GAGGTGCGCT	GGAGACATCC	AGGTGGTGCC	CATTGGAGTG
	GGCCCTAATG			
4901	CCAACGTGCA	GGAGCTGGAG	AGGATTGGCT	GGCCCAATGC
	CCCTATCCTC			
4951	ATCCAGGACT	TTGAGACGCT	CCCCGAGAG	GCTCCTGACC
	TGGTGCTGCA			
5001	GAGGTGCTGC	TCCGGAGAGG	GGCTGCAGAT	CCCCACCCTC
	TCCCCTGCAC			
5051	CTGACTGCAG	CCAGCCCCTG	GACGTGATCC	TTCTCCTGGA
	TGGCTCCTCC			
5101	AGTTTCCCAG	CTTCTTATTT	TGATGAAATG	AAGAGTTTCG
	CCAAGGCTTT			
5151	CATTTCAAAA	GCCAATATAG	GGCCTCGTCT	CACTCAGGTG
	TCAGTGCTGC			
5201	AGTATGGAAG	CATCACCACC	ATTGACGTGC	CATGGAACGT
	GGTCCCGGAG			
5251	AAAGCCCATT	TGCTGAGCCT	TGTGGACGTC	ATGCAGCGGG
	AGGGAGGCC			
5301	CAGCCAAATC	GGGGATGCCT	TGGGCTTTGC	TGTGCGATAC
	TTGACTTCAG			
5351	AAATGCATGG	TGCCAGGCCG	GGAGCCTCAA	AGGCGGTGGT
	CATCCTGGTC			
5401	ACGGACGTCT	CTGTGGATTC	AGTGGATGCA	GCAGCTGATG
	CCGCCAGGTC			

5451	CAACAGAGTG	ACAGTGTTCC	CTATTGGAAT	TGGAGATCGC
	TACGATGCAG			
5501	CCCAGCTACG	GATCTTGGCA	GGCCCAGCAG	GCGACTCCAA
	CGTGGTGAAG			
5551	CTCCAGCGAA	TCGAAGACCT	CCCTACCATG	GTCACCTTGG
	GCAATTCCCTT			
5601	CCTCCACAAA	CTGTGCTCTG	GATTTGTTAG	GATTTGCATG
	GATGAGGATG			
5651	GGAATGAGAA	GAGGCCCGGG	GACGTCTGGA	CCTTGCCAGA
	CCAGTGCCAC			
5701	ACCGTGACTT	GCCAGCCAGA	TGGCCAGACC	TTGCTGAAGA
	GTCATCGGGT			
5751	CAACTGTGAC	CGGGGGCTGA	GGCCTTCGTG	CCCTAACAGC
	CAGTCCCCTG			
5801	TTAAAGTGGA	AGAGACCTGT	GGCTGCCGCT	GGACCTGCCC
	CTGYGTGTGC			
5851	ACAGGCAGCT	CCACTCGGCA	CATCGTGACC	TTTGATGGGC
	AGAATTTCAA			
5901	GCTGACTGGC	AGCTGTTCTT	ATGTCCTATT	TCAAAACAAG
	GAGCAGGACC			
5951	TGGAGGTGAT	TCTCCATAAT	GGTGCCTGCA	GCCCTGGAGC
	AAGGCAGGGC			
6001	TGCATGAAAT	CCATCGAGGT	GAAGCACAGT	GCCCTCTCCG
	TCGAGSTGCA			
6051	CAGTGACATG	GAGGTGACGG	TGAATGGGAG	ACTGGTCTCT
	GTTCTTTACG			
6101	TGGGTGGGAA	CATGGAAGTC	AACGTTTATG	GTGCCATCAT
	GCATGAGGTC			
6151	AGATTCAATC	ACCTTGGTCA	CATCTTCACA	TTCCTCCAC
	AAAACAATGA			
6201	GTTCCAATG	CAGCTCAGCC	CCAAGACTTT	TGCTTCAAAG
	ACGTATGGTC			
6251	TGTGTGGGAT	CTGTGATGAG	AACGGAGCCA	ATGACTTCAT
	GCTGAGGGAT			
6301	GGCACAGTCA	CCACAGACTG	GAAAACACTT	GTTCAGGAAT
	GGAATGTGCA			
6351	GCGGCCAGGG	CAGACGTGCC	AGCCCATCCT	GGAGGAGCAG
	TGTCTTGTCC			
6401	CCGACAGCTC	CCACTGCCAG	GTCCTCCTCT	TACCACTGTT
	TGCTGAATGC			
6451	CACAAGGTCC	TGGCTCCAGC	CACATTCTAT	GCCATCTGCC
	AGCAGGACAG			
6501	TTGCCACCAG	GAGCAAGTGT	GTGAGGTGAT	CGCCTCTTAT
	GCCCACCTCT			
6551	GTGGGACCAA	CGGGGTCTGC	GTTGACTGGA	GGACACCTGA
	TTTCTGTGCT			
6601	ATGTCATGCC	CACCATCTCT	GGTCTACAAC	CACTGTGAGC
	ATGGCTGTCC			
6651	CCGGCACTGT	GATGGCAACG	TGAGCTCCTG	TGGGGACCAT
	CCCTCCGAAG			
6701	GCTGTTTCTG	CCCTCCAGAT	AAAGTCATGT	TGGAAGGCAG
	CTGTGTCCCT			
6751	GAAGAGGCCT	GCACTCAGTG	CATTGGTGAG	GATGGAGTCC
	AGCACCAGTT			
6801	CCTGGAAGCC	TGGGTCCCGG	ACCACCAGCC	CTGTCAGATC
	TGCACATGCC			
6851	TCAGCGGGCG	GAAGGTCAAC	TGCACAACGC	AGCCCTGCCC
	CACGGCCAAA			
6901	GCTCCCACGT	GTGGCCTGTG	TGAAGTAGCC	CGCCTCCGCC
	AGAATGCAGA			
6951	CCAGTGCTGC	CCCAGTATG	AGTGTGTGTG	TGACCCAGTG
	AGCTGTGACC			
7001	TGCCCCCAGT	GCCTCACTGT	GAACGTGGCC	TCCAGCCCAC
	ACTGACCAAC			
7051	CCTGGCGAGT	GCAGACCCAA	CTTCACCTGC	GCCTGCAGGA
	AGGAGGAGTG			

7101	CAAAAGAGTG	TCCCCACCCT	CCTGCCCCCC	GCACCGTTTG
	CCCACCCTTC			
7151	GGAAGACCCA	GTGCTGTGAT	GAGTATGAGT	GTGCCTGCAA
	CTGTGTCAAC			
7201	TCCACAGTGA	GCTGTCCCCCT	TGGGTACTTG	GCCTCAACCG
	CCACCAATGA			
7251	CTGTGGCTGT	ACCACAACCA	CCTGCCTTCC	CGACAAGGTG
	TGTGTCCACC			
7301	GAAGCACCAT	CTACCCTGTG	GGCCAGTTCT	GGGAGGAGGG
	CTGCGATGTG			
7351	TGCACCTGCA	CCGACATGGA	GGATGCCGTG	ATGGGCCTCC
	GCGTGGCCCA			
7401	GTGCTCCAG	AAGCCCTGTG	AGGACAGCTG	TCGGTCGGGC
	TTCACTTACG			
7451	TTCTGCATGA	AGGCGAGTGC	TGTGGAAGGT	GCCTGCCATC
	TGCCTGTGAG			
7501	GTGGTGACTG	GCTCACCGCG	GGGGGACTCC	CAGTCTTCCT
	GGAAGAGTGT			
7551	CGGCTCCAG	TGGGCCTCCC	CGGAGAACCC	CTGCCTCATC
	AATGAGTGTG			
7601	TCCGAGTGAA	GGAGGAGGTC	TTTATACAAC	AAAGGAACGT
	CTCCTGCCCC			
7651	CAGCTGGAGG	TCCCTGTCTG	CCCCTCGGGC	TTTCAGCTGA
	GCTGTAAGAC			
7701	CTCAGCGTGC	TGCCCAAGCT	GTCGCTGTGA	GCGCATGGAG
	GCCTGCATGC			
7751	TCAATGGCAC	TGTCATTGGG	CCCGGGAAGA	CTGTGATGAT
	CGATGTGTGC			
7801	ACGACCTGCC	GCTGCATGGT	GCAGGTGGGG	GTCATCTCTG
	GATTCAAGCT			
7851	GGAGTGCAGG	AAGACCACCT	GCAACCCCTG	CCCCCTGGGT
	TACAAGGAAG			
7901	AAAATAACAC	AGGTGAATGT	TGTGGGAGAT	GTTTGCCCTAC
	GGCTTGCACC			
7951	ATTCAGCTAA	GAGGAGGACA	GATCATGACA	CTGAAGCGTG
	ATGAGACGCT			
8001	CCAGGATGGC	TGTGATACTC	ACTTCTGCAA	GGTCAATGAG
	AGAGGAGAGT			
8051	ACTTCTGGGA	GAAGAGGGTC	ACAGGCTGCC	CACCCTTTGA
	TGAACACAAG			
8101	TGTCTTGCTG	AGGGAGGTAA	AATTATGAAA	ATTCCAGGCA
	CCTGCTGTGA			
8151	CACATGTGAG	GAGCCTGAGT	GCAACGACAT	CACTGCCAGG
	CTGCAGTATG			
8201	TCAAGGTGGG	AAGCTGTAAG	TCTGAAGTAG	AGGTGGATAT
	CCACTACTGC			
8251	CAGGGCAAAT	GTGCCAGCAA	AGCCATGTAC	TCCATTGACA
	TCAACGATGT			
8301	GCAGGACCAG	TGCTCCTGCT	GCTCTCCGAC	ACGGACGGAG
	CCCATGCAGG			
8351	TGGCCCTGCA	CTGCACCAAT	GGCTCTGTTG	TGTACCATGA
	GGTTCTCAAT			
8401	GCCATGGAGT	GCAAATGCTC	CCCCAGGAAG	TGCAGCAAGT GA

[0129] The VWF protein as used herein can be a VWF fragment comprising a D' domain and a D3 domain of VWF, wherein the VWF fragment binds to Factor VIII (FVIII) and inhibits binding of endogenous VWF (full-length VWF) to FVIII. The VWF fragment comprising the D' domain and the D3 domain can further comprise a VWF domain selected from the group consisting of an A1 domain, an A2 domain, an A3 domain, a D1 domain, a D2 domain, a D4 domain, a B1

domain, a B2 domain, a B3 domain, a C1 domain, a C2 domain, a CK domain, one or more fragments thereof, and any combinations thereof. In one embodiment, a VWF fragment comprises, consists essentially of, or consists of: (1) the D' and D3 domains of VWF or fragments thereof; (2) the D1, D', and D3 domains of VWF or fragments thereof; (3) the D2, D', and D3 domains of VWF or fragments thereof; (4) the D1, D2, D', and D3 domains of VWF or fragments thereof; or (5) the D1, D2, D', D3, and A1 domains of VWF or fragments thereof. The VWF fragment described herein does not contain a site binding to a VWF clearance receptor. In another embodiment, the VWF fragment described herein is not amino acids 764 to 1274 of SEQ ID NO: 21. The VWF fragment of the present invention can comprise any other sequences linked to or fused to the VWF fragment. For example, a VWF fragment described herein can further comprise a signal peptide.

[0130] In one embodiment, the VWF fragment comprising a D' domain and a D3 domain binds to or is associated with a FVIII protein. By binding to or associating with a FVIII protein, a VWF fragment of the invention protects FVIII from protease cleavage and FVIII activation, stabilizes the heavy chain and light chain of FVIII, and prevents clearance of FVIII by scavenger receptors. In another embodiment, the VWF fragment binds to or associates with a FVIII protein and blocks or prevents binding of the FVIII protein to phospholipid and activated Protein C. By preventing or inhibiting binding of the FVIII protein with endogenous, full-length VWF, the VWF fragment of the invention reduces the clearance of FVIII by VWF clearance receptors and thus extends half-life of the chimeric protein. The half-life extension of a chimeric protein is thus due to the binding of or associating with the VWF fragment lacking a VWF clearance receptor binding site to the FVIII protein and shielding or protecting of the FVIII protein by the VWF fragment from endogenous VWF which contains the VWF clearance receptor binding site. The FVIII protein bound to or protected by the VWF fragment can also allow recycling of a FVIII protein. By eliminating the VWF clearance pathway receptor binding sites contained in the full length VWF molecule, the FVIII/VWF heterodimers of the invention are shielded from the VWF clearance pathway, further extending FVIII half-life.

[0131] In one embodiment, a VWF protein useful for the present invention comprises a D' domain and a D3 domain of VWF, wherein the D' domain is at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 866 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. In another embodiment, a VWF protein comprises the D' domain and the D3 domain of VWF, wherein the D3 domain is at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 867 to 1240 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. In some embodiments, a VWF protein described herein

comprises, consists essentially of, or consists of the D' domain and D3 domain of VWF, which are at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 1240 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. In other embodiments, a VWF protein comprises, consists essentially of, or consists of the D1, D2, D', and D3 domains at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 23 to 1240 of SEQ ID NO: 21, wherein the VWF protein prevents or inhibits binding of endogenous VWF to FVIII. In still other embodiments, the VWF protein further comprises a signal peptide operably linked thereto.

[0132] In some embodiments, a VWF protein useful for the invention consists essentially of or consists of (1) the D'D3 domain, the D1D'D3 domain, D2D'D3 domain, or D1D2D'D3 domain and (2) an additional VWF sequence up to about 10 amino acids (*e.g.*, any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1250 of SEQ ID NO: 21), up to about 15 amino acids (*e.g.*, any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1255 of SEQ ID NO: 21), up to about 20 amino acids (*e.g.*, any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1260 of SEQ ID NO: 21), up to about 25 amino acids (*e.g.*, any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1265 of SEQ ID NO: 21), or up to about 30 amino acids (*e.g.*, any sequences from amino acids 764 to 1240 of SEQ ID NO: 21 to amino acids 764 to 1260 of SEQ ID NO: 21). In a particular embodiment, the VWF protein comprising or consisting essentially of the D' domain and the D3 domain is neither amino acids 764 to 1274 of SEQ ID NO: 21 nor the full-length mature VWF. In some embodiments, the D1D2 domain is expressed in trans with the D'D3 domain. In some embodiments, the D1D2 domain is expressed in cis with the D'D3 domain.

[0133] In other embodiments, the VWF protein comprising the D'D3 domains linked to the D1D2 domains further comprises an intracellular cleavage site, *e.g.*, (a cleavage site by PACE (furin) or PC5), allowing cleavage of the D1D2 domains from the D'D3 domains upon expression. Non-limiting examples of the intracellular cleavage site are disclosed elsewhere herein.

[0134] In yet other embodiments, a VWF protein comprises a D' domain and a D3 domain, but does not comprise an amino acid sequence selected from the group consisting of (1) amino acids 1241 to 2813 corresponding to SEQ ID NO: 21, (2) amino acids 1270 to amino acids 2813 corresponding to SEQ ID NO: 21, (3) amino acids 1271 to amino acids 2813 corresponding to SEQ ID NO: 21, (4) amino acids 1272 to amino acids 2813 corresponding to SEQ ID NO: 21, (5) amino acids 1273 to amino acids 2813 corresponding to SEQ ID NO: 21, (6) amino acids 1274 to amino acids 2813 corresponding to SEQ ID NO: 21, and any combinations thereof.

- [0135] In still other embodiments, a VWF protein of the present invention comprises, consists essentially of, or consists of an amino acid sequence corresponding to the D' domain, D3 domain, and A1 domain, wherein the amino acid sequence is at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acid 764 to 1479 of SEQ ID NO: 21, wherein the VWF protein prevents binding of endogenous VWF to FVIII. In a particular embodiment, the VWF protein is not amino acids 764 to 1274 of SEQ ID NO: 21.
- [0136] In some embodiments, a VWF protein of the invention comprises a D' domain and a D3 domain, but does not comprise at least one VWF domain selected from the group consisting of (1) an A1 domain, (2) an A2 domain, (3) an A3 domain, (4) a D4 domain, (5) a B1 domain, (6) a B2 domain, (7) a B3 domain, (8) a C1 domain, (9) a C2 domain, (10) a CK domain, (11) a CK domain and C2 domain, (12) a CK domain, a C2 domain, and a C1 domain, (13) a CK domain, a C2 domain, a C1 domain, a B3 domain, (14) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, (15) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, and a B1 domain, (16) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, and a D4 domain, (17) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, and an A3 domain, (18) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, an A3 domain, and an A2 domain, (19) a CK domain, a C2 domain, a C1 domain, a B3 domain, a B2 domain, a B1 domain, a D4 domain, an A3 domain, an A2 domain, and an A1 domain, and (20) any combinations thereof.
- [0137] In yet other embodiments, the VWF protein comprises the D'D3 domains and one or more domains or modules. Examples of such domains or modules include, but are not limited to, the domains and modules disclosed in Zhou et al., Blood published online April 6, 2012: DOI 10.1182/blood-2012-01-405134, which is incorporated herein by reference in its entirety. For example, the VWF protein can comprise the D'D3 domain and one or more domains or modules selected from the group consisting of A1 domain, A2 domain, A3 domain, D4N module, VWD4 module, C8-4 module, TIL-4 module, C1 module, C2 module, C3 module, C4 module, C5 module, C5 module, C6 module, and any combinations thereof.
- [0138] In still other embodiments, the VWF protein is linked to a heterologous moiety, wherein the heterologous moiety is linked to the N-terminus or the C-terminus of the VWF protein or inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites) in the VWF protein. For example, the insertion sites for the heterologous moiety in the VWF protein can be in the D' domain, the D3 domain, or both. The heterologous moiety can be a half-life extender.

[0139] In certain embodiments, a VWF protein useful for the invention forms a multimer, *e.g.*, dimer, trimer, tetramer, pentamer, hexamer, heptamer, or the higher order multimers. In other embodiments, the VWF protein is a monomer having only one VWF protein. In some embodiments, the VWF protein of the present invention can have one or more amino acid substitutions, deletions, additions, or modifications. In one embodiment, the VWF protein can include amino acid substitutions, deletions, additions, or modifications such that the VWF protein is not capable of forming a disulfide bond or forming a dimer or a multimer. In another embodiment, the amino acid substitution is within the D' domain and the D3 domain. In a particular embodiment, a VWF protein useful for the invention contains at least one amino acid substitution at a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 corresponding to SEQ ID NO: 21. The at least one amino acid substitution can be any amino acids that are not occurring naturally in the wild type VWF. For example, the amino acid substitution can be any amino acids other than cysteine, *e.g.*, Isoleucine, Alanine, Leucine, Asparagine, Lysine, Aspartic acid, Methionine, Phenylalanine, Glutamic acid, Threonine, Glutamine, Tryptophan, Glycine, Valine, Proline, Serine, Tyrosine, Arginine, or Histidine. In another example, the amino acid substitution has one or more amino acids that prevent or inhibit the VWF proteins from forming multimers.

[0140] In certain embodiments, the VWF protein useful herein can be further modified to improve its interaction with FVIII, *e.g.*, to improve binding affinity to FVIII. As a non-limiting example, the VWF protein comprises a serine residue at the residue corresponding to amino acid 764 of SEQ ID NO: 21 and a lysine residue at the residue corresponding to amino acid 773 of SEQ ID NO: 21. Residues 764 and/or 773 can contribute to the binding affinity of the VWF proteins to FVIII. In other embodiments, The VWF proteins useful for the invention can have other modifications, *e.g.*, the protein can be pegylated, glycosylated, hesylated, or polysialylated.

II. B. XTEN Sequences

[0141] As used herein "XTEN sequence" refers to extended length polypeptides with non-naturally occurring, substantially non-repetitive sequences that are composed mainly of small hydrophilic amino acids, with the sequence having a low degree or no secondary or tertiary structure under physiologic conditions. As a chimeric protein partner, XTENS can serve as a carrier, conferring certain desirable pharmacokinetic, physicochemical and pharmaceutical properties when linked to a VWF protein or a FVIII sequence of the invention to create a chimeric protein. Such desirable properties include but are not limited to enhanced pharmacokinetic parameters and solubility characteristics. As used herein, "XTEN" specifically excludes antibodies or antibody fragments such as single-chain antibodies or Fc fragments of a light chain or a heavy chain.

[0142] The present invention provides that a shorter XTEN sequence provides an improved half-life extending property compared to a longer XTEN sequence when the XTEN sequence is fused to a VWF protein and/or the second Ig constant region or a portion thereof. Therefore, the XTEN sequence fused to a VWF protein and/or the second Ig constant region or a portion thereof contains less than 288 amino acids in length, i.e., is shorter than 288 amino acids. In one embodiment, the XTEN sequence fused to a VWF protein and/or the second Ig constant region or a portion thereof consists of an amino acid sequence having a length of between 12 amino acids and 287 amino acids. In another embodiment, the XTEN sequence fused to a VWF protein and/or the second Ig constant region or a portion thereof comprise at least about 36 amino acids, at least about 42 amino acids, at least about 72 amino acids, or at least about 144 amino acids, but less than 288 amino acids. In other embodiments, the XTEN sequence fused to a VWF protein and/or the second Ig constant region or a portion thereof is selected from AE36, AG36, AE42, AG42, AE72, AG72, AE144, or AG144. In one embodiment, the XTEN sequence fused to a VWF protein and/or the second Ig constant region or a portion thereof is an amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 14, wherein the chimeric protein exhibits an improved half-life compared to a chimeric protein without the XTEN sequence.

[0143] The chimeric protein of the invention can further comprise an additional (second, third, or more) XTEN sequences. The additional XTEN sequence can further be fused to the FVIII protein or the first Ig constant region or a portion thereof. The additional XTEN sequences can be any length. For example, the additional XTEN sequence fused to the FVIII protein or the first Ig constant region or a portion thereof is a peptide or a polypeptide having greater than about 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1200, 1400, 1600, 1800, or 2000 amino acid residues. In certain embodiments, the additional XTEN sequence is a peptide or a polypeptide having greater than about 20 to about 3000 amino acid residues, greater than about 30 to about 2500 residues, greater than about 40 to about 2000 residues, greater than about 50 to about 1500 residues, greater than about 60 to about 1000 residues, greater than about 70 to about 900 residues, greater than about 80 to about 800 residues, greater than about 90 to about 700 residues, greater than about 100 to about 600 residues, greater than about 110 to about 500 residues, or greater than about 120 to about 400 residues.

[0144] The XTEN sequences (i.e., the XTEN sequence fused to the VWF protein and/or the second Ig constant region or a portion thereof or the XTEN sequence fused to the FVIII protein and/or the first Ig constant region or a portion thereof or inserted at one or more insertion sites within the FVIII protein) can comprise one or more sequence motif of 9 to 14 amino acid residues

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

[0145] In some embodiments, the XTEN sequence comprises non-overlapping sequence motifs in which at least about 80%, or at least about 85%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% or about 100% of the sequence consists of multiple units of non-overlapping sequences selected from a single motif family selected from Table 2A, resulting in a family sequence. As used herein, "family" means that the XTEN has motifs selected only from a single motif category from Table 2A; *i.e.*, AD, AE, AF, AG, AM, AQ, BC, or BD XTEN, and that any other amino acids in the XTEN not from a family motif are selected to achieve a needed property, such as to permit incorporation of a restriction site by the encoding nucleotides, incorporation of a cleavage sequence, or to achieve a better linkage to FVIII or VWF. In some embodiments of XTEN families, an XTEN sequence comprises multiple units of non-overlapping sequence motifs of the AD motif family, or of the AE motif family, or of the AF motif family, or of the AG motif family, or of the AM motif family, or of the AQ motif family, or of the BC family, or of the BD family, with the resulting XTEN exhibiting the range of homology described above. In other embodiments, the XTEN comprises multiple units of motif sequences from two or more of the motif families of Table 2A. These sequences can be selected to achieve desired physical/chemical characteristics, including such properties as net charge, hydrophilicity, lack of secondary structure, or lack of repetitiveness that are conferred by the amino acid composition of the motifs, described more fully below. In the embodiments hereinabove described in this paragraph, the motifs incorporated into the XTEN can be selected and assembled using the methods described herein to achieve an XTEN of about 36 to about 3000 amino acid residues.

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

Motif Family*	MOTIF SEQUENCE
AD	GESPGGSSGSES (SEQ ID NO: 24)
AD	GSEGSSGPGESS (SEQ ID NO: 25)
AD	GSSESGSSEGGP (SEQ ID NO: 26)
AD	GSGGEPSESGSS (SEQ ID NO: 27)
AE, AM	GSPAGSPTSTEE (SEQ ID NO: 28)
AE, AM, AQ	GSEPATSGSETP (SEQ ID NO: 29)
AE, AM, AQ	GTSESATPESGP (SEQ ID NO: 30)
AE, AM, AQ	GTSTEPSEGSAP (SEQ ID NO: 31)
AF, AM	GSTSESPSGTAP (SEQ ID NO: 32)

Motif Family*	MOTIF SEQUENCE
AF, AM	GTSTPESGSASP (SEQ ID NO: 33)
AF, AM	GTSPSGESSTAP (SEQ ID NO: 34)
AF, AM	GSTSSTAESPGP (SEQ ID NO: 35)
AG, AM	GTPGSGTASSSP (SEQ ID NO: 36)
AG, AM	GSSTPSGATGSP (SEQ ID NO: 37)
AG, AM	GSSPSASTGTGP (SEQ ID NO: 38)
AG, AM	GASPGTSSTGSP (SEQ ID NO: 39)
AQ	GEPAGSPTSTSE (SEQ ID NO: 40)
AQ	GTGEPSSTPASE (SEQ ID NO: 41)
AQ	GSGPSTESAPTE (SEQ ID NO: 42)
AQ	GSETPSGPSETA (SEQ ID NO: 43)
AQ	GPSETSTSEPGA (SEQ ID NO: 44)
AQ	GSPSEPTEGTSA (SEQ ID NO: 45)
BC	GSGASEPTSTEP (SEQ ID NO: 46)
BC	GSEPATSGTEPS (SEQ ID NO: 47)
BC	GTSEPSTSEPGA (SEQ ID NO: 48)
BC	GTSTEPSEPGSA (SEQ ID NO: 49)
BD	GSTAGSETSTE A (SEQ ID NO: 50)
BD	GSETATSGSETA (SEQ ID NO: 51)
BD	GTSESATSESGA (SEQ ID NO: 52)
BD	GTSTEASEGSAS (SEQ ID NO: 53)

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

[0146] In some embodiments, the XTEN sequence used in the invention is at least 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a sequence selected from the group consisting of AE42, AG42, AE48, AM48, AE72, AG72, AE108, AG108, AE144, AF144, AG144, AE180, AG180, AE216, AG216, AE252, AG252, AE288, AG288, AE324, AG324, AE360, AG360, AE396, AG396, AE432, AG432, AE468, AG468, AE504, AG504, AF504, AE540, AG540, AF540, AD576, AE576, AF576, AG576, AE612, AG612, AE624, AE648, AG648, AG684, AE720, AG720, AE756, AG756, AE792, AG792, AE828, AG828, AD836, AE864, AF864, AG864, AM875, AE912, AM923, AM1318, BC864, BD864, AE948, AE1044, AE1140, AE1236, AE1332, AE1428, AE1524, AE1620, AE1716, AE1812, AE1908, AE2004A, AG948, AG1044, AG1140, AG1236, AG1332, AG1428, AG1524, AG1620, AG1716, AG1812, AG1908, and AG2004. *See* US 2010-0239554 A1.

[0147] In one embodiment, the XTEN sequence is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of AE42 (SEQ ID NO: 9), AE72 (SEQ ID NO: 10), AE144_2A (SEQ ID NO: 55), AE144_3B (SEQ ID NO: 56), AE144_4A (SEQ ID NO: 57), AE144_5A (SEQ ID NO: 58), AE144_6B (SEQ ID NO: 59), AG144_A (SEQ ID NO: 60), AG144_B (SEQ ID NO: 61), AG144_C (SEQ ID NO: 62), AG144_F (SEQ ID NO: 63), AE864 (SEQ ID NO: 15), AE576 (SEQ ID NO: 16), AE288

(SEQ ID NO: 8), AE288_2 (SEQ ID NO: 54), AE144 (SEQ ID NO: 11), AG864 (SEQ ID NO: 17), AG576 (SEQ ID NO: 18), AG288 (SEQ ID NO: 19), AG144 (SEQ ID NO: 14), and any combinations thereof. In another embodiment, the XTEN sequence is selected from the group consisting of AE42 (SEQ ID NO: 9), AE72 (SEQ ID NO: 10), AE144_2A (SEQ IDNO: 55), AE144_3B (SEQ ID NO: 56), AE144_4A (SEQ ID NO: 57), AE144_5A (SEQ IDNO: 58), AE144_6B (SEQ ID NO: 59), AG144_A (SEQ ID NO: 60), AG144_B (SEQ ID NO: 61), AG144_C (SEQ ID NO: 62), AG144_F (SEQ IDNO: 63), AE864 (SEQ ID NO: 15), AE576 (SEQ ID NO: 16), AE288 (SEQ ID NO: 8), AE288_2 (SEQ ID NO: 54), AE144 (SEQ ID NO: 11), AG864 (SEQ ID NO: 17), AG576 (SEQ ID NO: 18), AG288 (SEQ ID NO: 19), AG144 (SEQ ID NO: 14), and any combinations thereof. In a specific embodiment, the XTEN sequence is AE288. The amino acid sequences for certain XTEN sequences of the invention are shown in Table 2B.

TABLE 2B. XTEN Sequences

XTEN	Amino Acid Sequence
AE42 SEQ ID NO: 9	GAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPASS
AE72 SEQ ID NO: 10	GAP TSESATPESG PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG TSTEPSEGSAPGASS
AE144 SEQ ID NO: 11	GSEPATSGSETPGTSESATPESGPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEG SAPGSEPATSGSETPGSEPATSGSETPGSEPATSGSETPGTSTEPSEGSAPGTSESA PESGPGSEPATSGSETPGTSTEPSEGSAP
AE144_2A (SEQ ID NO: 55)	TSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTS TEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSTEPSEGSAPGTSTEPSEGSAPGTSES ATPESGPGTSESATPESGPG
AE144_3B (SEQ ID NO: 56)	SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTS TEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPG
AE144_4A (SEQ ID NO: 57)	TSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTS TEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGSPAGSPTSTEEGTSES ATPESGPGTSTEPSEGSAPG
AE144_5A (SEQ ID NO: 58)	TSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTS TEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAG SPTSTEEGSPAGSPTSTEEG
AE144_6B (SEQ ID NO: 59)	TSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSEPATSGSETPGSE PATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSES ATPESGPGTSTEPSEGSAPG

AG144 SEQ ID NO:14	GTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSST GSPGASPGTSSTGSPGSSTPSGATGSPGSSPSASTGTGPGASPGTSSTGSPGSSPSA STGTGPGTTPGSGTASSSPGSSTPSGATGSP
AG144_A (SEQ ID NO: 60)	GASPGTSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTTPGSGTASSSPGSSTPSGATGSPGS SPSASTGTGPGASPGTSSTGSPGTTPGSGTASSSPGSSTPSGATGSPGTTPGSGTASSSPGASP GTSSTGSPGASPGTSSTGSP
AG144_B (SEQ ID NO: 61)	GTPGSGTASSSPGSSTPSGATGSPGASPGTSSTGSPGTTPGSGTASSSPGSSTPSGATGSPGS SPSASTGTGPGSSPSASTGTGPGSSSTPSGATGSPGSSSTPSGATGSPGASPGTSSTGSPGASP GTSSTGSPGASPGTSSTGSP
AG144_C (SEQ ID NO: 62)	GTPGSGTASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGT TPGSGTASSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSSTPSGATGSPGSST PSGATGSPGASPGTSSTGSP
AG144_F (SEQ ID NO: 63)	GSSPSASTGTGPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGSSTPSGATGSPGS SPSASTGTGPGASPGTSSTGSPGSSPSASTGTGPGTTPGSGTASSSPGSSTPSGATGSPGSST PSGATGSPGASPGTSSTGSP
AE288 SEQ ID NO: 8	GTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESG PGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPES GPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPE SGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSE GSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP
AE288_2 (SEQ ID NO: 54)	GSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGT STEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPA GSPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSEPAT SGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSPAGSPT STEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAP
AG288 SEQ ID NO:19	PGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASS SPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGSSSTPSGATGSPGSSPSASTG TGPSSPSASTGTGPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSPSAST GTGPSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGSSSTPSGATGSPGSSPSAS TGTGPGASPGTSSTGSPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGS
AE576 SEQ ID NO:16	GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGSAP PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTST EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEG SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSEPATSG SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGSPAGSP TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSTEP SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSE ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSP AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAP
AG576 SEQ ID NO:18	PGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSSTPSGATG SPGSSTPSGATGSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSPSASTGTGPGTTPGSGTA SSSPGASPGTSSTGSPGASPGTSSTGSPGASPGTSSTGSPGSSSTPSGATGSPGSSSTPSG ATGSPGASPGTSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSSTPSGATGSPGSSSTPS GATGSPGSSPSASTGTGPGASPGTSSTGSPGASPGTSSTGSPGTPGSGTASSSPGASPG

	TSSTGSPGASPGTSSSTGSPGASPGTSSSTGSPGASPGTSSSTGSPGTPGSGTASSSPGSST PSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGSS TPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSSTGSPGTPGSGTASSSPGS STPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSSTGS
AE864 SEQ ID NO:15	GSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGS PGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTST EEGTSESATPESGPGTSTEPSEGSAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEG SAPGTSTEPSEGSAPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSEPATSG SETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGSPAGSP TSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSTEP SEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSE ATSGSETPGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGSPAGSPTSTEEGSP AGSPTSTEEGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGTSESATPESGPGS EPATSGSETPGTSESATPESGPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPG SPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEE GSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESG PGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGS APGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAP
AG864 SEQ ID NO:17	GASPGTSSSTGSPGSSPSASTGTGPGSSPSASTGTGPGTPGSGTASSSPGSSTPSGATGS PGSSPSASTGTGPGASPGTSSSTGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASS SPGASPGTSSSTGSPGASPGTSSSTGSPGTPGSGTASSSPGSSTPSGATGSPGASPGTSST GSPGTPGSGTASSSPGSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGSSPSGA TGSPGSSSTPSGATGSPGASPGTSSSTGSPGASPGTSSSTGSPGASPGTSSSTGSPGTPGSGT ASSSPGASPGTSSSTGSPGASPGTSSSTGSPGASPGTSSSTGSPGSSPSASTGTGPGTPGSG TASSSPGASPGTSSSTGSPGASPGTSSSTGSPGASPGTSSSTGSPGSSSTPSGATGSPGSSSTP SGATGSPGASPGTSSSTGSPGTPGSGTASSSPGSSTPSGATGSPGSSSTPSGATGSPGSSST PSGATGSPGSSPSASTGTGPGASPGTSSSTGSPGASPGTSSSTGSPGTPGSGTASSSPGAS PGTSSSTGSPGASPGTSSSTGSPGASPGTSSSTGSPGASPGTSSSTGSPGTPGSGTASSSPGS STPSGATGSPGTPGSGTASSSPGSSTPSGATGSPGTPGSGTASSSPGSSTPSGATGSPG SSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSSTGSPGTPGSGTASSSP GSSTPSGATGSPGSSPSASTGTGPGSSPSASTGTGPGASPGTSSSTGSPGASPGTSSSTGS PGSSTPSGATGSPGSSPSASTGTGPGASPGTSSSTGSPGSSPSASTGTGPGTPGSGTASS SPGSSSTPSGATGSPGSSSTPSGATGSPGASPGTSSSTGSP

[0148] In those embodiments wherein the XTEN component(s) have less than 100% of its amino acids consisting of 4, 5, or 6 types of amino acid selected from glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P), or less than 100% of the sequence consisting of the sequence motifs from Table 3 or the XTEN sequences of Tables 4, and 13-17, the other amino acid residues of the XTEN are selected from any of the other 14 natural L-amino acids, but are preferentially selected from hydrophilic amino acids such that the XTEN sequence contains at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% hydrophilic amino acids. The XTEN amino acids that are not glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) are either interspersed throughout the XTEN sequence, are located within or between the sequence motifs, or are concentrated in one or more short stretches of the XTEN sequence, e.g., to create a linker between the XTEN and the FVIII or VWF components. In such cases where the XTEN component comprises amino acids other than glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P), it is preferred that less than about 2% or less than about 1% of the amino acids be hydrophobic residues such

that the resulting sequences generally lack secondary structure, e.g., not having more than 2% alpha helices or 2% beta-sheets, as determined by the methods disclosed herein. Hydrophobic residues that are less favored in construction of XTEN include tryptophan, phenylalanine, tyrosine, leucine, isoleucine, valine, and methionine. Additionally, one can design the XTEN sequences to contain less than 5% or less than 4% or less than 3% or less than 2% or less than 1% or none of the following amino acids: cysteine (to avoid disulfide formation and oxidation), methionine (to avoid oxidation), asparagine and glutamine (to avoid desamidation). Thus, in some embodiments, the XTEN component comprising other amino acids in addition to glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) have a sequence with less than 5% of the residues contributing to alpha-helices and beta-sheets as measured by the Chou-Fasman algorithm and have at least 90%, or at least about 95% or more random coil formation as measured by the GOR algorithm.

[0149] In further embodiments, the XTEN sequence used in the invention affects the physical or chemical property, e.g., pharmacokinetics, of the chimeric protein of the present invention. The XTEN sequence used in the present invention can exhibit one or more of the following advantageous properties: conformational flexibility, enhanced aqueous solubility, high degree of protease resistance, low immunogenicity, low binding to mammalian receptors, or increased hydrodynamic (or Stokes) radii. In a specific embodiment, the XTEN sequence linked to a FVIII protein in this invention increases pharmacokinetic properties such as longer terminal half-life or increased area under the curve (AUC), so that the chimeric protein described herein stays *in vivo* for an increased period of time compared to wild type FVIII. In further embodiments, the XTEN sequence used in this invention increases pharmacokinetic properties such as longer terminal half-life or increased area under the curve (AUC), so that FVIII protein stays *in vivo* for an increased period of time compared to wild type FVIII.

[0150] One embodiment of the present invention is a FVIII/VWF fusion protein comprising a FVIII portion fused to an Fc region and a VWF portion fused to an Fc region, wherein an XTEN sequence (e.g., AE288) is inserted within the FVIII portion, and wherein an XTEN sequence having less than 288 amino acids (e.g., AE144) is inserted between the VWF portion and the Fc portion. As described in the examples, insertion of an XTEN having less than 288 amino acids between the VWF portion and the Fc portion has a greater effect on the pharmacokinetics of the chimeric protein than the insertion of an XTEN having 288 amino acids between the VWF portion and the Fc portion. For example, insertion of an XTEN sequence having less than 288 amino acids between the VWF portion and the Fc portion in FVIII/VWF fusion protein can increase the terminal half-life of the chimeric protein compared to an XTEN having 288 amino acids. In some embodiments, the terminal half-life is increased by at least about 5%, at least about

10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30%, relative to the insertion of an XTEN sequence having 288 amino acids. In one particular embodiment, the terminal half-life is increased by at least about 35% relative to the insertion of an XTEN having 288 amino acids. Insertion of an XTEN sequence having less than 288 amino acids can also increase the AUC value of the chimeric protein. In some embodiments, AUC is increased by at least about 50%, at least about 100%, or at least about 200% relative to the insertion of an XTEN having 288 amino acids. In one particular embodiment, AUC is increased by about two-fold. Insertion of an XTEN sequence having less than 288 amino acids can also reduce the clearance of the chimeric protein. For example, clearance can be decreased by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30%, relative to the insertion of an XTEN sequence having 288 amino acids. Insertion of an XTEN sequence having less than 288 amino acids can increase mean residence time (MRT) and/or decrease the apparent volume of distribution at steady state (V_{ss}) relative to the insertion of an XTEN having 288 amino acids.

[0151] A variety of methods and assays can be employed to determine the physical/chemical properties of proteins comprising the XTEN sequence. Such methods include, but are not limited to analytical centrifugation, EPR, HPLC-ion exchange, HPLC-size exclusion, HPLC-reverse phase, light scattering, capillary electrophoresis, circular dichroism, differential scanning calorimetry, fluorescence, HPLC-ion exchange, HPLC-size exclusion, IR, NMR, Raman spectroscopy, refractometry, and UV/Visible spectroscopy. Additional methods are disclosed in Amau *et al.*, *Prot Expr and Purif* 48, 1-13 (2006).

[0152] Additional examples of XTEN sequences that can be used according to the present invention and are disclosed in US Patent Publication Nos. 2010/0239554 A1, 2010/0323956 A1, 2011/0046060 A1, 2011/0046061 A1, 2011/0077199 A1, or 2011/0172146 A1, or International Patent Publication Nos. WO 2010091122 A1, WO 2010144502 A2, WO 2010144508 A1, WO 2011028228 A1, WO 2011028229 A1, WO 2011028344 A2, or WO 20130122617 A1.

II.C. Factor VIII (FVIII) Protein

[0153] "A FVIII protein" as used herein means a functional FVIII polypeptide in its normal role in coagulation, unless otherwise specified. The term a FVIII protein includes a functional fragment, variant, analog, or derivative thereof that retains the function of full-length wild-type Factor VIII in the coagulation pathway. "A FVIII protein" is used interchangeably with FVIII polypeptide (or protein) or FVIII. Examples of the FVIII functions include, but not limited to, an ability to activate coagulation, an ability to act as a cofactor for factor IX, or an ability to form a tenase complex with factor IX in the presence of Ca^{2+} and phospholipids, which then converts Factor X to the activated form Xa. The FVIII protein can be the human, porcine, canine, rat, or

murine FVIII protein. In addition, comparisons between FVIII from humans and other species have identified conserved residues that are likely to be required for function (Cameron *et al.*, *Thromb. Haemost.* 79:317-22 (1998); US 6,251,632).

[0154] A number of tests are available to assess the function of the coagulation system: activated partial thromboplastin time (aPTT) test, chromogenic assay, ROTEM assay, prothrombin time (PT) test (also used to determine INR), fibrinogen testing (often by the Clauss method), platelet count, platelet function testing (often by PFA-100), TCT, bleeding time, mixing test (whether an abnormality corrects if the patient's plasma is mixed with normal plasma), coagulation factor assays, antiphospholipid antibodies, D-dimer, genetic tests (*e.g.*, factor V Leiden, prothrombin mutation G20210A), dilute Russell's viper venom time (dRVVT), miscellaneous platelet function tests, thromboelastography (TEG or Sonoclot), thromboelastometry (TEM[®], *e.g.*, ROTEM[®]), or euglobulin lysis time (ELT).

[0155] The aPTT test is a performance indicator measuring the efficacy of both the "intrinsic" (also referred to the contact activation pathway) and the common coagulation pathways. This test is commonly used to measure clotting activity of commercially available recombinant clotting factors, *e.g.*, FVIII or FIX. It is used in conjunction with prothrombin time (PT), which measures the extrinsic pathway.

[0156] ROTEM analysis provides information on the whole kinetics of haemostasis: clotting time, clot formation, clot stability and lysis. The different parameters in thromboelastometry are dependent on the activity of the plasmatic coagulation system, platelet function, fibrinolysis, or many factors which influence these interactions. This assay can provide a complete view of secondary haemostasis.

[0157] The FVIII polypeptide and polynucleotide sequences are known, as are many functional fragments, mutants and modified versions. Examples of human FVIII sequences (full-length) are shown below.

TABLE 3. Amino Acid Sequence of Full-length Factor VIII

(Full-length FVIII (FVIII signal peptide underlined; FVIII heavy chain is double underlined; B domain is italicized; and FVIII light chain is in plain text))

Signal Peptide: (SEQ ID NO: 64)

MQIELSTCFFLCLLRCFCS

Mature Factor VIII (SEQ ID NO: 65)*

ATRRYYLGAVELSWDYMQSDLGELVDARFPRRVPKSFFPNTSVVYKKTLEVEFTDHLFNIAKPRPPWMGLL
GPTIQAEVYDTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDOTSQREKEDDKVFPGGSHTYVWQVLKEN
GPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGSLAKEKTQTLHKFILLFAVFDEGKSWHSETKNSL
MQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEI

SPITFLTAQTLMLDLGQFLLFCHISSHQHDGMEAYVKVDSCEEPQLRMKNNEEAEDYDDDLTDSEMDVVRF
 DDDNSPSFIQIRSVAKKHPKTWVHYIAAEEEDWDYAPLVLAPDDRSYKSOYLNNGPORIGRKYKKVRFMAYT
 DETFKTREAIOHESGILGPLLYGEVGDITLLIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPIL
 PGEIFKYKWTVTVEDGPTKSDPRCLTRYSSFVNMERDLASGLIGPLLYCYKESVDQRGNOIMSDKRNVLIF
 SVFDENRSWYLTENIORFLNPAGVOLEDPEFOASNIMHSINGYVFDLSQLSVCLHEVAYWYILSIGAOTDF
 LSVFFSGYTFFKHVMYEDTLTLFPFSGETVFMSENPGLWLILGCHNSDFRNRGMTALLKVSSCDKNTGDYIE
 DSYEDISAYLLSKNNAIEPRSFSONSRHPSTRQKQFNATTIPENDIEKTDPWFHRTMPKIQNVSSDLLM
 LLRQSPFPHGLSLSDLQEAKEYETFSDDPSPGAIDSNNSLSEMTFRPQLHHSQDMVFTPESGQLRLNEKLG
 TTAATELKKLDFKVSSTSNLLISTIPSDNLAAGTDNTSSLGPPSMFVHYDSQLDITLFGKKSSPLTESGGPL
 SLSEENNDKLLSGLMNSQESSWGKNVSSSTESGRLFKGKRAHGFPALLTKDNALFKVSISSLKTNKTSNNSA
 TNRKTHIDGPSLLIENSPPSVQONILESDETFKKVTPLIHDMMLMDKNATALRLNHSNKTSSKNMEMVQOK
 KEGPIPPDAQNPDMSFFKMLFLPESARWIQRTHGKNSLNSGGQGPSFKQLVSLGPEKSVEGQNFLSEKNKVVV
 GKGEFTKDVGLKEMVFPSSRNLFELTNLDNLHENNTHNQEKKIQEEIEKKETLIQENVVLPQIHTVTGTNFM
 KNLELLSTRQNVESYDGAYAPVLQDFRSLNDSTNRKHTAHFSKKGEENLEGLGNQTKQIVEKYACTTR
 ISPNTSQQNFVTQRSKRALKQFRLPLEETELEKRIIVDDTSTQWSKNMKHLTPSTLTQIDYNEKEKGAITQS
 PLSDCLTRSHSIPQANRSLPLAKVSSFPISIRPIYLTRVLFQDNSSHLPAASYRKKDSGVQESSHFLQGAKK
 NNLSLAILTLEMTGDQREVGSLGTSATNSVTYKKVENTVLKPKDLPKTSGKVELLPKVHIYQKDLFPTETSN
 GSPGHLDLVEGSLQGTGEGAIKWNEANRPGKVPFLRVATESAKTPSKLLDPLAWDNHYGTQIPKEEWSQE
 KSPEKTAFFKKKDTILSLNACESNHAIAAINEGONKPEIEVTWAKQGRTERLCSQNPVPLKRHQREITRTTLO
 SDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQKKTRHYFIAAVERLWDYGMSSSPHVLNRNAQSGSVP
 QFKKVVFQFTDGSFTQPLYRGELNEHLGLLPYIRAEVEDNIMVTFRNQASRPYSFYSSLISYEEDQRQGA
 EPRKNFVKPNETKTYFWKVQHHMAPTKDEFDCWAYFSDVDLEKDVHSLIGLPLLVCHTNTLNPAGHRQVT
 VQEFALFFTIFDETKSWYFTENMERNCRAPCNIQMEDPTFKENYRFHAINGYIMDTLPLGLVMAQDQIRIRWYL
 LSMGNSNENIHSIHFSGHVFTVRKKEEYKMALYNLYPGVFETVEMLPSKAGIWRVECLIGEHLHAGMSTLFLV
 YSNKCQTPLGMAAGHIRDFQITASQYQGWAPKLARLHYSGSINAWSTKEPFSWIKVDLLAPMI IHGIKTQG
 ARQKFSSLYISQFIIMYSLDGKKWQTYRGNSTGTLMVFFGNVDSSGIKHNI FNPIIARYIRLHPHTHSIRS
 TLRMELMGCDLNSCSMPLGMESKAISDAQITASSYFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQV
 DFQKTMKVTVGTTQGVKSLTSMYVKEFLISSQDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTR
 YLRIHPQSVWHQIALRMEVLGCEAQDLY

TABLE 4. Nucleotide Sequence Encoding Full-Length FVIII (SEQ ID NO: 66)*

				ATG	CAAATAGAGC	TCTCCACCTG
661						
721	CTTCTTTCTG	TGCCTTTTGC	GATTCTGCTT	TAGTGCCACC	AGAAGATACT	ACCTGGGTGC
781	AGTGGAACCTG	TCATGGGACT	ATATGCAAAG	TGATCTCGGT	GAGCTGCCTG	TGGACGCAAG
841	ATTTCTCCT	AGAGTGCCAA	AATCTTTTCC	ATTCAACACC	TCAGTCGTGT	ACAAAAAGAC
901	TCTGTTTGT	GAATTCACGG	ATCACCTTTT	CAACATCGCT	AAGCCAAGGC	CACCTGGAT
961	GGGTCTGCTA	GGTCCTACCA	TCCAGGCTGA	GGTTTATGAT	ACAGTGGTCA	TTACACTTAA
1021	GAACATGGCT	TCCCATCCTG	TCAGTCTTCA	TGCTGTTGGT	GTATCCTACT	GGAAAGCTTC
1081	TGAGGGAGCT	GAATATGATG	ATCAGACCAG	TCAAAGGGAG	AAAGAAGATG	ATAAAGCTCT
1141	CCCTGGTGGA	AGCCATACAT	ATGTCTGGCA	GGTCTGAAA	GAGAATGGTC	CAATGGCCTC
1201	TGACCCACTG	TGCCTTACCT	ACTCATATCT	TTCTCATGTG	GACCTGGTAA	AAGACTTGAA
1261	TTCAGGCCTC	ATTGGAGCCC	TACTAGTATG	TAGAGAAGGG	AGTCTGGCCA	AGGAAAAGAC
1321	ACAGACCTTG	CACAAATTTA	TACTACTTTT	TGCTGTATTT	GATGAAGGGA	AAAGTTGGCA
1381	CTCAGAAACA	AAGAACTCCT	TGATGCAGGA	TAGGGATGCT	GCATCTGCTC	GGGCCTGGCC
1441	TAAAATGCAC	ACAGTCAATG	GTTATGTAAA	CAGGTCTCTG	CCAGGTCTGA	TTGGATGCCA
1501	CAGGAAATCA	GTCTATTGGC	ATGTGATTGG	AATGGGCACC	ACTCCTGAAG	TGCACTCAAT
1561	ATTCTCGAA	GGTCACACAT	TTCTTGTGAG	GAACCATCGC	CAGGCGTCTC	TGGAAATCTC
1621	GCCAATAACT	TTCTTACTG	CTCAAACACT	CTTGATGGAC	CTTGGACAGT	TTCTACTGTT
1681	TTGTCATATC	TCTTCCCACC	AACATGATGG	CATGGAAGCT	TATGTCAAAG	TAGACAGCTG
1741	TCCAGAGGAA	CCCCAACTAC	GAATGAAAAA	TAATGAAGAA	GCGGAAGACT	ATGATGATGA
1801	TCTTACTGAT	TCTGAAAATGG	ATGTGGTCAG	GTTTGATGAT	GACAACCTCTC	CTTCCTTTAT
1861	CCAAATTGCG	TCAGTTGCCA	AGAAGCATCC	TAAAACTTGG	GTACATTACA	TTGCTGCTGA

1921	AGAGGAGGAC	TGGGACTATG	CTCCCTTAGT	CCTCGCCCCC	GATGACAGAA	GTTATAAAAG
1981	TCAATATTTG	AACAATGGCC	CTCAGCGGAT	TGGTAGGAAG	TACAAAAAAG	TCCGATTTAT
2041	GGCATACACA	GATGAAACCT	TTAAGACTCG	TGAAGCTATT	CAGCATGAAT	CAGGAATCTT
2101	GGGACCTTTA	CTTTATGGGG	AAGTTGGAGA	CACACTGTTG	ATTATATTTA	AGAATCAAGC
2161	AAGCAGACCA	TATAACATCT	ACCCTCACGG	AATCACTGAT	GTCCGTCCTT	TGTATTCAAG
2221	GAGATTACCA	AAAGGTGTAA	AACATTTGAA	GGATTTTCCA	ATTCTGCCAG	GAGAAATATT
2281	CAAAATATAA	TGGACAGTGA	CTGTAGAAGA	TGGGCCAACT	AAATCAGATC	CTCGGTGCCT
2341	GACCCGCTAT	TACTCTAGTT	TCGTTAATAT	GGAGAGAGAT	CTAGCTTCAG	GACTCATTGG
2401	CCCTCTCCTC	ATCTGCTACA	AAGAATCTGT	AGATCAAAGA	GGAAACCAGA	TAATGTCAGA
2461	CAAGAGGAAT	GTCATCCTGT	TTTCTGTATT	TGATGAGAAC	CGAAGCTGGT	ACCTCACAGA
2521	GAATATACAA	CGCTTTCTCC	CCAATCCAGC	TGGAGTGCAG	CTTGAGGATC	CAGAGTTCCA
2581	AGCCTCCAAC	ATCATGCACA	GCATCAATGG	CTATGTTTTT	GATAGTTTGC	AGTTGTCAGT
2641	TTGTTTGCAT	GAGGTGGCAT	ACTGGTACAT	TCTAAGCATT	GGAGCACAGA	CTGACTTCCT
2701	TTCTGTCTTC	TTCTCTGGAT	ATACCTTCAA	ACACAAAATG	GTCTATGAAG	ACACACTCAC
2761	CCTATTCCCA	TTCTCAGGAG	AACTGTCTT	CATGTCGATG	GAAAACCCAG	GTCTATGGAT
2821	TCTGGGGTGC	CACAACCTCAG	ACTTTCGGAA	CAGAGGCATG	ACCGCCTTAC	TGAAGGTTTC
2881	TAGTTGTGAC	AAGAACACTG	GTGATTATTA	CGAGGACAGT	TATGAAGATA	TTTCAGCATA
2941	CTTGCTGAGT	AAAAACAATG	CCATTGAACC	AAGAAGCTTC	TCCCAGAATT	CAAGACACCC
3001	TAGCACTAGG	CAAAAAGCAAT	TTAATGCCAC	CACAATTCCA	GAAAATGACA	TAGAGAAGAC
3061	TGACCCTTGG	TTTGCACACA	GAACACCTAT	GCCTAAAATA	CAAAATGTCT	CCTCTAGTGA
3121	TTTGTGTGATG	CTCTTGCGAC	AGAGTCCTAC	TCCACATGGG	CTATCCTTAT	CTGATCTCCA
3181	AGAAGCCAAA	TATGAGACTT	TTTCTGATGA	TCCATCACCT	GGAGCAATAG	ACAGTAATAA
3241	CAGCCTGTCT	GAAATGACAC	ACTTCAGGCC	ACAGCTCCAT	CACAGTGGGG	ACATGGTATT
3301	TACCCCTGAG	TCAGGCCCTCC	AATTAAGATT	AAATGAGAAA	CTGGGGACAA	CTGCAGCAAC
3361	AGAGTTGAAG	AAACTTGATT	TCAAAGTTTC	TAGTACATCA	AATAATCTGA	TTTCAACAAT
3421	TCCATCAGAC	AATTTGGCAG	CAGGTA CTGA	TAATACAAGT	TCCTTAGGAC	CCCCAAGTAT
3481	GCCAGTTCAT	TATGATAGTC	AATTAGATAC	CACTCTATTT	GGCAAAAAGT	CATCTCCCCT
3541	TACTGAGTCT	GGTGGACCTC	TGAGCTTGAG	TGAAGAAAAT	AATGATTCAA	AGTTGTTAGA
3601	ATCAGGTTTA	ATGAATAGCC	AAGAAAGTTC	ATGGGGAAAA	AATGTATCGT	CAACAGAGAG
3661	TGGTAGGTTA	TTTAAAGGGA	AAAGAGCTCA	TGGACCTGCT	TTGTTGACTA	AAGATAATGC
3721	CTTATTCAAA	GTTAGCATCT	CTTTGTTAAA	GACAAACAAA	ACTTCCAATA	ATTACAGCAAC
3781	TAATAGAAAAG	ACTCACATTG	ATGGCCCATC	ATTATTAATT	GAGAATAGTC	CTCAGTCTG
3841	GCAAAAATATA	TTAGAAAAGTG	ACACTGAGTT	TAAAAAAGTG	ACACCTTTGA	TTCATGACAG
3901	AATGCTTATG	GACAAAAAATG	CTACAGCTTT	GAGGCTAAAT	CATATGTCAA	ATAAAACTAC
3961	TTCATCAAAA	AACATGGAAA	TGGTCCAACA	GAAAAAAGAG	GGCCCCATTC	CACCAGATGC
4021	ACAAAATCCA	GATATGTCGT	TCTTTAAGAT	GCTATTCTTG	CCAGAATCAG	CAAGGTGGAT
4081	ACAAAGGACT	CATGGAAAAGA	ACTCTCTGAA	CTCTGGGCAA	GGCCCCAGTC	CAAAGCAATT
4141	AGTATCCTTA	GGACCAGAAA	AATCTGTGGA	AGGTCAGAAT	TTCTTGCTCTG	AGAAAAACAA
4201	AGTGGTAGTA	GGAAAGGGTG	AATTTACAAA	GGACGTAGGA	CTCAAAGAGA	TGGTTTTTCC
4261	AAGCAGCAGA	AACCTATTTT	TTACTAACTT	GGATAATTTA	CATGAAAATA	ATACACACAA
4321	TCAAGAAAAA	AAAATTCAGG	AAGAAATAGA	AAAGAAGGAA	ACATTAATCC	AAGAGAATGT
4381	AGTTTTGCCT	CAGATACATA	CAGTGA CTGG	CAC TAAGAAT	TTCATGAAGA	ACCTTTTCTT
4441	ACTGAGCACT	AGGCAAAAATG	TAGAAGGTTT	ATATGACGGG	GCATATGCTC	CAGTACTTCA
4501	AGATTTTAGG	TCATTAAATG	ATTCAACAAA	TAGAACAAAG	AAACACACAG	CTCATTTCTC
4561	AAAAAAAGGG	GAGGAAGAAA	ACTTGGAAGG	CTTGGGAAAT	CAAACCAAGC	AAATTGTAGA
4621	GAAATATGCA	TGCACCACAA	GGATATCTCC	TAATACAAGC	CAGCAGAATT	TTGTCACGCA
4681	ACGTAGTAAG	AGAGCTTTGA	AACAATT CAG	ACTCCCCTA	GAAGAAACAG	AACTTGAAAA
4741	AAGGATAATT	GTGGATGACA	CCTCAACCCA	GTGGTCCAAA	AACATGAAAC	ATTTGACCCC
4801	GAGCACCTTC	ACACAGATAG	ACTACAATGA	GAAGGAGAAA	GGGGCCATTA	CTCAGTCTCC
4861	CTTATCAGAT	TGCCTTACGA	GGAGTCATAG	CATCCCTCAA	GCAAATAGAT	TCCATTACC
4921	CATTGCAAAAG	GTATCATCAT	TTCCATCTAT	TAGACCTATA	TATCTGACCA	GGGTCTTATT
4981	CCAAGACAAC	TCTTCTCATC	TTCCAGCAGC	ATCTTATAGA	AAGAAAGATT	CTGGGGTCCA
5041	AGAAAGCAGT	CATTTCTTAC	AAGGAGCCAA	AAAAAATAAC	CTTTCTTTAG	CCATTCTAAC
5101	CTTGAGATG	ACTGGTGATC	AAAGAGAGGT	TGGCTCCCTG	GGGACAAGTG	CCACAAATTC
5161	AGTCACATAC	AAGAAAGTTG	AGAACACTGT	TCTCCCGAAA	CCAGACTTGC	CCAAAACATC
5221	TGGCAAAGTT	GAATTGCTTC	CAAAAGTTCA	CATTTATCAG	AAGGACCTAT	TCCCTACGGA
5281	AACTAGCAAT	GGGTCTCCTG	GCCATCTGGA	TCTCGTGGAA	GGGAGCCTTC	TTCAGGGAAC
5341	AGAGGGAGCG	ATTAAGTGGA	ATGAAGCAAA	CAGACCTGGA	AAAGTTCCCT	TTCTGAGAGT
5401	AGCAACAGAA	AGCTCTGCAA	AGACTCCCTC	CAAGCTATTG	GATCCTCTTG	CTTGGGATAA

```

5461   CCACTATGGT ACTCAGATAC CAAAAGAAGA GTGGAAATCC CAAGAGAAGT CACCAGAAAA
5521   AACAGCTTTT AAGAAAAAGG ATACCATTTT GTCCCTGAAC GCTTGTGAAA GCAATCATGC
5581   AATAGCAGCA ATAAATGAGG GACAAAATAA GCCCGAAATA GAAGTCACCT GGGCAAAGCA
5641   AGGTAGGACT GAAAGGCTGT GCTCTCAAAA CCCACCAGTC TTGAAACGCC ATCAACGGGA
5701   AATAACTCGT ACTACTCTTC AGTCAGATCA AGAGGAAATT GACTATGATG ATACCATATC
5761   AGTTGAAATG AAGAAGGAAG ATTTTGACAT TTATGATGAG GATGAAAATC AGAGCCCCCG
5821   CAGCTTTCAA AAGAAAACAC GACACTATTT TATTGCTGCA GTGGAGAGGC TCTGGGATTA
5881   TGGGATGAGT AGCTCCCCAC ATGTTCTAAG AAACAGGGCT CAGAGTGGCA GTGTCCCTCA
5941   GTTCAAGAAA GTTGTTTTCC AGGAATTTAC TGATGGCTCC TTTACTCAGC CCTTATACCG
6001   TGGAGAACTA AATGAACATT TGGGACTCCT GGGGCCATAT ATAAGAGCAG AAGTTGAAGA
6061   TAATATCATG GTAACTTTCA GAAATCAGGC CTCTCGTCCC TATTCCTTCT ATTCTAGCCT
6121   TATTTCTTAT GAGGAAGATC AGAGGCAAGG AGCAGAACCT AGAAAAAATC TTGTCAAGCC
6181   TAATGAAACC AAAACTTACT TTTGGAAAAGT GCAACATCAT ATGGCACCCA CTAAAGATGA
6241   GTTTGACTGC AAAGCCTGGG CTTATTTCTC TGATGTTGAC CTGGAAAAAG ATGTGCACTC
6301   AGGCCTGATT GGACCCCTTC TGGTCTGCCA CACTAACACA CTGAACCTTG CTCATGGGAG
6361   ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTCACCATC TTTGATGAGA CCAAAAGCTG
6421   GTACTTCACT GAAAAATATGG AAAGAAACTG CAGGGCTCCC TGCAATATCC AGATGGAAGA
6481   TCCCACTTTT AAAGAGAATT ATCGCTTCCA TGCAATCAAT GGCTACATAA TGGATACACT
6541   ACCTGGCTTA GTAATGGCTC AGGATCAAAG GATTCGATGG TATCTGCTCA GCATGGGCAG
6601   CAATGAAAAC ATCCATTCTA TTCATTTTCA TGGACATGTG TTCACTGTAC GAAAAAAGA
6661   GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT TTTGAGACAG TGGAAATGTT
6721   ACCATCCAAA GCTGGAATTT GCGGGTGGA ATGCCTTATT GGCGAGCATC TACATGCTGG
6781   GATGAGCACA CTTTTTCTGG TGTACAGCAA TAAGTGTGAG ACTCCCCTGG GAATGGCTTC
6841   TGGACACATT AGAGATTTTC AGATTACAGC TTCAGGACAA TATGGACAGT GGGCCCCAAA
6901   GCTGGCCAGA CTTCAATTAT CCGGATCAAT CAATGCCTGG AGCACCAAGG AGCCCTTTTC
6961   TTGGATCAAG GTGGATCTGT TGGCACCAAT GATTATTAC GGCATCAAGA CCCAGGGTGC
7021   CCGTCAGAAG TTCTCCAGCC TCTACATCTC TCAGTTTATC ATCATGTATA GTCTTGATGG
7081   GAAGAAGTGG CAGACTTATC GAGGAAATTC CACTGGAACC TTAATGGTCT TCTTTGGCAA
7141   TGTGGATTCA TCTGGGATAA AACACAATAT TTTTAACCCT CCAATTATTG CTCGATACAT
7201   CCGTTTGCAC CCAACTCATT ATAGCATTCG CAGCACTCTT CGCATGGAGT TGATGGGCTG
7261   TGATTTAAAT AGTTGCAGCA TGCCATTGGG AATGGAGAGT AAAGCAATAT CAGATGCACA
7321   GATTACTGCT TCATCCTACT TTACCAATAT GTTTGCCACC TGGTCTCCTT CAAAAGCTCG
7381   ACTTCACTC CAAGGGAGGA GTAATGCCTG GAGACCTCAG GTGAATAATC CAAAAGAGTG
7441   GCTGCAAGTG GACTTCCAGA AGACAATGAA AGTCACAGGA GTAACACTC AGGGAGTAAA
7501   ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC TCCAGCAGTC AAGATGGCCA
7561   TCAGTGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTCAGGGAA ATCAAGACTC
7621   CTTACACCT GTGGTGAAC CTCTAGACCC ACCGTTACTG ACTCGCTACC TTCGAATTCA
7681   CCCCAGAGT TGGGTGCACC AGATTGCCCT GAGGATGGAG GTTCTGGGCT GCGAGGCACA
7741   GGACCTCTAC

```

*The underlined nucleic acids encode a signal peptide.

[0158] FVIII polypeptides include full-length FVIII, full-length FVIII minus Met at the N-terminus, mature FVIII (minus the signal sequence), mature FVIII with an additional Met at the N-terminus, and/or FVIII with a full or partial deletion of the B domain. In certain embodiments, FVIII variants include B domain deletions, whether partial or full deletions.

[0159] The sequence of native mature human FVIII is presented as SEQ ID NO: 65. A native FVIII protein has the following formula: A1-a1-A2-a2-B-a3-A3-C1-C2, where A1, A2, and A3 are the structurally-related "A domains," B is the "B domain," C1 and C2 are the structurally-related "C domains," and a1, a2 and a3 are acidic spacer regions. Referring to the primary amino acid sequence position in SEQ ID NO:65, the A1 domain of human FVIII extends from Ala1 to about Arg336, the a1 spacer region extends from about Met337 to about Val374, the A2 domain

extends from about Ala375 to about Tyr719, the a2 spacer region extends from about Glu720 to about Arg740, the B domain extends from about Ser741 to about Arg 1648, the a3 spacer region extends from about Glu1649 to about Arg1689, the A3 domain extends from about Ser1690 to about Leu2025, the C1 domain extends from about Gly2026 to about Asn2072, and the C2 domain extends from about Ser2073 to Tyr2332. Other than specific proteolytic cleavage sites, designation of the locations of the boundaries between the domains and regions of FVIII can vary in different literature references. The boundaries noted herein are therefore designated as approximate by use of the term "about."

[0160] The human FVIII gene was isolated and expressed in mammalian cells (Toole, J. J., *et al.*, *Nature* 312:342-347 (1984); Gitschier, J., *et al.*, *Nature* 312:326-330 (1984); Wood, W. I., *et al.*, *Nature* 312:330-337 (1984); Vehar, G. A., *et al.*, *Nature* 312:337-342 (1984); WO 87/04187; WO 88/08035; WO 88/03558; and U.S. Pat. No. 4,757,006). The FVIII amino acid sequence was deduced from cDNA as shown in U.S. Pat. No. 4,965,199. In addition, partially or fully B-domain deleted FVIII is shown in U.S. Pat. Nos. 4,994,371 and 4,868,112. In some embodiments, the human FVIII B-domain is replaced with the human Factor V B-domain as shown in U.S. Pat. No. 5,004,803. The cDNA sequence encoding human Factor VIII and amino acid sequence are shown in SEQ ID NOs: 1 and 2, respectively, of US Application Publ. No. 2005/0100990.

[0161] The porcine FVIII sequence is published in Toole, J. J., *et al.*, *Proc. Natl. Acad. Sci. USA* 83:5939-5942 (1986). Further, the complete porcine cDNA sequence obtained from PCR amplification of FVIII sequences from a pig spleen cDNA library has been reported in Healey, J. F., *et al.*, *Blood* 88:4209-4214 (1996). Hybrid human/porcine FVIII having substitutions of all domains, all subunits, and specific amino acid sequences were disclosed in U.S. Pat. No. 5,364,771 by Lollar and Runge, and in WO 93/20093. More recently, the nucleotide and corresponding amino acid sequences of the A1 and A2 domains of porcine FVIII and a chimeric FVIII with porcine A1 and/or A2 domains substituted for the corresponding human domains were reported in WO 94/11503. U.S. Pat. No. 5,859,204, Lollar, J. S., also discloses the porcine cDNA and deduced amino acid sequences. U.S. Pat. No. 6,458,563 discloses a B-domain-deleted porcine FVIII.

[0162] U.S. Pat. No. 5,859,204 to Lollar, J. S. reports functional mutants of FVIII having reduced antigenicity and reduced immunoreactivity. U.S. Pat. No. 6,376,463 to Lollar, J. S. also reports mutants of FVIII having reduced immunoreactivity. US Appl. Publ. No. 2005/0100990 to Saenko *et al.* reports functional mutations in the A2 domain of FVIII.

[0163] In one embodiment, the FVIII (or FVIII portion of a chimeric protein) may be at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a FVIII amino acid

sequence of amino acids 1 to 1438 of SEQ ID NO: 67 or amino acids 1 to 2332 of SEQ ID NO: 65 (without a signal sequence) or a FVIII amino acid sequence of amino acids 1 to 19 of SEQ ID NO: 64 and 1 to 1438 of SEQ ID NO: 67 or amino acids 1 to 19 of SEQ ID NO: 64 and amino acids 1 to 2332 of SEQ ID NO: 65 (with a signal sequence), wherein the FVIII has a clotting activity, *e.g.*, activates Factor IX as a cofactor to convert Factor X to activated Factor X. The FVIII (or FVIII portion of a chimeric protein) may be identical to a FVIII amino acid sequence of amino acids 1 to 1438 of SEQ ID NO: 67 or amino acids 1 to 2332 of SEQ ID NO: 65 (without a signal sequence). The FVIII may further comprise a signal sequence.

[0164] The "B-domain" of FVIII, as used herein, is the same as the B-domain known in the art that is defined by internal amino acid sequence identity and sites of proteolytic cleavage, *e.g.*, residues Ser741-Arg1648 of full-length human FVIII. The other human FVIII domains are defined by the following amino acid residues: A1, residues Ala1-Arg372; A2, residues Ser373-Arg740; A3, residues Ser1690-Asn2019; C1, residues Lys2020-Asn2172; C2, residues Ser2173-Tyr2332. The A3-C1-C2 sequence includes residues Ser1690-Tyr2332. The remaining sequence, residues Glu1649-Arg1689, is usually referred to as the a3 acidic region. The locations of the boundaries for all of the domains, including the B-domains, for porcine, mouse and canine FVIII are also known in the art. In one embodiment, the B domain of FVIII is deleted ("B-domain-deleted factor VIII" or "BDD FVIII"). An example of a BDD FVIII is REFACTO® (recombinant BDD FVIII), which has the same sequence as the Factor VIII portion of the sequence in Table 5. (BDD FVIII heavy chain is double underlined; B domain is italicized; and BDD FVIII light chain is in plain text). A nucleotide sequence encoding Table 6 (SEQ ID NO: 68) is shown in Table 6.

TABLE 5. Amino Acid Sequence of B-domain Deleted Factor VIII (BDD FVIII)

BDD FVIII (SEQ ID NO: 67)

ATRRYYLGAVELSWDYMOSDLGELPVDAREFPVRPKSFPPNTSVVYKKTLEVEFTDHLFNIAKPRPPWMGLL
GPTIQAEVYDVTVVITLKNMASHPVSLHAVGVSYWKASEGAEYDDOTSQREKEDDKVFPGGSHTYVWQVLKEN
GPMASDPLCLTYSYLSHVDLVKDLNSGLIGALLVCREGLAKEKTQTLHKFILLFAVFDEGKSWHSETKNSL
MQDRDAASARAWPKMHTVNGYVNRSLPGLIGCHRKSVYWHVIGMGTTPEVHSIFLEGHTFLVRNHRQASLEI
SPITFLTAQTLTLLMDLGQFLLEFCHISSHQHDMGEAYVKVDSCPEEPQLRMKNNEEAEDYDDDLTDSEMDVVR
DDDNPSPSFIQIRSVAKKHPKTWVHYIAABEEDWDYAPLV LAPDDRSYKSOYLNNGPORIGRKYKKVRFMAYT
DETFTKTRAIQHESGILGPLYGEVGDTLIIIFKNQASRPYNIYPHGITDVRPLYSRRLPKGVKHLKDFPIL
PGEIFKYKWTVTVEDGPTKSDPRCLTRYSSFVNMERDLASGLIGPLLYCYKESVDQGNQIMSDKRNILF
SVFDENRSWYL TENIQRFLNPAGVQLEDPEFQASNIMHSINGYVFDLSQLSVCLHEVAYWYILSIGAQTDF
LSVFFSGYTFKHKMVEYEDTLTLFPFSGETVFMSENPLWLILGCHNSDFRNRGMTALLKVSSCDKNTGDYYE
DSYEDISAYLLSKNNAIEPRSFQNPVVKRHRQREITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQ
SPRSFQKKTRHYFIAAVERLWDYGMSSSPHVLNRNAQSGSVQFVKVVFQEF TDGSFTQPLYRGELNEHLGL
LGPYIRAEVEDNIMVTFRNQASRPYSFYSSLSIYEEDQRQGAEPKKNFVKPNETKTYFWKVQHMAPTKDEF
DCKAWAYFSDVDLEKDVHSGLIGPLLVCNTNTLNPAHGRQVTVQEFALFFTIFDETSWYFTENMERNCRAP
CNIQMEDPTFKENYRFHAINGYIMDTLPGLVMAQDQRIRWYLLSMGSNENIHSIHFSGHVFTVRKKEEYKMA
LYNLYPGVFETVEMLPKAGIWRVECLIGEHLHAGMSTFLVYSNKCQTPMGASGHIRDFQITASGQYQGW
APKLARLHYSGSINAWSTKEPFSWIKVDLLAPMI IHGIKTQGARQKFSSLYISQFIIMYSLDGKKWQTYRGN
STGTLMVFFGNVDSSGIKHNIFNPPIIARYIRLHPHTHSIRSTLRMELMGCDLNSCSMPLGMESKAISDAQI

TASSYFTNMFATWSPSKARLHLQGRSNAWRPQVNNPKEWLQVDFQKTMKVTGVTTQGVKSLLTSMYVKEFLI
SSSQDGHQWTLFFQNGKVKVFQGNQDSFTPVVNSLDPPLLTRYLRIHPQSWVHQIALRMEVLGCEAQDLY

TABLE 6. Nucleotide Sequence Encoding BDD FVIII (SEQ ID NO: 68)*

661	A TGCAAATAGA GCTCTCCACC TGCTTCTTTC					
721	TGTGCCTTTT	GCGATTCTGC	TTTAGTGCCA	CCAGAAGATA	CTACCTGGGT	GCAGTGGAAC
781	TGTCATGGGA	CTATATGCAA	AGTGATCTCG	GTGAGCTGCC	TGTGGACGCA	AGATTTCCTC
841	CTAGAGTGCC	AAAATCTTTT	CCATTCAACA	CCTCAGTCGT	GTACAAAAAG	ACTCTGTTTG
901	TAGAATTAC	GGATCACCTT	TTCAACATCG	CTAAGCCAAG	GCCACCCTGG	ATGGGTCTGC
961	TAGGTCCTAC	CATCCAGGCT	GAGGTTTATG	ATACAGTGGT	CATTACACTT	AAGAACATGG
1021	CTTCCCATCC	TGTCAGTCTT	CATGCTGTTG	GTGTATCCTA	CTGGAAAGCT	TCTGAGGGAG
1081	CTGAATATGA	TGATCAGACC	AGTCAAAGGG	AGAAAAGAAG	TGATAAAGTC	TTCCCTGGTG
1141	GAAGCCATAC	ATATGTCTGG	CAGGTCCCTGA	AAGAGAATGG	TCCAATGGCC	TCTGACCCAC
1201	TGTGCCTTAC	CTACTCATAT	CTTTCTCATG	TGGACCTGGT	AAAAGACTTG	AATTCAGGCC
1261	TCATTGGAGC	CCTACTAGTA	TGTAGAGAAG	GGAGTCTGGC	CAAGGAAAAG	ACACAGACCT
1321	TGCACAAATT	TATACTACTT	TTTGCTGTAT	TTGATGAAGG	GAAAAGTTGG	CACTCAGAAA
1381	CAAAGAATC	CTTGATGCAG	GATAGGGATG	CTGCATCTGC	TCGGGCCCTG	CCTAAAATGC
1441	ACACAGTCAA	TGGTTATGTA	AACAGGTCTC	TGCCAGGTCT	GATTGGATGC	CACAGGAAAT
1501	CAGTCTATTG	GCATGTGATT	GGAATGGGCA	CCACTCCTGA	AGTGCACCTA	ATATTCTCTG
1561	AAGGTCACAC	ATTTCTTGTG	AGGAACCATC	GCCAGGCGTC	CTTGGAATC	TCGCCAATAA
1621	CTTTCCCTTAC	TGCTCAAACA	CTCTTGATGG	ACCTTGACAC	GTTTCTACTG	TTTTGTCTATA
1681	TCTCTTCCCA	CCAACATGAT	GGCATGGAAG	CTTATGTCAA	AGTAGACAGC	TGTCCAGAGG
1741	AACCCCAACT	ACGAATGAAA	AATAATGAAG	AAGCGGAAGA	CTATGATGAT	GATCTTACTG
1801	ATTCTGAAAT	GGATGTGGTC	AGGTTTGTATG	ATGACAACCTC	TCCTTCCTTT	ATCCAAATTC
1861	GCTCAGTTGC	CAAGAAGCAT	CCTAAAACTT	GGGTACATTA	CATTGCTGCT	GAAGAGGAGG
1921	ACTGGGACTA	TGCTCCCTTA	GTCCTCGCCC	CCGATGACAG	AAGTTATAAA	AGTCAATATT
1981	TGAACAATGG	CCCTCAGCGG	ATTGGTAGGA	AGTACAAAAA	AGTCCGATTT	ATGGCATAACA
2041	CAGATGAAAC	CTTTAAGACT	CGTGAAGCTA	TTTACAGATGA	ATCAGGAATC	TTGGGACCTT
2101	TACTTTTATGG	GGAAGTTGGA	GACACACTGT	TGATTATATT	TAAGAATCAA	GCAAGCAGAC
2161	CATATAACAT	CTACCCCTCAC	GGAATCACTG	ATGTCCGTCC	TTTGTTATTC	AGGAGATTAC
2221	CAAAAGGTGT	AAAACATTTG	AAGGATTTTC	CAATTCTGCC	AGGAGAAATA	TTCAAATATA
2281	AATGGACAGT	GACTGTAGAA	GATGGGGCCAA	CTAAATCAGA	TCCTCGGTGC	CTGACCCGCT
2341	ATTACTCTAG	TTTCGTTAAT	ATGGAGAGAG	ATCTAGCTTC	AGGACTCATT	GGCCCTCTCC
2401	TCATCTGCTA	CAAAGAATCT	GTAGATCAAA	GAGGAAACCA	GATAATGTCA	GACAAGAGGA
2461	ATGTCATCCT	GTTTTCTGTA	TTTGATGAGA	ACCGAAGCTG	GTACCTCACA	GAGAATATAC
2521	AACGCTTTCT	CCCCAATCCA	GCTGGAGTGC	AGCTTGAGGA	TCCAGAGTTC	CAAGCCTCCA
2581	ACATCATGCA	CAGCATCAAT	GGCTATGTTT	TTGATAGTTT	GCAGTTGTCA	GTTTGTGTTGC
2641	ATGAGGTGGC	ATACTGGTAC	ATTCTAAGCA	TTGGAGCACA	GACTGACTTC	CTTTCTGTCT
2701	TCTTCTCTGG	ATATACCTTC	AAACACAAAA	TGGTCTATGA	AGACACACTC	ACCCTATTCC
2761	CATTCTCAGG	AGAAACTGTC	TTCATGTCTGA	TGGAAAACCC	AGGTCTATGG	ATTCTGGGGT
2821	GCCACAATC	AGACTTTTCGG	AACAGAGGCA	TGACCGCCTT	ACTGAAGGTT	TCTAGTTGTG
2881	ACAAGAACAC	TGGTGATTAT	TACGAGGACA	GTTATGAAGA	TATTTTCAGCA	TACTTGCTGA
2941	GTAAAAACAA	TGCCATTGAA	CCAAGAAGCT	TCTCTCAAAA	CCCACCAGTC	TTGAAACGCC
3001	ATCAACGGGA	AATAACTCGT	ACTACTCTTC	AGTCAGATCA	AGAGGAAATT	GACTATGATG
3061	ATACCATATC	AGTTGAAATG	AAGAAGGAAG	ATTTTGACAT	TTATGATGAG	GATGAAAATC
3121	AGAGCCCCCG	CAGCTTTC	AAGAAAACAC	GACACTATTT	TATTGCTGCA	GTGGAGAGGC
3181	TCTGGGATTA	TGGGATGAGT	AGCTCCCCAC	ATGTTCTAAG	AAACAGGGCT	CAGATGGCA
3241	GTGTCCTCA	GTTCAAGAAA	GTGTTTTC	AGGAATTTAC	TGATGGCTCC	TTTACTCAGC
3301	CCTTATACCG	TGGAGAACTA	AATGAACATT	TGGGACTCCT	GGGGCCATAT	ATAAGAGCAG
3361	AAGTTGAAGA	TAATATCATG	GTAACCTTTC	GAAATCAGGC	CTCTCGTCCC	TATTCCTTCT
3421	ATTCTAGCCT	TATTTCTTAT	GAGGAAGATC	AGAGGCAAGG	AGCAGAACCT	AGAAAAACT
3481	TTGTCAAGCC	TAATGAAACC	AAAACTTACT	TTTGGAAAGT	GCAACATCAT	ATGGCACCCA
3541	CTAAAGATGA	GTTTGACTGC	AAAGCCTGGG	CTTATTTCTC	TGATGTTGAC	CTGGAAAAAG
3601	ATGTGCACTC	AGGCCTGATT	GGACCCCTTC	TGGTCTGCCA	CACTAACACA	CTGAACCCTG

```

3661   CTCATGGGAG ACAAGTGACA GTACAGGAAT TTGCTCTGTT TTTACCATC TTTGATGAGA
3721   CCAAAAGCTG GTACTTCACT GAAAATATGG AAAGAAACTG CAGGGCTCCC TGCAATATCC
3781   AGATGGAAGA TCCCACTTTT AAAGAGAAAT ATCGCTTCCA TGCAATCAAT GGCTACATAA
3841   TGGATACACT ACCTGGCTTA GTAATGGCTC AGGATCAAAG GATTTCGATGG TATCTGCTCA
3901   GCATGGGCAG CAATGAAAAC ATCCATTCTA TTCATTTTCTG TGGACATGTG TTTACTGTAC
3961   GAAAAAAGA GGAGTATAAA ATGGCACTGT ACAATCTCTA TCCAGGTGTT TTTGAGACAG
4021   TGGAAATGTT ACCATCCAAA GCTGGAATTT GGCGGGTGGG ATGCCTTATT GGCGAGCATC
4081   TACATGCTGG GATGAGCACA CTTTTTCTGG TGTACAGCAA TAAGTGTCTAG ACTCCCCTGG
4141   GAATGGCTTC TGGACACATT AGAGATTTTC AGATTACAGC TTCAGGACAA TATGGACAGT
4201   GGGCCCCAAA GCTGGCCAGA CTTCATTATT CCGGATCAAT CAATGCCTGG AGCACCAAGG
4261   AGCCCTTTTC TTGGATCAAG GTGGATCTGT TGGCACCAAT GATTATTCAC GGCATCAAGA
4321   CCCAGGGTGC CCGTCAGAAG TTCTCCAGCC TCTACATCTC TCAGTTTATC ATCATGTATA
4381   GTCTTGATGG GAAGAAGTGG CAGACTTATC GAGGAAATTC CACTGGAACC TTAATGGTCT
4441   TCTTTGGCAA TGTGGATTCA TCTGGGATAA AACACAATAT TTTTAACCCT CCAATTATTG
4501   CTCGATACAT CCGTTTGCAC CCACTCATT ATAGCATTCG CAGCACTCTT CGCATGGAGT
4561   TGATGGGCTG TGATTTAAAT AGTTGCAGCA TGCCATTGGG AATGGAGAGT AAAGCAATAT
4621   CAGATGCACA GATTACTGCT TCATCCTACT TTACCAATAT GTTTGCCACC TGGTCTCCTT
4681   CAAAAGCTCG ACTTCACCTC CAAGGGAGGA GTAATGCCTG GAGACCTCAG GTGAATAATC
4741   CAAAAGAGTG GCTGCAAGTG GACTTCCAGA AGACAATGAA AGTCACAGGA GTAACTACTC
4801   AGGGAGTAAA ATCTCTGCTT ACCAGCATGT ATGTGAAGGA GTTCCTCATC TCCAGCAGTC
4861   AAGATGGCCA TCAGTGGACT CTCTTTTTTC AGAATGGCAA AGTAAAGGTT TTTCAGGGAA
4921   ATCAAGACTC CTTCACACCT GTGGTGAACT CTCTAGACCC ACCGTTACTG ACTCGCTACC
4981   TTCGAATTCA CCCCAGAGT TGGGTGCACC AGATTGCCCT GAGGATGGAG GTTCTGGGCT
5041   GCGAGGCACA GGACCTCTAC

```

*The underlined nucleic acids encode a signal peptide.

[0165] A "B-domain-deleted FVIII" may have the full or partial deletions disclosed in U.S. Pat. Nos. 6,316,226, 6,346,513, 7,041,635, 5,789,203, 6,060,447, 5,595,886, 6,228,620, 5,972,885, 6,048,720, 5,543,502, 5,610,278, 5,171,844, 5,112,950, 4,868,112, and 6,458,563. In some embodiments, a B-domain-deleted FVIII sequence of the present invention comprises any one of the deletions disclosed at col. 4, line 4 to col. 5, line 28 and Examples 1-5 of U.S. Pat. No. 6,316,226 (also in US 6,346,513). In another embodiment, a B-domain deleted Factor VIII is the S743/Q1638 B-domain deleted Factor VIII (SQ BDD FVIII) (*e.g.*, Factor VIII having a deletion from amino acid 744 to amino acid 1637, *e.g.*, Factor VIII having amino acids 1-743 and amino acids 1638-2332 of SEQ ID NO: 65, *i.e.*, SEQ ID NO: 67). In some embodiments, a B-domain-deleted FVIII of the present invention has a deletion disclosed at col. 2, lines 26-51 and examples 5-8 of U.S. Patent No. 5,789,203 (also US 6,060,447, US 5,595,886, and US 6,228,620). In some embodiments, a B-domain-deleted Factor VIII has a deletion described in col. 1, lines 25 to col. 2, line 40 of US Patent No. 5,972,885; col. 6, lines 1-22 and example 1 of U.S. Patent no. 6,048,720; col. 2, lines 17-46 of U.S. Patent No. 5,543,502; col. 4, line 22 to col. 5, line 36 of U.S. Patent no. 5,171,844; col. 2, lines 55-68, figure 2, and example 1 of U.S. Patent No. 5,112,950; col. 2, line 2 to col. 19, line 21 and table 2 of U.S. Patent No. 4,868,112; col. 2, line 1 to col. 3, line 19, col. 3, line 40 to col. 4, line 67, col. 7, line 43 to col. 8, line 26, and col. 11, line 5 to col. 13, line 39 of U.S. Patent no. 7,041,635; or col. 4, lines 25-53, of U.S. Patent No. 6,458,563. In some embodiments, a B-domain-deleted FVIII has a deletion of most of the B domain, but still contains

amino-terminal sequences of the B domain that are essential for *in vivo* proteolytic processing of the primary translation product into two polypeptide chain, as disclosed in WO 91/09122. In some embodiments, a B-domain-deleted FVIII is constructed with a deletion of amino acids 747-1638, *i.e.*, virtually a complete deletion of the B domain. Hoebe R.C., *et al. J. Biol. Chem.* 265 (13): 7318-7323 (1990). A B-domain-deleted Factor VIII may also contain a deletion of amino acids 771-1666 or amino acids 868-1562 of FVIII. Meulien P., *et al. Protein Eng.* 2(4): 301-6 (1988). Additional B domain deletions that are part of the invention include: deletion of amino acids 982 through 1562 or 760 through 1639 (Toole *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* (1986) 83, 5939-5942)), 797 through 1562 (Eaton, *et al. Biochemistry* (1986) 25:8343-8347)), 741 through 1646 (Kaufman (PCT published application No. WO 87/04187)), 747-1560 (Sarver, *et al.*, *DNA* (1987) 6:553-564)), 741 through 1648 (Pasek (PCT application No.88/00831)), or 816 through 1598 or 741 through 1648 (Lagner (Behring Inst. Mitt. (1988) No 82:16-25, EP 295597)). In other embodiments, BDD FVIII includes a FVIII polypeptide containing fragments of the B-domain that retain one or more N-linked glycosylation sites, *e.g.*, residues 757, 784, 828, 900, 963, or optionally 943, which correspond to the amino acid sequence of the full-length FVIII sequence. Examples of the B-domain fragments include 226 amino acids or 163 amino acids of the B-domain as disclosed in Miao, H.Z., *et al.*, *Blood* 103(a): 3412-3419 (2004), Kasuda, A, *et al.*, *J. Thromb. Haemost.* 6: 1352-1359 (2008), and Pipe, S.W., *et al.*, *J. Thromb. Haemost.* 9: 2235-2242 (2011) (*i.e.*, the first 226 amino acids or 163 amino acids of the B domain are retained). In still other embodiments, BDD FVIII further comprises a point mutation at residue 309 (from Phe to Ser) to improve expression of the BDD FVIII protein. See Miao, H.Z., *et al.*, *Blood* 103(a): 3412-3419 (2004). In still other embodiments, the BDD FVIII includes a FVIII polypeptide containing a portion of the B-domain, but not containing one or more furin cleavage sites (*e.g.*, Arg1313 and Arg 1648). See Pipe, S.W., *et al.*, *J. Thromb. Haemost.* 9: 2235-2242 (2011). Each of the foregoing deletions may be made in any FVIII sequence.

[0166] In some embodiments, the FVIII has a partial B-domain. In some embodiments, the FVIII protein with a partial B-domain is FVIII198. FVIII198 is a partial B-domain containing single chain FVIII₁₉₈ molecule-226N6. Number 226 represents the N-terminus 226 amino acid of the FVIII B-domain, and N6 represents six N-glycosylation sites in the B-domain.

[0167] In one embodiment, FVIII is cleaved right after Arginine at amino acid 1648 (in full-length Factor VIII or SEQ ID NO: 65), amino acid 754 (in the S743/Q1638 B-domain deleted Factor VIII or SEQ ID NO: 67), or the corresponding Arginine residue (in other variants), thereby resulting in a heavy chain and a light chain. In another embodiment, FVIII comprises a heavy chain and a light chain, which are linked or associated by a metal ion-mediated non-covalent bond.

- [0168] In other embodiments, FVIII is a single chain FVIII that has not been cleaved right after Arginine at amino acid 1648 (in full-length FVIII or SEQ ID NO: 65), amino acid 754 (in the S743/Q1638 B-domain-deleted FVIII or SEQ ID NO: 67), or the corresponding Arginine residue (in other variants). A single chain FVIII may comprise one or more amino acid substitutions. In one embodiment, the amino acid substitution is at a residue corresponding to residue 1648, residue 1645, or both of full-length mature Factor VIII polypeptide (SEQ ID NO: 65) or residue 754, residue 751, or both of SQ BDD Factor VIII (SEQ ID NO: 67). The amino acid substitution can be any amino acids other than Arginine, *e.g.*, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, selenocysteine, serine, tyrosine, histidine, ornithine, pyrrolysine, or taurine.
- [0169] FVIII can further be cleaved by thrombin and then activated as FVIIIa, serving as a cofactor for activated Factor IX (FIXa). And the activated FIX together with activated FVIII forms a Xase complex and converts Factor X to activated Factor X (FXa). For activation, FVIII is cleaved by thrombin after three Arginine residues, at amino acids 372, 740, and 1689 (corresponding to amino acids 372, 740, and 795 in the B-domain deleted FVIII sequence), the cleavage generating FVIIIa having the 50kDa A1, 43kDa A2, and 73kDa A3-C1-C2 chains. In one embodiment, the FVIII protein useful for the present invention is non-active FVIII. In another embodiment, the FVIII protein is an activated FVIII.
- [0170] The protein having FVIII polypeptide linked to or associated with the VWF protein can comprise a sequence at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 65 or 67, wherein the sequence has the FVIII clotting activity, *e.g.*, activating Factor IX as a cofactor to convert Factor X to activated Factor X (FXa).
- [0171] "Hybrid" or "chimeric" polypeptides and proteins, as used herein, includes a combination of a first polypeptide chain, *e.g.*, the VWF protein fused to an XTEN sequence having less than 288 amino acids and a first Ig constant region or a portion thereof, with a second polypeptide chain, *e.g.*, a FVIII protein fused to a second Ig constant region or a portion thereof, thereby forming a heterodimer. In one embodiment, the first polypeptide and the second polypeptide in a hybrid are associated with each other via protein-protein interactions, such as charge-charge or hydrophobic interactions. In another embodiment, a first polypeptide comprises a VWF protein-XTEN-Fc fusion protein, and a second polypeptide comprises FVIII-Fc fusion protein, making the hybrid a heterodimer, wherein the XTEN contains less than 288 amino acids. In other embodiments, the first polypeptide comprises a VWF protein-XTEN-Fc fusion protein, and the second polypeptide comprises FVIII(X)-Fc fusion protein, making the hybrid a heterodimer, wherein the XTEN contains less than 288 amino acids. The first polypeptide and the second

polypeptide can be associated through a covalent bond, *e.g.*, a disulfide bond, between the first Fc region and the second Fc region. The first polypeptide and the second polypeptide can further be associated with each other by binding between the VWF fragment and the FVIII protein.

[0172] A FVIII protein useful in the present invention can include FVIII having one or more additional XTEN sequences, which do not affect the FVIII coagulation activity. Such XTEN sequences can be fused to the C-terminus or N-terminus of the FVIII protein or inserted between one or more of the two amino acid residues in the FVIII protein while the insertions do not affect the FVIII coagulation activity or FVIII function. In one embodiment, the insertions improve pharmacokinetic properties of the FVIII protein (*e.g.*, half-life). In another embodiment, the insertions can be multiple insertions, *e.g.*, more than two, three, four, five, six, seven, eight, nine, or ten insertions. Examples of the insertion sites include, but are not limited to, the sites listed in Tables 7, 8, 9, 10, 11, 12, 13, 14, 15 or any combinations thereof.

[0173] The FVIII protein linked to one or more XTEN sequences can be represented as FVIII(X2) or FVIII_(a→b)-X-FVIII_(c→d), wherein FVIII_(a→b) comprises, consists essentially of, or consists of a first portion of a FVIII protein from amino acid residue "a" to amino acid residue "b"; X2 comprises, consists essentially of, or consists of one or more XTEN sequences, FVIII_(c→d) comprises, consists essentially of, or consists of a second portion of a FVIII protein from amino acid residue "c" to amino acid residue "d";

a is the N-terminal amino acid residue of the first portion of the FVIII protein,

b is the C-terminal amino acid residue of the first portion of the FVIII protein but is also the N-terminal amino acid residue of the two amino acids of an insertion site in which the XTEN sequence is inserted,

c is the N-terminal amino acid residue of the second portion of the FVIII protein but is also the C-terminal amino acid residue of the two amino acids of an insertion site in which the XTEN sequence is inserted, and

d is the C-terminal amino acid residue of the FVIII protein, and

wherein the first portion of the FVIII protein and the second portion of the FVIII protein are not identical to each other and are of sufficient length together such that the FVIII protein has a FVIII coagulation activity.

[0174] In one embodiment, the first portion of the FVIII protein and the second portion of the FVIII protein are fragments of SEQ ID NO: 65 [full length mature FVIII sequence] or SEQ ID NO: 67 [B-domain deleted FVIII], *e.g.*, N-terminal portion and C-terminal portion, respectively. In certain embodiments, the first portion of the FVIII protein comprises the A1 domain and the A2 domain of the FVIII protein. The second portion of the FVIII protein comprises the A3 domain, the C1 domain, and optionally the C2 domain. In yet other embodiments, the first

portion of the FVIII protein comprises the A1 domain and A2 domain, and the second portion of the FVIII protein comprises a portion of the B domain, the A3 domain, the C1 domain, and optionally the C2 domain. In still other embodiments, the first portion of the FVIII protein comprises the A1 domain, A2 domain, and a portion of the B domain of the FVIII protein, and the second portion of the FVIII protein comprises the A3 domain, the C1 domain, and optionally the C2 domain. In still other embodiments, the first portion of the FVIII protein comprises the A1 domain, A2 domain, and a first portion of the B domain of the FVIII protein. The second portion of the FVIII protein comprises a second portion of the B domain, the A3 domain, the C1 domain, and optionally the C2 domain. In some embodiments, the two amino acids ("b" and "c") can be any one or more of the amino acid residues insertion sites shown in Tables 7, 8, 9, 10, 11, 12, 13, 14, and 15. For example, "b" can be the amino acid residue immediately upstream of the site in which one or more XTEN sequences are inserted or linked, and "c" can be the amino acid residue immediately downstream of the site in which the one or more XTEN sequences are inserted or linked. In some embodiments, "a" is the first mature amino acid sequence of a FVIII protein, and "d" is the last amino acid sequence of a FVIII protein. For example, FVIII_(a→b) can be an amino acid sequence at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 1 to 745 of SEQ ID NO: 67 [B domain deleted FVIII amino acid sequence] or SEQ ID NO: 65 [full length FVIII] and FVIII_(c→d) can be amino acids 746 to 1438 of SEQ ID NO: 67 or amino acids 1641 to 2332 of SEQ ID NO: 65, respectively.

[0175] In some aspects, the insertion site in the FVIII protein is located in one or more domains of the FVIII protein, which is the N-terminus, the A1 domain, the A2 domain, the A3 domain, the B domain, the C1 domain, the C2 domain, the C-terminus, or two or more combinations thereof or between two domains of the FVIII protein, which are the A1 domain and a1 acidic region, and the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, and the A3 domain and C1 domain, the C1 domain and C2 domain, or any combinations thereof. For example, the insertion sites in which the XTEN sequence can be inserted are selected from the group consisting of the N-terminus and A1 domain, the N-terminus and A2 domain, the N-terminus and A3 domain, the N-terminus and B domain, the N-terminus and C1 domain, the N-terminus and C2 domain, the N-terminus and the C-terminus, the A1 and A2 domains, the A1 and A3 domains, the A1 and B domains, the A1 and C1 domains, the A1 and C2 domains, the A1 domain and the C-terminus, the A2 and A3 domains, the A2 and B domains, the A2 and C1 domains, the A2 and C2 domains, the A2 domain and the C-terminus, the A3 and B domains, the A3 and C1 domains, the A3 and C2 domains, the A3 domain and the C-terminus, the B and C1 domains, the B and C2 domains, the B domain and the C-terminus, the C1 and C2 domains, the C1 and the C-terminus, the C2 domain, and the C-

terminus, and two or more combinations thereof. Non-limiting examples of the insertion sites are listed in Tables 7, 8, 9, 10, 11, 12, 13, 14, and 15.

[0176] The FVIII protein, in which the XTEN sequence is inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites) in the FVIII protein or linked at the C-terminus or the N-terminus, retains the FVIII activity after linkage to or insertion by the XTEN sequence. The XTEN sequence can be inserted in the FVIII protein once or more than once, twice, three times, four times, five times, or six times such that the insertions do not affect the FVIII activity (*i.e.*, the FVIII protein still retains the coagulation property).

[0177] The FVIII protein useful in the present invention can be linked to one or more XTEN polypeptides at the N-terminus or C-terminus of the FVIII protein by an optional linker or inserted immediately downstream of one or more amino acids (*e.g.*, one or more XTEN insertion sites) in the FVIII protein by one or more optional linkers. In one embodiment, the two amino acid residues in which the XTEN sequence is inserted or the amino acid residue to which the XTEN sequence is linked correspond to the two or one amino acid residues of SEQ ID NO: 65 [full length mature FVIII] selected from the group consisting of the residues in Table 7, Table 8, Table 9, and Table 10 and any combinations thereof.

[0178] In other embodiments, at least one XTEN sequence is inserted in any one or more XTEN insertion sites disclosed herein or any combinations thereof. In one aspect, at least one XTEN sequence is inserted in one or more XTEN insertion sites disclosed in one or more amino acids disclosed in Table 7.

TABLE 7: Exemplary XTEN Insertion Sites

No.	XTEN Insertion Point*	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain
1	0	(N-terminus)	ATR	A1
2	3	R	RYY	A1
3	17	M	QSD	A1
4	18	Q	SDL	A1
5	22	G	ELP	A1
6	24	L	PVD	A1
7	26	V	DAR	A1
8	28	A	RFP	A1
9	32	P	RVP	A1
10	38	F	PFN	A1
11	40	F	NTS	A1
12	41	N	TSV	A1
13	60	N	IAK	A1
14	61	I	AKP	A1
15	65	R	PPW	A1
16	81	Y	DTV	A1
17	111	G	AEY	A1
18	116	D	QTS	A1
19	119	S	QRE	A1

No.	XTEN Insertion Point*	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain
20	120	Q	REK	A1
21	128	V	FPG	A1
22	129	F	PGG	A1
23	130	P	GGG	A1
24	182	G	SLA	A1
25	185	A	KEK	A1
26	188	K	TQT	A1
27	205	G	KSW	A1
28	210	S	ETK	A1
29	211	E	TKN	A1
30	216	L	MQD	A1
31	220	R	DAA	A1
32	222	A	ASA	A1
33	223	A	SAR	A1
34	224	S	ARA	A1
35	230	K	MHT	A1
36	243	P	GLI	A1
37	244	G	LIG	A1
38	250	R	KSV	A1
39	318	D	GME	A1
40	333	P	QLR	A1
42	334	Q	LRM	A1
43	336	R	MKN	a1
44	339	N	NEE	a1
45	345	D	YDD	a1
46	357	V	VRF	a1
47	367	S	FIQ	a1
48	370	S	RPY	a1
49	375	A	KKH	A2
50	376	K	KHP	A2
51	378	H	PKT	A2
52	399	V	LAP	A2
53	403	D	DRS	A2
54	405	R	SYK	A2
55	409	S	QYL	A2
56	416	P	QRI	A2
57	434	E	TFK	A2
58	438	T	REA	A2
59	441	A	IQH	A2
60	442	I	QHE	A2
61	463	I	IFK	A2
62	487	Y	SRR	A2
63	490	R	LPK	A2
64	492	P	KGV	A2
65	493	K	GVK	A2
66	494	G	VKH	A2
67	500	D	FPI	A2
68	506	G	EIF	A2
69	518	E	DGP	A2
70	556	K	ESV	A2
71	565	Q	IMS	A2
72	566	I	MSD	A2
73	598	P	AGV	A2

No.	XTEN Insertion Point*	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain
74	599	A	GVQ	A2
75	603	L	EDP	A2
76	616	S	ING	A2
77	686	G	LWI	A2
78	713	K	NTG	A2
79	719	Y	EDS	A2
80	730	L	LSK	A2
81	733	K	NNA	A2
82	745	N	PPV**	B
83	1640	P	PVL	B
84	1652	R	TTL	B
85	1656	Q	SDQ	A3
86	1685	N	QSP	A3
87	1711	M	SSS	A3
88	1713	S	SPH	A3
89	1720	N	RAQ	A3
90	1724	S	GSV	A3
91	1725	G	SVP	A3
92	1726	S	VPQ	A3
93	1741	G	SFT	A3
94	1744	T	QPL	A3
95	1749	R	GEL	A3
96	1773	V	TFR	A3
97	1792	Y	EED	A3
98	1793	E	EDQ	A3
99	1796	Q	RQG	A3
100	1798	Q	GAE	A3
101	1799	G	AEP	A3
102	1802	P	RKN	A3
103	1803	R	KNF	A3
104	1807	V	KPN	A3
105	1808	K	PNE	A3
106	1827	K	DEF	A3
107	1844	E	KDV	A3
108	1861	N	TLN	A3
109	1863	L	NPA	A3
110	1896	E	RNC	A3
111	1900	R	APC	A3
112	1904	N	IQM	A3
113	1905	I	QME	A3
114	1910	P	TFK	A3
115	1920	A	ING	A3
116	1937	D	QRI	A3
117	1981	G	VFE	A3
118	2019	N	KCQ	A3
119	2020	K	CQT	C1
120	2044	G	QWA	C1
121	2068	F	SWI	C1
122	2073	V	DLL	C1
123	2090	R	QKF	C1
124	2092	K	FSS	C1
125	2093	F	SSL	C1
126	2111	K	WQT	C1

No.	XTEN Insertion Point*	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain
127	2115	Y	RGN	C1
128	2120	T	GTL	C1
129	2125	V	FFG	C1
130	2171	L	NSC	C1
131	2173	S	CSM	C2
132	2188	A	QIT	C2
133	2223	V	NNP	C2
134	2224	N	NPK	C2
135	2227	K	EWL	C2
136	2268	G	HQW	C2
137	2277	N	GKV	C2
138	2278	G	KVK	C2
139	2290	F	TPV	C2
140	2332	Y	C terminus of FVIII	CT

* Indicates an insertion point for XTEN based on the amino acid number of mature full-length human FVIII, wherein the insertion could be either on the N- or C-terminal side of the indicated amino acid.

[0179] In some embodiments, one or more XTEN sequences are inserted within about six amino acids up or down from amino acids 32, 220, 224, 336, 339, 399, 416, 603, 1656, 1711, 1725, 1905, or 1910, corresponding to SEQ ID NO: 65 or any combinations thereof.

TABLE 8. Exemplary XTEN Insertion Ranges

No.	XTEN Insertion Point	Insertion Residue	FVIII BDD Downstream Sequence	FVIII Domain	Distance from insertion residue*
9	32	P	RVP	A1	-3, +6
31	220	R	DAA	A1	-
34	224	S	ARA	A1	+5
43	336	R	MKN	a1	-1, +6
44	339	N	NEE	a1	-4, +5
52	399	V	LAP	A2	-6, +3
56	416	P	QRI	A2	+6
75	603	L	EDP	A2	-6, +6
85	1656	Q	SDQ	B	-3, +6
87	1711	M	SSS	A3	-6, +1
91	1725	G	SVP	A3	+6
113	1905	I	QME	A3	+6
114	1910	P	TFK	A3	-5, +6

*Distance from insertion residue refers to the relative number of amino acids away from the N-terminus (negative numbers) or C-terminus (positive numbers) of the designated insertion residue (residue "0") where an insertion may be made. The designation "-x" refers to an insertion site which is x amino acids away on the N-terminal side of the designated insertion residue. Similarly, the designation "+x" refers to an insertion site which is x amino acids away on the C-terminal side of the designated insertion residue.

For example, "-1, +2" indicates that the insertion is made at the N-terminus or C-terminus of amino acid residues denoted -1, 0, +1 or +2.

[0180] In other embodiments, one or more XTEN sequences are inserted immediately downstream of one or more amino acids corresponding to the full-length mature human FVIII selected from the group consisting of one or more insertion sites in Table 9.

TABLE 9. Exemplary XTEN Insertion Sites or Ranges

No.	XTEN Insertion Point Range*	First Insertion Residue	FVIII Domain
3	18-32	Q	A1
8	40	F	A1
18	211-224	E	A1
27	336-403	R	A1, A2
43	599	A	A2
47	745-1640	N	B
50	1656-1728	Q	B, a3, A3
57	1796-1804	R	A3
65	1900-1912	R	A3
81	2171-2332	L	C1, C2

* indicates range of insertion sites numbered relative to the amino acid number of mature human FVIII

[0181] In yet other embodiments, one or more XTENs are inserted in the B domain of FVIII. In one example, an XTEN is inserted between amino acids 740 and 1640 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 740 and 1640 is optionally not present. In another example, an XTEN is inserted between amino acids 741 and 1690 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 740 and 1690 is optionally not present. In other examples, an XTEN is inserted between amino acids 741 and 1648 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 741 and 1648 is optionally not present. In yet other examples, an XTEN is inserted between amino acids 743 and 1638 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 743 and 1638 is optionally not present. In still other examples, an XTEN is inserted between amino acids 745 and 1656 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1656 is optionally not present. In some examples, an XTEN is inserted between amino acids 745 and 1657 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1657 is optionally not present. In certain examples, an XTEN is inserted between amino acids 745 and 1667 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1667 is optionally not present. In still other examples, an XTEN is inserted between amino acids 745 and 1686 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 745 and 1686 is optionally not present. In some other examples, an XTEN is inserted between amino acids 747 and 1642 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 747 and 1642 is optionally not present. In still

other examples, an XTEN is inserted between amino acids 751 and 1667 corresponding to SEQ ID NO: 65, wherein the FVIII sequence between amino acids 751 and 1667 is optionally not present.

[0182] In some embodiments, one or more XTENs are inserted in one or more amino acids immediately downstream of an amino acid of an insertion site selected from the group consisting of the amino acid residues in Table 10.

TABLE 10: FVIII XTEN insertion sites and construct designations

Construct Number	Domain	Upstream Residue No.*	Downstream Residue No.*	Upstream Sequence	Downstream Sequence
F8X-1	A1	3	4	ATR	RYY
F8X-2	A1	18	19	YMQ	SDL
F8X-3	A1	22	23	DLG	ELP
F8X-4	A1	26	27	LPV	DAR
F8X-5	A1	40	41	FPF	NTS
F8X-6	A1	60	61	LFN	IAK
F8X-7	A1	116	117	YDD	QTS
F8X-8	A1	130	131	VFP	GGs
F8X-9	A1	188	189	KEK	TQT
F8X-10	A1	216	217	NSL	MQD
F8X-11	A1	230	231	WPK	MHT
F8X-12	A1	333	334	EEP	QLR
F8X-13	A2	375	376	SVA	KKH
F8X-14	A2	403	404	APD	DRS
F8X-15	A2	442	443	EAI	QHE
F8X-16	A2	490	491	RRL	PKG
F8X-17	A2	518	519	TVE	DGP
F8X-18	A2	599	600	NPA	GVQ
F8X-19	A2	713	714	CDK	NTG
F8X-20	BD	745	746	SQN	PPV
F8X-21	BD	745	746	SQN	PPV
F8X-22	BD**	745	746	SQN	PPV
F8X-23	A3	1720	1721	APT	KDE
F8X-24	A3	1796	1797	EDQ	RQG
F8X-25	A3	1802	1803	AEP	RKN
F8X-26	A3	1827	1828	PTK	DEF
F8X-27	A3	1861	1862	HTN	TLN
F8X-28	A3	1896	1897	NME	RNC
F8X-29	A3	1900	1901	NCR	APC
F8X-30	A3	1904	1905	PCN	IQM
F8X-31	A3	1937	1938	AQD	QRI
F8X-32	C1	2019	2020	YSN	KCQ
F8X-33	C1	2068	2069	EPF	SWI
F8X-34	C1	2111	2112	GKK	WQT
F8X-35	C1	2120	2121	NST	GTL
F8X-36	C2	2171	2172	CDL	NSC
F8X-37	C2	2188	2189	SDA	QIT

F8X-38	C2	2227	2228	NPK	EWL
F8X-39	C2	2277	2278	FQN	GKV
F8X-40	CT	2332	NA	DLY	NA
F8X-41	CT	2332	NA	DLY	NA
F8X-42	A1	3	4	ATR	ATR
pSD0001	A2	403	404		
pSD0002	A2	599	600		
pSD0021	N-term	0	1		
pSD0022	A1	32	33		
pSD0023	A1	65	66		
pSD0024	A1	81	82		
pSD0025	A1	119	120		
pSD0026	A1	211	212		
pSD0027	A1	220	221		
pSD0028	A1	224	225		
pSD0029	A1	336	337		
pSD0030	A1	339	340		
pSD0031	A2	378	379		
pSD0032	A2	399	400		
pSD0033	A2	409	410		
pSD0034	A2	416	417		
pSD0035	A2	487	488		
pSD0036	A2	494	495		
pSD0037	A2	500	501		
pSD0038	A2	603	604		
pSD0039	A3	1656	1657		
pSD0040	A3	1711	1712		
pSD0041	A3	1725	1726		
pSD0042	A3	1749	1750		
pSD0043	A3	1905	1906		
pSD0044	A3	1910	1911		
pDS0062	A3	1900	1901		

* Indicates the amino acid number of the mature FVIII protein

[0183] In one embodiment, the one or more XTEN insertion sites are located within one or more surface-exposed, flexible loop structure of the FVIII protein (e.g., a permissive loop). For example, at least one XTEN sequence can be inserted in each FVIII "A" domain comprising at least two "permissive loops" into which at least one XTEN polypeptide can be inserted without eliminating procoagulant activity of the recombinant protein, or the ability of the recombinant proteins to be expressed *in vivo* or *in vitro* in a host cell. The permissive loops are regions that allow insertion of at least one XTEN sequence with, among other attributes, high surface or solvent exposure and high conformational flexibility. The A1 domain comprises a permissive loop-1 (A1-1) region and a permissive loop-2 (A1-2) region, the A2 domain comprises a permissive loop-1 (A2-1) region and a permissive loop-2 (A2-2) region, the A3 domain comprises a permissive loop-1 (A3-1) region and a permissive loop-2 (A3-2) region.

[0184] In one aspect, a first permissive loop in the FVIII A1 domain (A1-1) is located between beta strand 1 and beta strand 2, and a second permissive loop in the FVIII A2 domain (A1-2) is located between beta strand 11 and beta strand 12. A first permissive loop in the FVIII A2 domain (A2-1) is located between beta strand 22 and beta strand 23, and a second permissive loop in the FVIII A2 domain (A2-2) is located between beta strand 32 and beta strand 33. A first permissive loop in the FVIII A3 domain (A3-1) is located between beta strand 38 and beta strand 39, and a second permissive loop in the FVIII A3 (A3-2) is located between beta strand 45 and beta strand 46. In certain aspects, the surface-exposed, flexible loop structure comprising A1-1 corresponds to a region in native mature human FVIII from about amino acid 15 to about amino acid 45 of SEQ ID NO: 65, *e.g.*, from about amino acid 18 to about amino acid 41 of SEQ ID NO: 65. In other aspects, the surface-exposed, flexible loop structure comprising A1-2 corresponds to a region in native mature human FVIII from about amino acid 201 to about amino acid 232 of SEQ ID NO: 65, *e.g.*, from about amino acid 218 to about amino acid 229 of SEQ ID NO: 65. In yet other aspects, the surface-exposed, flexible loop structure comprising A2-1 corresponds to a region in native mature human FVIII from about amino acid 395 to about amino acid 421 of SEQ ID NO: 65, *e.g.* from about amino acid 397 to about amino acid 418 of SEQ ID NO: 65. In still other embodiments, the surface-exposed, flexible loop structure comprising A2-2 corresponds to a region in native mature human FVIII from about amino acid 577 to about amino acid 635 of SEQ ID NO: 65, *e.g.*, from about amino acid 595 to about amino acid 607 of SEQ ID NO: 65. In certain aspects the surface-exposed, flexible loop structure comprising A3-1 corresponds to a region in native mature human FVIII from about amino acid 1705 to about amino acid 1732 of SEQ ID NO: 65, *e.g.*, from about amino acid 1711 to about amino acid 1725 of SEQ ID NO: 65. In yet other aspects, the surface-exposed, flexible loop structure comprising A3-2 corresponds to a region in native mature human FVIII from about amino acid 1884 to about amino acid 1917 of SEQ ID NO: 65, *e.g.*, from about amino acid 1899 to about amino acid 1911 of SEQ ID NO: 65.

[0185] In another embodiment, the one or more amino acids in which at least one XTEN sequence is inserted is located within a3 domain, *e.g.*, amino acids 1649 to 1689, corresponding to full-length mature FVIII polypeptide. In a particular embodiment, an XTEN sequence is inserted between amino acids 1656 and 1657 of SEQ ID NO: 65 (full-length mature FVIII). In a specific embodiment, a FVIII protein comprising an XTEN sequence inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 65 further comprises a deletion from amino acid 745 to amino acid 1656 corresponding to SEQ ID NO: 65.

[0186] In some embodiments, the one or more insertion sites for one or more XTEN insertions are immediately downstream of one or more amino acids corresponding to mature full-length FVIII, selected from the group consisting of:

- (1) amino acid 3, (2) amino acid 18, (3) amino acid 22,
 (4) amino acid 26, (5) amino acid 32, (6) amino acid 40,
 (7) amino acid 60, (8) amino acid 65, (9) amino acid 81,
 (10) amino acid 116, (11) amino acid 119, (12) amino acid 130,
 (13) amino acid 188, (14) amino acid 211, (15) amino acid 216,
 (16) amino acid 220, (17) amino acid 224, (18) amino acid 230,
 (19) amino acid 333, (20) amino acid 336, (21) amino acid 339,
 (22) amino acid 375, (23) amino acid 399, (24) amino acid 403,
 (25) amino acid 409, (26) amino acid 416, (26) amino acid 442,
 (28) amino acid 487, (29) amino acid 490, (30) amino acid 494,
 (31) amino acid 500, (32) amino acid 518, (33) amino acid 599,
 (34) amino acid 603, (35) amino acid 713, (36) amino acid 745,
 (37) amino acid 1656, (38) amino acid 1711, (39) amino acid 1720,
 (40) amino acid 1725, (41) amino acid 1749, (42) amino acid 1796,
 (43) amino acid 1802, (44) amino acid 1827, (45) amino acid 1861,
 (46) amino acid 1896, (47) amino acid 1900, (48) amino acid 1904,
 (49) amino acid 1905, (50) amino acid 1910, (51) amino acid 1937,
 (52) amino acid 2019, (53) amino acid 2068, (54) amino acid 2111,
 (55) amino acid 2120, (56) amino acid 2171, (57) amino acid 2188,
 (58) amino acid 2227, (59) amino acid 2277, and
 (60) two or more combinations thereof.

[0187] In one embodiment, a FVIII protein useful for the invention comprises two XTEN sequences, a first XTEN sequence inserted into a first XTEN insertion site and a second XTEN inserted into a second XTEN insertion site. Non-limiting examples of the first XTEN insertion site and the second XTEN insertion site are listed in Table 11.

TABLE 11. Exemplary Insertion Sites for Two XTENS

Insertion 1		Insertion 2	
Insertion Site	Domain	Insertion Site	Domain
745	B	2332	CT
26	A1	403	A2
40	A1	403	A2
18	A1	403	A2
26	A1	599	A2
40	A1	599	A2
18	A1	599	A2
1720	A3	1900	A3
1725	A3	1900	A3
1711	A3	1905	A3

Insertion 1		Insertion 2	
Insertion Site	Domain	Insertion Site	Domain
1720	A3	1905	A3
1725	A3	1905	A3
1656	A3	26	A1
1656	A3	18	A1
1656	A3	40	A1
1656	A3	399	A2
1656	A3	403	A2
1656	A3	1725	A3
1656	A3	1720	A3
1900	A3	18	A1
1900	A3	26	A1
1900	A3	40	A1
1905	A3	18	A1
1905	A3	40	A1
1905	A3	26	A1
1910	A3	26	A1
18	A1	399	A2
26	A1	399	A2
40	A1	399	A2
18	A1	403	A2
1656	A3	1900	A3
1656	A3	1905	A3
1711	A3	40	A1
1711	A3	26	A1
1720	A3	26	A1
1720	A3	40	A1
1720	A3	18	A1
1725	A3	26	A1
1725	A3	40	A1
1725	A3	18	A1
1720	A3	403	A2
1720	A3	399	A2
1711	A3	403	A2
1720	A3	403	A2
1725	A3	403	A2
1725	A3	399	A2
1711	A3	403	A2
1900	A3	399	A2
1900	A3	403	A2
1905	A3	403	A2
1905	A3	399	A2
1910	A3	403	A2

[0188] The two XTENs inserted or linked to the FVIII protein can be identical or different. In some embodiments, a FVIII protein useful for the invention comprises two XTEN sequences inserted in the FVIII protein, a first XTEN sequence inserted immediately downstream of amino acid 745 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately

downstream of amino acid 2332 corresponding to SEQ ID NO: 65 (the C-terminus). In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, 40, 1656, or 1720 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 403 corresponding to SEQ ID NO: 65. In yet other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 599 corresponding to SEQ ID NO: 65. In still other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 18, 26, 40, 399, 403, 1725, 1720, 1900, 1905, or 2332 corresponding to SEQ ID NO: 65. In certain embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1900 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 65. In some embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 399 corresponding to SEQ ID NO: 65. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 18, 26, or 40 corresponding to SEQ ID NO: 65. In still other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 1720 corresponding to SEQ ID NO: 65, and a second XTEN sequence inserted immediately downstream of amino acid 18 corresponding to SEQ ID NO: 65. In a particular embodiment, the FVIII protein comprising two XTEN sequences, a first XTEN sequence inserted immediately downstream of amino acid 745 corresponding to SEQ ID NO: 65 and a second XTEN sequence inserted immediately downstream of amino acid 2332 corresponding to SEQ ID NO: 65, wherein the FVIII protein further has a deletion from amino acid 745 corresponding to SEQ ID NO: 65 to amino acid 1685 corresponding to SEQ ID NO: 65, a mutation or substitution at amino acid 1680 corresponding to SEQ ID NO: 65, *e.g.*, Y1680F, a mutation or substitution at amino acid 1648 corresponding to SEQ ID NO: 65, *e.g.*, R1648A, or at least two mutations or substitutions at amino acid 1648 corresponding to SEQ ID NO: 65, *e.g.*, R1648A, and amino acid 1680 corresponding to SEQ ID NO: 65, *e.g.*, Y1680F. In a specific embodiment, the FVIII protein comprises two XTEN sequences, a first XTEN inserted immediately downstream of amino acid 1656 corresponding to SEQ ID NO: 65 and a second XTEN sequence inserted immediately downstream of amino acid 2332 of SEQ ID NO: 65, wherein the FVIII protein further has a deletion from amino acid 745 to amino acid 1656 corresponding to SEQ ID NO: 65.

[0189] In certain embodiments, a FVIII protein comprises three XTEN sequences, a first XTEN sequence inserted into a first XTEN insertion site, a second XTEN sequence inserted into a second XTEN sequence, and a third XTEN sequence inserted into a third XTEN insertion site. The first, second, or third XTEN sequences can be identical or different. The first, second, and third insertion sites can be selected from the group of any one of the insertion sites disclosed herein. In some embodiments, the FVIII protein comprising three XTEN sequences can further comprise a mutation or substitution, *e.g.*, amino acid 1648 corresponding to SEQ ID NO: 65, *e.g.*, R1648A. For example, non-limiting examples of the first, second, and third XTEN insertion sites are listed in Table 12.

TABLE 12. Exemplary Insertion Sites for Three XTENs

Insertion 1		Insertion 2		Insertion 3	
Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain
26	A1	403	A2	1656	A3
26	A1	403	A2	1720	A3
26	A1	403	A2	1900	A3
26	A1	1656	A3	1720	A3
26	A1	1656	A3	1900	A3
26	A1	1720	A3	1900	A3
403	A2	1656	A3	1720	A3
403	A2	1656	A3	1900	A3
403	A2	1720	A3	1900	A3
1656	A3	1720	A3	1900	A3
745	B	1900		2332	
18	A1	745	B	2332	CT
26	A1	745	B	2332	CT
40	A1	745	B	2332	CT
18	A1	745	B	2332	CT
40	A1	745	B	2332	CT
403	A2	745	B	2332	CT
399	A2	745	B	2332	CT
1725	A3	745	B	2332	CT
1720	A3	745	B	2332	CT
1711	A3	745	B	2332	CT
1900	A3	745	B	2332	CT
1905	A3	745	B	2332	CT
1910	A3	745	B	2332	CT

[0190] In some embodiments, a FVIII protein comprises three XTEN sequences, a first XTEN sequence inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 65, a second XTEN sequence inserted downstream of amino acid 403 corresponding to SEQ ID NO: 65, and a third XTEN sequence inserted downstream of amino acid 1656, 1720, or 1900 corresponding to SEQ ID NO: 65. In other embodiments, the first XTEN sequence is inserted

immediately downstream of amino acid 26 corresponding to SEQ ID NO: 65, a second XTEN sequence is inserted downstream of amino acid 1656 corresponding to SEQ ID NO: 65, and a third XTEN sequence is inserted downstream of amino acid 1720 or 1900 corresponding to SEQ ID NO: 65. In yet other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 26 corresponding to SEQ ID NO: 65, a second XTEN sequence is inserted downstream of amino acid 1720 corresponding to SEQ ID NO: 65, and a third XTEN sequence is inserted downstream of amino acid 1900 corresponding to SEQ ID NO: 65. In still other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 403 corresponding to SEQ ID NO: 65, a second XTEN sequence is inserted downstream of amino acid 1656 corresponding to SEQ ID NO: 65, and a third XTEN sequence is inserted downstream of amino acid 1720 or 1900 corresponding to SEQ ID NO: 65. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 403 or 1656 corresponding to SEQ ID NO: 65, a second XTEN sequence is inserted downstream of amino acid 1720 corresponding to SEQ ID NO: 65, and a third XTEN sequence is inserted downstream of amino acid 1900 corresponding to SEQ ID NO: 65. In other embodiments, the first XTEN sequence is inserted immediately downstream of amino acid 18, 26, 40, 399, 403, 1711, 1720, 1725, 1900, 1905, or 1910 corresponding to SEQ ID NO: 65, a second XTEN sequence is inserted downstream of amino acid 745 corresponding to SEQ ID NO: 65, and a third XTEN sequence is inserted downstream of amino acid 2332 corresponding to SEQ ID NO: 65.

[0191] In other embodiments, a FVIII protein in the invention comprises four XTEN sequences, a first XTEN sequence inserted into a first insertion site, a second XTEN sequence inserted into a second insertion site, a third XTEN sequence inserted into a third insertion site, and a fourth XTEN sequence inserted into a fourth insertion site. The first, second, third, and fourth XTEN sequences can be identical, different, or combinations thereof. In some embodiments, the FVIII protein comprising four XTEN sequences can further comprise a mutation or substitution, *e.g.*, amino acid 1648 corresponding to SEQ ID NO: 65, *e.g.*, R1648A. Non-limiting examples of the first, second, third, and fourth XTEN insertion sites are listed in Table 13.

TABLE 13. Exemplary Insertion Sites for Four XTENS

Insertion 1		Insertion 2		Insertion 3		Insertion 4	
Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain
26	A1	403	A2	1656	a3	1720	A3
26	A1	403	A2	1656	a3	1900	A3
26	A1	403	A2	1720	A3	1900	A3
26	A1	1656	a3	1720	A3	1900	A3
403	A2	1656	a3	1720	A3	1900	A3
0040	A1	0403	A2	745	B	2332	CT

Insertion 1		Insertion 2		Insertion 3		Insertion 4	
Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain
0040	A1	0403	A2	745	B	2332	CT
0018	A1	0409	A2	745	B	2332	CT
0040	A1	0409	A2	745	B	2332	CT
0040	A1	0409	A2	745	B	2332	CT
0018	A1	0409	A2	745	B	2332	CT
0040	A1	1720	A3	745	B	2332	CT
0026	A1	1720	A3	745	B	2332	CT
0018	A1	1720	A3	745	B	2332	CT
0018	A1	1720	A3	745	B	2332	CT
0018	A1	1720	A3	745	B	2332	CT
0026	A1	1720	A3	745	B	2332	CT
0018	A1	1720	A3	745	B	2332	CT
0018	A1	1900	A3	745	B	2332	CT
0018	A1	1900	A3	745	B	2332	CT
0026	A1	1900	A3	745	B	2332	CT
0040	A1	1900	A3	745	B	2332	CT
0040	A1	1905	A3	745	B	2332	CT
0018	A1	1905	A3	745	B	2332	CT
0040	A1	1905	A3	745	B	2332	CT
0026	A1	1905	A3	745	B	2332	CT
0018	A1	1905	A3	745	B	2332	CT
0018	A1	1905	A3	745	B	2332	CT
0018	A1	1910	A3	745	B	2332	CT
0018	A1	1910	A3	745	B	2332	CT
0040	A1	1910	A3	745	B	2332	CT
0026	A1	1910	A3	745	B	2332	CT
0018	A1	1910	A3	745	B	2332	CT
0026	A1	1910	A3	745	B	2332	CT
0040	A1	1910	A3	745	B	2332	CT
0018	A1	1910	A3	745	B	2332	CT
0409	A2	1720	A3	745	B	2332	CT
0403	A2	1720	A3	745	B	2332	CT
0409	A2	1720	A3	745	B	2332	CT
0403	A2	1720	A3	745	B	2332	CT
0403	A2	1720	A3	745	B	2332	CT
0403	A2	1900	A3	745	B	2332	CT
0403	A2	1900	A3	745	B	2332	CT
0409	A2	1900	A3	745	B	2332	CT
0403	A2	1900	A3	745	B	2332	CT
0403	A2	1900	A3	745	B	2332	CT
0409	A2	1900	A3	745	B	2332	CT
0409	A2	1905	A3	745	B	2332	CT
0403	A2	1905	A3	745	B	2332	CT
0403	A2	1905	A3	745	B	2332	CT
0403	A2	1905	A3	745	B	2332	CT
0409	A2	1905	A3	745	B	2332	CT
0403	A2	1905	A3	745	B	2332	CT

Insertion 1		Insertion 2		Insertion 3		Insertion 4	
Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain	Insertion Site	Domain
0409	A2	1910	A3	745	B	2332	CT
0403	A2	1910	A3	745	B	2332	CT
0403	A2	1910	A3	745	B	2332	CT
0403	A2	1910	A3	745	B	2332	CT
0403	A2	1910	A3	745	B	2332	CT
1720	A3	1900	A3	745	B	2332	CT
1720	A3	1905	A3	745	B	2332	CT
1720	A3	1910	A3	745	B	2332	CT
1720	A3	1910	A3	745	B	2332	CT
0403	A2	1656	a3	1720	A3	2332	CT
0403	A2	1656	a3	1900	A3	2332	CT
0403	A2	1720	A3	1900	A3	2332	CT
1656	a3	1720	A3	1900	A3	2332	CT
0018	A1	0403	A2	1656	a3	2332	CT
0018	A1	0403	A2	1720	A3	2332	CT
0018	A1	0403	A2	1900	A3	2332	CT
0018	A1	1656	a3	1720	A3	2332	CT
0018	A1	1656	a3	1900	A3	2332	CT
0018	A1	1720	A3	1900	A3	2332	CT
0018	A1	0403	A2	0745	B	2332	CT
0018	A1	0745	B	1720	A3	2332	CT
0018	A1	0745	B	1900	A3	2332	CT
0403	A2	0745	B	1720	A3	2332	CT
0403	A2	0745	B	1900	A3	2332	CT
0745	B	1720	A3	1900	A3	2332	CT
0188	A1	1900	A3	0745	B	2332	CT
0599		1900	A3	0745	B	2332	CT
2068		1900	A3	0745	B	2332	CT
2171		1900	A3	0745	B	2332	CT
2227		1900	A3	0745	B	2332	CT
2277		1900	A3	0745	B	2332	CT

[0192] In some embodiments, a FVIII protein comprises five XTEN sequences, a first XTEN sequence inserted into a first insertion site, a second XTEN sequence inserted into a second insertion site, a third XTEN sequence inserted into a third XTEN insertion site, a fourth XTEN sequence inserted into a fourth XTEN insertion site, and a fifth XTEN sequence inserted into a fifth XTEN insertion site. The first, second, third, fourth, or fifth XTEN sequences can be identical, different, or combinations thereof. Non-limiting examples of the first, second, third, fourth, and fifth insertion sites are listed in Table 14.

TABLE 14. Exemplary Insertion Sites for Five XTENs

XTEN Insertion 1	XTEN insertion 2	XTEN Insertion 3	XTEN Insertion 4	XTEN Insertion 5
0403	1656	1720	1900	2332

0018	0403	1656	1720	2332
0018	0403	1656	1900	2332
0018	0403	1720	1900	2332
0018	1656	1720	1900	2332
0018	0403	0745	1720	2332
0018	0403	0745	1900	2332
0018	0745	1720	1900	2332
0403	0745	1720	1900	2332

[0193] In certain embodiments, a FVIII protein comprises six XTEN sequences, a first XTEN sequence inserted into a first XTEN insertion site, a second XTEN sequence inserted into a second XTEN insertion site, a third XTEN sequence inserted into a third XTEN insertion site, a fourth XTEN sequence inserted into a fourth XTEN insertion site, a fifth XTEN sequence inserted into a fifth XTEN insertion site, and a sixth XTEN sequence inserted into a sixth XTEN insertion site. The first, second, third, fourth, fifth, or sixth XTEN sequences can be identical, different, or combinations thereof. Examples of the six XTEN insertion sites include, but are not limited to the insertion sites listed in Table 15.

TABLE 15. Exemplary XTEN Insertion Sites for Six XTENs

XTEN Insertion 1	XTEN insertion 2	XTEN Insertion 3	XTEN Insertion 4	XTEN Insertion 5	XTEN Insertion 5
0018	0403	1656	1720	1900	2332
0018	0403	0745	1720	1900	2332

[0194] In a particular example, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 65, and a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65 (full-length mature FVIII). In another example, a first XTEN is inserted between amino acids 403 and 404 corresponding to SEQ ID NO: 65, and a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65. In some examples, a first XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 65, and a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65. In other examples, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 65, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 65, and a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65. In yet other embodiments, a first XTEN is inserted between amino acids 403 and 404 corresponding to SEQ ID NO: 65, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 65, and a

third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65. In still other embodiments, a first XTEN is inserted between amino acids 403 and 404 corresponding to SEQ ID NO: 65, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 65, and a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65. In certain embodiments, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 65, a second XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65, and a third XTEN is inserted between amino acids 1900 and 1901 corresponding to SEQ ID NO: 65. In some embodiments, a first XTEN is inserted between amino acids 26 and 27 corresponding to SEQ ID NO: 65, a second XTEN is inserted between amino acids 1656 and 1657 corresponding to SEQ ID NO: 65, a third XTEN is inserted between amino acids 1720 and 1721 corresponding to SEQ ID NO: 65, and a fourth XTEN is inserted between 1900 and 1901 corresponding to SEQ ID NO: 65.

[0195] In a particular embodiment, an XTEN sequence is inserted between amino acids 745 and 746 of a full-length Factor VIII or the corresponding insertion site of the B-domain deleted Factor VIII.

[0196] In some embodiments, a chimeric protein of the invention comprises two polypeptide sequences, a first polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from FVIII-161 (SEQ ID NO: 69), FVIII-169 (SEQ ID NO: 70), FVIII-170 (SEQ ID NO: 71), FVIII-173 (SEQ ID NO: 72); FVIII-195 (SEQ ID NO: 73); FVIII-196 (SEQ ID NO: 74), FVIII-199 (SEQ ID NO: 75), FVIII-201 (SEQ ID NO: 76); FVIII-203 (SEQ ID NO: 77), FVIII-204 (SEQ ID NO: 78), FVIII-205 (SEQ ID NO: 79), FVIII-266 (SEQ ID NO: 80), FVIII-267 (SEQ ID NO: 81), FVIII-268 (SEQ ID NO: 82), FVIII-269 (SEQ ID NO: 83), FVIII-271 (SEQ ID NO: 84) or FVIII-272 (SEQ ID NO: 85) and a second polypeptide sequence comprising an amino acid sequence at least about 80%, 90%, 95%, or 100% identical to a sequence selected from VWF031 (SEQ ID NO: 86), VWF034 (SEQ ID NO: 87), or VWF-036.

II.D. Ig Constant Region or a portion thereof

[0197] The chimeric protein of the invention also includes two Ig constant region or a portion thereof, a first Ig constant region or a portion thereof fused to a FVIII protein by an optional linker and a second Ig constant region or a portion thereof fused to a VWF protein through the XTEN sequence having less than 288 amino acids. The Ig constant region or a portion thereof can improve pharmacokinetic or pharmacodynamic properties of the chimeric protein in combination with the XTEN sequence and the VWF protein. In certain embodiments, the Ig

constant region or a portion thereof extends a half-life of a molecule fused to the Ig constant region or a portion thereof.

[0198] An Ig constant region is comprised of domains denoted CH (constant heavy) domains (CH1, CH2, etc.). Depending on the isotype, (*i.e.* IgG, IgM, IgA, IgD, or IgE), the constant region can be comprised of three or four CH domains. Some isotypes (*e.g.* IgG) constant regions also contain a hinge region. See Janeway *et al.* 2001, *Immunobiology*, Garland Publishing, N.Y., N.Y.

[0199] An Ig constant region or a portion thereof for producing the chimeric protein of the present invention may be obtained from a number of different sources. In some embodiments, an Ig constant region or a portion thereof is derived from a human Ig. It is understood, however, that the Ig constant region or a portion thereof may be derived from an Ig of another mammalian species, including for example, a rodent (*e.g.* a mouse, rat, rabbit, guinea pig) or non-human primate (*e.g.* chimpanzee, macaque) species. Moreover, the Ig constant region or a portion thereof may be derived from any Ig class, including IgM, IgG, IgD, IgA, and IgE, and any Ig isotype, including IgG1, IgG2, IgG3, and IgG4. In one embodiment, the human isotype IgG1 is used.

[0200] A variety of the Ig constant region gene sequences (*e.g.*, human constant region gene sequences) are available in the form of publicly accessible deposits. Constant region domains sequence can be selected having a particular effector function (or lacking a particular effector function) or with a particular modification to reduce immunogenicity. Many sequences of antibodies and antibody-encoding genes have been published and suitable Ig constant region sequences (*e.g.*, hinge, CH2, and/or CH3 sequences, or portions thereof) can be derived from these sequences using art recognized techniques. The genetic material obtained using any of the foregoing methods may then be altered or synthesized to obtain polypeptides of the present invention. It will further be appreciated that the scope of this invention encompasses alleles, variants and mutations of constant region DNA sequences.

[0201] The sequences of the Ig constant region or a portion thereof can be cloned, *e.g.*, using the polymerase chain reaction and primers which are selected to amplify the domain of interest. To clone a sequence of the Ig constant region or a portion thereof from an antibody, mRNA can be isolated from hybridoma, spleen, or lymph cells, reverse transcribed into DNA, and antibody genes amplified by PCR. PCR amplification methods are described in detail in U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; 4,965,188; and in, *e.g.*, "PCR Protocols: A Guide to Methods and Applications" Innis *et al.* eds., Academic Press, San Diego, CA (1990); Ho *et al.* 1989. *Gene* 77:51; Horton *et al.* 1993. *Methods Enzymol.* 217:270). PCR may be initiated by consensus constant region primers or by more specific primers based on the published heavy and light chain DNA and amino acid sequences. As discussed above, PCR also may be used to isolate DNA

clones encoding the antibody light and heavy chains. In this case the libraries may be screened by consensus primers or larger homologous probes, such as mouse constant region probes. Numerous primer sets suitable for amplification of antibody genes are known in the art (*e.g.*, 5' primers based on the N-terminal sequence of purified antibodies (Benhar and Pastan. 1994. *Protein Engineering* 7:1509); rapid amplification of cDNA ends (Ruberti, F. et al. 1994. *J. Immunol. Methods* 173:33); antibody leader sequences (Larrick et al. 1989 *Biochem. Biophys. Res. Commun.* 160:1250). The cloning of antibody sequences is further described in Newman et al., U.S. Pat. No. 5,658,570, filed January 25, 1995, which is incorporated by reference herein.

[0202] An Ig constant region used herein can include all domains and the hinge region or portions thereof. In one embodiment, the Ig constant region or a portion thereof comprises CH2 domain, CH3 domain, and a hinge region, *i.e.*, an Fc region or an FcRn binding partner.

[0203] As used herein, the term "Fc region" is defined as the portion of a polypeptide which corresponds to the Fc region of native Ig, *i.e.*, as formed by the dimeric association of the respective Fc domains of its two heavy chains. A native Fc region forms a homodimer with another Fc region. In contrast, the term "genetically-fused Fc region" or "single-chain Fc region" (scFc region), as used herein, refers to a synthetic dimeric Fc region comprised of Fc domains genetically linked within a single polypeptide chain (*i.e.*, encoded in a single contiguous genetic sequence).

[0204] In one embodiment, the "Fc region" refers to the portion of a single Ig heavy chain beginning in the hinge region just upstream of the papain cleavage site (*i.e.* residue 216 in IgG, taking the first residue of heavy chain constant region to be 114) and ending at the C-terminus of the antibody. Accordingly, a complete Fc domain comprises at least a hinge domain, a CH2 domain, and a CH3 domain.

[0205] The Fc region of an Ig constant region, depending on the Ig isotype can include the CH2, CH3, and CH4 domains, as well as the hinge region. Chimeric proteins comprising an Fc region of an Ig bestow several desirable properties on a chimeric protein including increased stability, increased serum half-life (see Capon *et al.*, 1989, *Nature* 337:525) as well as binding to Fc receptors such as the neonatal Fc receptor (FcRn) (U.S. Pat. Nos. 6,086,875, 6,485,726, 6,030,613; WO 03/077834; US2003-0235536A1), which are incorporated herein by reference in their entireties.

[0206] An Ig constant region or a portion thereof can be an FcRn binding partner. FcRn is active in adult epithelial tissues and expressed in the lumen of the intestines, pulmonary airways, nasal surfaces, vaginal surfaces, colon and rectal surfaces (U.S. Pat. No. 6,485,726). An FcRn binding partner is a portion of an Ig that binds to FcRn.

- [0207] The FcRn receptor has been isolated from several mammalian species including humans. The sequences of the human FcRn, monkey FcRn, rat FcRn, and mouse FcRn are known (Story et al. 1994, J. Exp. Med. 180:2377). The FcRn receptor binds IgG (but not other Ig classes such as IgA, IgM, IgD, and IgE) at relatively low pH, actively transports the IgG transcellularly in a luminal to serosal direction, and then releases the IgG at relatively higher pH found in the interstitial fluids. It is expressed in adult epithelial tissue (U.S. Pat. Nos. 6,485,726, 6,030,613, 6,086,875; WO 03/077834; US2003-0235536A1) including lung and intestinal epithelium (Israel et al. 1997, Immunology 92:69) renal proximal tubular epithelium (Kobayashi et al. 2002, Am. J. Physiol. Renal Physiol. 282:F358) as well as nasal epithelium, vaginal surfaces, and biliary tree surfaces.
- [0208] FcRn binding partners useful in the present invention encompass molecules that can be specifically bound by the FcRn receptor including whole IgG, the Fc fragment of IgG, and other fragments that include the complete binding region of the FcRn receptor. The region of the Fc portion of IgG that binds to the FcRn receptor has been described based on X-ray crystallography (Burmeister et al. 1994, Nature 372:379). The major contact area of the Fc with the FcRn is near the junction of the CH2 and CH3 domains. Fc-FcRn contacts are all within a single Ig heavy chain. The FcRn binding partners include whole IgG, the Fc fragment of IgG, and other fragments of IgG that include the complete binding region of FcRn. The major contact sites include amino acid residues 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. References made to amino acid numbering of Igs or Ig fragments, or regions, are all based on Kabat et al. 1991, Sequences of Proteins of Immunological Interest, U.S. Department of Public Health, Bethesda, Md.
- [0209] Fc regions or FcRn binding partners bound to FcRn can be effectively shuttled across epithelial barriers by FcRn, thus providing a non-invasive means to systemically administer a desired therapeutic molecule. Additionally, fusion proteins comprising an Fc region or an FcRn binding partner are endocytosed by cells expressing the FcRn. But instead of being marked for degradation, these fusion proteins are recycled out into circulation again, thus increasing the *in vivo* half-life of these proteins. In certain embodiments, the portions of Ig constant regions are an Fc region or an FcRn binding partner that typically associates, via disulfide bonds and other non-specific interactions, with another Fc region or another FcRn binding partner to form dimers and higher order multimers.
- [0210] Two FcRn receptors can bind a single Fc molecule. Crystallographic data suggest that each FcRn molecule binds a single polypeptide of the Fc homodimer. In one embodiment, linking the FcRn binding partner, *e.g.*, an Fc fragment of an IgG, to a biologically active molecule provides a means of delivering the biologically active molecule orally, buccally, sublingually,

rectally, vaginally, as an aerosol administered nasally or via a pulmonary route, or via an ocular route. In another embodiment, the chimeric protein can be administered invasively, *e.g.*, subcutaneously, intravenously.

[0211] An FcRn binding partner region is a molecule or a portion thereof that can be specifically bound by the FcRn receptor with consequent active transport by the FcRn receptor of the Fc region. Specifically bound refers to two molecules forming a complex that is relatively stable under physiologic conditions. Specific binding is characterized by a high affinity and a low to moderate capacity as distinguished from nonspecific binding which usually has a low affinity with a moderate to high capacity. Typically, binding is considered specific when the affinity constant KA is higher than $10^6 M^{-1}$, or higher than $10^8 M^{-1}$. If necessary, non-specific binding can be reduced without substantially affecting specific binding by varying the binding conditions. The appropriate binding conditions such as concentration of the molecules, ionic strength of the solution, temperature, time allowed for binding, concentration of a blocking agent (*e.g.* serum albumin, milk casein), etc., may be optimized by a skilled artisan using routine techniques.

[0212] In certain embodiments, a chimeric protein of the invention comprises one or more truncated Fc regions that are nonetheless sufficient to confer Fc receptor (FcR) binding properties to the Fc region. For example, the portion of an Fc region that binds to FcRn (*i.e.*, the FcRn binding portion) comprises from about amino acids 282-438 of IgG1, EU numbering (with the primary contact sites being amino acids 248, 250-257, 272, 285, 288, 290-291, 308-311, and 314 of the CH2 domain and amino acid residues 385-387, 428, and 433-436 of the CH3 domain. Thus, an Fc region of the invention may comprise or consist of an FcRn binding portion. FcRn binding portions may be derived from heavy chains of any isotype, including IgG1, IgG2, IgG3 and IgG4. In one embodiment, an FcRn binding portion from an antibody of the human isotype IgG1 is used. In another embodiment, an FcRn binding portion from an antibody of the human isotype IgG4 is used.

[0213] In another embodiment, the "Fc region" includes an amino acid sequence of an Fc domain or derived from an Fc domain. In certain embodiments, an Fc region comprises at least one of: a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain (about amino acids 216-230 of an antibody Fc region according to EU numbering), a CH2 domain (about amino acids 231-340 of an antibody Fc region according to EU numbering), a CH3 domain (about amino acids 341-438 of an antibody Fc region according to EU numbering), a CH4 domain, or a variant, portion, or fragment thereof. In other embodiments, an Fc region comprises a complete Fc domain (*i.e.*, a hinge domain, a CH2 domain, and a CH3 domain). In some embodiments, an Fc region comprises, consists essentially of, or consists of a hinge domain (or a portion thereof) fused to a CH3 domain (or a portion thereof), a hinge domain (or a portion thereof) fused to a CH2 domain (or a portion

thereof), a CH2 domain (or a portion thereof) fused to a CH3 domain (or a portion thereof), a CH2 domain (or a portion thereof) fused to both a hinge domain (or a portion thereof) and a CH3 domain (or a portion thereof). In still other embodiments, an Fc region lacks at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). In a particular embodiment, an Fc region comprises or consists of amino acids corresponding to EU numbers 221 to 447.

[0214] The Fc regions denoted as F, F1, or F2 herein may be obtained from a number of different sources. In one embodiment, an Fc region of the polypeptide is derived from a human Ig. It is understood, however, that an Fc region may be derived from an Ig of another mammalian species, including for example, a rodent (*e.g.* a mouse, rat, rabbit, or guinea pig) or non-human primate (*e.g.* chimpanzee, macaque) species. Moreover, the polypeptide of the Fc domains or portions thereof may be derived from any Ig class, including IgM, IgG, IgD, IgA and IgE, and any Ig isotype, including IgG1, IgG2, IgG3 and IgG4. In another embodiment, the human isotype IgG1 is used.

[0215] In certain embodiments, the Fc variant confers a change in at least one effector function imparted by an Fc region comprising said wild-type Fc domain (*e.g.*, an improvement or reduction in the ability of the Fc region to bind to Fc receptors (*e.g.* FcγRI, FcγRII, or FcγRIII) or complement proteins (*e.g.* C1q), or to trigger antibody-dependent cytotoxicity (ADCC), phagocytosis, or complement-dependent cytotoxicity (CDCC)). In other embodiments, the Fc variant provides an engineered cysteine residue.

[0216] The Fc regions of the invention may employ art-recognized Fc variants which are known to impart a change (*e.g.*, an enhancement or reduction) in effector function and/or FcR or FcRn binding. Specifically, a binding molecule of the invention may include, for example, a change (*e.g.*, a substitution) at one or more of the amino acid positions disclosed in International PCT Publications WO88/07089A1, WO96/14339A1, WO98/05787A1, WO98/23289A1, WO99/51642A1, WO99/58572A1, WO00/09560A2, WO00/32767A1, WO00/42072A2, WO02/44215A2, WO02/060919A2, WO03/074569A2, WO04/016750A2, WO04/029207A2, WO04/035752A2, WO04/063351A2, WO04/074455A2, WO04/099249A2, WO05/040217A2, WO04/044859, WO05/070963A1, WO05/077981A2, WO05/092925A2, WO05/123780A2, WO06/019447A1, WO06/047350A2, and WO06/085967A2; US Patent Publication Nos. US2007/0231329, US2007/0231329, US2007/0237765, US2007/0237766, US2007/0237767, US2007/0243188, US20070248603, US20070286859, US20080057056 ; or US Patents 5,648,260; 5,739,277; 5,834,250; 5,869,046; 6,096,871; 6,121,022; 6,194,551; 6,242,195; 6,277,375; 6,528,624; 6,538,124; 6,737,056; 6,821,505; 6,998,253; 7,083,784; 7,404,956, and 7,317,091, each of which is incorporated by reference herein. In one embodiment, the specific change (*e.g.*, the specific substitution of one or more amino acids disclosed in the art) may be

made at one or more of the disclosed amino acid positions. In another embodiment, a different change at one or more of the disclosed amino acid positions (*e.g.*, the different substitution of one or more amino acid position disclosed in the art) may be made.

[0217] The Fc region or FcRn binding partner of IgG can be modified according to well recognized procedures such as site directed mutagenesis and the like to yield modified IgG or Fc fragments or portions thereof that will be bound by FcRn. Such modifications include modifications remote from the FcRn contact sites as well as modifications within the contact sites that preserve or even enhance binding to the FcRn. For example, the following single amino acid residues in human IgG1 Fc (Fc γ 1) can be substituted without significant loss of Fc binding affinity for FcRn: P238A, S239A, K246A, K248A, D249A, M252A, T256A, E258A, T260A, D265A, S267A, H268A, E269A, D270A, E272A, L274A, N276A, Y278A, D280A, V282A, E283A, H285A, N286A, T289A, K290A, R292A, E293A, E294A, Q295A, Y296F, N297A, S298A, Y300F, R301A, V303A, V305A, T307A, L309A, Q311A, D312A, N315A, K317A, E318A, K320A, K322A, S324A, K326A, A327Q, P329A, A330Q, P331A, E333A, K334A, T335A, S337A, K338A, K340A, Q342A, R344A, E345A, Q347A, R355A, E356A, M358A, T359A, K360A, N361A, Q362A, Y373A, S375A, D376A, A378Q, E380A, E382A, S383A, N384A, Q386A, E388A, N389A, N390A, Y391F, K392A, L398A, S400A, D401A, D413A, K414A, R416A, Q418A, Q419A, N421A, V422A, S424A, E430A, N434A, T437A, Q438A, K439A, S440A, S444A, and K447A, where for example P238A represents wild type proline substituted by alanine at position number 238. As an example, a specific embodiment incorporates the N297A mutation, removing a highly conserved N-glycosylation site. In addition to alanine other amino acids may be substituted for the wild type amino acids at the positions specified above. Mutations may be introduced singly into Fc giving rise to more than one hundred Fc regions distinct from the native Fc. Additionally, combinations of two, three, or more of these individual mutations may be introduced together, giving rise to hundreds more Fc regions. Moreover, one of the Fc region of a construct of the invention may be mutated and the other Fc region of the construct not mutated at all, or they both may be mutated but with different mutations.

[0218] Certain of the above mutations may confer new functionality upon the Fc region or FcRn binding partner. For example, one embodiment incorporates N297A, removing a highly conserved N-glycosylation site. The effect of this mutation is to reduce immunogenicity, thereby enhancing circulating half-life of the Fc region, and to render the Fc region incapable of binding to Fc γ RI, Fc γ RIIA, Fc γ RIIB, and Fc γ RIIIA, without compromising affinity for FcRn (Routledge et al. 1995, Transplantation 60:847; Friend et al. 1999, Transplantation 68:1632; Shields et al. 1995, J. Biol. Chem. 276:6591). As a further example of new functionality arising from mutations

described above affinity for FcRn may be increased beyond that of wild type in some instances. This increased affinity may reflect an increased "on" rate, a decreased "off" rate or both an increased "on" rate and a decreased "off" rate. Examples of mutations believed to impart an increased affinity for FcRn include, but not limited to, T256A, T307A, E380A, and N434A (Shields et al. 2001, J. Biol. Chem. 276:6591).

[0219] Additionally, at least three human Fc gamma receptors appear to recognize a binding site on IgG within the lower hinge region, generally amino acids 234-237. Therefore, another example of new functionality and potential decreased immunogenicity may arise from mutations of this region, as for example by replacing amino acids 233-236 of human IgG1 "ELLG" to the corresponding sequence from IgG2 "PVA" (with one amino acid deletion). It has been shown that FcγRI, FcγRII, and FcγRIII, which mediate various effector functions will not bind to IgG1 when such mutations have been introduced. Ward and Ghetie 1995, Therapeutic Immunology 2:77 and Armour et al. 1999, Eur. J. Immunol. 29:2613.

[0220] In one embodiment, the Ig constant region or a portion thereof, *e.g.*, an Fc region, is a polypeptide including the sequence PKNSSMISNTP (SEQ ID NO: 89 or SEQ ID NO: 3 of U.S. Pat. No. 5,739,277) and optionally further including a sequence selected from HQSLGTQ (SEQ ID NO: 90), HQNLSDGK (SEQ ID NO: 91), HQNISDGK (SEQ ID NO: 92), or VISSHLGQ (SEQ ID NO: 93) (or SEQ ID NOs: 11, 1, 2, and 31, respectively of U.S. Pat. No. 5,739,277).

[0221] In another embodiment, the immunoglobulin constant region or a portion thereof comprises an amino acid sequence in the hinge region or a portion thereof that forms one or more disulfide bonds with another immunoglobulin constant region or a portion thereof. The disulfide bond by the immunoglobulin constant region or a portion thereof places the first polypeptide comprising FVIII and the second polypeptide comprising the VWF fragment together so that endogenous VWF does not replace the VWF fragment and does not bind to the FVIII. Therefore, the disulfide bond between the first immunoglobulin constant region or a portion thereof and a second immunoglobulin constant region or a portion thereof prevents interaction between endogenous VWF and the FVIII protein. This inhibition of interaction between the VWF and the FVIII protein allows the half-life of the chimeric protein to go beyond the two fold limit. The hinge region or a portion thereof can further be linked to one or more domains of CH1, CH2, CH3, a fragment thereof, and any combinations thereof. In a particular embodiment, the immunoglobulin constant region or a portion thereof is a hinge region and CH2.

[0222] In certain embodiments, the Ig constant region or a portion thereof is hemi-glycosylated. For example, the chimeric protein comprising two Fc regions or FcRn binding partners may contain a first, glycosylated, Fc region (*e.g.*, a glycosylated CH2 region) or FcRn binding partner and a second, aglycosylated, Fc region (*e.g.*, an aglycosylated CH2 region) or FcRn binding

partner. In one embodiment, a linker may be interposed between the glycosylated and aglycosylated Fc regions. In another embodiment, the Fc region or FcRn binding partner is fully glycosylated, *i.e.*, all of the Fc regions are glycosylated. In other embodiments, the Fc region may be aglycosylated, *i.e.*, none of the Fc moieties are glycosylated.

[0223] In certain embodiments, a chimeric protein of the invention comprises an amino acid substitution to an Ig constant region or a portion thereof (*e.g.*, Fc variants), which alters the antigen-independent effector functions of the Ig constant region, in particular the circulating half-life of the protein.

[0224] Such proteins exhibit either increased or decreased binding to FcRn when compared to proteins lacking these substitutions and, therefore, have an increased or decreased half-life in serum, respectively. Fc variants with improved affinity for FcRn are anticipated to have longer serum half-lives, and such molecules have useful applications in methods of treating mammals where long half-life of the administered polypeptide is desired, *e.g.*, to treat a chronic disease or disorder (*see, e.g.*, US Patents 7,348,004, 7,404,956, and 7,862,820). In contrast, Fc variants with decreased FcRn binding affinity are expected to have shorter half-lives, and such molecules are also useful, for example, for administration to a mammal where a shortened circulation time may be advantageous, *e.g.* for in vivo diagnostic imaging or in situations where the starting polypeptide has toxic side effects when present in the circulation for prolonged periods. Fc variants with decreased FcRn binding affinity are also less likely to cross the placenta and, thus, are also useful in the treatment of diseases or disorders in pregnant women. In addition, other applications in which reduced FcRn binding affinity may be desired include those applications in which localization the brain, kidney, and/or liver is desired. In one exemplary embodiment, the chimeric protein of the invention exhibit reduced transport across the epithelium of kidney glomeruli from the vasculature. In another embodiment, the chimeric protein of the invention exhibit reduced transport across the blood brain barrier (BBB) from the brain, into the vascular space. In one embodiment, a protein with altered FcRn binding comprises at least one Fc region or FcRn binding partner (*e.g.*, one or two Fc regions or FcRn binding partners) having one or more amino acid substitutions within the "FcRn binding loop" of an Ig constant region. The FcRn binding loop is comprised of amino acid residues 280-299 (according to EU numbering) of a wild-type, full-length, Fc region. In other embodiments, an Ig constant region or a portion thereof in a chimeric protein of the invention having altered FcRn binding affinity comprises at least one Fc region or FcRn binding partner having one or more amino acid substitutions within the 15 Å FcRn "contact zone." As used herein, the term 15 Å FcRn "contact zone" includes residues at the following positions of a wild-type, full-length Fc moiety: 243-261, 275-280, 282-293, 302-319, 336- 348, 367, 369, 372-389, 391, 393, 408, 424, 425-440 (EU numbering). In other

embodiments, a Ig constant region or a portion thereof of the invention having altered FcRn binding affinity comprises at least one Fc region or FcRn binding partner having one or more amino acid substitutions at an amino acid position corresponding to any one of the following EU positions: 256, 277-281, 283-288, 303-309, 313, 338, 342, 376, 381, 384, 385, 387, 434 (*e.g.*, N434A or N434K), and 438. Exemplary amino acid substitutions which altered FcRn binding activity are disclosed in International PCT Publication No. WO05/047327 which is incorporated by reference herein.

[0225] An Fc region or FcRn binding partner used in the invention may also comprise an art recognized amino acid substitution which alters the glycosylation of the chimeric protein. For example, the Fc region or FcRn binding partner of the chimeric protein linked to a VWF fragment or a FVIII protein may comprise an Fc region having a mutation leading to reduced glycosylation (*e.g.*, N- or O-linked glycosylation) or may comprise an altered glycoform of the wild-type Fc moiety (*e.g.*, a low fucose or fucose-free glycan).

[0226] In one embodiment, an unprocessed chimeric protein of the invention may comprise a genetically fused Fc region (*i.e.*, scFc region) having two or more of its constituent Ig constant region or a portion thereof independently selected from the Ig constant region or a portion thereof described herein. In one embodiment, the Fc regions of a dimeric Fc region are the same. In another embodiment, at least two of the Fc regions are different. For example, the Fc regions or FcRn binding partners of the proteins of the invention comprise the same number of amino acid residues or they may differ in length by one or more amino acid residues (*e.g.*, by about 5 amino acid residues (*e.g.*, 1, 2, 3, 4, or 5 amino acid residues), about 10 residues, about 15 residues, about 20 residues, about 30 residues, about 40 residues, or about 50 residues). In yet other embodiments, the Fc regions or FcRn binding partners of the protein of the invention may differ in sequence at one or more amino acid positions. For example, at least two of the Fc regions or FcRn binding partners may differ at about 5 amino acid positions (*e.g.*, 1, 2, 3, 4, or 5 amino acid positions), about 10 positions, about 15 positions, about 20 positions, about 30 positions, about 40 positions, or about 50 positions).

II.E. Linkers

[0227] The chimeric protein of the present invention further comprises one or more linkers. One type of the linkers is a cleavable linker, which can be cleaved by various proteases when administered to a subject in vivo, *e.g.*, at a site of coagulation. In one embodiment, the cleavable linker allows cleavage of moiety, *e.g.*, a VWF protein, from the XTEN sequence, thus from the chimeric protein at the site of the coagulation cascade, thereby allowing activated FVIII (FVIIIa) to have its FVIIIa activity. Another type of the linkers is a processable linker, which contains an

intracellular cleavage site and thus can be cleaved by an intracellular processing enzyme in a host cell, allowing convenient expression of a polypeptide and formation of a chimeric protein.

[0228] One or more linkers can be present between any two proteins in the chimeric protein. In one embodiment, a chimeric protein comprises a first polypeptide which comprises (i) a FVIII protein and (ii) a first Ig constant region or a portion thereof and a second polypeptide which comprises (iii) a VWF protein, (iv) a linker (e.g., a cleavable linker), (v) an XTEN sequence, and (vi) a second Ig constant region or a portion thereof. In another embodiment, a chimeric protein comprises a first polypeptide which comprises (i) a FVIII protein and (ii) a first Ig constant region or a portion thereof and a second polypeptide which comprises (iii) a VWF protein, (iv) an XTEN sequence, (v) a linker (e.g., a cleavable linker), and (vi) a second Ig constant region or a portion thereof. In other embodiments, a chimeric protein comprises a first polypeptide which comprises (i) a FVIII protein and (ii) a first Ig constant region or a portion thereof and a second polypeptide which comprises (iii) a VWF protein, (iv) a first linker (e.g., a cleavable linker), (v) an XTEN sequence, (vi) a second linker (e.g., a cleavable linker), and (vii) a second Ig constant region or a portion thereof. In some embodiments, the first polypeptide further comprises a linker, e.g., a cleavable linker between the FVIII protein and the first Ig constant region.

[0229] In certain embodiments, a chimeric protein comprises a single chain comprising (i) a FVIII protein, (ii) a first Ig constant region or a portion thereof, (iii) a linker (e.g., a processable linker), (iv) a VWF protein, (v) an XTEN sequence, and (vi) a second Ig constant region or a portion thereof. In other embodiments, a chimeric protein comprises a single chain comprising (i) a FVIII protein, (ii) a first Ig constant region or a portion thereof, (iii) a first linker (e.g., a processable linker), (iv) a VWF protein, (v) a second linker (e.g., a cleavable linker), (vi) an XTEN sequence, and (vii) a second Ig constant region or a portion thereof. The processable linker can be processed after the chimeric protein is expressed in the host cell; thus the chimeric protein produced in the host cell can be in the final form comprising two or three polypeptide chains.

[0230] The linker useful in the present invention can comprise any organic molecule. In one embodiment, the linker comprises a polymer, e.g., polyethylene glycol (PEG) or hydroxyethyl starch (HES). In another embodiment, the linker comprises an amino acids sequence. The 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. The 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, or 900-1000 amino acids. In one embodiment, the linker comprises an XTEN sequence.

Additional examples of XTEN can be used according to the present invention and are disclosed in US Patent Publication Nos. 2010/0239554 A1, 2010/0323956 A1, 2011/0046060 A1, 2011/0046061 A1, 2011/0077199 A1, or 2011/0172146 A1, or International Patent Publication Nos. WO 2010091122 A1, WO 2010144502 A2, WO 2010144508 A1, WO 2011028228 A1, WO 2011028229 A1, or WO 2011028344 A2. In another embodiment, the linker is a PAS sequence.

[0231] In one embodiment, the linker is a polymer, *e.g.*, polyethylene glycol (PEG) or hydroxyethyl starch (HES). In another embodiment, the linker is an amino acid sequence. The 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. The 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, or 900-1000 amino acids.

[0232] Examples of linkers are well known in the art. In one embodiment, the linker comprises the sequence G_n . The linker can comprise the sequence $(GA)_n$. The linker can comprise the sequence $(GGS)_n$. In other embodiments, the linker comprises $(GGGS)_n$ (SEQ ID NO: 101). In still other embodiments, the linker comprises the sequence $(GGS)_n(GGGGS)_n$ (SEQ ID NO: 95). In these instances, n may be an integer from 1-100. In other instances, n may 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: 96), GGSGGSGGSGGSGGG (SEQ ID NO: 97), GGSGGSGGGSGGGGS (SEQ ID NO: 98), GGSGGSGGSGGSGGSGGS (SEQ ID NO: 99), or GGGGSGGGSGGGGS (SEQ ID NO: 100). The linker does not eliminate or diminish the VWF protein activity or the clotting activity of Factor VIII. Optionally, the linker enhances the VWF protein activity or the clotting activity of Factor VIII protein, *e.g.*, by further diminishing the effects of steric hindrance and making the VWF protein or Factor VIII portion more accessible to its target binding site.

[0233] In one embodiment, the linker useful for the chimeric protein is 15-25 amino acids long. In another embodiment, the linker useful for the chimeric protein is 15-20 amino acids long. In some embodiments, the linker for the chimeric protein is 10-25 amino acids long. In other embodiments, the linker for the chimeric protein is 15 amino acids long. In still other embodiments, the linker for the chimeric protein is $(GGGGS)_n$ (SEQ ID NO: 94) where G represents glycine, S represents serine and n is an integer from 1-20.

II. F. Cleavage Sites

[0234] A cleavable linkers can incorporate a moiety capable of being cleaved either chemically (*e.g.*, hydrolysis of an ester bond), enzymatically (*i.e.*, incorporation of a protease cleavage

sequence), or photolytically (*e.g.*, a chromophore such as 3-amino-3-(2-nitrophenyl) propionic acid (ANP)) in order to release one molecule from another.

[0235] In one embodiment, a cleavable linker comprises one or more cleavage sites at the N-terminus or C-terminus or both. In another embodiment, the cleavable linker consists essentially of or consists of one or more cleavable sites. In other embodiments, the cleavable linker comprises heterologous amino acid linker sequences described herein or polymers and one or more cleavable sites.

[0236] In certain embodiments, a cleavable linker comprises one or more cleavage sites that can be cleaved in a host cell (*i.e.*, intracellular processing sites). Non limiting examples of the cleavage site include RRRR (SEQ ID NO: 102), RKRRKR (SEQ ID NO: 103), and RRRRS (SEQ ID NO: 104).

[0237] In some embodiments, a cleavable linker comprises an a1 region from FVIII, an a2 region from FVIII, an a3 region from FVIII, a thrombin cleavable site which comprises X-V-P-R (SEQ ID NO: 105) and a PAR1 exosite interaction motif, wherein X is an aliphatic amino acid, or any combinations thereof. comprises the a2 region which comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to Glu720 to Arg740 corresponding to full-length FVIII, wherein the a2 region is capable of being cleaved by thrombin. In a particular embodiment, a cleavable linker useful for the invention comprises an a2 region which comprises ISDKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 106). In other embodiments, a cleavable linker for the invention comprises the a1 region which comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to Met337 to Arg372 corresponding to full-length FVIII, wherein the a1 region is capable of being cleaved by thrombin. In a particular embodiment, the a1 region comprises ISMKNNEEAEDYDDDLTDSEMDVVRFDNNSPSFIQIRSV (SEQ ID NO: 107). In some embodiments, a cleavable linker of the invention comprises the a3 region which comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to Glu1649 to Arg1689 corresponding to full-length FVIII, wherein the a3 region is capable of being cleaved by thrombin. In a specific embodiment, a cleavable linker for the invention comprises an a3 region comprises ISEITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQ (SEQ ID NO: 108).

[0238] In other embodiments, a cleavable linker comprises the thrombin cleavage site which comprises X-V-P-R (SEQ ID NO: 105) and the PAR1 exosite interaction motif and wherein the PAR1 exosite interaction motif comprises S-F-L-L-R-N (SEQ ID NO: 109). The PAR1 exosite interaction motif can further comprise an amino acid sequence selected from P, P-N, P-N-D, P-N-D-K (SEQ ID NO: 110), P-N-D-K-Y (SEQ ID NO: 111), P-N-D-K-Y-E (SEQ ID NO: 112), P-N-

D-K-Y-E-P (SEQ ID NO: 113), P-N-D-K-Y-E-P-F (SEQ ID NO: 114), P-N-D-K-Y-E-P-F-W (SEQ ID NO: 115), P-N-D-K-Y-E-P-F-W-E (SEQ ID NO: 116), P-N-D-K-Y-E-P-F-W-E-D (SEQ ID NO: 117), P-N-D-K-Y-E-P-F-W-E-D-E (SEQ ID NO: 118), P-N-D-K-Y-E-P-F-W-E-D-E-E (SEQ ID NO: 119), P-N-D-K-Y-E-P-F-W-E-D-E-E-S (SEQ ID NO: 120), or any combination thereof. In some embodiments, the aliphatic amino acid is selected from Glycine, Alanine, Valine, Leucine, or Isoleucine.

[0239] In other embodiments, a cleavable linker comprises one or more cleavage sites that are cleaved by a protease after a chimeric protein comprising the cleavable linker is administered to a subject. In one embodiment, the cleavage site is cleaved by a protease selected from the group consisting of factor XIa, factor XIIa, kallikrein, factor VIIa, factor IXa, factor Xa, factor IIa (thrombin), Elastase-2, MMP-12, MMP-13, MMP-17, and MMP-20. In another embodiment, the cleavage site is selected from the group consisting of a FXIa cleavage site (*e.g.*, KLTR↓AET (SEQ ID NO: 121)), a FXIa cleavage site (*e.g.*, DFTR↓VVG (SEQ ID NO: 122)), a FXIIa cleavage site (*e.g.*, TMTR↓IVGG (SEQ ID NO: 123)), a Kallikrein cleavage site (*e.g.*, SPFR↓STGG (SEQ ID NO: 124)), a FVIIa cleavage site (*e.g.*, LQVR↓IVGG (SEQ ID NO: 125)), a FIXa cleavage site (*e.g.*, PLGR↓IVGG (SEQ ID NO: 126)), a FXa cleavage site (*e.g.*, IEGR↓TVGG (SEQ ID NO: 127)), a FIIa (thrombin) cleavage site (*e.g.*, LTPR↓SLLV (SEQ ID NO: 128)), a Elastase-2 cleavage site (*e.g.*, LGPV↓SGVP (SEQ ID NO: 129)), a Granzyme-B cleavage site (*e.g.*, VAGD↓SLEE (SEQ ID NO: 130)), a MMP-12 cleavage site (*e.g.*, GPAG↓LGGA (SEQ ID NO: 131)), a MMP-13 cleavage site (*e.g.*, GPAG↓LRGA (SEQ ID NO: 132)), a MMP-17 cleavage site (*e.g.*, APLG↓LRLR (SEQ ID NO: 133)), a MMP-20 cleavage site (*e.g.*, PALP↓LVAQ (SEQ ID NO: 134)), a TEV cleavage site (*e.g.*, ENLYFQ↓G (SEQ ID NO: 135)), a Enterokinase cleavage site (*e.g.*, DDDK↓IVGG (SEQ ID NO: 136)), a Protease 3C (PRESCISSION™) cleavage site (*e.g.*, LEVLFFQ↓GP (SEQ ID NO: 137)), and a Sortase A cleavage site (*e.g.*, LPKT↓GSES) (SEQ ID NO: 138). In certain embodiments, the FXIa cleavage sites include, but are not limited to, *e.g.*, TQSFNDFTR (SEQ ID NO: 1) and SVSQTSLTR (SEQ ID NO: 3). Non-limiting exemplary thrombin cleavage sites include, *e.g.*, DFLAEGGGVR (SEQ ID NO: 4), TTKIKPR (SEQ ID NO: 5), LVPRG (SEQ ID NO: 6), DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), or IEPRSFS (SEQ ID NO: 194), and a sequence comprising, consisting essentially of, or consisting of ALRPR (SEQ ID NO: 7) (*e.g.*, ALRPRVVGGA (SEQ ID NO: 145)).

[0240] In a specific embodiment, the cleavage site is TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 146). In another embodiment, the cleavage site comprises DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88) or a fragment thereof. In one particular embodiment, the cleavage site comprises IEPRSFS (SEQ ID NO: 194). In another

embodiment, the cleavage site comprises EPRSFS (SEQ ID NO: 195), wherein the cleavage site is not the full-length a2 region of FVIII. In still another embodiment, the cleavage site comprises IEPR (SEQ ID NO: 200). In another embodiment, the cleavage site comprises IEPR (SEQ ID NO: 200), wherein the cleavage site is not the full-length a2 region of FVIII or does not comprise the full-length a2 region of FVIII. In other embodiments, the cleavage site comprises DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), KNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 139), NTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 140), TGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 141), GDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 142), DYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 143), YYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 144), YEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 176), EDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 177), DSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 178), SYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 179), YEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 180), EDISAYLLSKNNAIEPRSFS (SEQ ID NO: 181), DISAYLLSKNNAIEPRSFS (SEQ ID NO: 182), ISAYLLSKNNAIEPRSFS (SEQ ID NO: 183), SAYLLSKNNAIEPRSFS (SEQ ID NO: 184), AYLLSKNNAIEPRSFS (SEQ ID NO: 185), YLLSKNNAIEPRSFS (SEQ ID NO: 186), LLSKNNAIEPRSFS (SEQ ID NO: 187), LSKNNAIEPRSFS (SEQ ID NO: 188), SKNNAIEPRSFS (SEQ ID NO: 189), KNNAIEPRSFS (SEQ ID NO: 190), NNAIEPRSFS (SEQ ID NO: 191), NAIIEPRSFS (SEQ ID NO: 192), AIEPRSFS (SEQ ID NO: 193), or IEPRSFS (SEQ ID NO: 194). In other embodiments, the cleavage site comprises DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88), KNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 139), NTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 140), TGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 141), GDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 142), DYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 143), YYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 144), YEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 176), EDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 177), DSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 178), SYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 179), YEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 180), EDISAYLLSKNNAIEPRSFS (SEQ ID NO: 181), DISAYLLSKNNAIEPRSFS (SEQ ID NO: 182), ISAYLLSKNNAIEPRSFS (SEQ ID NO: 183), SAYLLSKNNAIEPRSFS (SEQ ID NO: 184), AYLLSKNNAIEPRSFS (SEQ ID NO: 185), YLLSKNNAIEPRSFS (SEQ ID NO: 186), LLSKNNAIEPRSFS (SEQ ID NO: 187), LSKNNAIEPRSFS (SEQ ID NO: 188), SKNNAIEPRSFS (SEQ ID NO: 189), KNNAIEPRSFS (SEQ ID NO: 190), NNAIEPRSFS (SEQ ID NO: 191), NAIIEPRSFS (SEQ ID NO: 192), AIEPRSFS (SEQ ID NO: 193), or IEPRSFS (SEQ ID NO: 194).

186), LLSKNNAIEPRSFS (SEQ ID NO: 187), LSKNNAIEPRSFS (SEQ ID NO: 188), SKNNAIEPRSFS (SEQ ID NO: 189), KNNNAIEPRSFS (SEQ ID NO: 190), NNAIEPRSFS (SEQ ID NO: 191), NAIIEPRSFS (SEQ ID NO: 192), AIEPRSFS (SEQ ID NO: 193), or IEPRSFS (SEQ ID NO: 194), wherein the cleavage site is not the full-length FVIII a2 region. In certain embodiments the cleavable linker is cleavable in a thrombin cleavage assay as provided herein or as known in the art.

III. Polynucleotides, Vectors, and Host cells

[0241] Also provided in the invention is a polynucleotide encoding a chimeric protein of the invention. In one embodiment, the first polypeptide chain and the second polypeptide chain can be encoded by a single polynucleotide chain. In another embodiment, the first polypeptide chain and the second polypeptide chain are encoded by two different polynucleotides, *i.e.*, a first nucleotide sequence and a second nucleotide sequence. In another embodiment, the first nucleotide sequence and the second nucleotide sequence are on two different polynucleotides (*e.g.*, different vectors).

[0242] The invention includes a polynucleotide encoding a single polypeptide chain (*e.g.*, FVIII(X2)-F1-L3-F2-L2-X1-L1-V), wherein FVIII(X2) comprises a FVIII protein in which an XTEN sequence is inserted at one or more insertion sites, F1 comprises a first Ig constant region or a portion thereof, *e.g.*, a first Fc region, L1 comprises a first linker, V comprises a VWF protein, X1 comprises an XTEN sequence having less than 288 amino acids in length, L2 comprises a second linker, L3 comprises a third linker, and F2 comprises a second Ig constant region or a portion thereof, *e.g.*, a second Fc region. The invention also includes two polynucleotides, a first polynucleotide sequence encoding a first polypeptide which comprises a FVIII protein fused to a first Ig constant region or a portion thereof and a second polynucleotide sequence encoding a second polypeptide which comprises a VWF protein, an XTEN sequence having less than 288 amino acids in length, and a second Ig constant region or a portion thereof. In some embodiments, a chimeric protein comprising two polypeptide chains or three polypeptide chains can be encoded by a single polynucleotide chain, and then processed into two or three (or more) polypeptide chains. In yet other embodiments, a chimeric protein comprising these polypeptide chains can be encoded by two or three polynucleotide chains.

[0243] In other embodiments, the set of the polynucleotides further comprises an additional nucleotide chain (*e.g.*, a second nucleotide chain when the chimeric polypeptide is encoded by a single polynucleotide chain or a third nucleotide chain when the chimeric protein is encoded by two polynucleotide chains) which encodes a protein convertase. The protein convertase can be selected from the group consisting of proprotein convertase subtilisin/kexin type 5 (PCSK5 or PC5), proprotein convertase subtilisin/kexin type 7 (PCSK7 or PC5), a yeast Kex 2, proprotein

convertase subtilisin/kexin type 3 (PACE or PCSK3), and two or more combinations thereof. In some embodiments, the protein convertase is PACE, PC5, or PC7. In a specific embodiment, the protein convertase is PC5 or PC7. See International Application no. PCT/US2011/043568.

[0244] As used herein, an expression vector refers to any nucleic acid construct which contains the necessary elements for the transcription and translation of an inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation, when introduced into an appropriate host cell. Expression vectors can include plasmids, phagemids, viruses, and derivatives thereof.

[0245] Expression vectors of the invention will include polynucleotides encoding the chimeric protein described herein. In one embodiment, one or more of the coding sequences for the first polypeptide comprising a FVIII protein and a first Ig constant region, the second polypeptide comprising a VWF protein, an XTEN sequence having less than 288 amino acids, and a second Ig constant region or a portion thereof, or both are operably linked to an expression control sequence. As used herein, two nucleic acid sequences are operably linked when they are covalently linked in such a way as to permit each component nucleic acid sequence to retain its functionality. A coding sequence and a gene expression control sequence are said to be operably linked when they are covalently linked in such a way as to place the expression or transcription and/or translation of the coding sequence under the influence or control of the gene expression control sequence. Two DNA sequences are said to be operably linked if induction of a promoter in the 5' gene expression sequence results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequence, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a gene expression sequence would be operably linked to a coding nucleic acid sequence if the gene expression sequence were capable of effecting transcription of that coding nucleic acid sequence such that the resulting transcript is translated into the desired protein or polypeptide.

[0246] A gene expression control sequence as used herein is any regulatory nucleotide sequence, such as a promoter sequence or promoter-enhancer combination, which facilitates the efficient transcription and translation of the coding nucleic acid to which it is operably linked. The gene expression control sequence may, for example, be a mammalian or viral promoter, such as a constitutive or inducible promoter. Constitutive mammalian promoters include, but are not limited to, the promoters for the following genes: hypoxanthine phosphoribosyl transferase (HPRT), adenosine deaminase, pyruvate kinase, beta-actin promoter, and other constitutive promoters. Exemplary viral promoters which function constitutively in eukaryotic cells include, for example,

promoters from the cytomegalovirus (CMV), simian virus (*e.g.*, SV40), papilloma virus, adenovirus, human immunodeficiency virus (HIV), Rous sarcoma virus, cytomegalovirus, the long terminal repeats (LTR) of Moloney leukemia virus, and other retroviruses, and the thymidine kinase promoter of herpes simplex virus. Other constitutive promoters are known to those of ordinary skill in the art. The promoters useful as gene expression sequences of the invention also include inducible promoters. Inducible promoters are expressed in the presence of an inducing agent. For example, the metallothionein promoter is induced to promote transcription and translation in the presence of certain metal ions. Other inducible promoters are known to those of ordinary skill in the art.

[0247] In general, the gene expression control sequence shall include, as necessary, 5' non-transcribing and 5' non-translating sequences involved with the initiation of transcription and translation, respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribing sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined coding nucleic acid. The gene expression sequences optionally include enhancer sequences or upstream activator sequences as desired.

[0248] Viral vectors include, but are not limited to, nucleic acid sequences from the following viruses: retrovirus, such as Moloney murine leukemia virus, Harvey murine sarcoma virus, murine mammary tumor virus, and Rous sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyomaviruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors well-known in the art. Certain viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses, the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (*i.e.*, capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high efficiency transduction of genes *in vivo*. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell line with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, M., Gene Transfer and Expression, A Laboratory Manual, W.H. Freeman Co., New York (1990) and Murry, E. J., Methods in Molecular Biology, Vol. 7, Humana Press, Inc., Clifton, N.J. (1991).

[0249] In one embodiment, the virus is an adeno-associated virus, a double-stranded DNA virus. The adeno-associated virus can be engineered to be replication-deficient and is capable of infecting a wide range of cell types and species. It further has advantages such as heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hematopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion.

[0250] Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well-known to those of skill in the art. See, *e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989. In the last few years, plasmid vectors have been found to be particularly advantageous for delivering genes to cells *in vivo* because of their inability to replicate within and integrate into a host genome. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operably encoded within the plasmid. Some commonly used plasmids available from commercial suppliers include pBR322, pUC18, pUC19, various pcDNA plasmids, pRC/CMV, various pCMV plasmids, pSV40, and pBlueScript. Additional examples of specific plasmids include pcDNA3.1, catalog number V79020; pcDNA3.1/hygro, catalog number V87020; pcDNA4/myc-His, catalog number V86320; and pBudCE4.1, catalog number V53220, all from Invitrogen (Carlsbad, CA.). Other plasmids are well-known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using standard molecular biology techniques to remove and/or add specific fragments of DNA.

[0251] In one insect expression system that may be used to produce the proteins of the invention, *Autographa californica* nuclear polyhidrosis virus (AcNPV) is used as a vector to express the foreign genes. The virus grows in *Spodoptera frugiperda* cells. A coding sequence may be cloned into non-essential regions (for example, the polyhedron gene) of the virus and placed under control of an ACNPV promoter (for example, the polyhedron promoter). Successful insertion of a coding sequence will result in inactivation of the polyhedron gene and production of non-occluded recombinant virus (*i.e.*, virus lacking the proteinaceous coat coded for by the polyhedron gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (see, *e.g.*, Smith *et al.* (1983) *J Virol* 46:584; U.S. Pat. No. 4,215,051).

Further examples of this expression system may be found in Ausubel *et al.*, eds. (1989) Current Protocols in Molecular Biology, Vol. 2, Greene Publish. Assoc. & Wiley Interscience.

[0252] Another system which can be used to express the proteins of the invention is the glutamine synthetase gene expression system, also referred to as the "GS expression system" (Lonza Biologics PLC, Berkshire UK). This expression system is described in detail in U.S. Pat. No. 5,981,216.

[0253] In mammalian host cells, a number of viral based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing peptide in infected hosts. See, *e.g.*, Logan & Shenk (1984) *Proc Natl Acad Sci USA* 81:3655). Alternatively, the vaccinia 7.5 K promoter may be used. See, *e.g.*, Mackett *et al.* (1982) *Proc Natl Acad Sci USA* 79:7415; Mackett *et al.* (1984) *J Virol* 49:857; Panicali *et al.* (1982) *Proc Natl Acad Sci USA* 79:4927.

[0254] To increase efficiency of production, the polynucleotides can be designed to encode multiple units of the protein of the invention separated by enzymatic cleavage sites. The resulting polypeptide can be cleaved (*e.g.*, by treatment with the appropriate enzyme) in order to recover the polypeptide units. This can increase the yield of polypeptides driven by a single promoter. When used in appropriate viral expression systems, the translation of each polypeptide encoded by the mRNA is directed internally in the transcript; *e.g.*, by an internal ribosome entry site, IRES. Thus, the polycistronic construct directs the transcription of a single, large polycistronic mRNA which, in turn, directs the translation of multiple, individual polypeptides. This approach eliminates the production and enzymatic processing of polyproteins and may significantly increase the yield of polypeptides driven by a single promoter.

[0255] Vectors used in transformation will usually contain a selectable marker used to identify transformants. In bacterial systems, this can include an antibiotic resistance gene such as ampicillin or kanamycin. Selectable markers for use in cultured mammalian cells include genes that confer resistance to drugs, such as neomycin, hygromycin, and methotrexate. The selectable marker may be an amplifiable selectable marker. One amplifiable selectable marker is the dihydrofolate reductase (DHFR) gene. Simonsen C C *et al.* (1983) *Proc Natl Acad Sci USA* 80:2495-9. Selectable markers are reviewed by Thilly (1986) Mammalian Cell Technology, Butterworth Publishers, Stoneham, Mass., and the choice of selectable markers is well within the level of ordinary skill in the art.

- [0256] Selectable markers may be introduced into the cell on a separate plasmid at the same time as the gene of interest, or they may be introduced on the same plasmid. If on the same plasmid, the selectable marker and the gene of interest may be under the control of different promoters or the same promoter, the latter arrangement producing a dicistronic message. Constructs of this type are known in the art (for example, U.S. Pat. No. 4,713,339).
- [0257] The expression vectors can encode for tags that permit easy purification of the recombinantly produced protein. Examples include, but are not limited to, vector pUR278 (Ruther *et al.* (1983) *EMBO J* 2:1791), in which coding sequences for the protein to be expressed may be ligated into the vector in frame with the lac z coding region so that a tagged fusion protein is produced; pGEX vectors may be used to express proteins of the invention 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 (thrombin or Factor Xa protease or PRESCISSON PROTEASETM (Pharmacia, Peapack, N.J.)) for easy removal of the tag after purification.
- [0258] The expression vector or vectors are then transfected or co-transfected into a suitable target cell, which will express the polypeptides. Transfection techniques known in the art include, but are not limited to, calcium phosphate precipitation (Wigler *et al.* (1978) *Cell* 14:725), electroporation (Neumann *et al.* (1982) *EMBO J* 1:841), and liposome-based reagents. A variety of host-expression vector systems may be utilized to express the proteins described herein including both prokaryotic and eukaryotic cells. These include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli*) transformed with recombinant bacteriophage DNA or plasmid DNA expression vectors containing an appropriate coding sequence; yeast or filamentous fungi transformed with recombinant yeast or fungi expression vectors containing an appropriate coding sequence; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing an appropriate coding sequence; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus or tobacco mosaic virus) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing an appropriate coding sequence; or animal cell systems, including mammalian cells (*e.g.*, HEK 293, CHO, Cos, HeLa, HKB11, and BHK cells).
- [0259] In one embodiment, the host cell is a eukaryotic cell. As used herein, a eukaryotic cell refers to any animal or plant cell having a definitive nucleus. Eukaryotic cells of animals include cells of vertebrates, *e.g.*, mammals, and cells of invertebrates, *e.g.*, insects. Eukaryotic cells of plants specifically can include, without limitation, yeast cells. A eukaryotic cell is distinct from a prokaryotic cell, *e.g.*, bacteria.

- [0260] In certain embodiments, the eukaryotic cell is a mammalian cell. A mammalian cell is any cell derived from a mammal. Mammalian cells specifically include, but are not limited to, mammalian cell lines. In one embodiment, the mammalian cell is a human cell. In another embodiment, the mammalian cell is a HEK 293 cell, which is a human embryonic kidney cell line. HEK 293 cells are available as CRL-1533 from American Type Culture Collection, Manassas, VA, and as 293-H cells, Catalog No. 11631-017 or 293-F cells, Catalog No. 11625-019 from Invitrogen (Carlsbad, Calif.). In some embodiments, the mammalian cell is a PER.C6[®] cell, which is a human cell line derived from retina. PER.C6[®] cells are available from Crucell (Leiden, The Netherlands). In other embodiments, the mammalian cell is a Chinese hamster ovary (CHO) cell. CHO cells are available from American Type Culture Collection, Manassas, VA. (*e.g.*, CHO-K1; CCL-61). In still other embodiments, the mammalian cell is a baby hamster kidney (BHK) cell. BHK cells are available from American Type Culture Collection, Manassas, Va. (*e.g.*, CRL-1632). In some embodiments, the mammalian cell is a HKB11 cell, which is a hybrid cell line of a HEK293 cell and a human B cell line. Mei *et al.*, *Mol. Biotechnol.* 34(2): 165-78 (2006).
- [0261] In one embodiment, a plasmid including a FVIII(X2)-Fc fusion coding sequence, a VWF protein-L1-X1-L2-Fc coding sequence, or both and a selectable marker, *e.g.*, zeocin resistance, are transfected into HEK 293 cells, for production of a chimeric protein.
- [0262] In another embodiment, a plasmid including a FVIII-Fc fusion coding sequence, a VWF protein-L1-X-L2-Fc coding sequence, or both and a selectable marker, *e.g.*, zeocin resistance, are transfected into HEK 293 cells, for production of a chimeric protein.
- [0263] In some embodiments, a first plasmid including a FVIII(X2)-Fc fusion coding sequence and a first selectable marker, *e.g.*, a zeocin resistance gene, and a second plasmid including a VWF protein-L1-X1-L2-Fc coding sequence and a second selectable marker, *e.g.*, a neomycin resistance gene, and a third plasmid including a protein convertase coding sequence and a third selectable marker, *e.g.*, a hygromycin resistance gene, are cotransfected into HEK 293 cells, for production of the chimeric protein. The first and second plasmids can be introduced in equal amounts (*i.e.*, 1:1 molar ratio), or they can be introduced in unequal amounts.
- [0264] In still other embodiments, a first plasmid including a FVIII-Fc fusion coding sequence and a first selectable marker, *e.g.*, a zeocin resistance gene, and a second plasmid including a VWF protein-L1-X-L2-Fc coding sequence and a second selectable marker, *e.g.*, a neomycin resistance gene, and a third plasmid including a protein convertase coding sequence and a third selectable marker, *e.g.*, a hygromycin resistance gene, are cotransfected into HEK 293 cells, for production of the chimeric protein. The first and second plasmids can be introduced in equal amounts (*i.e.*, 1:1 molar ratio), or they can be introduced in unequal amounts.

- [0265] In yet other embodiments, a first plasmid including a FVIII(X2)-Fc fusion coding sequence and a first selectable marker, *e.g.*, a zeocin resistance gene, and a second plasmid including a VWF protein-L1-X1-L2-Fc fusion coding sequence and a second selectable marker, *e.g.*, a neomycin resistance gene, and a third plasmid including a protein convertase coding sequence and a third selectable marker, *e.g.*, a hygromycin resistance gene, are cotransfected into HEK 293 cells, for production of the chimeric protein. The first and second plasmids can be introduced in equal amounts (*i.e.*, 1:1 molar ratio), or they can be introduced in unequal amounts.
- [0266] In certain embodiments, a first plasmid, including a chimeric protein encoding FVIII (with or without XTEN)-F1-L3-F2-L2-X-L1-V coding sequence and a first selectable marker, *e.g.*, a zeocin resistance gene, and a second plasmid including a protein convertase coding sequence and a second selectable marker, *e.g.*, a hygromycin resistance gene, are cotransfected into HEK 293 cells, for production of the chimeric protein. The promoters for the FVIII(X)-F1 coding sequence and the V-L2-X-L1-F2 coding sequence can be different or they can be the same.
- [0267] In still other embodiments, transfected cells are stably transfected. These cells can be selected and maintained as a stable cell line, using conventional techniques known to those of skill in the art.
- [0268] Host cells containing DNA constructs of the protein are grown in an appropriate growth medium. As used herein, the term "appropriate growth medium" means a medium containing nutrients required for the growth of cells. Nutrients required for cell growth may include a carbon source, a nitrogen source, essential amino acids, vitamins, minerals, and growth factors. Optionally, the media can contain one or more selection factors. Optionally the media can contain bovine calf serum or fetal calf serum (FCS). In one embodiment, the media contains substantially no IgG. The growth medium will generally select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker on the DNA construct or co-transfected with the DNA construct. Cultured mammalian cells are generally grown in commercially available serum-containing or serum-free media (*e.g.*, MEM, DMEM, DMEM/F12). In one embodiment, the medium is CD293 (Invitrogen, Carlsbad, CA.). In another embodiment, the medium is CD17 (Invitrogen, Carlsbad, CA.). Selection of a medium appropriate for the particular cell line used is within the level of those ordinary skilled in the art.
- [0269] In order to co-express the two polypeptide chains of the chimeric protein, the host cells are cultured under conditions that allow expression of both chains. As used herein, culturing refers to maintaining living cells *in vitro* for at least a definite time. Maintaining can, but need not include, an increase in population of living cells. For example, cells maintained in culture can be

static in population, but still viable and capable of producing a desired product, *e.g.*, a recombinant protein or recombinant fusion protein. Suitable conditions for culturing eukaryotic cells are well known in the art and include appropriate selection of culture media, media supplements, temperature, pH, oxygen saturation, and the like. For commercial purposes, culturing can include the use of any of various types of scale-up systems including shaker flasks, roller bottles, hollow fiber bioreactors, stirred-tank bioreactors, airlift bioreactors, Wave bioreactors, and others.

[0270] The cell culture conditions are also selected to allow association of the VWF fragment with the FVIII protein. Conditions that allow expression of the VWF fragment and/or the FVIII protein may include the presence of a source of vitamin K. For example, in one embodiment, stably transfected HEK 293 cells are cultured in CD293 media (Invitrogen, Carlsbad, CA) or OptiCHO media (Invitrogen, Carlsbad, CA) supplemented with 4 mM glutamine.

[0271] In one aspect, the present invention is directed to a method of expressing, making, or producing the chimeric protein of the invention comprising a) transfecting a host cell comprising a polynucleotide encoding the chimeric protein and b) culturing the host cell in a culture medium under a condition suitable for expressing the chimeric protein, wherein the chimeric protein is expressed.

[0272] In further embodiments, the protein product containing the FVIII protein linked to a first Ig constant region or a portion thereof and/or the VWF protein fused to a second Ig constant region or a portion thereof by an XTEN sequence is secreted into the media. Media is separated from the cells, concentrated, filtered, and then passed over two or three affinity columns, *e.g.*, a protein A column and one or two anion exchange columns.

[0273] In certain aspects, the present invention relates to the chimeric protein produced by the methods described herein.

[0274] *In vitro* production allows scale-up to give large amounts of the desired altered polypeptides of the invention. Techniques for mammalian cell cultivation under tissue culture conditions are known in the art and include homogeneous suspension culture, *e.g.* in an airlift reactor or in a continuous stirrer reactor, or immobilized or entrapped cell culture, *e.g.* in hollow fibers, microcapsules, on agarose microbeads or ceramic cartridges. If necessary and/or desired, the solutions of polypeptides can be purified by the customary chromatography methods, for example gel filtration, ion-exchange chromatography, hydrophobic interaction chromatography (HIC, chromatography over DEAE-cellulose or affinity chromatography).

IV. Pharmaceutical Composition

[0275] Compositions containing the chimeric protein of the present invention may contain a suitable pharmaceutically acceptable carrier. For example, they may contain excipients and/or

auxiliaries that facilitate processing of the active compounds into preparations designed for delivery to the site of action.

[0276] The pharmaceutical composition can be formulated for parenteral administration (*i.e.* intravenous, subcutaneous, or intramuscular) by bolus injection. Formulations for injection can be presented in unit dosage form, *e.g.*, in ampoules or in multidose containers with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, *e.g.*, pyrogen free water.

[0277] Suitable formulations for parenteral administration also include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, including, for example, sodium carboxymethyl cellulose, sorbitol and dextran. Optionally, the suspension may also contain stabilizers. Liposomes also can be used to encapsulate the molecules of the invention for delivery into cells or interstitial spaces. Exemplary pharmaceutically acceptable carriers are physiologically compatible solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like. In some embodiments, the composition comprises isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride. In other embodiments, the compositions comprise pharmaceutically acceptable substances such as wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the active ingredients.

[0278] Compositions of the invention may be in a variety of forms, including, for example, liquid (*e.g.*, injectable and infusible solutions), dispersions, suspensions, semi-solid and solid dosage forms. The preferred form depends on the mode of administration and therapeutic application.

[0279] The composition can be formulated as a solution, micro emulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active ingredient in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active ingredient into a sterile vehicle that 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 that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution. The proper fluidity of a solution 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. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0280] The active ingredient can be formulated with a controlled-release formulation or device. Examples of such formulations and devices include implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, for example, ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for the preparation of such formulations and devices are known in the art. See *e.g.*, Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0281] Injectable depot formulations can be made by forming microencapsulated matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the polymer employed, the rate of drug release can be controlled. Other exemplary biodegradable polymers are polyorthoesters and polyanhydrides. Depot injectable formulations also can be prepared by entrapping the drug in liposomes or microemulsions.

[0282] Supplementary active compounds can be incorporated into the compositions. In one embodiment, the chimeric protein of the invention is formulated with another clotting factor, or a variant, fragment, analogue, or derivative thereof. For example, the clotting factor includes, but is not limited to, factor V, factor VII, factor VIII, factor IX, factor X, factor XI, factor XII, factor XIII, prothrombin, fibrinogen, von Willebrand factor or recombinant soluble tissue factor (rsTF) or activated forms of any of the preceding. The clotting factor of hemostatic agent can also include anti-fibrinolytic drugs, *e.g.*, epsilon-amino-caproic acid, tranexamic acid.

[0283] Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. See, *e.g.*, Remington's Pharmaceutical Sciences (Mack Pub. Co., Easton, Pa. 1980).

- [0284] In addition to the active compound, the liquid dosage form may contain inert ingredients such as water, ethyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols, and fatty acid esters of sorbitan.
- [0285] Non-limiting examples of suitable pharmaceutical carriers are also described in Remington's Pharmaceutical Sciences by E. W. Martin. Some examples of excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. The composition can also contain pH buffering reagents, and wetting or emulsifying agents.
- [0286] 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.
- [0287] For buccal administration, the composition may take the form of tablets or lozenges according to conventional protocols.
- [0288] For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of a nebulized aerosol with or without excipients or in the form of an aerosol spray from a pressurized pack or nebulizer, with optionally a propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoromethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.
- [0289] 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.
- [0290] In one embodiment, a pharmaceutical composition comprises a chimeric protein, the polynucleotide encoding the chimeric protein, the vector comprising the polynucleotide, or the host cell comprising the vector, and a pharmaceutically acceptable carrier. The FVIII protein in a chimeric protein has extended half-life compared to wild type FVIII protein or the corresponding

FVIII protein without the VWF fragment. In one embodiment, wherein the half-life of the chimeric protein is extended 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 wild type FVIII. In another embodiment, the half-life of Factor VIII is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours.

[0291] In some embodiments, the composition is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration. The parenteral administration can be intravenous or subcutaneous administration.

[0292] In other embodiments, the composition is used to treat a bleeding disease or condition in a subject in need thereof. The bleeding disease or condition is selected from the group consisting of a bleeding coagulation disorder, 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 and any combinations thereof. In still other embodiments, the subject is scheduled to undergo a surgery. In yet other embodiments, the treatment is prophylactic or on-demand.

V. Gene Therapy

[0293] A chimeric protein thereof of the invention can be produced in vivo in a mammal, *e.g.*, a human patient, using a gene therapy approach to treatment of a bleeding disease or disorder selected from the group consisting of a bleeding coagulation disorder, 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, and bleeding in the iliopsoas sheath would be therapeutically beneficial. In one embodiment, the bleeding disease or disorder is hemophilia. In another embodiment, the bleeding disease or disorder is hemophilia A. This involves

administration of a suitable chimeric protein-encoding nucleic acid operably linked to suitable expression control sequences. In certain embodiment, these sequences are incorporated into a viral vector. Suitable viral vectors for such gene therapy include adenoviral vectors, lentiviral vectors, baculoviral vectors, Epstein Barr viral vectors, papovaviral vectors, vaccinia viral vectors, herpes simplex viral vectors, and adeno associated virus (AAV) vectors. The viral vector can be a replication-defective viral vector. In other embodiments, an adenoviral vector has a deletion in its E1 gene or E3 gene. When an adenoviral vector is used, the mammal may not be exposed to a nucleic acid encoding a selectable marker gene. In other embodiments, the sequences are incorporated into a non-viral vector known to those skilled in the art.

VI. Methods of Using Chimeric Protein

[0294] The present invention is directed to a method of using a chimeric protein described herein to prevent or inhibit endogenous VWF binding to a FVIII protein. The present invention is also directed to a method of using a chimeric protein having a FVIII protein linked to XTEN and an Ig constant region or a portion thereof.

[0295] One aspect of the present invention is directed to preventing or inhibiting FVIII interaction with endogenous VWF by blocking or shielding the VWF binding site on the FVIII from endogenous VWF and at the same time extending half-life of the chimeric protein using an XTEN sequence in combination with an Ig constant region or a portion thereof, which can also be a half-life extender. In one embodiment, the invention is directed to a method of constructing a FVIII protein having half-life longer than wild-type FVIII. The chimeric protein useful in the method includes any one or more chimeric protein described herein.

[0296] Another aspect of the invention includes a method of administering to a subject in need thereof a chimeric protein comprising a FVIII protein having half-life longer than wild-type FVIII, wherein the method comprises administering the chimeric protein described herein to the subject.

[0297] In one embodiment, the invention is directed to a method of using an XTEN sequence and an Ig constant region or a portion thereof to improve a half-life of a chimeric protein comprising FVIII protein and a VWF protein, which prevents or inhibits endogenous VWF interaction with a FVIII protein. A FVIII protein linked to an XTEN sequence (*e.g.*, FVIII(X)) and then bound to or associated with a VWF protein fused to an XTEN and an Ig constant region or a portion thereof is shielded or protected from the clearance pathway of VWF and thus has reduced clearance compared to the FVIII protein not bound to the VWF protein. The shielded FVIII protein thus has maximum extension of a half-life compared to a FVIII protein not bound to or associated with the XTEN sequence and the VWF protein. In certain embodiments, the FVIII protein associated with or protected by a VWF protein and linked to an XTEN sequence is not cleared by a VWF

clearance receptor. In other embodiments, the FVIII protein associated with or protected by a VWF protein and linked to an XTEN sequence is cleared from the system slower than the FVIII protein that is not associated with or protected by the VWF protein and linked to the XTEN sequence.

[0298] In one aspect, the chimeric protein comprising the FVIII protein linked to an XTEN sequence or the FVIII protein bound to or associated with a VWF protein linked to XTEN has reduced clearance from circulation as the VWF protein does not contain a VWF clearance receptor binding site. The VWF protein prevents or inhibits clearance of FVIII bound to or associated with the VWF protein from the system through the VWF clearance pathway. The VWF proteins useful for the present invention can also provide at least one or more VWF-like FVIII protection properties that are provided by endogenous VWF. In certain embodiments, the VWF protein or the XTEN sequence can also mask one or more FVIII clearance receptor binding site, thereby preventing clearance of FVIII by its own clearance pathway.

[0299] In some embodiments, the prevention or inhibition of a FVIII protein binding to endogenous VWF by the VWF protein or the XTEN sequence can be *in vitro* or *in vivo*.

[0300] Also provided is a method of increasing the half-life of a chimeric protein comprising administering the chimeric protein described herein to a subject in need thereof. The half-life of non-activated FVIII bound to or associated with full-length VWF is about 12 to 14 hours in plasma. In VWD type 3, wherein there is almost no VWF in circulation, the half-life of FVIII is only about six hours, leading to symptoms of mild to moderate hemophilia A in such patients due to decreased concentrations of FVIII. The half-life of the chimeric protein linked to or associated with the VWF fragment or the XTEN sequence of the present invention can increase at least about 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2.0 times, 2.1 times, 2.2 times, 2.3 times, 2.4 times, 2.6 times, 2.7. times, 2.8 times, 2.9 times, 3.0 times, 3.1 times, 3.2 times, 3.3 times, 3.4 times, 3.5 times, 3.6 times, 3.7 times, 3.8 times, 3.9 times, or 4.0 times higher than the half-life of the non-activated FVIII bound to or associated with full-length VWF.

[0301] In one embodiment, a chimeric protein comprising a first polypeptide comprising a FVIII protein and a first Ig constant region or a portion thereof and a second polypeptide comprising a VWF protein, an XTEN having less than 288 amino acids, and an Ig constant region or a portion thereof exhibits a half-life at least about 2 times, 2.5 times, 3.0 times, 3.5 times, 4.0 times, 4.5 times, 5.0 times, 5.5 times, 6.0 times, 7 times, 8 times, 9 times, or 10 times higher than a corresponding chimeric protein comprising the same first polypeptide and the second polypeptide without the XTEN sequence or wild type FVIII. In another embodiment, a chimeric protein comprising a first polypeptide comprising a FVIII protein and a first Ig constant region or a portion thereof and a second polypeptide comprising a VWF protein, an XTEN having less than

288 amino acids, and an Ig constant region or a portion thereof exhibits a half-life about 2 to about 5 times, about 3 to about 10 times, about 5 to about 15 times, about 10 to about 20 times, about 15 to about 25 times, about 20 to about 30 times, about 25 to about 35 times, about 30 to about 40 times, about 35 to about 45 times higher than a corresponding chimeric protein comprising the same first polypeptide and the second polypeptide without the XTEN sequence or wild type FVIII. In a specific embodiment, the half-life of a chimeric protein of the invention increases at least about 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 times higher than the half-life of the wild type FVIII in a FVIII and VWF double knockout mouse.

- [0302] In certain embodiments, a chimeric protein exhibits a half-life of about 40 hours in mice.
- [0303] In some embodiments, the half-life of a chimeric protein is longer than the half-life of a FVIII associated with endogenous VWF. In other embodiments, the half-life of the chimeric protein is at least about 1.5 times, 2 times, 2.5 times, 3.5 times, 3.6 times, 3.7 times, 3.8 times, 3.9 times, 4.0 times, 4.5 times, or 5.0 times the half-life of wild type FVIII or a FVIII protein associated with endogenous VWF.
- [0304] In some embodiments, as a result of the invention the half-life of the chimeric protein is extended compared to a FVIII protein without the VWF protein or wild-type FVIII. The half-life of the chimeric protein of the invention 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 half-life of a chimeric protein without the VWF protein or wild-type FVIII. In one embodiment, the half-life of FVIII 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 half-life of wild-type FVIII. In another embodiment, the half-life of the FVIII 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 wild-type FVIII or a FVIII protein without the VWF protein. In other embodiments, the half-life of the chimeric protein of the invention is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at

least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 40 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours. In still other embodiments, the half-life of the chimeric protein of the invention is about 15 hours to about two weeks, about 16 hours to about one week, about 17 hours to about one week, about 18 hours to about one week, about 19 hours to about one week, about 20 hours to about one week, about 21 hours to about one week, about 22 hours to about one week, about 23 hours to about one week, about 24 hours to about one week, about 36 hours to about one week, about 48 hours to about one week, about 60 hours to about one week, about 24 hours to about six days, about 24 hours to about five days, about 24 hours to about four days, about 24 hours to about three days, or about 24 hours to about two days.

[0305] In some embodiments, the average half-life of the chimeric protein of the invention per subject is about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours (1 day), about 25 hours, about 26 hours, about 27 hours, about 28 hours, about 29 hours, about 30 hours, about 31 hours, about 32 hours, about 33 hours, about 34 hours, about 35 hours, about 36 hours, about 40 hours, about 44 hours, about 48 hours (2 days), about 54 hours, about 60 hours, about 72 hours (3 days), about 84 hours, about 96 hours (4 days), about 108 hours, about 120 hours (5 days), about six days, about seven days (one week), about eight days, about nine days, about 10 days, about 11 days, about 12 days, about 13 days, or about 14 days.

[0306] In addition, the invention provides a method of treating or preventing a bleeding disease or disorder comprising administering an effective amount of a chimeric protein. In one embodiment, the bleeding disease or disorder is selected from the group consisting of a bleeding coagulation disorder, 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, and bleeding in the iliopsoas sheath. In a specific embodiment, the bleeding disease or disorder is hemophilia A.

[0307] The chimeric protein comprising an XTEN sequence and an Ig constant region or a portion thereof in combination with a VWF protein described herein, that prevents or inhibits interaction of the FVIII protein with endogenous VWF prepared by the invention, has many uses as will be recognized by one skilled in the art, including, but not limited to methods of treating a subject having a hemostatic disorder and methods of treating a subject in need of a general

hemostatic agent. In one embodiment, the invention relates to a method of treating a subject having a hemostatic disorder comprising administering a therapeutically effective amount of the chimeric protein.

[0308] The FVIII protein portion in the chimeric protein treats or prevents a hemostatic disorder by serving as a cofactor to Factor IX on a negatively charged phospholipid surface, thereby forming a Xase complex. The binding of activated coagulation factors to a phospholipid surface localizes this process to sites of vascular damage. On a phospholipid surface, Factor VIIIa increases the maximum velocity of Factor X activation by Factor IXa, by approximately 200,000-fold, leading to the large second burst of thrombin generation.

[0309] The chimeric protein of the invention can be used to treat any hemostatic disorder. The hemostatic disorders that may be treated by administration of the chimeric protein of the invention include, but are not limited to, hemophilia A, as well as deficiencies or structural abnormalities relating to Factor VIII. In one embodiment, the hemostatic disorder is hemophilia A.

[0310] The chimeric protein of the invention can be used prophylactically to treat a subject with a hemostatic disorder. The chimeric protein of the invention can be used to treat an acute bleeding episode in a subject with a hemostatic disorder. In another embodiment, the hemostatic disorder can be the result of a defective clotting factor, *e.g.*, von Willebrand's factor. In one embodiment, the hemostatic disorder is an inherited disorder. In another embodiment, the hemostatic disorder is 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).

[0311] The invention also relates to methods of treating a subject that does not have a congenital hemostatic disorder, but has a secondary disease or condition resulting in acquisition of a hemostatic disorder, *e.g.*, due to development of an anti-FVIII antibody or a surgery. 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 the chimeric protein prepared by the present methods.

[0312] The present invention is also related to methods of reducing immunogenicity of FVIII or inducing less immunogenicity against FVIII comprising administering an effective amount of the chimeric proteins described herein, or the polynucleotides encoding the same.

[0313] In one embodiment, the subject in need of a general hemostatic agent is undergoing, or is about to undergo, surgery. The chimeric protein of the invention can be administered prior to, during, or after surgery as a prophylactic regimen. The chimeric protein of the invention can be administered prior to, during, or after surgery to control an acute bleeding episode..

- [0314] The chimeric protein 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. Non limiting examples of bleeding episodes include a bleeding coagulation disorder, 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, and any combinations thereof.
- [0315] In prophylactic applications, one or more compositions containing the chimeric protein 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 reduce symptoms associated with a disease or disorder. Such an amount is defined to be a "prophylactic effective dose." In therapeutic applications, a relatively high dosage (*e.g.*, from about 1 to 400 mg/kg of polypeptide per dose, with dosages of from 5 to 25 mg being more commonly used for radioimmuno conjugates and higher doses for cytotoxin-drug modified polypeptides) at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.
- [0316] In some embodiments, a chimeric protein or a composition of the invention is used for on-demand treatment, which includes treatment for a bleeding episode, hemarthrosis, muscle bleed, oral bleed, hemorrhage, hemorrhage into muscles, oral hemorrhage, trauma, trauma capitis (head trauma), gastrointestinal bleeding, intracranial hemorrhage, intra-abdominal hemorrhage, intrathoracic hemorrhage, bone fracture, central nervous system bleeding, bleeding in the retropharyngeal space, bleeding in the retroperitoneal space, or bleeding in the iliopsoas sheath. The subject may be in need of surgical prophylaxis, peri-operative management, or treatment for surgery. Such surgeries include, *e.g.*, minor surgery, major surgery, tooth extraction, tonsillectomy, inguinal herniotomy, synovectomy, total knee replacement, craniotomy, osteosynthesis, trauma surgery, intracranial surgery, intra-abdominal surgery, intrathoracic surgery, or joint replacement surgery.
- [0317] In one embodiment, the chimeric protein of the present invention is administered intravenously, subcutaneously, intramuscularly, or via any mucosal surface, *e.g.*, orally, sublingually, buccally, nasally, rectally, vaginally or via pulmonary route. The chimeric protein comprising a VWF fragment and a FVIII protein of the present invention can be implanted within or linked to a biopolymer solid support that allows for the slow release of the chimeric protein to

the site of bleeding or implanted into bandage/dressing. The dose of the chimeric protein will vary depending on the subject and upon the particular route of administration used. Dosages can range from 0.1 to 100,000 µg/kg body weight. In one embodiment, the dosing range is 0.1-1,000 µg/kg. In another embodiment, the dosing range is 0.1-500 µg/kg. The protein can be administered continuously or at specific timed intervals. *In vitro* assays may be employed to determine optimal dose ranges and/or schedules for administration. *In vitro* assays that measure clotting factor activity are known in the art, *e.g.*, STA-CLOT VIIa-rTF clotting assay or ROTEM clotting assay. Additionally, effective doses may be extrapolated from dose-response curves obtained from animal models, *e.g.*, a hemophiliac dog (Mount *et al.* 2002, *Blood* 99(8):2670).

[0318] 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, publications, and articles referred to herein are expressly and specifically incorporated herein by reference.

Examples

[0319] Throughout the examples, the following materials and methods were used unless otherwise stated.

Materials and Methods

[0320] In general, the practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, biophysics, molecular biology, recombinant DNA technology, immunology (especially, *e.g.*, antibody technology), and standard techniques in electrophoresis. See, *e.g.*, Sambrook, Fritsch and Maniatis, *Molecular Cloning*: Cold Spring Harbor Laboratory Press (1989); *Antibody Engineering Protocols* (Methods in Molecular Biology), 510, Paul, S., Humana Pr (1996); *Antibody Engineering: A Practical Approach* (Practical Approach Series, 169), McCafferty, Ed., Irl Pr (1996); *Antibodies: A Laboratory Manual*, Harlow *et al.*, CS.H.L. Press, Pub. (1999); and *Current Protocols in Molecular Biology*, eds. Ausubel *et al.*, John Wiley & Sons (1992).

Example 1: FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers

[0321] The present invention is directed to generate a chimeric FVIII molecule which is coupled to D'D3 domain of von Willebrand Factor (VWF) protein via Fc domain of IgG. Attached D'D3 domain prevents the interaction of FVIII with endogenous VWF multimers. This molecule serves as a platform to incorporate other half- life extension technologies in order to improve the

pharmacokinetics of the chimeric protein. XTEN sequences were incorporated into the FVIII B-domain and in between D'D3 and Fc region to increase the half-life of FVIII/VWF heterodimer.

[0322] Thrombin cleavage site in between D'D3 and Fc allows the release of D'D3 domain upon the activation of FVIII molecule by thrombin.

Example 2: Plasmid construction of FVIII-XTEN-Fc/D'D3-Fc heterodimers

Cloning of VWF050- IHH triple mutation in VWF031

[0323] IHH triple mutation in Fc prevents interaction with FcRn, thus there is no recycling of Fc containing molecule by FcRn pathway. The 3 mutations in Fc are I253A, H310A, H435A.

[0324] VWF050 was generated by swapping the Fc region of VWF031 plasmid with Fc fragment containing IHH triple mutation between the RsRII and Not 1 restriction sites.

Cloning of VWF057- Cloning VWF-Fc with 144 AE XTEN +35aa thrombin cleavable linker.

Oligos

ESC 155- Oligo for 144 AE XTEN in VWF034-rev

CCCCGCCACCGGATCCCCGCCACCGGATCCCCGCCACCGGATCCCCGCCACCGGAACCTC
CACCGCCGCTCGAGGCACCTTCTTCAGTGCTGGTGGGCGAGCCCGCTGGTGACCCTTCCTC

ESC 156-Oligo for 144 AE XTEN- GS linker in VWF034-rev

GGGGAAGAGGAAGACTGACGGTCCGCCAGGAGTTCTGGAGCTGGGCACGGTGGGCATGTGT
GAGTTTTGTGCGCTCCGCTGCCCCGGGGGACCAGGGATCCCCGCCACCGGATCCCCGCCAC
CGGATCCCCGCCACCGGATCCCCCGCC

ESC 157-Oligo for 144 AE XTEN in VWF031-Fwd

GTGAAGCCTGCCAGGAGCCGATATCGGGCGCGCCAACATCAGAGAGCGCCACCCCTGAAAGT
GGTCCCGGGAGCGAGCCAGC

[0325] PCR was done twice to obtain the 144 AE-XTEN + 35 aa GS linker with thrombin cleavage site.

[0326] First PCR reaction was done using 144- AE XTEN coding DNA as template and ESC 157/ESC155 primer pair. About 550 bp long PCR product obtained from this reaction was used as template for second PCR reaction and was amplified using ESC 157/156 primer pair. This reaction gave ~ 700 bp long product. This 700 bp PCR product and VWF034 plasmid was then digested with EcoRV-HF and RsRII. Plasmid backbone from digested.

[0327] VWF034 was then used to ligate 700bp PCR product.

Cloning of VWF058- IHH triple mutation in VWF034

[0328] IHH triple mutation in Fc prevents interaction with FcRn, thus there is no recycling of Fc containing molecule by FcRn pathway. The 3 mutations in Fc are I253A, H310A, H435A.

[0329] VWF058 was generated by swapping the Fc region of VWF034 plasmid with Fc fragment containing IHH triple mutation between the RsRII and Not 1 restriction sites.

Cloning of FVIII-263- FVIII 205 with IHH triple mutation

[0330] IHH triple mutation in Fc prevents interaction with FcRn, thus there is no recycling of Fc containing molecule by FcRn pathway. The 3 mutations in Fc are I253A, H310A, H435A.

[0331] FVIII-263 was generated by swapping the Fc region of FVIII 205 plasmid with Fc fragment containing IHH triple mutation between the RsRII and Not 1 restriction sites.

Cloning of FVIII-282- FVIII-Fc with 144 AE XTEN in B-domain

ESC 158-Oligo for 144 AE XTEN in B-domain-fwd

AAGAAGCTTCTCTCAAAACGGCGCGCCAACATCAGAGAGCGCCACCCCTGAAAGTGGTCCCG
GGAGCGAGCCAGCCACATCTGGGTCTGGAAACGCCAGGC

ESC 159-Oligo for 144 AE XTEN in B-domain-rev

GGTATCATCATAATCGATTTCCTCTTGATCTGACTGAAGAGTAGTACGAGTTATTTTCAGCTTGA
TGGCGTTTCAAGACTGGTGGGCTCGAGGCACCTTCTTCAGTGCTGGTGGGCGAGCCCGCTGGT
GACCCTTCCTCAGTGGACGTAGG

[0332] First PCR reaction was done using 144- AE XTEN coding DNA as template and ESC 158/ESC159 primer pair. About 550 bp long PCR product obtained from this reaction and FVIII 169 plasmid was then digested with AscI and Cla1. Plasmid backbone from digested FVIII 169 was then used to ligate 550 bp PCR product in order to obtain FVIII 282.

Cloning of FVIII-283- FVIII 169 with IHH triple mutation

[0333] IHH triple mutation in Fc prevents interaction with FcRn, thus there is no recycling of Fc containing molecule by FcRn pathway. The 3 mutations in Fc are I253A, H310A, H435A.

[0334] FVIII-283 was generated by swapping the Fc region of FVIII 169 plasmid with Fc fragment containing IHH triple mutation between the RsRII and Not 1 restriction sites.

Example 3: Production of FVIII-XTEN-Fc/D'D3-XTEN-Fc in HEK293 cells

[0335] Figure 2. Schematic diagram showing the expression of FVIII-XTEN-Fc/D'D3-XTEN-Fc construct. Three plasmids co-transfection was done in HEK293 cells using Polyethylenimine (PEI). First plasmid derives the expression of FVIII-XTEN-Fc, second plasmid expresses D1D2D'D3-XTEN-Fc and the third plasmid expression PACE/furin, which is required to enzymatically remove propeptide, i.e., D1D2 domain from D1D2D'D3-XTEN-Fc. Products of this three plasmid expression system includes of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimer, D'D3-XTEN-Fc homodimer and traces of FVIII-XTEN-Fc hemizygous looking species.

Example 4: Purification of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers

[0336] To purify the FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers, a tangential flow filtration (TFF) step was used to first concentrate the conditioned media by 10 fold. Products in the filtrate were then further purified using affinity chromatography follow by a desalting column. Purity of the molecule was acceptable by HPLC-SEC and was further confirmed by western blotting. The specific activity of the molecule was comparable to B-domain deleted FVIII, as measured by FVIII activity assay (example 5) and OD280 measurement.

Example 5: Specific activity of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers

[0337] The activity of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers was measure by FVIII chromogenic assay and activated Partial Thromboplastin Time (aPTT) assay. The specific chromogenic activity and specific aPTT activity of SQ BDD-FVIII, rFVIII169/VWF034 and rFVIII169/VWF057 were listed in Table 16. Compared to SQ BDD-FVIII, we have observed comparable specific chromogenic activities and 60% reduction on the specific aPTT activity for rFVIII169/VWF034 and rFVIII169/VWF057.

Table 16: Specific activity of heterodimer variants

FVIII	SQ BDD-FVIII	rFVIII169/VWF034	rFVIII160/VWF057
Specific Chromogenic Activity (IU/pmol)	0.9 – 2.0	1.1-1.2	0.8-1.6
Specific aPTT Activity (IU/pmol)	0.75-1.7	0.4	0.3-0.6

FVIII chromogenic assay

[0338] The FVIII activity was measured using the COATEST SP FVIII kit from DiaPharma (produce #: K824086) and all incubations were performed on a 37°C plate heater with shaking.

[0339] The WHO 8th International Standard for Blood Coagulation Factor VIII:C, Concentrate, coded 07/350 was used as assay standard, the range of the standard was from 100 mIU/mL to 0.78 mIU/mL. A pooled normal human plasma assay control and testing samples (diluted with 1X Coatest buffer) were added into Immulon 2HB 96-well plates in duplicate (25 µL/well). Freshly prepared IXa/FX/Phospholipid mix (50 µL), 25 µL of 25mM CaCl₂, and 50 µL of FXa substrate were added sequentially into each well with 5 minutes incubation between each addition. After incubating with the substrate, 25 µL of 20% Acetic Acid was added to terminate the color reaction, and the absorbance of OD405 was measured with a SpectraMAX plus (Molecular

Devices) instrument. Data were analyzed with SoftMax Pro software (version 5.2). The Lowest Level of Quantification (LLOQ) is 7.8 mIU/mL.

FVIII aPTT assay

[0340] The FVIII aPTT assay was performed on the Sysmex CA-1500 coagulation analyzer as follows: First, 50 uL of manually diluted samples, standards and Controls in aPTT buffer (50 mM Tris, 100 mM NaCl, 1% HSA, pH 7.4) were added by the instrument into the reaction cuvette, followed by adding 50 uL of FVIII-deficient plasma (George King Bio-Medical, product #: 0800). Following incubation at 37°C for 1 minute, 50 uL of aPTT reagent (Actin® FSL activated cephaloplastin reagent - Dade Behring, reference # B4219-2) was added to the reaction mixture, and incubated at 37° C for 4 minutes. Subsequently, 50 ul of 20 mM CaCl₂ (Dade Behring, reference # ORFO37) was added, and the reaction cuvette was immediately transferred to one of four spectrophotometer channel positions to measure the amount of refracted light in the mixture, which was converted to the onset of clotting by the instrument's software algorithm. Reported clotting time was the length of time from the addition of CaCl₂ until the onset of clot formation. Assay standard was generated by diluting the WHO 8th International FVIII Standard into aPTT buffer in a range from 100 mIU/ml to 0.78 mIU/ml. The standard curve was plotted as the clotting time (in seconds) as Y-axis versus the log (base 10) of the FVIII activity (mIU/mL) as X-axis in MS Excel, and the activity of the individual samples was calculated using the formula for the linear regression line of this standard curve. Based on the assay performance, the lower limit of quantization (LLOQ) was 7.8 mIU/mL.

Example 6: Additive effect of XTEN insertions on the half-life extension of heterodimer

[0341] XTEN insertions were incorporated into the heterodimers for half-life extension. Insertion of a single 288 amino acid (aa) AE-XTEN at FVIII B-domain resulted in a 16.7 hrs half-life of the heterodimer in HemA mice, as demonstrated by rFVIII169/VWF031 in Figure 3. To further improve the half-life of the heterodimer, a second XTEN insertion at 144 aa or 288 aa length was incorporated into FVIII169/VWF031 either in the FVIII A1 domain or immediate down stream of D'D3 fragment respectively, the heterodimer variants were named as FVIII205/VWF031 and FVIII169/VWF034.

[0342] The half-life of rFVIII169/VWF031, rFVIII205/VWF031 and rFVIII169/VWF034 were evaluated in FVIII deficient (HemA) mice by a single intravenous administration of test molecules at 200 IU/kg dose. Plasma samples were collected at designate time points as indicated

in Figure 3, the FVIII activity of the samples were determined by FVIII chromogenic assay, the PK parameters were calculated using WinNonlin-Phoenix program and listed in Table 17.

[0343] As shown in Figure 3 and Table 17, the addition of the second XTEN insertion either at A1 domain of FVIII or down stream of D'D3 further improves the half-life of heterodimer to 29.45 or 31.10 respectively. Furthermore, more than 2-fold improvements on clearance and AUC were also observed from both XTEN insertions.

Table 17: PK parameter of heterodimers in Hema mice

FVIII	XTEN Insertions		T _{1/2} (hr)	MRT (hr)	Cl (mL/hr/kg)	Vss (mL/kg)	AUC_D (kg*hr/mL)
	Insertion 1	Insertion 2					
rFVIII169/VWF031	B*-AE288		16.65	18.44	3.57	85.72	0.28
rFVIII205/VWF031	B*-AE288	A1-AE144	29.45	36.02	1.76	63.56	0.57
rFVIII169/VWF034	B*-AE288	D'D3-AE288	31.10	34.57	1.73	59.77	0.58

Example 7: 144 aa AE-XTEN confers better half-life benefit than 288 aa AE-XTEN when inserted in between D'D3 and Fc domains.

[0344] Another heterodimer-FVIII169/VWF057 was constructed in the effort of identifying the optimal length of XTEN insertion within the D'D3-XTEN-Fc chain, in which the length of XTEN insertion was reduced to 144aa from 288aa. As shown in Figure 4, compared to rFVIII169/VWF034, the half-life of rFVIII169/VWF057 was increased from 31 hrs to 42 hrs. Improved clearance and AUC were also observed for rFVIII169/VWF057, data was listed in Table 18. Thus, 144aa AE-XTEN insertion is more optimal than AE-288aa XTEN when inserted between D'D3 and Fc domain of the FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers.

Table 18: PK parameters of rFVIII169/VWF034 and rFVIII169/VWF057 in Hema mice

FVIII	T _{1/2} (hr)	MRT (hr)	Cl (mL/hr/kg)	Vss (mL/kg)	AUC_D (kg*hr/mL)
rFVIII169/VWF034	31.10	34.57	1.73	59.77	0.58
rFVIII169/VWF057	42.23	53.24	0.97	51.44	1.03

Example 8: Fc domain extends the half-life of heterodimer

[0345] Fc domains extent its fusion protein's half-life through FcRn mediated recycling pathway. To confirm the necessity of the Fc domain on the half-life extension of the heterodimer, the wild-type Fc domains were replaced by a triple mutant (I253A/H310A/H435A; IHH) in

rFVIII205/VWF031 to form rFVIII263/VWF050, and complete elimination of FcRn binding was confirmed by Surface Plasmon Resonance (Biacore) assay for rFVIII263/VWF050. The half-life of FVIII263/VWF050 was evaluated in HemA mice in comparison with rFVIII205/VWF031. Increased clearance rate, as well as reduced half-life and AUC were observed for rFVIII263/VWF050 as shown in Figure 5 and Table 19. This result demonstrated that in addition to ensure the covalent binding of FVIII and D'D3, the Fc domains is also necessary for the half-life improvement of the heterodimer.

Table 19: PK parameters of rFVIII205/VWF031 and rFVIII263/VWF040 in HemA mice

FVIII	Mutation in Fc domain	T_{1/2} (hr)	MRT (hr)	Cl (mL/hr/kg)	Vss (mL/kg)	AUC_D (kg*hr/mL)
rFVIII205/VWF031	None	29.45	36.02	1.76	63.56	0.57
rFVIII263/VWF050	IHH	22.96	26.15	2.36	61.69	0.42

Example 9: Acute efficacy of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers in HemA mouse tail clip bleeding model

[0346] The acute efficacy of lead heterodimer candidates were evaluated using HemA mouse tail clip bleeding model.

[0347] 8-12 weeks old male HemA mice were randomized into 4 treatment groups, and treated with a single intravenous administration of SQ BDD-FVIII, rFVIII169/VWF034, rFVIII169/VWF057 or vehicle solution respectively. In order to mimic the episodic treatment of FVIII (to reconstitute 50-100% of normal FVIII plasma level), the selected FVIII treatment dose is 75 IU/kg as measured by FVIII aPTT activity. At this dose level, all testing FVIII variants will reconstitute ~70% of normal murine plasma FVIII activity 5min post dosing.

[0348] Blood loss volume from each individual animal in the study was plotted in Figure 6. Significant reduction on blood loss volume was observed for all FVIII treatment groups compared to vehicle treated animals. Within the three FVIII treatment groups, no statistical significant different were found on blood loss reduction, suggesting the heterodimer molecules could potentially as efficacious as SQ BDD-FVIII for on demand treatment.

[0349] Blood loss volume from each individual animal in the study was plotted in Figure 6. Significant reduction on blood loss volume was observed for all FVIII treatment groups compared to vehicle treated animals. Within the three FVIII treatment groups, no statistical significant different were found on blood loss reduction, suggesting the heterodimer molecules could potentially as efficacious as SQ BDD-FVIII for on demand treatment.

[0350] In addition, HemA mice were treated with a lower dose (37.5 IU/kg) of rBDD-FVIII or rFVIII169/VWF034, and the results are shown in Figure 6B. Same as the 75 IU/kg dose, rFVIII169/VWF034 provided similar protection as BDD-FVIII to HemA mice post tail clip injury, indicating the molecule was still efficacious to treat severe bleeding episodes at ~35% of normal murine circulating FVIII level in HemA mice.

[0351] The Tail Clip procedure was carried out as follows. Briefly, mice were anesthetized with a 50 mg/kg Ketamine/0.5 mg/kg Dexmedetomidine cocktail prior to tail injury and placed on a 37°C heating pad to help maintain the body temperature. The tails of the mice were then be immersed in 37°C saline for 10 minutes to dilate the lateral vein. After vein dilation, FVIII variants or vehicle solution were injected via the tail vein and the distal 5 mm of the tail was then cut off using a straight edged #11 scalpel 5min post dosing. The shed blood was collected into 13 ml of 37°C saline for 30 minutes and blood loss volume was determined by the weight change of the blood collection tube: blood loss volume = (collection tube end weight - beginning weight + 0.10) ml. Statistical analysis were conducted using t test (Mann Whitney test) and one way ANOVA (KRUSKAL-Wallis test, posttest: Dunns multiple comparison test).

Example 10: Prophylactic efficacy of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimer in HemA mouse tail vein transection bleeding model

[0352] The prophylactic efficacy of FVIII169/VWF057 was tested in HemA mouse tail vein transection (TVT) model. The TVT model induces bleeding by introducing injury to the lateral vein of the mouse tail, which mimics the spontaneous bleeding episodes in patients with hemophilia bleeding disorder.

[0353] 8-10 weeks old male HemA mice were randomized into four treatment groups, and treated with either FVIII169/VWF057 at 72hr prior of the tail vein injury, or SQ BDD-FVIII at 24hr or 48hr before the injury. Vehicle treated animal were used as negative control. Events of re-bleeding or euthanasia due to the excessive blood loss within 24 hrs post injury were plotted in Figure 7.

[0354] As shown in Figure 7, unlike mice treated with SQ BDD-FVIII at 48hr prior to TVT, of whom only limited protection was observed post injury, mice that received rFVIII169/VWF057 at 72hr prior the tail injury had similar protection on re-bleeding and survival compared to the mice that received SQ BDD-FVIII treatment 24hr before TVT, indicating rFVIII169/VWF057 can provide at least 3-fold or more (e.g., 4-fold) longer-protection to HemA mice in TVT model. Therefor rFVIII169/VWF057 might significantly reduce the treatment frequency of the current FVIII prophylaxis.

[0355] Similarly, HemA mice were treated with FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers: rFVIII169/VWF034 and rFVIII169/VWF057 at 24 or 96 hours prior to the tail vein injury. The rebleeding and survival data of the treatments were compared with the data by the rBDD-FVIII at 24 or 48 hours prior to the injury and vehicle. While the rebleeding in mice treated with rBDD-FVIII at 24 hours prior to the tail vein injury was similar to the mice treated with vehicle, the rebleeding data of mice treated with the heterodimers at 24hr before the injury are significantly better than the vehicle treatment group. Furthermore, the rebleeding data of mice treated with the heterodimers at 96hr before the injury were comparable to mice received rBDD-FVIII at 24hr before the injury. As for the survival rate at 24hr post the TVT injury, in contrast of the less than 50% survival rate of mice treated with rBDD-FVIII, more than 90% of the mice survived the TVT injury with FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers treatment when FVIII molecules were administered at 24hr before the injury. In addition, the survival in mice treated with the FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimers at 96 hours prior to the tail vein injury were better (in the case of rFVIII169/VWF034) or comparable (in the case of rFVIII169/VWF057) when compared with the mice that received rBDD-FVIII treatment at 24 hours prior to the injury. Both rebleeding and survival data had indicated a 4-fold efficacy prolongation of FVIII-XTEN-Fc/D'D3-XTEN-Fc heterodimer treatment vs. rBDD-FVIII treatment.

HemA mouse tail vein transection model

[0356] The tail vein transection procedure was conducted as follows. Mice were anesthetized with a cocktail containing 50 mg/kg of Ketamine, 0.125 mg/kg of Dexmedetomidine, and 0.1 mg/kg of Buprenex. At an adequate anesthetic depth, the lateral tail vein of the mice was transected with straight edged number 11 surgical blade at an area where the diameter of the tail is approximately 2.7 mm. The shedding blood was washed away with warm saline to ensure clear observation of the wound. The treated mice were then single housed in a clean cage with white paper bedding for the next 24 hours. Tail re-bleed and the mouse's physical activity were observed and recorded hourly up to 12 hours post tail injury. Moribund mice were euthanized immediately, and a final observation was performed at 24 hours post tail injury. To mimic the bleeding situation in hemophilia patients and to ensure the animal's completely recovery from anesthesia, 1 mg/kg of Atipamezole solution was given to reverse Dexmedetomidine effect at the beginning of the Tail Vein Transection. An additional dose of 0.1 mg/kg Buprenex was administered at the end of the 12 hour observation period for overnight pain management. The survival curve of Time to Re-bleed and Time to Euthanasia was generated for data analysis, and Log-rank (Mantel-COX) test was used for statistic evaluation.

Example 11: Preparation of FVIII169/VWF059 and other constructs

pSYN FVIII 310 cloning:

[0357] A synthetic DNA fragment flanked with BamH1 site at the N-terminus and Cla 1 site at the C-terminus was commercially made. This synthetic DNA was used to replace the BamH1 to Cla1 region in pSYN FVIII 169 construct (SEQ ID NO: 155). Both synthetic DNA and pSYN FVIII 169 DNA were double digested with BamH1 and Cla1, digested synthetic DNA was inserted into digested pSYN FVIII 169 to create pSYN FVIII 310 (SEQ ID NO:168; Table 20).

Cloning pSYN FVIII 312:

[0358] A synthetic DNA fragment flanked with BamH1 site at the N-terminus and Afe 1 site at the C-terminus was commercially made. This synthetic DNA was used to replace the BamH1 to Afe1 region in pSYN FVIII 169 construct (SEQ ID NO: 155). Both synthetic DNA and pSYN FVIII 169 DNA were double digested with BamH1 and Afe1, digested synthetic DNA was inserted into digested pSYN FVIII 169 to create pSYN FVIII 312 (SEQ ID NO: 169; Table 20). pSYN FVIII 312A (SEQ ID NO: 2; Table 20) was created from pSYN FVIII312 to remove AscI site which codes for amino acid residues GAP at the junction of FVIII and XTEN.

Table 20: Synthetic FVIII constructs.

Construct	Protein Sequence
pSYN FVIII 169	<u>PRSF</u> <u>SQNG</u> <u>APGT</u> <u>SESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSG</u> <u>SETP</u> <u>GTSESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSGS</u> <u>ETP</u> <u>GTSESAT</u> <u>PESG</u> <u>PGT</u> <u>STEP</u> <u>SEGS</u> <u>APG</u> <u>SPAG</u> <u>SPTST</u> <u>EEGT</u> <u>SESAT</u> <u>PESG</u> <u>PGSE</u> <u>PAT</u> <u>ATSG</u> <u>SETP</u> <u>GTSESAT</u> <u>PESG</u> <u>PGSP</u> <u>AGSPT</u> <u>STEEG</u> <u>SPAG</u> <u>SPTST</u> <u>EEGT</u> <u>STEP</u> <u>SEGS</u> <u>A</u> <u>PGTSESAT</u> <u>PESG</u> <u>PGTSESAT</u> <u>PESG</u> <u>PGTSESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSG</u> <u>SETP</u> <u>GPSE</u> <u>PAT</u> <u>SGSETP</u> <u>GSPAG</u> <u>SPTST</u> <u>EEGT</u> <u>STEP</u> <u>SEGS</u> <u>APGT</u> <u>STEP</u> <u>SEGS</u> <u>APG</u> <u>SEP</u> <u>PATSG</u> <u>SETP</u> <u>G</u> <u>TSESAT</u> <u>PESG</u> <u>PGTSTEP</u> <u>SEGS</u> <u>APASS</u> <u>PPVL</u> <u>KRHQ</u> <u>AEITR</u> (SEQ ID NO: 167) <i>(Underlined = XTEN residues; not underlined = FVIII residues)</i>
pSYN FVIII 310	<u>PRSF</u> <u>GAPGT</u> <u>SESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSG</u> <u>SETP</u> <u>GTSESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSG</u> <u>SETP</u> <u>GTSESAT</u> <u>PESG</u> <u>PGTSTEP</u> <u>SEGS</u> <u>APG</u> <u>SPAG</u> <u>SPTST</u> <u>EEGT</u> <u>SESAT</u> <u>PESG</u> <u>PGSE</u> <u>PAT</u> <u>GSETP</u> <u>GTSESAT</u> <u>PESG</u> <u>PGSP</u> <u>AGSPT</u> <u>STEEG</u> <u>SPAG</u> <u>SPTST</u> <u>EEGT</u> <u>STEP</u> <u>SEGS</u> <u>APGT</u> <u>SESAT</u> <u>PESG</u> <u>PGTSESAT</u> <u>PESG</u> <u>PGTSESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSG</u> <u>SETP</u> <u>GPSE</u> <u>PATSGS</u> <u>ETP</u> <u>GSPAG</u> <u>SPTST</u> <u>EEGT</u> <u>STEP</u> <u>SEGS</u> <u>APGT</u> <u>STEP</u> <u>SEGS</u> <u>APG</u> <u>SEP</u> <u>PATSG</u> <u>SETP</u> <u>GTSE</u> <u>SATPESG</u> <u>PGTSTEP</u> <u>SEGS</u> <u>APASSE</u> <u>ITR</u> (SEQ ID NO: 168) <i>(Underlined = XTEN residues; not underlined = FVIII residues)</i>
pSYN FVIII 312	<u>PRSF</u> <u>SQNG</u> <u>APGT</u> <u>SESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSG</u> <u>SETP</u> <u>GTSESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSGS</u> <u>ETP</u> <u>GTSESAT</u> <u>PESG</u> <u>PGTSTEP</u> <u>SEGS</u> <u>APG</u> <u>SPAG</u> <u>SPTST</u> <u>EEGT</u> <u>SESAT</u> <u>PESG</u> <u>PGSE</u> <u>PAT</u> <u>ATSG</u> <u>SETP</u> <u>GTSESAT</u> <u>PESG</u> <u>PGSP</u> <u>AGSPT</u> <u>STEEG</u> <u>SPAG</u> <u>SPTST</u> <u>EEGT</u> <u>STEP</u> <u>SEGS</u> <u>A</u> <u>PGTSESAT</u> <u>PESG</u> <u>PGTSESAT</u> <u>PESG</u> <u>PGTSESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSG</u> <u>SETP</u> <u>GPSE</u> <u>PAT</u> <u>SGSETP</u> <u>GSPAG</u> <u>SPTST</u> <u>EEGT</u> <u>STEP</u> <u>SEGS</u> <u>APGT</u> <u>STEP</u> <u>SEGS</u> <u>APG</u> <u>SEP</u> <u>PATSG</u> <u>SETP</u> <u>G</u> <u>TSESAT</u> <u>PESG</u> <u>PGTSTEP</u> <u>SEGS</u> <u>APASSE</u> <u>ITR</u> (SEQ ID NO: 169) <i>(Underlined = XTEN residues; not underlined = FVIII residues)</i>
pSYN FVIII	<u>PRSF</u> <u>SQNG</u> <u>TSESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSG</u> <u>SETP</u> <u>GTSESAT</u> <u>PESG</u> <u>PGSE</u> <u>PATSG</u> <u>SETP</u>

312A	<u>GTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSESATPESGPGSEPATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSEPATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSETPGTSESATPESGPGTSTEPSEGSAPASSEITR</u> (SEQ ID NO: 2) <i>(similar sequence as pSYN FVIII312 just residues corresponding to AscI site i.e GAP are removed) (Underlined = XTEN residues; not underlined = FVIII residues)</i>
------	--

Cloning pSYN VWF059 and VWF073:

[0359] Various synthetic DNA fragments coding for different linker regions between D'D3-XTEN and Fc were made. These synthetic DNA fragments were flanked with AscI site at N-terminus and NotI site at the C-terminus. These synthetic DNAs were used to replace the AscI to NotI region in pSYN VWF057 construct (SEQ ID NO: 152). The pSYN VWF059 construct (Table 21) comprises a linker region (SEQ ID NO: 13), which includes the entire FVIII acidic region 2 (a2). This site is reported to be cleaved by thrombin, and upon FVIII activation D'D3XTEN is released. The pSYN VWF073 construct (Table 21) contains only the thrombin cleavage site of FVIII acidic region 2 (a2) (*i.e.*, IEPRSFSS) (SEQ ID NO: 23). Both synthetic DNA and pSYN VWF057 DNA were double digested with AscI and NotI. Digested synthetic DNA was inserted into digested pSYN VWF057 to create pSYN VWF059 and pSYN VWF073. The pSYN VWF59A construct (Table 21) was generated from pSYN VWF059 by removing the EcoRV restriction site. FVIII169/VWF057 and FVIII169/VWF059 heterodimer proteins were generated by co-expression of FVIII169 and VWF057 or VWF059 in HEK293 cells.

Table 21: Synthetic VWF constructs – Cleavable Linker Regions.

Construct	Protein Sequence
pSYN VWF057	TSTEEGASS <u>GGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSLVPR</u> SGGDKTH (SEQ ID NO: 12) Italics and underlined sequence shows GS linker and LVPR thrombin cleavage site (also shaded).
pSYN VWF059	TSTEEGASISD <u>KNTGDYYEDSYEDISAYLLSKNNAIEPRSFSS</u> DKTH (SEQ ID NO: 13) Italics and underlined sequence shows 32 aa from FVIII acidic region 2 (a2). Shaded sequence shows thrombin cleavage site used in pSYN VWF059A.
pSYN VWF059A	TSTEEGASSD <u>KNTGDYYEDSYEDISAYLLSKNNAIEPRSFSS</u> DKTH (SEQ ID NO: 22) Italics and underlined sequence shows 32 aa from FVIII acidic region 2 (a2). This sequence is similar sequence to VWF059, except that residues corresponding to the EcoRV site (<i>i.e.</i> , IS) are removed.
pSYN VWF073	TSTEEGASS <u>GGGGSGGGSGGGSGGGSGGGSGGGSGGGSLVPR</u> SGGDKTH (SEQ ID NO: 23)

	Italics and underlined sequence shows GS linker with truncated thrombin cleavage site from FVIII acidic region 2 (shaded 7 amino acids-IEPRSFS).
--	--

Example 12: Thrombin digestion of FVIII heterodimer to analyze the release of D'D3 from Fc

[0360] Two FVIII heterodimer proteins were tested in thrombin digestion experiments and their rate of cleavage by thrombin was examined. The two heterodimer constructs used in this experiment were FVIII169/VWF057 heterodimer and FVIII169/VWF059 heterodimer along with FVIII_hFc. The FVIII169/VWF057 and FVIII169/VWF059 heterodimers are described above. Three digestion reactions were carried out: i) FVIII_hFc ii) FVIII169/VWF057 (FIG. 11), and iii) FVIII 169/VWF059 (FIG. 12). Test samples were treated with human α -thrombin at a molar ratio of FVIII:thrombin of approximately 22:1. Each reaction was incubated in a 37°C water bath. At each indicated time point (t = 5, 15, 30, 45, 60 minutes), a 22.5 μ L sample was withdrawn, stopped with 22.5 μ L non-reducing 2x SDS loading dye, and heated for 3 minutes. The digested protein was then run on an SDS-PAGE gel. Western blotting was performed using anti-FVIII heavy chain (GMA012) and anti-VWF-D3 (Ab96340) antibodies using a LICOR system.

[0361] As shown in figure 11, exposure of FVIII169/VWF057 to thrombin resulted in a gradual decrease in the detected level of D'D3-XTEN-Fc, correlating with an increase in the level of D'D3-144 XTEN, the cleaved product. Un-cleaved FVIII169/VWF057 remained after 15 minutes. Conversely, figure 12 shows that FVIII 169/VWF059 is cleaved more rapidly by thrombin, as evidenced by little to no detectable un-cleaved FVIII 169/VWF059 after 5 minutes. Accordingly, FVIII 169/VWF059 showed better release of D'D3 from Fc upon thrombin activation as compared to FVIII169/VWF057.

[0362] Parallel experiments were done to investigate thrombin cleavage using mass spectroscopy (MS). By MS, FVIII 169/VWF059 again showed better release of D'D3 from Fc as compared to VWF057.

Example 13: *In vivo* evaluation of FVIII169/VWF059 in HemA mice

[0363] To further evaluate the pharmacokinetic profile and *in vivo* potency of FVIII169/VWF059, HemA mice were treated with FVIII169/VWF059 through intravenous administration at 150 IU/kg dose. Plasma samples were collected via vena cava blood collection at 5 minutes, 24, 48, 72, 96 and 120 hours post injection. FVIII activity in plasma samples were measured by FVIII chromogenic assay and PK parameters were calculated using Phoenix

program. A similar PK profile of FVIII169/VWF059 was observed in comparison with FVIII169/VWF057, as shown in Table 22, indicating that the a2 thrombin cleavage linker has no negative effect on the PK profile of the heterodimer.

Table 22: PK profile of FVIII169/VWF057 and FVIII169/VWF059 in HemA mice

Heterodimer	T _{1/2} (hr)	AUC/D (hr*kg*mIU/mL/mIU)	Cl (mL/hr/kg)	MRT (hr)	Vss (mL/kg)
FVIII169/VWF057	38.53	0.80	1.26	44.92	56.38
FVIII169/VWF059	40.51	0.74	1.35	49.22	66.26

[0364] The acute efficacy of FVIII169/VWF059 was evaluated in a HemA mouse tail clip model (described in Example 9) in comparison with wild type BDD-FVIII. HemA mice were treated with 75 IU/kg of either FVIII169/VWF059 or BDD-FVIII, and blood loss volume of each experimental mouse was plotted in Figure 13. Compared to BDD-FVIII, FVIII169/VWF059 provided the same degree of protection to HemA mice ($p=0.9883$), indicating that FVIII169/VWF059 is fully functional *in vivo*.

Plasmid construction of FVIII-XTEN-Fc/D'D3-Fc heterodimers

VWF031 nucleotide Sequence (SEQ ID NO: 147)

```

1   ATGAT TCCTG CCAGA TTTGC CGGGG TGCTG CTTGC TCTGG CCCTC ATTTT
51  GCCAG GGACC CTTTG TGCAG AAGGA ACTCG CGGCA GGTCA TCCAC GGCCC
101 GATGC AGCCT TTTCG GAAGT GACTT CGTCA ACACC TTTGA TGGGA GCATG
151 TACAG CTTTG CGGGA TACTG CAGTT ACCTC CTGGC AGGGG GCTGC CAGAA
201 ACGCT CCTTC TCGAT TATTG GGGAC TTCCA GAATG GCAAG AGAGT GAGCC
251 TCTCC GTGTA TCTTG GGGAA TTTT T GACA TCCAT TTGTT TGTCA ATGGT
301 ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
351 GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
401 ATGGC TTTGT GGCCA GGATC GATGG CAGCG GCAAC TTTCA AGTCC TGCTG
451 TCAGA CAGAT ACTTC AACAA GACCT GCGGG CTGTG TGGCA ACTTT AACAT
501 CTTTG CTGAA GATGA CTTTA TGACC CAAGA AGGGA CCTTG ACCTC GGACC
551 CTTAT GACTT TGCCA ACTCA TGGGC TCTGA GCAGT GGAGA ACAGT GGTGT
601 GAACG GGCAT CTCC T CCCAG CAGCT CATGC AACAT CTCCT CTGGG GAAAT
651 GCAGA AGGGC CTGTG GGAGC AGTGC CAGCT TCTGA AGAGC ACCTC GGTGT
701 TTGCC CGCTG CCACC CTCTG GTGGA CCCC G AGCCT TTTGT GGCCC TGTGT
751 GAGAA GACTT TGTGT GAGTG TGCTG GGGGG CTGGA GTGCG CCTGC CCTGC
801 CCTCC TGGAG TACGC CCGGA CCTGT GCCCA GGAGG GAATG GTGCT GTACG
851 GCTGG ACCGA CCACA GCGCG TGCAG CCCAG TGTGC CCTGC TGGTA TGGAG
901 TATAG GCAGT GTGTG TCCCC TTGCG CCAGG ACCTG CCAGA GCCTG CACAT
951 CAATG AAATG TGTCA GGAGC GATGC GTGGA TGGCT GCAGC TGCCC TGAGG
1001 GACAG CTCCT GGATG AAGGC CTCTG CGTGG AGAGC ACCGA GTGTC CCTGC
1051 GTGCA TTCCG GAAAG CGCTA CCCTC CCGGC ACCTC CCTCT CTGCA GACTG
1101 CAACA CCTGC ATTTG CCGAA ACAGC CAGTG GATCT GCAGC AATGA AGAAT
1151 GTCCA GGGGA GTGCC TTGTC ACTGG TCAAT CCCAC TTCAA GAGCT TTGAC
1201 AACAG ATACT TCACC TTCAG TGGGA TCTGC CAGTA CCTGC TGGCC CGGGA

```

```

1251   TTGCC AGGAC CACTC CTTCT CCATT GTCAT TGAGA CTGTC CAGTG TGCTG
1301   ATGAC CGCGA CGCTG TGTGC ACCCG CTCCG TCACC GTCCG GCTGC CTGGC
1351   CTGCA CAACA GCCTT GTGAA ACTGA AGCAT GGGGC AGGAG TTGCC ATGGA
1401   TGGCC AGGAC ATCCA GCTCC CCCTC CTGAA AGGTG ACCTC CGCAT CCAGC
1451   ATACA GTGAC GGCCCT CCGTG CGCCT CAGCT ACGGG GAGGA CCTGC AGATG
1501   GACTG GGATG GCCGC GGGAG GCTGC TGGTG AAGCT GTCCC CCGTC TATGC
1551   CGGGA AGACC TGCGG CCTGT GTGGG AATTA CAATG GCAAC CAGGG CGACG
1601   ACTTC CTTAC CCCCT CTGGG CTGGC GGAGC CCCGG GTGGA GGACT TCGGG
1651   AACGC CTGGA AGCTG CACGG GGACT GCCAG GACCT GCAGA AGCAG CACAG
1701   CGATC CCTGC GCCCT CAACC CGCGC ATGAC CAGGT TCTCC GAGGA GCGCT
1751   GCGCG GTCTT GACGT CCCCC ACATT CGAGG CCTGC CATCG TGCCG TCAGC
1801   CCGCT GCCCT ACCTG CGGAA CTGCC GCTAC GACGT GTGCT CCTGC TCGGA
1851   CGGCC GCGAG TGCTT GTGCG GCGCC CTGGC CAGCT ATGCC GCGCG CTGCG
1901   CGGGG AGAGG CGTGC GCGTG GCGTG GCGCG AGCCA GGCCG CTGTG AGCTG
1951   AACTG CCCGA AAGGC CAGGT GTACC TGCAG TCGGG GAGCC CCTGC AACCT
2001   GACCT CCCGC TCTCT CTCTT ACCCG GATGA GGAAT GCAAT GAGGC CTGCC
2051   TGGAG GGCTG CTTCT GCCCC CCAGG GCTCT ACATG GATGA GAGGG GGGAC
2101   TGCGT GCCCA AGGCC CAGTG CCCCT GTTAC TATGA CGGTG AGATC TTCCA
2151   GCCAG AAGAC ATCTT CTCAG ACCAT CACAC CATGT GCTAC TGTGA GGATG
2201   GCTTC ATGCA CTGTA CCATG AGTGG AGTCC CCGGA AGCTT GCTGC CTGAC
2251   GCTGT CCTCA GCAGT CCCCT GTCTC ATCGC AGCAA AAGGA GCCTA TCCTG
2301   TCGGC CCCCC ATGGT CAAGC TGGTG TGTCC CGCTG ACAAC CTGCG GGCTG
2351   AAGGG CTCGA GTGTA CAAA ACGTG CCAGA ACTAT GACCT GGAGT GCATG
2401   AGCAT GGGCT GTGTC TCTGG CTGCC TCTGC CCCCC GGGCA TGGTC CGGCA
2451   TGAGA ACAGA TGTGT GGCCC TGGAA AGGTG TCCCT GCTTC CATCA GGGCA
2501   AGGAG TATGC CCCTG GAGAA ACAGT GAAGA TTGGC TGCAA CACTT GTGTC
2551   TGTCG GGACC GGAAG TGGAA CTGCA CAGAC CATGT GTGTG ATGCC ACGTG
2601   CTCCA CGATC GGCAT GGCCC ACTAC CTCAC CTTCG ACGGG CTCAA ATACC
2651   TGTTT CCCGG GGAGT GCCAG TACGT TCTGG TGCAG GATTA CTGCG GCAGT
2701   AACCC TGGGA CCTTT CGGAT CCTAG TGGGG AATAA GGGAT GCAGC CACCC
2751   CTCAG TGAAA TGCAA GAAAC GGGTC ACCAT CCTGG TGGAG GGAGG AGAGA
2801   TTGAG CTGTT TGACG GGGAG GTGAA TGTGA AGAGG CCCAT GAAGG ATGAG
2851   ACTCA CTTTG AGGTG GTGGA GTCTG GCCGG TACAT CATTC TGCTG CTGGG
2901   CAAAG CCTTC TCCGT GGTCT GGGAC CGCCA CCTGA GCATC TCCGT GGTCC
2951   TGAAG CAGAC ATACC AGGAG AAAGT GTGTG GCCTG TGTGG GAATT TTGAT
3001   GGCAT CCAGA ACAAT GACCT CACCA GCAGC AACCT CCAAG TGGAG GAAGA
3051   CCCTG TGGAC TTTGG GAACT CCTGG AAAGT GAGCT CGCAG TGTGC TGACA
3101   CCAGA AAAGT GCCTC TGGAC TCATC CCCTG CCACC TGCCA TAACA ACATC
3151   ATGAA GCAGA CGATG GTGGA TTCCT CCTGT AGAAT CCTTA CCAGT GACGT
3201   CTTCC AGGAC TGCAA CAAGC TGGTG GACCC CGAGC CATAT CTGGA TGTCT
3251   GCATT TACGA CACCT GCTCC TGTGA GTCCA TTGGG GACTG CGCCG CATTC
3301   TGCGA CACCA TTGCT GCCTA TGCCC ACGTG TGTGC CCAGC ATGGC AAGGT
3351   GGTGA CCTGG AGGAC GGCCA CATTG TGCCC CCAGA GCTGC GAGGA GAGGA
3401   ATCTC CGGGA GAACG GGTAT GAGGC TGAGT GGCGC TATAA CAGCT GTGCA
3451   CCTGC CTGTC AAGTC ACGTG TCAGC ACCCT GAGCC ACTGG CCTGC CCTGT
3501   GCAGT GTGTG GAGGG CTGCC ATGCC CACTG CCCTC CAGGG AAAAT CCTGG
3551   ATGAG CTTTT GCAGA CCTGC GTTGA CCCTG AAGAC TGTC CAGTGT GTGAG
3601   GTGGC TGGCC GCGGT TTTGC CTCAG GAAAG AAAGT CACCT TGAAT CCCAG
3651   TGACC CTGAG CACTG CCAGA TTTGC CACTG TGATG TTGTC AACCT CACCT
3701   GTGAA GCCTG CCAGG AGCCG ATATC TGGCG GTGGA GGTTC CGGTG CCGGG
3751   GGATC CGGCG GTGGA GGTTC CGGCG GTGGA GGTTC CGGTG CCGGG GGATC
3801   CGGTG GCGGG GGATC CCTGG TCCCC CGGGG CAGCG GCGGT GGAGG TTCCG
3851   GTGGC GGGGG ATCCG AAAAA ACTCA CACAT GCCCA CCGTG CCCAG CTCCA
3901   GAACT CCTGG GCGGA CCGTC AGTCT TCCTC TTCCC CCCAA AACCC AAGGA
3951   CACCC TCATG ATCTC CCGGA CCCCT GAGGT CACAT GCGTG GTGGT GGACG
4001   TGAGC CACGA AGACC CTGAG GTCAA GTTCA ACTGG TACGT GGACG GCGTG
4051   GAGGT GCATA ATGCC AAGAC AAAGC CGCGG GAGGA GCAGT ACAAC AGCAC
4101   GTACC GTGTG GTCAG CGTCC TCACC GTCTT GCACC AGGAC TGGCT GAATG
4151   GCAAG GAGTA CAAGT GCAAG GTCTC CAACA AAGCC CTCCC AGCCC CCATC

```

```

4201   GAGAA AACCA TCTCC AAAGC CAAAG GGCAG CCCC GAGAAC CACAG GTGTA
4251   CACCC TGCCC CCATC CCGCG ATGAG CTGAC CAAGA ACCAG GTCAG CCTGA
4301   CCTGC CTGGT CAAAG GCTTC TATCC CAGCG ACATC GCCGT GGAGT GGGAG
4351   AGCAA TGGGC AGCCG GAGAA CAACT ACAAG ACCAC GCCTC CCGTG TTGGA
4401   CTCCG ACGGC TCCTT CTTCC TCTAC AGCAA GCTCA CCGTG GACAA GAGCA
4451   GGTGG CAGCA GGGGA ACGTC TTCTC ATGCT CCGTG ATGCA TGAGG CTCTG
4501   CACAA CCACT ACACG CAGAA GAGCC TCTCC CTGTC TCCGG GTAAA TGA

```

VWF031 protein Sequence (SEQ ID NO: 86)

```

1      MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFVNTFDGSM
      51      YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
      101     TVTQGDQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
      151     SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
      201     ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
      251     EKTLCCECAGG LECACPALLE YARTCAQEGM VLYGWDHSA CSPVCPAGME
      301     YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
      351     VHS GKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECIV TGQSHFKSFD
      401     NRYFTTFSGIC QYLLARDCQD HSF SIV IETV QCADDRDAVC TRSVTVRLPG
      451     LHNSLVKLKH GAGVAMDGQD IQLPLLKGD LRIQHTVTASV RLSYGEDLQM
      501     DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
      551     NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
      601     PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCEL
      651     NCPKGQVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
      701     CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
      751     AVLSSPLSHR SKRSLSCRPP MVKLVC PADN LRAEGLECTK TCQNYDLECM
      801     SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
      851     CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGEQ YVLVQDYCGS
      901     NPGTFRILVG NKGCSHPSVK CKKRV TILVE GGEIELFDGE VNVKRPMKDE
      951     THFEVVESGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
      1001    GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
      1051    MKQTMVDSSC RILTS DVFQD CNKLVDPEPY LDVCIYDTCS CESIGDCAAF
      1101    CDTIAAYAHV CAQH GKVV TW RTATLC PQSC EERNLRENGY EAEWRYNSCA
      1151    PACQVTCQHP EPLACP VQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
      1201    VAGRRFASGK KVT LNPSDPE HCQICHCDVV NLTCEACQEP ISGGGSGGG
      1251    GSGGGGSGGG GSGGGGSGGG GSLVPRGSGG GSGGGGSGDK THTCP PCPAP
      1301    ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
      1351    EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNKKEYKCK VSNKALPAPI
      1401    EKTISKAKGQ PREPQVY TLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE
      1451    SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
      1501    HNHYTQKSLS LSPGK*

```

VWF034 nucleotide Sequence (SEQ ID NO: 148)

```

      1      ATGAT TCCTG CCAGA TTTGC CGGGG TGCTG CTTGC TCTGG CCCTC ATTTT
      51      GCCAG GGACC CTTTG TGCAG AAGGA ACTCG CGGCA GGTCA TCCAC GGCCC
      101     GATGC AGCCT TTTCG GAAGT GACTT CGTCA ACACC TTTGA TGGGA GCATG
      151     TACAG CTTTG CGGGA TACTG CAGTT ACCTC CTGGC AGGGG GCTGC CAGAA
      201     ACGCT CCTTC TCGAT TATTG GGGAC TTCCA GAATG GCAAG AGAGT GAGCC
      251     TCTCC GTGTA TCTTG GGGAA TTTT TGACA TCCAT TTGTT TGTC AATGGT
      301     ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
      351     GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
      401     ATGGC TTTGT GGCCA GATC GATGG CAGCG GCAAC TTTCA AGTCC TGCTG
      451     TCAGA CAGAT ACTTC AACAA GACCT GCGGG CTGTG TGGCA ACTTT AACAT
      501     CTTTG CTGAA GATGA CTTTA TGACC CAAGA AGGGA CCTTG ACCTC GGACC
      551     CTTAT GACTT TGCCA ACTCA TGGGC TCTGA GCAGT GGAGA ACAGT GGTGT
      601     GAACG GGCAT CTCCT CCCAG CAGCT CATGC AACAT CTCCT CTGGG GAAAT

```

```

651   GCAGA AGGGC CTGTG GGAGC AGTGC CAGCT TCTGA AGAGC ACCTC GGTGT
701   TTGCC CGCTG CCACC CTCTG GTGGA CCCCC AGCCT TTTGT GGCCC TGTGT
751   GAGAA GACTT TGTGT GAGTG TGCTG GGGGG CTGGA GTGCG CCTGC CCTGC
801   CCTCC TGGAG TACGC CCGGA CCTGT GCCCA GGAGG GAATG GTGCT GTACG
851   GCTGG ACCGA CCACA GCGCG TGCAG CCCAG TGTGC CCTGC TGGTA TGGAG
901   TATAG GCAGT GTGTG TCCCC TTGCG CCAGG ACCTG CCAGA GCCTG CACAT
951   CAATG AAATG TGTCA GGAGC GATGC GTGGA TGGCT GCAGC TGCCC TGAGG
1001  GACAG CTCCT GGATG AAGGC CTCTG CGTGG AGAGC ACCGA GTGTC CCTGC
1051  GTGCA TTCCG GAAAAG CGCTA CCCTC CCGGC ACCTC CCTCT CTCGA GACTG
1101  CAACA CCTGC ATTTG CCGAA ACAGC CAGTG GATCT GCAGC AATGA AGAAT
1151  GTCCA GGGGA GTGCC TTGTC ACTGG TCAAT CCCAC TTCAA GAGCT TTGAC
1201  AACAG ATACT TCACC TTCAG TGGGA TCTGC CAGTA CTGTC TGGCC CGGGA
1251  TTGCC AGGAC CACTC CTTCT CCATT GTCAT TGAGA CTGTC CAGTG TGCTG
1301  ATGAC CGCGA CGCTG TGTGC ACCCG CTCCG TCACC GTCCG GCTGC CTGGC
1351  CTGCA CAACA GCCTT GTGAA ACTGA AGCAT GGGGC AGGAG TTGCC ATGGA
1401  TGGCC AGGAC ATCCA GCTCC CCCTC CTGAA AGGTG ACCTC TGCAT CCAGC
1451  ATACA GTGAC GGCCCT CCGTG CGCCT CAGCT ACGGG GAGGA CCTGC AGATG
1501  GACTG GGATG GCCGC GGGAG GCTGC TGGTG AAGCT GTCCC CCGTC TATGC
1551  CGGGA AGACC TGCGG CCTGT GTGGG AATTA CAATG GCAAC CAGGG CGACG
1601  ACTTC CTTAC CCCCT CTGGG CTGGC GGAGC CCCGG GTGGA GGACT TCGGG
1651  AACGC CTGGA AGCTG CACGG GGACT GCCAG GACCT GCAGA AGCAG CACAG
1701  CGATC CCTGC GCCCT CAACC CGCGC ATGAC CAGGT TCTCC GAGGA GGCCT
1751  GCGCG GTCCT GACGT CCCCC ACATT CGAGG CCTGC CATCG TGCCG TCAGC
1801  CCGCT GCCCT ACCTG CGGAA CTGCC GCTAC GACGT GTGCT CCTGC TCGGA
1851  CGGCC GCGAG TGCCCT GTGCG GCGCC CTGGC CAGCT ATGCC GCGGC CTGCG
1901  CGGGG AGAGG CGTGC GCGTC GCGTG GCGCG AGCCA GGCCG CTGTG AGCTG
1951  AACTG CCCGA AAGGC CAGGT GTACC TGCAG TGCGG GACCC CCTGC AACCT
2001  GACCT GCCGC TCTCT CTCTT ACCCG GATGA GGAAT GCAAT GAGGC CTGCC
2051  TGGAG GGCTG CTTCT GCCCC CCAGG GCTCT ACATG GATGA GAGGG GGGAC
2101  TGCGT GCCCA AGGCC CAGTG CCCCT GTTAC TATGA CGGTG AGATC TTCCA
2151  GCCAG AAGAC ATCTT CTCAG ACCAT CACAC CATGT GCTAC TGTGA GGATG
2201  GCTTC ATGCA CTGTA CCATG AGTGG AGTCC CCGGA AGCTT GCTGC CTGAC
2251  GCTGT CCTCA GCAGT CCCCT GTCTC ATCGC AGCAA AAGGA GCCTA TCCTG
2301  TCGGC CCCCC ATGGT CAAGC TGGTG TGTCC CGCTG ACAAC CTGCG GGCTG
2351  AAGGG CTCGA GTGTA CCAAA ACGTG CCAGA ACTAT GACCT GGAGT GCATG
2401  AGCAT GGGCT GTGTC TCTGG CTGCC TCTGC CCCCC GGGCA TGGTC CGGCA
2451  TGAGA ACAGA TGTGT GGCCC TGGAA AGGTG TCCCT GCTTC CATCA GGGCA
2501  AGGAG TATGC CCCTG GAGAA ACAGT GAAGA TTGGC TGCAA CACTT GTGTC
2551  TGTCG GGACC GGAAG TGGAA CTGCA CAGAC CATGT GTGTG ATGCC ACGTG
2601  CTCCA CGATC GGCAT GGCCC ACTAC CTCAC CTTCG ACGGG CTCAA ATACC
2651  TGTTT CCCGG GGAGT GCCAG TACGT TCTGG TGCAG GATTA CTGCG GCAGT
2701  AACCC TGGGA CCTTT CGGAT CCTAG TGGGG AATAA GGGAT GCAGC CACCC
2751  CTCAG TGAAA TGCAA GAAAC GGGTC ACCAT CCTGG TGGAG GGAGG AGAGA
2801  TTGAG CTGTT TGACG GGGAG GTGAA TGTGA AGAGG CCCAT GAAGG ATGAG
2851  ACTCA CTTTG AGGTG GTGGA GTCTG GCCGG TACAT CATTC TGCTG CTGGG
2901  CAAAG CCCTC TCCGT GGTCT GGGAC CGCCA CCTGA GCATC TCCGT GGTCC
2951  TGAAG CAGAC ATACC AGGAG AAAGT GTGTG GCCTG TGTGG GAATT TTGAT
3001  GGCAT CCAGA ACAAT GACCT CACCA GCAGC AACCT CCAAG TGGAG GAAGA
3051  CCCTG TGGAC TTTGG GAACT CCTGG AAAGT GAGCT CGCAC TGTGC TGACA
3101  CCAGA AAAGT GCCTC TGGAC TCATC CCCTG CCACC TGCCA TAACA ACATC
3151  ATGAA GCAGA CGATG GTGGA TTCCCT CCTGT AGAAT CCTTA CCAGT GACGT
3201  CTTCC AGGAC TGCAA CAAGC TGGTG GACCC CGAGC CATAT CTGGA TGTCT
3251  GCATT TACGA CACCT GCTCC TGTGA GTCCA TTGGG GACTG CGCCG CATTC
3301  TGCGA CACCA TTGCT GCCTA TGCCC ACGTG TGTGC CCAGC ATGGC AAGGT
3351  GGTGA CCTGG AGGAC GGCCA CATTG TGCCC CCAGA GCTGC GAGGA GAGGA
3401  ATCTC CGGGA GAACG GGTAT GAGGC TGAGT GGCGC TATAA CAGCT GTGCA
3451  CCTGC CTGTC AAGTC ACGTG TCAGC ACCCT GAGCC ACTGG CCTGC CCTGT
3501  GCAGT GTGTG GAGGG CTGCC ATGCC CACTG CCCTC CAGGG AAAAT CCTGG
3551  ATGAG CTTTT GCAGA CCTGC GTTGA CCCTG AAGAC TGTCC AGTGT GTGAG

```

```

3601   GTGGC TGGCC GGCCT TTTGC CTCAG GAAAG AAAGT CACCT TGAAT CCCAG
3651   TGACC CTGAG CACTG CCAGA TTTGC CACTG TGATG TTGTC AACCT CACCT
3701   GTGAA GCCTG CCAGG AGCCG ATATC GGGTA CCTCA GAGTC TGCTA CCCCC
3751   GAGTC AGGGC CAGGA TCAGA GCCAG CCACC TCCGG GTCTG AGACA CCCGG
3801   GACTT CCGAG AGTGC CACCC CTGAG TCCGG ACCCG GTTCC GAGCC CGCCA
3851   CTTCC GGCTC CGAAA CTCCC GGCAC AAGCG AGAGC GCTAC CCCAG AGTCA
3901   GGACC AGGAA CATCT ACAGA GCCCT CTGAA GGCTC CGCTC CAGGG TCCCC
3951   AGCCG GCAGT CCCAC TAGCA CCGAG GAGGG AACCT CTGAA AGCGC CACAC
4001   CCGAA TCAGG GCCAG GGTCT GAGCC TGCTA CCAGC GGCAG CGAGA CACCA
4051   GGCAC CTCTG AGTCC GCCAC ACCAG AGTCC GGACC CGGAT CTCCC GCTGG
4101   GAGCC CCACC TCCAC TGAGG AGGGA TCTCC TGCTG GCTCT CCAAC ATCTA
4151   CTGAG GAAGG TACCT CAACC GAGCC ATCCG AGGGA TCAGC TCCCG GCACC
4201   TCAGA GTCGG CAACC CCGGA GTCTG GACCC GGAAC TTCCG AAAGT GCCAC
4251   ACCAG AGTCC GGTCC CGGGA CTTCA GAATC AGCAA CACCC GAGTC CGGCC
4301   CTGGG TCTGA ACCCG CCACA AGTGG TAGTG AGACA CCAGG ATCAG AACCT
4351   GCTAC CTCAG GGTCA GAGAC ACCCG GATCT CCGGC AGGCT CACCA ACCTC
4401   CACTG AGGAG GGCAC CAGCA CAGAA CCAAG CGAGG GCTCC GCACC CGGAA
4451   CAAGC ACTGA ACCCA GTGAG GGTTC AGCAC CCGGC TCTGA GCCGG CCACA
4501   AGTGG CAGTG AGACA CCCGG CACTT CAGAG AGTGC CACCC CCGAG AGTGG
4551   CCCAG GCACT AGTAC CGAGC CCTCT GAAGG CAGTG CGCCA GATTC TGGCG
4601   GTGGA GGTTC CGGTG GCGGG GGATC CGGTG GCGGG GGATC CGGTG GCGGG
4651   GGATC CGGTG GCGGG GGATC CCTGG TCCCC CGGGG CAGCG GAGGC GACAA
4701   AACTC ACACA TGCCC ACCGT GCCCA GCTCC AGAAC TCCTG GCGGG ACCGT
4751   CAGTC TTCCT CTTCC CCCC AACC CAAGG ACACC CTCAT GATCT CCCGG
4801   ACCCC TGAGG TCACA TGCGT GGTGG TGGAC GTGAG CCACG AAGAC CCTGA
4851   GGTCA AGTTC AACTG GTACG TGGAC GGCCT GGAGG TGCAT AATGC CAAGA
4901   CAAAG CCGCG GGAGG AGCAG TACAA CAGCA CGTAC CGTGT GTTCA GCGTC
4951   CTCAC CGTCC TGCAC CAGGA CTGGC TGAAT GGCAA GGAGT ACAAG TGCAA
5001   GGTCT CCAAC AAAGC CCTCC CAGCC CCCAT CGAGA AAACC ATCTC CAAAG
5051   CCAAA GGGCA GCCCC GAGAA CCACA GGTGT ACACC CTGCC CCCAT CCCGG
5101   GATGA GCTGA CCAAG AACCA GGTCA GCCTG ACCTG CCTGG TCAA GGCTT
5151   CTATC CCAGC GACAT CGCCG TGGAG TGGGA GAGCA ATGGG CAGCC GGAGA
5201   ACAAC TACAA GACCA CGCCT CCCGT GTTGG ACTCC GACGG CTCCT TCTTC
5251   CTCTA CAGCA AGCTC ACCGT GGCAC AGAGC AGGTG GCAGC AGGGG AACGT
5301   CTTCT CATGC TCCGT GATGC ATGAG GCTCT GCACA ACCAC TACAC GCAGA
5351   AGAGC CTCTC CCTGT CTCCG GTTAA ATGA

```

VWF034 Protein Sequence (SEQ ID NO: 87)

```

1     MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNNTFDGSM
51    YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
101   TVTQGDQRV S MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
151   SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
201   ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
251   EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
301   YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTCEPC
351   VHSGKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECLV TGQSHFKSFD
401   NRYFTTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
451   LHNSLVKLKH GAGVAMDGQD IQLPLLKGD LRIQHTVTASV RLSYGEDLQM
501   DWDGRGRLLV KLSVPYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
551   NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
601   PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCLE
651   NCPKQGVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
701   CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
751   AVLSSPLSHR SKRSLSCRPP MVKLVC PADN LRAEGLECTK TCQNYDLECM
801   SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
851   CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS

```

901 NPGTFRILVG NKGCSHPSVK CKKRVTLVE GGEIELFDGE VNVKRPMKDE
 951 THFEVVESGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
 1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
 1051 MKQTMVDSSC RILTSDFVD CNKLVDPEPY LDVCIYDTCS CESIGDCAAF
 1101 CDTIAAYAHV CAQHGKVVW RTATLCPQSC EERNLRENGY EAEWRYNSCA
 1151 PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
 1201 VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP ISGTSESATP
 1251 ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP GTSESATPES
 1301 GPGTSTEPSE GSAPGSPAGS PTSTEEGTSE SATPESGPGS EPATSGSETP
 1351 GTSESATPES GPGSPAGSPT STEEGSPAGS PTSTEEGTST EPSEGSAPGT
 1401 SESATPESGP GTSESATPES GPGTSESATP ESGPGSEPAT SGSETPGSEP
 1451 ATSGSETPGS PAGSPTSTEE GTSTEPSEGS APGTSTEPSE GSAPGSEPAT
 1501 SGSETPGTSE SATPESGPGT STEPSEGSAP DIGGGGGSGG GGS�VPRGSG
 1551 GDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
 1601 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
 1651 KCKVSNKALP APIEKTISKA KGQPREPVY TLPPSRDELT KNQVSLTCLV
 1701 KGFYPSDIAV EWESNGQPN NYKTTPPVL SDGSFFLYSK LTVDKSRWQQ
 1751 GNVFSCSVMH EALHNHYTQK SLSLSPGK*

VWF050 nucleotide sequence (IHH triple mutant) (SEQ ID NO: 149)

1 ATGAT TCCTG CCAGA TTTGC CGGGG TGCTG CTTGC TCTGG CCCTC ATTTT
 51 GCCAG GGACC CTTTG TGCAG AAGGA ACTCG CGGCA GGTCA TCCAC GGCCC
 101 GATGC AGCCT TTTCG GAAGT GACTT CGTCA ACACC TTTGA TGGGA GCATG
 151 TACAG CTTTG CGGGA TACTG CAGTT ACCTC CTGGC AGGGG GCTGC CAGAA
 201 ACGCT CCTTC TCGAT TATTG GGGAC TTCCA GAATG GCAAG AGAGT GAGCC
 251 TCTCC GTGTA TCTTG GGGAA TTTT TGACA TCCAT TTGTT TGTC AATGGT
 301 ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
 351 GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
 401 ATGGC TTTGT GGCCA GGATC GATGG CAGCG GCAAC TTTCA AGTCC TGCTG
 451 TCAGA CAGAT ACTTC AACAA GACCT GCGGG CTGTG TGGCA ACTTT AACAT
 501 CTTTG CTGAA GATGA CTTTA TGACC CAAGA AGGGA CCTTG ACCTC GGACC
 551 CTTAT GACTT TGCCA ACTCA TGGGC TCTGA GCAGT GGAGA ACAGT GGTGT
 601 GAACG GGCAT CTCCT CCCAG CAGCT CATGC AACAT CTCCT CTGGG GAAAT
 651 GCAGA AGGGC CTGTG GGAGC AGTGC CAGCT TCTGA AGAGC ACCTC GGTGT
 701 TTGCC CGCTG CCACC CTCTG GTGGA CCCCAG AGCCT TTTGT GGCCC TGTGT
 751 GAGAA GACTT TGTGT GAGTG TGCTG GGGGG CTGGA GTGCG CCTGC CTGCG
 801 CCTCC TGGAG TACGC CCGGA CCTGT GCCCA GGAGG GAATG GTGCT GTACG
 851 GCTGG ACCGA CCACA GCGCG TGCAG CCCAG TGTGC CCTGC TGGTA TGGAG
 901 TATAG GCAGT GTGTG TCCCC TTGCG CCAGG ACCTG CCAGA GCCTG CACAT
 951 CAATG AAATG TGTC AAGAG GATGC GTGGA TGGCT GCAGC TGCCC TGAGG
 1001 GACAG CTCCT GGATG AAGGC CTCTG CGTGG AGAGC ACCGA GTGTC CCTGC
 1051 GTGCA TTCCG GAAAG CGCTA CCCTC CCGGC ACCTC CCTCT CTCGA GACTG
 1101 CAACA CCTGC ATTTG CCGAA ACAGC CAGTG GATCT GCAGC AATGA AGAAT
 1151 GTCCA GGGGA GTGCC TTGTC ACTGG TCAAT CCCAC TTCAA GAGCT TTGAC
 1201 AACAG ATACT TCACC TTCAG TGGGA TCTGC CAGTA CTGTC TGGCC CGGGA
 1251 TTGCC AGGAC CACTC CTTCT CCATT GTCAT TGAGA CTGTC CAGTG TGCTG
 1301 ATGAC CGCGA CGCTG TGTGA ACCCG CTCCG TCACC GTCCG GCTGC CTGGC
 1351 CTGCA CAACA GCCTT GTGAA ACTGA AGCAT GGGGC AGGAG TTGCC ATGGA
 1401 TGGCC AGGAC ATCCA GCTCC CCCTC CTGAA AGGTG ACCTC CGCAT CCAGC
 1451 ATACA GTGAC GGCCCT CCGTG CGCCT CAGCT ACGGG GAGGA CCTGC AGATG
 1501 GACTG GGATG GCCGC GGGAG GCTGC TGGTG AAGCT GTCCC CCGTC TATGC
 1551 CGGGA AGACC TGCGG CCTGT GTGGG AATTA CAATG GCAAC CAGGG CGACG
 1601 ACTTC CTTAC CCCCT CTGGG CTGGC GGAGC CCCGG GTGGA GGACT TCGGG
 1651 AACGC CTGGA AGCTG CACGG GGACT GCCAG GACCT GCAGA AGCAG CACAG
 1701 CGATC CCTGC GCCCT CAACC CGCGC ATGAC CAGGT TCTCC GAGGA GGCCT
 1751 GCGCG GTCCCT GACGT CCCCC ACATT CGAGG CCTGC CATCG TGCCG TCAGC
 1801 CCGCT GCCCT ACCTG CCGAA CTGCC GCTAC GACGT GTGCT CCTGC TCGGA

```

1851   CGGCC GCGAG TGCCT GTGCG GCGCC CTGGC CAGCT ATGCC GCGGC CTGCG
1901   CGGGG AGAGG CGTGC GCGTC GCGTG GCGCG AGCCA GGCCG CTGTG AGCTG
1951   AACTG CCCGA AAGGC CAGGT GTACC TGCAG TGC GG GACCC CCTGC AACCT
2001   GACCT GCCGC TCTCT CTCTT ACCCG GATGA GGAAT GCAAT GAGGC CTGCC
2051   TGGAG GGCTG CTTCT GCCCC CCAGG GCTCT ACATG GATGA GAGGG GGGAC
2101   TGCGT GCCCA AGGCC CAGTG CCCCT GTTAC TATGA CGGTG AGATC TTCCA
2151   GCCAG AAGAC ATCTT CTCAG ACCAT CACAC CATGT GCTAC TGTGA GGATG
2201   GCTTC ATGCA CTGTA CCATG AGTGG AGTCC CCGGA AGCTT GCTGC CTGAC
2251   GCTGT CCTCA GCAGT CCCCT GTCTC ATCGC AGCAA AAGGA GCCTA TCCTG
2301   TCGGC CCCCC ATGGT CAAGC TGGTG TGTCC CGCTG ACAAC CTGCG GGCTG
2351   AAGGG CTCGA GTGTA CCAAA ACGTG CCAGA ACTAT GACCT GGAGT GCATG
2401   AGCAT GGGCT GTGTC TCTGG CTGCC TCTGC CCCCC GGGCA TGGTC CGGCA
2451   TGAGA ACAGA TGTGT GGCCC TGGAA AGGTG TCCCT GCTTC CATCA GGGCA
2501   AGGAG TATGC CCCTG GAGAA ACAGT GAAGA TTGGC TGCAA CACTT GTGTC
2551   TGTCG GGACC GGAAG TGGAA CTGCA CAGAC CATGT GTGTG ATGCC ACGTG
2601   CTCCA CGATC GGCAT GGCCC ACTAC CTCAC CTTCTG ACGGG CTCAA ATACC
2651   TGTTC CCCGG GGAGT GCCAG TACGT TCTGG TGCAG GATTA CTGCG GCAGT
2701   AACCC TGGGA CCTTT CGGAT CCTAG TGGGG AATAA GGGAT GCAGC CACCC
2751   CTCAG TGAAA TGCAA GAAAC GGGTC ACCAT CCTGG TGGAG GGAGG AGAGA
2801   TTGAG CTGTT TGACG GGGAG GTGAA TGTGA AGAGG CCCAT GAAGG ATGAG
2851   ACTCA CTTTG AGGTG GTGGA GTCTG GCCGG TACAT CATTC TGCTG CTGGG
2901   CAAAG CCCTC TCCGT GGTCT GGGAC CGCCA CCTGA GCATC TCCGT GGTCC
2951   TGAAG CAGAC ATACC AGGAG AAAGT GTGTG GCCTG TGTGG GAATT TTGAT
3001   GGCAT CCAGA ACAAT GACCT CACCA GCAGC AACCT CCAAG TGGAG GAAGA
3051   CCCTG TGGAC TTTGG GAACT CCTGG AAAGT GAGCT CGCAG TGTGC TGACA
3101   CCAGA AAAGT GCCTC TGGAC TCATC CCCTG CCACC TGCCA TAACA ACATC
3151   ATGAA GCAGA CGATG GTGGA TTCCT CCTGT AGAAT CCTTA CCAGT GACGT
3201   CTTCC AGGAC TGCAA CAAGC TGGTG GACCC CGAGC CATAT CTGGA TGTCT
3251   GCATT TACGA CACCT GCTCC TGTGA GTCCA TTGGG GACTG CGCCG CATTC
3301   TGCGA CACCA TTGCT GCCTA TGCCC ACGTG TGTGC CCAGC ATGGC AAGGT
3351   GGTGA CCTGG AGGAC GGCCA CATTG TGCCC CCAGA GCTGC GAGGA GAGGA
3401   ATCTC CGGGA GAACG GGTAT GAGGC TGAGT GGCGC TATAA CAGT CTGCA
3451   CTGTC CTGTC AAGTC ACGTG TCAGC ACCCT GAGCC ACTGG CTGTC CTGCT
3501   GCAGT GTGTG GAGGG CTGCC ATGCC CACTG CCCTC CAGGG AAAAT CCTGG
3551   ATGAG CTTTT GCAGA CCTGC GTTGA CCCTG AAGAC TGTCC AGTGT GTGAG
3601   GTGGC TGGCC GGCGT TTTGC CTCAG GAAAG AAAGT CACCT TGAAT CCCAG
3651   TGACC CTGAG CACTG CCAGA TTTGC CACTG TGATG TTGTC AACCT CACCT
3701   GTGAA GCCTG CCAGG AGCCG ATATC TGGCG GTGGA GGTTC CGGTG GCGGG
3751   GGATC CGGCG GTGGA GGTTC CGGCG GTGGA GGTTC CGGTG GCGGG GGATC
3801   CGGTG GCGGG GGATC CCTGG TCCCC GGGGG CAGCG GCGGT GGAGG TTCCG
3851   GTGGC GGGGG ATCCG AAAAA ACTCA CACAT GCCCA CCGTG CCCAG CTCCA
3901   GAACT CCTGG GCGGA CCGTC AGTCT TCCTC TTCCC CCCAA AACCC AAGGA
3951   CACCC TCATG GCCTC CCGGA CCCCT GAGGT CACAT GCGTG GTGGT GGACG
4001   TGAGC CACGA AGACC CTGAG GTCAA GTTCA ACTGG TACGT GGACG GCGTG
4051   GAGGT GCATA ATGCC AAGAC AAAGC CGCGG GAGGA GCAGT ACAAC AGCAC
4101   GTACC GTGTG GTCAG CGTCC TCACC GTCC TGGCC AGGAC TGGCT GAATG
4151   GCAAG GAGTA CAAGT GCAAG GTCTC CAACA AAGCC CTCCC AGCCC CCATC
4201   GAGAA AACCA TCTCC AAAGC CAAAG GGCAG CCCCC AGAAC CACAG GTGTA
4251   CACCC TGCCC CCATC CCGCG ATGAG CTGAC CAAGA ACCAG GTGAG CCTGA
4301   CCTGC CTGGT CAAAG GCTTC TATCC CAGCG ACATC GCCGT GGAGT GGGAG
4351   AGCAA TGGGC AGCCG GAGAA CAACT ACAAG ACCAC GCCTC CCGTG TTGGA
4401   CTCCG ACGGC TCCTT CTTCC TCTAC AGCAA GCTCA CCGTG GACAA GAGCA
4451   GGTGG CAGCA GGGGA ACGTC TTCTC ATGCT CCGTG ATGCA TGAGG CTCTG
4501   CACAA CGCCT ACACG CAGAA GAGCC TCTCC CTGTC TCCGG GTAAA TGA

```

VWF050 protein sequence (IHH triple mutant) (SEQ ID NO: 150)

1 MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNFTDGSMS

51 YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
 101 TVTQGDQRVs MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
 151 SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
 201 ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
 251 EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWDHSA CSPVCPAGME
 301 YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC
 351 VHS GKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGECIV TGQSHFKSFD
 401 NRYFTTFSGIC QYLLARDCQD HSFSIVIVTV QCADDRDAVC TRSVTVRLPG
 451 LHNSLVKLKH GAGVAMDGDQD IQLPLLKGDL RIQHTVTASV RLSYGEDLQM
 501 DWDGRGRLLV KLSPVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
 551 NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
 601 PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRCCL
 651 NCPKGQVYLQ CGTPCNLTCT SLSYPDEECN EACLEGCFCP PGLYMDERGD
 701 CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
 751 AVLSSPLSHR SKRSLSCRPP MVKLVC PADN LRAEGLECTK TCQNYDLECM
 801 SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
 851 CRDRKWNCTD HVCDATCSTI GMAHYLTDFG LKYLFPGEQ YVLVQDYCGS
 901 NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMDKE
 951 THFEVVESGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
 1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
 1051 MKQTMVDSSC RILTSDFVD CNKLVDP EY LDVCIYDTCS CESIGDCAAF
 1101 CDTIAAYAHV CAQH GKVV TW RTATLCPQSC EERNLRENGY EAEWRYNSCA
 1151 PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
 1201 VAGRRFASGK KVTLNPSDPE HCQICHCDVV NLTCEACQEP ISGGGGSGGG
 1251 GSGGGGSGGG GSGGGGSGGG GSLVPRGSGG GSGGGGSGDK THTCP PCPAP
 1301 ELLGGPSVFL FPPKPKDTLM ASRTPEVTCV VVDVSHEDPE VKFNWYVDGV
 1351 EVHNAKTKPR EEQYNSTYRV VSVLTVLAQD WLNGKEYKCK VSNKALPAPI
 1401 EKTISKAKGQ PREPQVY TLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE
 1451 SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL
 1501 HNAYTQKSLs LSPGK*

VWF057 nucleotide sequence (SEQ ID NO: 151)

1 ATGAT TCCTG CCAGA TTTGC CGGGG TGCTG CTTGC TCTGG CCCTC ATTTT
 51 GCCAG GGACC CTTTG TGCAG AAGGA ACTCG CGGCA GGTCA TCCAC GGCCC
 101 GATGC AGCCT TTTCG GAAGT GACTT CGTCA ACACC TTTGA TGGGA GCATG
 151 TACAG CTTTG CGGGA TACTG CAGTT ACCTC CTGGC AGGGG GCTGC CAGAA
 201 ACGCT CCTTC TCGAT TATTG GGGAC TTCCA GAATG GCAAG AGAGT GAGCC
 251 TCTCC GTGTA TCTTG GGGAA TTTT TGACA TCCAT TTGTT TGTCA ATGGT
 301 ACCGT GACAC AGGGG GACCA AAGAG TCTCC ATGCC CTATG CCTCC AAAGG
 351 GCTGT ATCTA GAAAC TGAGG CTGGG TACTA CAAGC TGTCC GGTGA GGCCT
 401 ATGGC TTTGT GGCCA GGATC GATGG CAGCG GCAAC TTTCA AGTCC TGCTG
 451 TCAGA CAGAT ACTTC AACAA GACCT GCGGG CTGTG TGGCA ACTTT AACAT
 501 CTTTG CTGAA GATGA CTTTA TGACC CAAGA AGGGA CCTTG ACCTC GGACC
 551 CTTAT GACTT TGCCA ACTCA TGGGC TCTGA GCAGT GGAGA ACAGT GGTGT
 601 GAACG GGCAT CTCCT CCCAG CAGCT CATGC AACAT CTCCT CTGGG GAAAT
 651 GCAGA AGGGC CTGTG GGAGC AGTGC CAGCT TCTGA AGAGC ACCTC GGTGT
 701 TTGCC CGCTG CCACC CTCTG TGGA CCCC AGCCT TTTGT GGCCC TGTGT
 751 GAGAA GACTT TGTGT GAGTG TGTG GGGG CTGGA GTGCG CCTGC CCTGC
 801 CCTCC TGGAG TACGC CCGGA CCTGT GCCCA GGAGG GAATG GTGCT GTACG
 851 GCTGG ACCGA CCACA GCGCG TGCAG CCCAG TGTGC CCTGC TGGTA TGGAG
 901 TATAG GCAGT GTGTG TCCCC TTGCG CCAGG ACCTG CCAGA GCCTG CACAT
 951 CAATG AAATG TGTCA GGAGC GATGC GTGGA TGGCT GCAGC TGCCC TGAGG
 1001 GACAG CTCCT GGATG AAGGC CTCTG CGTGG AGAGC ACCGA GTGTC CCTGC
 1051 GTGCA TTCCG GAAAG CGCTA CCCTC CCGGC ACCTC CCTCT CTCGA GACTG
 1101 CAACA CCTGC ATTTG CCGAA ACAGC CAGTG GATCT GCAGC AATGA AGAAT

1151	GTCCA	GGGGA	GTGCC	TTGTC	ACTGG	TCAAT	CCCAC	TTCAA	GAGCT	TTGAC
1201	AACAG	ATACT	TCACC	TTTCT	TGGGA	TCTGC	CAGTA	CCTGC	TGGCC	CGGGA
1251	TTGCC	AGGAC	CACTC	CTTCT	CCATT	GTCAT	TGAGA	CTGTC	CAGTG	TGCTG
1301	ATGAC	CGCGA	CGCTG	TGTGC	ACCCG	CTCCG	TCACC	GTCCG	GCTGC	CTGGC
1351	CTGCA	CAACA	GCCTT	GTGAA	ACTGA	AGCAT	GGGGC	AGGAG	TTGCC	ATGGA
1401	TGGCC	AGGAC	ATCCA	GCTCC	CCCTC	CTGAA	AGGTG	ACCTC	CGCAT	CCAGC
1451	ATACA	GTGAC	GGCCT	CCGTG	CGCCT	CAGCT	ACGGG	GAGGA	CCTGC	AGATG
1501	GACTG	GGATG	GCCGC	GGGAG	GCTGC	TGGTG	AAGCT	GTCCC	CCGTC	TATGC
1551	CGGGA	AGACC	TGCGG	CCTGT	GTGGG	AATTA	CAATG	GCAAC	CAGGG	CGACG
1601	ACTTC	CTTAC	CCCCT	CTGGG	CTGGC	GGAGC	CCCGG	GTGGA	GGACT	TCGGG
1651	AACGC	CTGGA	AGCTG	CACGG	GGACT	GCCAG	GACCT	GCAGA	AGCAG	CACAG
1701	CGATC	CCTGC	GCCCT	CAACC	CGCGC	ATGAC	CAGGT	TCTCC	GAGGA	GGCGT
1751	GCGCG	GTCTT	GACGT	CCCCC	ACATT	CGAGG	CCTGC	CATCG	TGCCG	TCAGC
1801	CCGCT	GCCCT	ACCTG	CGGAA	CTGCC	GCTAC	GACGT	GTGCT	CCTGC	TCGGA
1851	CGGCC	CGGAG	TGCCT	GTGCG	GCGCC	CTGGC	CAGCT	ATGCC	CGGGC	CTGCG
1901	CGGGG	AGAGG	CGTGC	GCGTC	GCGTG	GCGCG	AGCCA	GGCCG	CTGTG	AGCTG
1951	AACTG	CCCGA	AAGGC	CAGGT	GTACC	TGCAG	TGCGG	GACCC	CCTGC	AACCT
2001	GACCT	GCCGC	TCTCT	CTCTT	ACCCG	GATGA	GGAAT	GCAAT	GAGGC	CTGCC
2051	TGGAG	GGCTG	CTTCT	GCCCC	CCAGG	GCTCT	ACATG	GATGA	GAGGG	GGGAC
2101	TGCGT	GCCCA	AGGCC	CAGTG	CCCCT	GTTAC	TATGA	CGGTG	AGATC	TTCCA
2151	GCCAG	AAGAC	ATCTT	CTCAG	ACCAT	CACAC	CATGT	GCTAC	TGTGA	GGATG
2201	GCTTC	ATGCA	CTGTA	CCATG	AGTGG	AGTCC	CCGGA	AGCTT	GCTGC	CTGAC
2251	GCTGT	CCTCA	GCAGT	CCCCT	GTCTC	ATCGC	AGCAA	AAGGA	GCCTA	TCCTG
2301	TCGGC	CCCCC	ATGGT	CAAGC	TGGTG	TGTCC	CGCTG	ACAAC	CTGCG	GGCTG
2351	AAGGG	CTCGA	GTGTA	CCAAA	ACGTG	CCAGA	ACTAT	GACCT	GGAGT	GCATG
2401	AGCAT	GGGCT	GTGTC	TCTGG	CTGCC	TCTGC	CCCCC	GGGCA	TGGTC	CGGCA
2451	TGAGA	ACAGA	TGTGT	GGCCC	TGGAA	AGGTG	TCCCT	GCTTC	CATCA	GGGCA
2501	AGGAG	TATGC	CCCTG	GAGAA	ACAGT	GAAGA	TTGGC	TGCAA	CACTT	GTGTC
2551	TGTCG	GGACC	GGAAG	TGGAA	CTGCA	CAGAC	CATGT	GTGTG	ATGCC	ACGTG
2601	CTCCA	CGATC	GGCAT	GGCCC	ACTAC	CTCAC	CTTCG	ACGGG	CTCAA	ATACC
2651	TGTTT	CCCGG	GGAGT	GCCAG	TACGT	TCTGG	TGCAG	GATTA	CTGCG	GCAGT
2701	AACCC	TGGGA	CCTTT	CGGAT	CCTAG	TGGGG	AATAA	GGGAT	GCAGC	CACCC
2751	CTCAG	TGAAA	TGCAA	GAAAC	GGGTC	ACCAT	CCTGG	TGGAG	GGAGG	AGAGA
2801	TTGAG	CTGTT	TGACG	GGGAG	TTGAA	TGTGA	AGAGG	CCCAT	GAAGG	ATGAG
2851	ACTCA	CTTTG	AGGTG	GTGGA	GTCTG	GCCGG	TACAT	CATTG	TGCTG	CTGGG
2901	CAAAG	CCCTC	TCCGT	GGTCT	GGGAC	CGCCA	CCTGA	GCATC	TCCGT	GGTCC
2951	TGAAG	CAGAC	ATACC	AGGAG	AAAGT	GTGTG	GCCTG	TGTGG	GAATT	TTGAT
3001	GGCAT	CCAGA	ACAAT	GACCT	CACCA	GCAGC	AACCT	CCAAG	TGGAG	GAAGA
3051	CCCTG	TGGAC	TTTGG	GAACT	CCTGG	AAAGT	GAGCT	CGCAG	TGTGC	TGACA
3101	CCAGA	AAAGT	GCCTC	TGGAC	TCATC	CCCTG	CCACC	TGCCA	TAACA	ACATC
3151	ATGAA	GCAGA	CGATG	GTGGA	TTCTT	CCTGT	AGAAT	CCTTA	CCAGT	GACGT
3201	CTTCC	AGGAC	TGCAA	CAAGC	TGGTG	GACCC	CGAGC	CATAT	CTGGA	TGTCT
3251	GCATT	TACGA	CACCT	GCTCC	TGTGA	GTCCA	TTGGG	GACTG	CGCCG	CATTG
3301	TGCGA	CACCA	TTGCT	GCCTA	TGCCC	ACGTG	TGTGC	CCAGC	ATGGC	AAGGT
3351	GGTGA	CCTGG	AGGAC	GGCCA	CATTG	TGCCC	CCAGA	GCTGC	GAGGA	GAGGA
3401	ATCTC	CGGGA	GAACG	GGTAT	GAGGC	TGAGT	GGCGC	TATAA	CAGCT	GTGCA
3451	CCTGC	CTGTC	AAGTC	ACGTG	TCAGC	ACCCCT	GAGCC	ACTGG	CCTGC	CCTGT
3501	GCAGT	GTGTG	GAGGG	CTGCC	ATGCC	CACTG	CCCTC	CAGGG	AAAAT	CCTGG
3551	ATGAG	CTTTT	GCAGA	CCTGC	GTTGA	CCCTG	AAGAC	TGTCC	AGTGT	GTGAG
3601	GTGGC	TGGCC	GGCGT	TTTGC	CTCAG	GAAAG	AAAGT	CACCT	TGAAT	CCCAG
3651	TGACC	CTGAG	CACTG	CCAGA	TTTGC	CACCTG	TGATG	TTGTC	AACCT	CACCT
3701	GTGAA	GCCTG	CCAGG	AGCCG	ATATC	GGGCG	CGCCA	ACATC	AGAGA	GCGCC
3751	ACCCC	TGAAA	GTGGT	CCCGG	GAGCG	AGCCA	GCCAC	ATCTG	GGTCG	GAAAC
3801	GCCAG	GCACA	AGTGA	GTCTG	CAACT	CCCGA	GTCCG	GACCT	GGCTC	CGAGC
3851	CTGCC	ACTAG	CGGCT	CCGAG	ACTCC	GGGAA	CTTCC	GAGAG	CGCTA	CACCA
3901	GAAAG	CGGAC	CCGGA	ACCAG	TACCG	AACCT	AGCGA	GGGCT	CTGCT	CCGGG
3951	CAGCC	CAGCC	GGCTC	TCCTA	CATCC	ACGGA	GGAGG	GCACT	TCCGA	ATCCG
4001	CCACC	CCGGA	GTCAG	GGCCA	GGATC	TGAAC	CCGCT	ACCTC	AGGCA	GTGAG
4051	ACGCC	AGGAA	CGAGC	GAGTC	CGCTA	CACCG	GAGAG	TGGGC	CAGGG	AGCCC

```

4101   TGCTG GATCT CCTAC GTCCA CTGAG GAAGG GTCAC CAGCG GGCTC GCCCA
4151   CCAGC ACTGA AGAAG GTGCC TCGAG CGGCG GTGGA GGTTC CGGTG GCGGG
4201   GGATC CGGTG GCGGG GGATC CGGTG GCGGG GGATC CGGTG GCGGG GGATC
4251   CCTGG TCCCC CGGGG CAGCG GAGGC GACAA AACTC ACACA TGCCC ACCGT
4301   GCCCA GCTCC AGAAC TCCTG GGCGG ACCGT CAGTC TTCCT CTTCC CCCCC
4351   AAACC CAAGG ACACC CTCAT GATCT CCCGG ACCCC TGAGG TCACA TGCGT
4401   GGTGG TGGAC GTGAG CCACG AAGAC CCTGA GGTCA AGTTC AACTG GTACG
4451   TGGAC GGCCT GGAGG TGCAT AATGC CAAGA CAAAG CCGCG GGAGG AGCAG
4501   TACAA CAGCA CGTAC CGTGT GGTC A GCGTC CTCAC CGTCC TGCAC CAGGA
4551   CTGGC TGAAT GGCAA GGAGT ACAAG TGCAA GGTCT CCAAC AAAGC CCTCC
4601   CAGCC CCCAT CGAGA AAACC ATCTC CAAAG CAAA GGGCA GCCCC GAGAA
4651   CCACA GGTGT ACACC CTGCC CCCAT CCCGG GATGA GCTGA CCAAG AACCA
4701   GGTCA GCCTG ACCTG CCTGG TCAAA GGCTT CTATC CCAGC GACAT CGCCG
4751   TGGAG TGGGA GAGCA ATGGG CAGCC GGAGA ACAAC TACAA GACCA CGCCT
4801   CCCGT GTTGG ACTCC GACGG CTCCT TCTTC CTCTA CAGCA AGCTC ACCGT
4851   GGACA AGAGC AGGTG GCAGC ATGGG AACGT CTTCT CATGC TCCGT GATGC
4901   ATGAG GCTCT GCACA ACCAC TACAC GCAGA AGAGC CTCTC CCTGT CTCCG
4951   GTAA ATGA

```

VWF057 protein sequence (SEQ ID NO: 152)

```

1     MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNFTFDGSM
51    YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
101   TVTQGDQRV S MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
151   SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
201   ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
251   EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
301   YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTTEPC
351   VHS GKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGEC LV TGQSHFKSFD
401   NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
451   LHN SLVKLKH GAGVAMDQD IQLPLLKGD L RIQHTVTASV RLSYGEDLQM
501   DWDGRGRLLV KLS PVYAGKT CGLCGNYNGN QGDDFLTPSG LAEPREDFG
551   NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TWEACHRAVS
601   PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWEPRGRCEL
651   NCPKQGVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
701   CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
751   AVLSSPLSHR SKRSLSCRPP MVKLVC PADN LRAEGLECTK TCQNYDLECM
801   SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
851   CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
901   NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
951   THFEVVE SGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
1001  GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
1051  MKQTMVDSSC RILTSDFVD CNKLVDPEPY LDVCIYDTC SCSIGDCAAF
1101  CDTIAAYAHV CAQH GKVV TW RTATLCPQSC EERNLRENGY EAEWRYN SCA
1151  PACQVTCQHP EPLACPVCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
1201  VAGRRFASGK KVT LNPSDPE HCQICHCDVV NLTCEACQEP ISGAPTSESA
1251  TPESGPGSEP ATSGSETPGT SESATPESGP GSEPATSGSE TPGTSESATP
1301  ESGPGTSTEP ESGPGSPA GSPTSTEEGT SESATPESGP GSEPATSGSE
1351  TPGTSESATP ESGPGSPA GSPTSTEEGT SESATPESGP GSEPATSGSE
1401  GSGGGGSGGG GSGGGGSLVP RSGGGDKTHT CPPCPAPELL GGPSVFLFPP
1451  KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ
1501  YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKQPRE
1551  PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYK TTP
1601  PVLDS DGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
1651  GK*

```

VWF058 nucleotide sequence (VWF034 with IHH mutation) (SEQ ID NO: 153)

```

1     ATGAT TCCTG CCAGA TTTGC CGGGG TGCTG CTTGC TCTGG CCCTC ATTTT

```

51	GCCAG	GGACC	CTTTG	TGCAG	AAGGA	ACTCG	CGGCA	GGTCA	TCCAC	GGCCC
101	GATGC	AGCCT	TTTCG	GAAGT	GACTT	CGTCA	ACACC	TTTGA	TGGGA	GCATG
151	TACAG	CTTTG	CGGGA	TACTG	CAGTT	ACCTC	CTGGC	AGGGG	GCTGC	CAGAA
201	ACGCT	CCTTC	TCGAT	TATTG	GGGAC	TTCCA	GAATG	GCAAG	AGAGT	GAGCC
251	TCTCC	GTGTA	TCTTG	GGGAA	TTTTT	TGACA	TCCAT	TTGTT	TGTCA	ATGGT
301	ACCGT	GACAC	AGGGG	GACCA	AAGAG	TCTCC	ATGCC	CTATG	CCTCC	AAAGG
351	GCTGT	ATCTA	GAAAC	TGAGG	CTGGG	TACTA	CAAGC	TGTCC	GGTGA	GGCCT
401	ATGGC	TTTGT	GGCCA	GGATC	GATGG	CAGCG	GCAAC	TTTCA	AGTCC	TGCTG
451	TCAGA	CAGAT	ACTTC	AACAA	GACCT	GCGGG	CTGTG	TGGCA	ACTTT	AACAT
501	CTTTG	CTGAA	GATGA	CTTTA	TGACC	CAAGA	AGGGA	CCTTG	ACCTC	GGACC
551	CTTAT	GACTT	TGCCA	ACTCA	TGGGC	TCTGA	GCAGT	GGAGA	ACAGT	GGTGT
601	GAACG	GGCAT	CTCCT	CCCAG	CAGCT	CATGC	AACAT	CTCCT	CTGGG	GAAAT
651	GCAGA	AGGGC	CTGTG	GGAGC	AGTGC	CAGCT	TCTGA	AGAGC	ACCTC	GGTGT
701	TTGCC	CGCTG	CCACC	CTCTG	GTGGA	CCCCG	AGCCT	TTTGT	GGCCC	TGTGT
751	GAGAA	GACTT	TGTGT	GAGTG	TGCTG	GGGGG	CTGGA	TGCGC	CCTGC	CCTGC
801	CCTCC	TGGAG	TACGC	CCGGA	CCTGT	GCCCA	GGAGG	GAATG	GTGCT	GTACG
851	GCTGG	ACCGA	CCACA	GCGCG	TGCAG	CCCAG	TGTGC	CCTGC	TGGTA	TGGAG
901	TATAG	GCAGT	GTGTG	TCCCC	TTGCG	CCAGG	ACCTG	CCAGA	GCCTG	CACAT
951	CAATG	AAATG	TGTCA	GGAGC	GATGC	GTGGA	TGGCT	GCAGC	TGCCC	TGAGG
1001	GACAG	CTCCT	GGATG	AAGGC	CTCTG	CGTGG	AGAGC	ACCGA	GTGTC	CCTGC
1051	GTGCA	TTCCG	GAAAG	CGCTA	CCCTC	CCGGC	ACCTC	CCTCT	CTCGA	GA CTG
1101	CAACA	CCTGC	ATTTG	CCGAA	ACAGC	CAGTG	GATCT	GCAGC	AATGA	AGAAT
1151	GTCCA	GGGGA	GTGCC	TTGTC	ACTGG	TCAAT	CCCAC	TTCAA	GAGCT	TTGAC
1201	AACAG	ATACT	TCACC	TTCAG	TGGGA	TCTGC	CAGTA	CCTGC	TGGCC	CGGGA
1251	TTGCC	AGGAC	CACTC	CTTCT	CCATT	GTCAT	TGAGA	CTGTC	CAGTG	TGCTG
1301	ATGAC	CGCGA	CGCTG	TGTGC	ACCCG	CTCCG	TCACC	GTCCG	GCTGC	CTGGC
1351	CTGCA	CAACA	GCCTT	GTGAA	ACTGA	AGCAT	GGGGC	AGGAG	TTGCC	ATGGA
1401	TGGCC	AGGAC	ATCCA	GCTCC	CCCTC	CTGAA	AGGTG	ACCTC	CGCAT	CCAGC
1451	ATACA	GTGAC	GGCCT	CCGTG	CGCCT	CAGCT	ACGGG	GAGGA	CCTGC	AGATG
1501	GACTG	GGATG	GCCGC	GGGAG	GCTGC	TGGTG	AAGCT	GTCCC	CCGTC	TATGC
1551	CGGGA	AGACC	TGCGG	CCTGT	GTGGG	AATTA	CAATG	GCAAC	CAGGG	CGACG
1601	ACTTC	CTTAC	CCCCT	CTGGG	CTGGC	GGAGC	CCCGG	GTGGA	GGACT	TCGGG
1651	AACGC	CTGGA	AGCTG	CACGG	GGACT	GCCAG	GACCT	GCAGA	AGCAG	CACAG
1701	CGATC	CCTGC	GCCCT	CAACC	GCGCG	ATGAC	CAGGT	TCTCC	GAGGA	GGCGT
1751	GCGCG	GTCTT	GACGT	CCCCC	ACATT	CGAGG	CCTGC	CATCG	TGCCG	TCAGC
1801	CCGCT	GCCCT	ACCTG	CGGAA	CTGCC	GCTAC	GACGT	GTGCT	CCTGC	TCGGA
1851	CGGCC	GCGAG	TGCCT	GTGCG	GCGCC	CTGGC	CAGCT	ATGCC	GCGGC	CTGCG
1901	CGGGG	AGAGG	CGTGC	GCGTC	GCGTG	GCGCG	AGCCA	GGCCG	CTGTG	AGCTG
1951	AACTG	CCCGA	AAGGC	CAGGT	GTACC	TGCAG	TGCGG	GACCC	CCTGC	AACCT
2001	GACCT	GCCGC	TCTCT	CTCTT	ACCCG	GATGA	GGAAT	GCAAT	GAGGC	CTGCC
2051	TGGAG	GGCTG	CTTCT	GCCCC	CCAGG	GCTCT	ACATG	GATGA	GAGGG	GGGAC
2101	TGCGT	GCCCA	AGGCC	CAGTG	CCCCT	GTTAC	TATGA	CGGTG	AGATC	TTCCA
2151	GCCAG	AAGAC	ATCTT	CTCAG	ACCAT	CACAC	CATGT	GCTAC	TGTGA	GGATG
2201	GCTTC	ATGCA	CTGTA	CCATG	AGTGG	AGTCC	CCGGA	AGCTT	GCTGC	CTGAC
2251	GCTGT	CCTCA	GCAGT	CCCCT	GTCTC	ATCGC	AGCAA	AAGGA	GCCTA	TCCTG
2301	TCGGC	CCCCC	ATGGT	CAAGC	TGGTG	TGTCC	CGCTG	ACAAC	CTGCG	GGCTG
2351	AAGGG	CTCGA	GTGTA	CCAAA	ACGTG	CCAGA	ACTAT	GACCT	GGAGT	GCATG
2401	AGCAT	GGGCT	GTGTC	TCTGG	CTGCC	TCTGC	CCCCC	GGGCA	TGGTC	CGGCA
2451	TGAGA	ACAGA	TGTGT	GGCCC	TGGAA	AGGTG	TCCCT	GCTTC	CATCA	GGGCA
2501	AGGAG	TATGC	CCCTG	GAGAA	ACAGT	GAAGA	TTGGC	TGCAA	CAC TT	GTGTC
2551	TGTCG	GGACC	GGAAG	TGGAA	CTGCA	CAGAC	CATGT	GTGTG	ATGCC	ACGTG
2601	CTCCA	CGATC	GGCAT	GGCCC	ACTAC	CTCAC	CTTCG	ACGGG	CTCAA	ATACC
2651	TGTTT	CCCGG	GGAGT	GCCAG	TACGT	TCTGG	TGCAG	GATTA	CTGCG	GCAGT
2701	AACCC	TGGGA	CCTTT	CGGAT	CCTAG	TGGGG	AATAA	GGGAT	GCAGC	CACCC
2751	CTCAG	TGAAA	TGCAA	GAAAC	GGGTC	ACCAT	CCTGG	TGGAG	GGAGG	AGAGA
2801	TTGAG	CTGTT	TGACG	GGGAG	GTGAA	TGTGA	AGAGG	CCCAT	GAAGG	ATGAG
2851	ACTCA	CTTTG	AGGTG	GTGGA	GTCTG	GCCGG	TACAT	CATTC	TGCTG	CTGGG
2901	CAAAG	CCCTC	TCCGT	GGTCT	GGGAC	CGCCA	CCTGA	GCATC	TCCGT	GGTCC
2951	TGAAG	CAGAC	ATACC	AGGAG	AAAGT	GTGTG	GCCTG	TGTGG	GAATT	TTGAT

```

3001   GGCAT CCAGA ACAAT GACCT CACCA GCAGC AACCT CCAAG TGGAG GAAGA
3051   CCCTG TGGAC TTTGG GAACT CCTGG AAAGT GAGCT CGCAG TGTGC TGACA
3101   CCAGA AAAGT GCCTC TGGAC TCATC CCCTG CCACC TGCCA TAACA ACATC
3151   ATGAA GCAGA CGATG GTGGA TTCCT CCTGT AGAAT CCTTA CCAGT GACGT
3201   CTTCC AGGAC TGCAA CAAGC TGGTG GACCC CGAGC CATAT CTGGA TGTCT
3251   GCATT TACGA CACCT GCTCC TGTGA GTCCA TTGGG GACTG CGCCG CATTC
3301   TGCGA CACCA TTGCT GCCTA TGCCC ACGTG TGTGC CCAGC ATGGC AAGGT
3351   GGTGA CCTGG AGGAC GGCCA CATTG TGCCC CCAGA GCTGC GAGGA GAGGA
3401   ATCTC CGGGA GAACG GGTAT GAGGC TGAGT GGCGC TATAA CAGCT GTGCA
3451   CCTGC CTGTC AAGTC ACGTG TCAGC ACCCT GAGCC ACTGG CCTGC CCTGT
3501   GCAGT GTGTG GAGGG CTGCC ATGCC CACTG CCCTC CAGGG AAAAT CCTGG
3551   ATGAG CTTTT GCAGA CCTGC GTTGA CCCTG AAGAC TGTCC AGTGT GTGAG
3601   GTGGC TGGCC GCGGT TTTGC CTCAG GAAAG AAAGT CACCT TGAAT CCCAG
3651   TGACC CTGAG CACTG CCAGA TTTGC CACTG TGATG TTGTC AACCT CACCT
3701   GTGAA GCGTG CCAGG AGCCG ATATC GGGTA CCTCA GAGTC TGTCA CCCCC
3751   GAGTC AGGGC CAGGA TCAGA GCCAG CCACC TCCGG GTCTG AGACA CCCCG
3801   GACTT CCGAG AGTGC CACCC CTGAG TCCGG ACCCG GGTCC GAGCC CGCCA
3851   CTTCC GGCTC CGAAA CTCCC GGCAC AAGCG AGAGC GCTAC CCCAG AGTCA
3901   GGACC AGGAA CATCT ACAGA GCCCT CTGAA GGCTC CGCTC CAGGG TCCCC
3951   AGCCG GCAGT CCCAC TAGCA CCGAG GAGGG AACCT CTGAA AGCGC CACAC
4001   CCGAA TCAGG GCCAG GGTCT GAGCC TGCTA CCAGC GGCAG CGAGA CACCA
4051   GGCAC CTCTG AGTCC GCCAC ACCAG AGTCC GGACC CGGAT CTCCC GCTGG
4101   GAGCC CCACC TCCAC TGAGG AGGGA TCTCC TGCTG GCTCT CCAAC ATCTA
4151   CTGAG GAAGG TACCT CAACC GAGCC ATCCG AGGGA TCAGC TCCCG GCACC
4201   TCAGA GTCGG CAACC CCGGA GTCTG GACCC GGAAC TTCCG AAAGT GCCAC
4251   ACCAG AGTCC GGTCC CGGGA CTTC AATC AGCAA CACCC GAGTC CGGCC
4301   CTGGG TCTGA ACCCG CCACA AGTGG TAGTG AGACA CCAGG ATCAG AACCT
4351   GCTAC CTCAG GGTCA GAGAC ACCCG GATCT CCGGC AGGCT CACCA ACCTC
4401   CACTG AGGAG GGCAC CAGCA CAGAA CCAAG CGAGG GCTCC GCACC CGGAA
4451   CAAGC ACTGA ACCCA GTGAG GGTTC AGCAC CCGGC TCTGA GCCGG CCACA
4501   AGTGG CAGTG AGACA CCCGG CACTT CAGAG AGTGC CACCC CCGAG AGTGG
4551   CCCAG GCACT AGTAC CGAGC CCTCT GAAGG CAGTG CGCCA GATTC TGGCG
4601   GTGGA GGTTC CGGTG GCGGG GGATC CGGTG GCGGG GGATC CGGTG GCGGG
4651   GGATC CGGTG GCGGG GGATC CTTGG TCCCC CCGGG CAGCG GAGGC GACAA
4701   AACTC ACACA TGCCC ACCGT GCCCA GCTCC AGAAC TCCTG GGCGG ACCGT
4751   CAGTC TTCCT CTTCC CCCCC AAACC CAAGG ACACC CTCAT GGCCT CCCGG
4801   ACCCC TGAGG TCACA TGCGT GGTGG TGGAC GTGAG CCACG AAGAC CCTGA
4851   GGTCA AGTTC AACTG GTACG TGGAC GGCGT GGAGG TGCAT AATGC CAAGA
4901   CAAAG CCGCG GGAGG AGCAG TACAA CAGCA CGTAC CGTGT GGTCA GCGTC
4951   CTCAC CGTCC TGGCC CAGGA CTGGC TGAAT GGCAA GGAGT ACAAG TGCAA
5001   GGTCT CCAAC AAAGC CCTCC CAGCC CCCAT CGAGA AAACC ATCTC CAAAG
5051   CAAA GGGCA GCCCC GAGAA CCACA GGTGT ACACC CTGCC CCCAT CCCGC
5101   GATGA GCTGA CCAAG AACCA GGTCA GCCTG ACCTG CCTGG TCAAA GGCTT
5151   CTATC CCAGC GACAT CGCCG TGGAG TGGGA GAGCA ATGGG CAGCC GGAGA
5201   ACAAC TACAA GACCA CGCCT CCCGT GTTGG ACTCC GACGG CTCCT TCTTC
5251   CTCTA CAGCA AGCTC ACCGT GGACA AGAGC AGGTG GCAGC AGGGG AACGT
5301   CTTCT CATGC TCCGT GATGC ATGAG GCTCT GCACA ACGCC TACAC GCAGA
5351   AGAGC CTCTC CCTGT CTCCG GTTAA ATGA

```

VWF058 protein sequence (VWF034 with IHH mutation) (SEQ ID NO: 154)

```

1     MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNNTFDGSM
51    YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
101   TVTQGDQRVs MPYASKGLYL ETEAGYYKLS GEAYGFVARI DSGSNFQVLL
151   SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
201   ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
251   EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWDHSA CSPVCPAGME
301   YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTTEPC

```

```

351  VHSGKRYPPG  TSLSRDCNTC  ICRNSQWICS  NEECPGECLV  TGQSHFKSFD
401  NRYFTFSGIC  QYLLARDCQD  HSFSIVIETV  QCADDRDAVC  TRSVTVRLPG
451  LHNSLVKCLKH  GAGVAMDQD  IQLPLLKGD  RIQHTVTASV  RLSYGEDLQM
501  DWDGRGRLLV  KLSFVYAGKT  CGLCGNYNGN  QGDDFLTSPG  LAEPRVEDFG
551  NAWKLHGDCQ  DLQKQHSDFC  ALNPRMTRFS  EEACAVLTSP  TFEACHRAVS
601  PLPYLRNCRY  DVCSCSDGRE  CLCGALASYA  AACAGRGVRV  AWREPRGCEL
651  NCPKGQVYLQ  CGTPCNLTCT  SLSYPDEECN  EACLEGCFCP  PGLYMDERGD
701  CVPKAQCPCY  YDGEIFQPED  IFSDHHTMCY  CEDGFMHCTM  SGVPGSLLPD
751  AVLSSPLSHR  SKRSLSCRPP  MVKLVCPADN  LRAEGLECTK  TCQNYDLECM
801  SMGCVSGCLC  PPGMVRHENR  CVALERCPCF  HQGKEYAPGE  TVKIGCNTCV
851  CRDRKWNCTD  HVCDATCSTI  GMAHYLTDFG  LKYLFPGEQ  YVLVQDYCGS
901  NPGTFRILVG  NKGCSHPSVK  CKKRVTLVE  GGEIELFDGE  VNVKRPMDKE
951  THFEVVESGR  YIILLGKAL  SVVWDRHLSI  SVVLKQTYQE  KVCGLCGNFD
1001  GIQNNDLTSS  NLQVEEDPVD  FGNSWKVSSQ  CADTRKVPLD  SSPATCHNNI
1051  MKQTMVDSSC  RILTSDFVQD  CNKLVDPEPY  LDVCIYDTC  CESIGDCAAF
1101  CDTIAAYAHV  CAQHGVVVTW  RTATLCPQSC  EERNLRENGY  EAEWRYNSCA
1151  PACQVTCQHP  EPLACPVQCV  EGCHAHCPPG  KILDELLQTC  VDPEDCPVCE
1201  VAGRRFASGK  KVTLNPSDPE  HCQICHCDVV  NLTCEACQEP  ISGTSESATP
1251  ESGPGSEPAT  SGSETPGTSE  SATPESGPGS  EPATSGSETP  GTSESATPES
1301  GPGTSTEPSE  GSAPGSPAGS  PTSTEEGTSE  SATPESGPGS  EPATSGSETP
1351  GTSESATPES  GPGSPAGSPT  STEEGSPAGS  PTSTEEGTST  EPSEGSAPGT
1401  SESATPESGP  GTSESATPES  GPGTSESATP  ESGPGSEPAT  SGSETPGSEP
1451  ATSGSETPGS  PAGSPTSTEE  GTSTEPSEGS  APGTSTEPSE  GSAPGSEPAT
1501  SGSETPGTSE  SATPESGPGT  STEPSEGSAP  DSGGGGSGGG  GSGGGGSGGG
1551  GSGGGGSLVP  RSGGGDKTHT  CPPCPAPELL  GGPSVFLFPP  KPKDTLMASR
1601  TPEVTCVVVD  VSHEDPEVKF  NWYVDGVEVH  NAKTKPREEQ  YNSTYRVVSV
1651  LTVLAQDWLN  GKEYKCKVSN  KALPAPIEKT  ISKAKGQPRE  PQVYTLPPSR
1701  DELTKNQVSL  TCLVKGFYPS  DIAVEWESNG  QPENNYKTP  PVLDSGDSFF
1751  LYSKLTVDKS  RWQQGNVFSC  SVMHEALHNA  YTQKSLSLSP  GK*

```

FVIII 169 nucleotide sequence (SEQ ID NO: 155)

```

1    ATGCA AATAG AGCTC TCCAC CTGCT TCTTT CTGTG CCTTT TCGCA TTCTG
51   CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
101  ACTAT ATGCA AAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCCT
151  CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG TGTAC AAAAA
201  GACTC TGTTT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
251  GGCCA CCCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT
301  GATAC AGTGG TCATT ACACT TAAGA ACATG GCTTC CCATC CTGTC AGTCT
351  TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
401  ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTCC CTGGT
451  GGAAG CCATA CATAT GTCTG GCAGG TCCTG AAAGA GAATG GTCCA ATGGC
501  CTCTG ACCCA CTGTG CCTTA CCTAC TCATA TCTTT CTCAT GTGGA CCTGG
551  TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
601  GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
651  TTTTG CTGTA TTTGA TGAAG GGAAA AGTTG GCACT CAGAA ACAA GAACT
701  CCTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG
751  CACAC AGTCA ATGGT TATGT AAACA GGTCT CTGCC AGGTC TGATT GGATG
801  CCACA GGAAT TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
851  AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
901  CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CTTA CTGCT CAAAC
951  ACTCT TGATG GACCT TGGAC AGTTT CTACT GTTTT GTCAT ATCTC TTCCC
1001  ACCAA CATGA TGGCA TGGAA GCTTA TGTC AAGTA GACAG CTGTC CAGAG
1051  GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
1101  TGATC TTAAT GATTC TGAAA TGGAT GTGGT CAGGT TTGAT GATGA CAACT
1151  CTCCT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAATG
1201  TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GGACT ATGCT CCCTT
1251  AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG

```

1301	GCCCT	CAGCG	GATTG	GTAGG	AAGTA	CAAAA	AAGTC	CGATT	TATGG	CATAC
1351	ACAGA	TGAAA	CCTTT	AAGAC	TCGTG	AAGCT	ATTCA	GCATG	AATCA	GGAAT
1401	CTTGG	GACCT	TTACT	TTATG	GGGAA	GTTGG	AGACA	CACTG	TTGAT	TATAT
1451	TTAAG	AATCA	AGCAA	GCAGA	CCATA	TAACA	TCTAC	CCTCA	CGGAA	TCACT
1501	GATGT	CCGTC	CTTTG	TATTC	AAGGA	GATTA	CCAAA	AGGTG	TAAAA	CATTT
1551	GAAGG	ATTTT	CCAAT	TCTGC	CAGGA	GAAAT	ATTCA	AATAT	AAATG	GACAG
1601	TGACT	GTAGA	AGATG	GGCCA	ACTAA	ATCAG	ATCCT	CGGTG	CCTGA	CCCGC
1651	TATTA	CTCTA	GTTTC	GTTAA	TATGG	AGAGA	GATCT	AGCTT	CAGGA	CTCAT
1701	TGGCC	CTCTC	CTCAT	CTGCT	ACAAA	GAATC	TGTAG	ATCAA	AGAGG	AAACC
1751	AGATA	ATGTC	AGACA	AGAGG	AATGT	CATCC	TGTTT	TCTGT	ATTTG	ATGAG
1801	AACCG	AAGCT	GGTAC	CTCAC	AGAGA	ATATA	CAACG	CTTTC	TCCCC	AATCC
1851	AGCTG	GAGTG	CAGCT	TGAGG	ATCCA	GAGTT	CCAAG	CCTCC	AACAT	CATGC
1901	ACAGC	ATCAA	TGGCT	ATGTT	TTTGA	TAGTT	TGCAG	TTGTC	AGTTT	GTTTG
1951	CATGA	GGTGG	CATAC	TGGTA	CATTC	TAAGC	ATTGG	AGCAC	AGACT	GACTT
2001	CCTTT	CTGTC	TTCTT	CTCTG	GATAT	ACCTT	CAAA	ACAAA	ATGGT	CATATG
2051	AAGAC	ACACT	CACCC	TATTC	CCATT	CTCAG	GAGAA	ACTGT	CTTCA	TGTCG
2101	ATGGA	AAACC	CAGGT	CTATG	GATTC	TGGGG	TGCCA	CAACT	CAGAC	TTTCG
2151	GAACA	GAGGC	ATGAC	CGCCT	TACTG	AAGGT	TTCTA	GTTGT	GACAA	GAACA
2201	CTGGT	GATTA	TTACG	AGGAC	AGTTA	TGAAG	ATATT	TCAGC	ATACT	TGCTG
2251	AGTAA	AAACA	ATGCC	ATTGA	ACCAA	GAAGC	TTCTC	TCAAA	ACGGC	GCGCC
2301	AGGTA	CCTCA	GAGTC	TGCTA	CCCCC	GAGTC	AGGGC	CAGGA	TCAGA	GCCAG
2351	CCACC	TCCGG	GTCTG	AGACA	CCCGG	GACTT	CCGAG	AGTGC	CACCC	CTGAG
2401	TCCGG	ACCCG	GGTCC	GAGCC	CGCCA	CTTCC	GGCTC	CGAAA	CTCCC	GGCAC
2451	AAGCG	AGAGC	GCTAC	CCCAG	AGTCA	GGACC	AGGAA	CATCT	ACAGA	GCCCT
2501	CTGAA	GGCTC	CGCTC	CAGGG	TCCCC	AGCCG	GCAGT	CCCAC	TAGCA	CCGAG
2551	GAGGG	AACCT	CTGAA	AGCGC	CACAC	CCGAA	TCAGG	GCCAG	GGTCT	GAGCC
2601	TGCTA	CCAGC	GGCAG	CGAGA	CACCA	GGCAC	CTCTG	AGTCC	GCCAC	ACCAG
2651	AGTCC	GGACC	CGGAT	CTCCC	GCTGG	GAGCC	CCACC	TCCAC	TGAGG	AGGGA
2701	TCTCC	TGCTG	GCTCT	CCAAC	ATCTA	CTGAG	GAAGG	TACCT	CAACC	GAGCC
2751	ATCCG	AGGGA	TCAGC	TCCCG	GCACC	TCAGA	GTCGG	CAACC	CCGGA	GTCTG
2801	GACCC	GGAAC	TTCCG	AAAGT	GCCAC	ACCAG	AGTCC	GGTCC	CGGGA	CTTCA
2851	GAATC	AGCAA	CACCC	GAGTC	CGGCC	CTGGG	TCTGA	ACCCG	CCACA	AGTGG
2901	TAGTG	AGACA	CCAGG	ATCAG	AACCT	GCTAC	CTCAG	GGTCA	GAGAC	ACCCG
2951	GATCT	CCGGC	AGGCT	CACCA	ACCTC	CAC TG	AGGAG	GGCAC	CAGCA	CAGAA
3001	CCAAG	CGAGG	GCTCC	GCACC	CGGAA	CAAGC	ACTGA	ACCCA	GTGAG	GGTTC
3051	AGCAC	CCGGC	TCTGA	GCCGG	CCACA	AGTGG	CAGTG	AGACA	CCCGG	CACTT
3101	CAGAG	AGTGC	CACCC	CCGAG	AGTGG	CCCAG	GCACT	AGTAC	CGAGC	CCTCT
3151	GAAGG	CAGTG	CGCCA	GCCTC	GAGCC	CACCA	GTCTT	GAAAC	GCCAT	CAAGC
3201	TGAAA	TAAC T	CGTAC	TACTC	TTCAG	TCAGA	TCAAG	AGGAA	ATCGA	TTATG
3251	ATGAT	ACCAT	ATCAG	TTGAA	ATGAA	GAAGG	AAGAT	TTTGA	CATTT	ATGAT
3301	GAGGA	TGAAA	ATCAG	AGCCC	CCGCA	GCTTT	CAAAA	GAAAA	CACGA	CAC TA
3351	TTTTA	TTGCT	GCAGT	GGAGA	GGCTC	TGGGA	TTATG	GGATG	AGTAG	CTCCC
3401	CACAT	GTTCT	AAGAA	ACAGG	GCTCA	GAGTG	GCAGT	GTCCC	TCAGT	TCAAG
3451	AAAGT	TGTTT	TCCAG	GAATT	TACTG	ATGGC	TCCTT	TACTC	AGCCC	TTATA
3501	CCGTG	GAGAA	CTAAA	TGAAC	ATTTG	GGACT	CCTGG	GGCCA	TATAT	AAGAG
3551	CAGAA	GTTGA	AGATA	ATATC	ATGGT	AACTT	TCAGA	AATCA	GGCCT	CTCGT
3601	CCCTA	TTCCT	TCTAT	TCTAG	CCTTA	TTTCT	TATGA	GGAAG	ATCAG	AGGCA
3651	AGGAG	CAGAA	CCTAG	AAAAA	ACTTT	GTCAA	GCCTA	ATGAA	ACCAA	AACTT
3701	ACTTT	TGGAA	AGTGC	AACAT	CATAT	GGCAC	CCACT	AAAGA	TGAGT	TTGAC
3751	TGCAA	AGCCT	GGGCT	TATTT	CTCTG	ATGTT	GACCT	GGAAA	AAGAT	GTGCA
3801	CTCAG	GCCTG	ATTGG	ACCCC	TTCTG	GTCTG	CCACA	CTAAC	ACACT	GAACC
3851	CTGCT	CATGG	GAGAC	AAGTG	ACAGT	ACAGG	AATTT	GCTCT	GTTTT	TCACC
3901	ATCTT	TGATG	AGACC	AAAAG	CTGGT	ACTTC	ACTGA	AAATA	TGGAA	AGAAA
3951	CTGCA	GGGCT	CCCTG	CAATA	TCCAG	ATGGA	AGATC	CCACT	TTTAA	AGAGA
4001	ATTAT	CGCTT	CCATG	CAATC	AATGG	CTACA	TAATG	GATAC	ACTAC	CTGGC
4051	TTAGT	AATGG	CTCAG	GATCA	AAGGA	TTCGA	TGGTA	TCTGC	TCAGC	ATGGG
4101	CAGCA	ATGAA	AACAT	CCATT	CTATT	CATTT	CAGTG	GACAT	GTGTT	CACTG
4151	TACGA	AAAAA	AGAGG	AGTAT	AAAAT	GGCAC	TGTAC	AATCT	CTATC	CAGGT
4201	GTTTT	TGAGA	CAGTG	GAAAT	GTTAC	CATCC	AAAGC	TGGAA	TTTGG	CGGGT

4251 GGAAT GCCTT ATTGG CGAGC ATCTA CATGC TGGGA TGAGC ACACT TTTTC
 4301 TGGTG TACAG CAATA AGTGT CAGAC TCCCC TGGGA ATGGC TTCTG GACAC
 4351 ATTAG AGATT TTCAG ATTAC AGCTT CAGGA CAATA TGGAC AGTGG GCCCC
 4401 AAAGC TGGCC AGACT TCATT ATTCC GGATC AATCA ATGCC TGGAG CACCA
 4451 AGGAG CCCTT TTCTT GGATC AAGGT GGATC TGTTG GCACC AATGA TTATT
 4501 CACGG CATCA AGACC CAGGG TGCCC GTCAG AAGTT CTCCA GCCTC TACAT
 4551 CTCTC AGTTT ATCAT CATGT ATAGT CTTGA TGGGA AGAAG TGGCA GACTT
 4601 ATCGA GGAAA TTCCA CTGGA ACCTT AATGG TCTTC TTTGG CAATG TGGAT
 4651 TCATC TGGGA TAAAA CACAA TATTT TTAAC CCTCC AATTA TTGCT CGATA
 4701 CATCC GTTTG CACCC AACTC ATTAT AGCAT TCGCA GCACT CTTCG CATGG
 4751 AGTTG ATGGG CTGTG ATTTA AATAG TTGCA GCATG CCATT GGGAA TGGAG
 4801 AGTAA AGCAA TATCA GATGC ACAGA TTACT GCTTC ATCCT ACTTT ACCAA
 4851 TATGT TTGCC ACCTG GTCTC CTTCA AAAGC TCGAC TTCAC CTCCA AGGGA
 4901 GGAGT AATGC CTGGA GACCT CAGGT GAATA ATCCA AAAGA GTGGC TGCAA
 4951 TGGGA CTTCC AGAAG ACAAT GAAAG TCACA GGAGT AACTA CTCAG GGAGT
 5001 AAAAT CTCTG CTTAC CAGCA TGTAT GTGAA GGAGT TCCTC ATCTC CAGCA
 5051 GTCAA GATGG CCATC AGTGG ACTCT CTTTT TTCAG AATGG CAAAG TAAAG
 5101 GTTTT TCAGG GAAAT CAAGA CTCCT TCACA CCTGT GGTGA ACTCT CTAGA
 5151 CCCAC CGTTA CTGAC TCGCT ACCTT CGAAT TCACC CCCAG AGTTG GGTGC
 5201 ACCAG ATTGC CCTGA GGATG GAGGT TCTGG GCTGC GAGGC ACAGG ACCTC
 5251 TACGA CAAAA CTCAC ACATG CCCAC CGTGC CCAGC TCCAG AACTC CTGGG
 5301 CGGAC CGTCA GTCTT CCTCT TCCCC CAAA ACCCA AGGAC ACCCT CATGA
 5351 TCTCC CGGAC CCCTG AGGTC ACATG CGTGG TGGTG GACGT GAGCC ACGAA
 5401 GACCC TGAGG TCAAG TTCAA CTGGT ACGTG GACGG CGTGG AGGTG CATAA
 5451 TGCCA AGACA AAGCC GCGGG AGGAG CAGTA CAACA GCACG TACCG TGTGG
 5501 TCAGC GTCCT CACCG TCCTG CACCA GGACT GGCTG AATGG CAAGG AGTAC
 5551 AAGTG CAAGG TCTCC AACAA AGCCC TCCCA GCCCC CATCG AGAAA ACCAT
 5601 CTCCA AAGCC AAAGG GCAGC CCCGA GAACC ACAGG TGTAC ACCCT GCCCC
 5651 CATCC CGGGA TGAGC TGACC AAGAA CCAGG TCAGC CTGAC CTGCC TGGTC
 5701 AAAGG CTTCT ATCCC AGCGA CATCG CCGTG GAGTG GGAGA GCAAT GGGCA
 5751 GCCGG AGAAC AACTA CAAGA CCACG CCTCC CGTGT TGGAC TCCGA CGGCT
 5801 CCTTC TTCCT CTACA GCAAG CTCAC CGTGG ACAAG AGCAG GTGGC AGCAG
 5851 GGGAA CGTCT TCTCA TGCTC CGTGA TGCAT GAGGC TCTGC ACAAC CACTA
 5901 CACGC AGAAG AGCCT CTCCC TGTCT CCGGG TAAAT GA

FVIII 169 protein sequence (SEQ ID NO: 70)

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
 51 PRVPKSFPPN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
 151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
 301 RQASLEISPI TFLTAQTLIM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 451 TDETFKTREA IQHESGILGP LLYGEVGDIT LIIFKNQASR PYNIYPHGIT
 501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 601 NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 701 MENPGLWLIG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 751 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 801 SGPGSEPATs GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 851 EGTSESATPE SGPGSEPATs GSETPGTSES ATPESGPGSP AGSPTSTEEG
 901 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 951 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
 1001 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
 1051 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD

```

1101 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPOFK
1151 KVVVFQFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1201 PYSFYSSLIS YEEDQROGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1251 CKAWAYFSDV DLEKDVHSGI IGPLLVCHTN TLNPAHGRQV TVQEFALFFT
1301 IFDETKSWYF TENMERN CRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1351 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
1401 VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
1451 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMI I
1501 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
1551 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
1601 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
1651 VDFQKTMKVT GVTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
1701 VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
1751 YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
1801 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
1851 KCKVSNKALP APIEKTISKA KGQPREPVY TLPPSRDEL TKNQVSLTCLV
1901 KGFYPSDIAV EWESNGQPEN NYKTTPPVL SDGSFFLYSK LTVDKSRWQQ
1951 GNVFSCSVMH EALHNHYTQK SLSLSPGK*

```

FVIII 263 nucleotide sequence (IHH triple mutant) (SEQ ID NO: 156)

```

1 ATGCA AATAG AGCTC TCCAC CTGCT TCTTT CTGTG CCTTT TGC GA TTCTG
51 CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
101 ACTAT ATGCA AGGCG GCCCA ACATC AGAGA GCGCC ACCCC TGAAA GTGGT
151 CCCGG GAGCG AGCCA GCCAC ATCTG GGTCG GAAAC GCCAG GCACA AGTGA
201 GTCTG CAACT CCCGA GTCCG GACCT GGCTC CGAGC CTGCC ACTAG CGGCT
251 CCGAG ACTCC GGGAA CTTCC GAGAG CGCTA CACCA GAAAG CGGAC CCGGA
301 ACCAG TACCG AACCT AGCGA GGGCT CTGCT CCGGG CAGCC CAGCC GGCTC
351 TCCTA CATCC ACGGA GGAGG GCACT TCCGA ATCCG CCACC CCGGA GTCAG
401 GGCCA GGATC TGAAC CCGCT ACCTC AGGCA GTGAG ACGCC AGGAA CGAGC
451 GAGTC CGCTA CACCG GAGAG TGGCG CAGGG AGCCC TGCTG GATCT CCTAC
501 GTCCA CTGAG GAAGG GTCAC CAGCG GGCTC GCCCA CCAGC ACTGA AGAAG
551 GTGCC TCGAG CAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCTT
601 CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG TGTAC AAAAA
651 GACTC TGTTT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
701 GGCCA CCCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT
751 GATAC AGTGG TCATT ACACT TAAGA ACATG GCTTC CCATC CTGTC AGTCT
801 TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
851 ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTCC CTGGT
901 GGAAG CCATA CATAT GTCTG GCAGG TCCTG AAAGA GAATG GTCCA ATGGC
951 CTCTG ACCCA CTGTG CCTTA CCTAC TCATA TCTTT CTCAT GTGGA CCTGG
1001 TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
1051 GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
1101 TTTTG CTGTA TTTGA TGAAG GGAAA AGTTG GCACT CAGAA ACAA GAACT
1151 CCTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG
1201 CACAC AGTCA ATGGT TATGT AAACA GGTC CTGCC AGGTC TGATT GGATG
1251 CCACA GGAAA TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
1301 AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
1351 CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CCTTA CTGCT CAAAC
1401 ACTCT TGATG GACCT TGGAC AGTTT CTACT GTTTT GTCAT ATCTC TTCCC
1451 ACCAA CATGA TGGCA TGGAA GCTTA GTTCA AAGTA GACAG CTGTC CAGAG
1501 GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
1551 TGATC TTA CT GATTG TGA AAA TGGAT GTGGT CAGGT TTGAT GATGA CAACT
1601 CTCCT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAATG
1651 TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GGACT ATGCT CCCTT
1701 AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG
1751 GCCCT CAGCG GATTG GTAGG AAGTA CAAAA AAGTC CGATT TATGG CATAC
1801 ACAGA TGA AAA CCTTT AAGAC TCGTG AAGCT ATTCA GCATG AATCA GGAAT
1851 CTTGG GACCT TTA CT TTATG GGGAA GTTGG AGACA CACTG TTGAT TATAT

```

1901	TTAAG	AATCA	AGCAA	GCAGA	CCATA	TAACA	TCTAC	CCTCA	CGGAA	TCACT
1951	GATGT	CCGTC	CTTTG	TATTC	AAGGA	GATTA	CCAAA	AGGTG	TAAAA	CATTT
2001	GAAGG	ATTTT	CCAAT	TCTGC	CAGGA	GAAAT	ATTCA	AATAT	AAATG	GACAG
2051	TGACT	GTAGA	AGATG	GGCCA	ACTAA	ATCAG	ATCCT	CGGTG	CCTGA	CCCGC
2101	TATTA	CTCTA	GTTTC	GTTAA	TATGG	AGAGA	GATCT	AGCTT	CAGGA	CTCAT
2151	TGGCC	CTCTC	CTCAT	CTGCT	ACAAA	GAATC	TGTAG	ATCAA	AGAGG	AAACC
2201	AGATA	ATGTC	AGACA	AGAGG	AATGT	CATCC	TGTTT	TCTGT	ATTTG	ATGAG
2251	AACCG	AAGCT	GGTAC	CTCAC	AGAGA	ATATA	CAACG	CTTTC	TCCCC	AATCC
2301	AGCTG	GAGTG	CAGCT	TGAGG	ATCCA	GAGTT	CCAAG	CCTCC	AACAT	CATGC
2351	ACAGC	ATCAA	TGGCT	ATGTT	TTTGA	TAGTT	TGCAG	TTGTC	AGTTT	GTTTG
2401	CATGA	GGTGG	CATAC	TGGTA	CATTC	TAAGC	ATTGG	AGCAC	AGACT	GACTT
2451	CCTTT	CTGTC	TTCTT	CTCTG	GATAT	ACCTT	CAAAC	ACAAA	ATGGT	CTATG
2501	AAGAC	ACACT	CACCC	TATTC	CCATT	CTCAG	GAGAA	ACTGT	CTTCA	TGTCG
2551	ATGGA	AAACC	CAGGT	CTATG	GATTC	TGGGG	TGCCA	CAACT	CAGAC	TTTCG
2601	GAACA	GAGGC	ATGAC	CGCCT	TACTG	AAGGT	TTCTA	GTTGT	GACAA	GAACA
2651	CTGGT	GATTA	TTACG	AGGAC	AGTTA	TGAAG	ATATT	TCAGC	ATACT	TGCTG
2701	AGTAA	AAACA	ATGCC	ATTGA	ACCAA	GAAGC	TTCTC	TCAAA	ACGGC	GCGCC
2751	AGGTA	CCTCA	GAGTC	TGCTA	CCCCC	GAGTC	AGGGC	CAGGA	TCAGA	GCCAG
2801	CCACC	TCCGG	GTCTG	AGACA	CCCGG	GACTT	CCGAG	AGTGC	CACCC	CTGAG
2851	TCCGG	ACCCG	GGTCC	GAGCC	CGCCA	CTTCC	GGCTC	CGAAA	CTCCC	GGCAC
2901	AAGCG	AGAGC	GCTAC	CCCAG	AGTCA	GGACC	AGGAA	CATCT	ACAGA	GCCCT
2951	CTGAA	GGCTC	CGCTC	CAGGG	TCCCC	AGCCG	GCAGT	CCCAC	TAGCA	CCGAG
3001	GAGGG	AACCT	CTGAA	AGCGC	CACAC	CCGAA	TCAGG	GCCAG	GGTCT	GAGCC
3051	TGCTA	CCAGC	GGCAG	CGAGA	CACCA	GGCAC	CTCTG	AGTCC	GCCAC	ACCAG
3101	AGTCC	GGACC	CGGAT	CTCCC	GCTGG	GAGCC	CCACC	TCCAC	TGAGG	AGGGA
3151	TCTCC	TGCTG	GCTCT	CCAAC	ATCTA	CTGAG	GAAGG	TACCT	CAACC	GAGCC
3201	ATCCG	AGGGA	TCAGC	TCCCG	GCACC	TCAGA	GTCGG	CAACC	CCGGA	GTCTG
3251	GACCC	GGAAC	TTCCG	AAAAGT	GCCAC	ACCAG	AGTCC	GGTCC	CGGGA	CTTCA
3301	GAATC	AGCAA	CACCC	GAGTC	CGGCC	CTGGG	TCTGA	ACCCG	CCACA	AGTGG
3351	TAGTG	AGACA	CCAGG	ATCAG	AACCT	GCTAC	CTCAG	GGTCA	GAGAC	ACCCG
3401	GATCT	CCGGC	AGGCT	CACCA	ACCTC	CACTG	AGGAG	GGCAC	CAGCA	CAGAA
3451	CCAAG	CGAGG	GCTCC	GCACC	CGGAA	CAAGC	ACTGA	ACCCA	GTGAG	GGTTC
3501	AGCAC	CCGGC	TCTGA	CCCGG	CCACA	AGTGG	CAGTG	AGACA	CCCGG	CACCT
3551	CAGAG	AGTGC	CACCC	CCGAG	AGTGG	CCCAG	GCACT	AGTAC	CGAGC	CCTCT
3601	GAAGG	CAGTG	CGCCA	GCCTC	GAGCC	CACCA	GTCTT	GAAAC	GCCAT	CAAGC
3651	TGAAA	TAACT	CGTAC	TACTC	TTTCA	TCAGA	TCAAG	AGGAA	ATCGA	TTATG
3701	ATGAT	ACCAT	ATCAG	TTGAA	ATGAA	GAAGG	AAGAT	TTTGA	CATTT	ATGAT
3751	GAGGA	TGAAA	ATCAG	AGCCC	CCGCA	GCTTT	CAAAA	GAAAA	CACGA	CACTA
3801	TTTTA	TTGCT	GCAGT	GGAGA	GGCTC	TGGGA	TTATG	GGATG	AGTAG	CTCCC
3851	CACAT	GTTCT	AAGAA	ACAGG	GCTCA	GAGTG	GCAGT	GTCCC	TCAGT	TCAAG
3901	AAAGT	TGTTT	TCCAG	GAATT	TACTG	ATGGC	TCCTT	TACTC	AGCCC	TTATA
3951	CCGTG	GAGAA	CTAAA	TGAAC	ATTTG	GGACT	CCTGG	GGCCA	TATAT	AAGAG
4001	CAGAA	GTTGA	AGATA	ATATC	ATGGT	AACTT	TCAGA	AATCA	GGCCT	CTCGT
4051	CCCTA	TTCCT	TCTAT	TCTAG	CCTTA	TTTCT	TATGA	GGAAG	ATCAG	AGGCA
4101	AGGAG	CAGAA	CCTAG	AAAAA	ACTTT	GTCAA	GCCTA	ATGAA	ACCAA	AACTT
4151	ACTTT	TGGAA	AGTGC	AACAT	CATAT	GGCAC	CAACT	AAAGA	TGAGT	TTGAC
4201	TGCAA	AGCCT	GGGCT	TATTT	CTCTG	ATGTT	GACCT	GGAAA	AAGAT	GTGCA
4251	CTCAG	GCCTG	ATTGG	ACCCC	TTCTG	GTCTG	CCACA	CTAAC	ACACT	GAACC
4301	CTGCT	CATGG	GAGAC	AAGTG	ACAGT	ACAGG	AATTT	GCTCT	GTTTT	TCACC
4351	ATCTT	TGATG	AGACC	AAAAG	CTGGT	ACTTC	ACTGA	AAATA	TGGAA	AGAAA
4401	CTGCA	GGGCT	CCCTG	CAATA	TCCAG	ATGGA	AGATC	CCACT	TTTAA	AGAGA
4451	ATTAT	CGCTT	CCATG	CAATC	AATGG	CTACA	TAATG	GATAC	ACTAC	CTGGC
4501	TTAGT	AATGG	CTCAG	GATCA	AAGGA	TTCGA	TGGTA	TCTGC	TCAGC	ATGGG
4551	CAGCA	ATGAA	AACAT	CCATT	CTATT	CATTT	CAGTG	GACAT	GTGTT	CACTG
4601	TACGA	AAAAA	AGAGG	AGTAT	AAAAT	GGCAC	TGTAC	AATCT	CTATC	CAGGT
4651	GTTTT	TGAGA	CAGTG	GAAAT	GTTAC	CATCC	AAAGC	TGGAA	TTTGG	CGGGT
4701	GGAAT	GCCTT	ATTGG	CGAGC	ATCTA	CATGC	TGGGA	TGAGC	ACACT	TTTTC
4751	TGGTG	TACAG	CAATA	AGTGT	CAGAC	TCCCC	TGGGA	ATGGC	TTCTG	GACAC
4801	ATTAG	AGATT	TTCAG	ATTAC	AGCTT	CAGGA	CAATA	TGGAC	AGTGG	GCCCC

4851 AAAGC TGGCC AGACT TCATT ATTCC GGATC AATCA ATGCC TGGAG CACCA
 4901 AGGAG CCCTT TTCTT GGATC AAGGT GGATC TGTTG GCACC AATGA TTATT
 4951 CACGG CATCA AGACC CAGGG TGCCC GTCAG AAGTT CTCCA GCCTC TACAT
 5001 CTCTC AGTTT ATCAT CATGT ATAGT CTTGA TGGGA AGAAG TGGCA GACTT
 5051 ATCGA GGAAA TTCCA CTGGA ACCTT AATGG TCTTC TTTGG CAATG TGGAT
 5101 TCATC TGGGA TAAAA CACAA TATTT TTAAC CCTCC AATTA TTGCT CGATA
 5151 CATCC GTTTG CACCC AACTC ATTAT AGCAT TCGCA GCACCT CTTCCG CATGG
 5201 AGTTG ATGGG CTGTG ATTTA AATAG TTGCA GCATG CCATT GGGAA TGGAG
 5251 AGTAA AGCAA TATCA GATGC ACAGA TTACT GCTTC ATCCT ACTTT ACCAA
 5301 TATGT TTGCC ACCTG GTCTC CTTCA AAAGC TCGAC TTCAC CTCCA AGGGA
 5351 GGAGT AATGC CTGGA GACCT CAGGT GAATA ATCCA AAAGA GTGGC TGCAA
 5401 GTGGA CTTCC AGAAG ACAAT GAAAG TCACA GGAGT AACTA CTCAG GGAGT
 5451 AAAAT CTCTG CTTAC CAGCA TGTAT GTGAA GGAGT TCCTC ATCTC CAGCA
 5501 GTCAA GATGG CCATC AGTGG ACTCT CTTTT TTCAG AATGG CAAAG TAAAG
 5551 GTTTT TCAGG GAAAT CAAGA CTCTT TCACA CCTGT GGTGA ACTCT CTAGA
 5601 CCCAC CGTTA CTGAC TCGCT ACCTT CGAAT TCACC CCCAG AGTTG GGTGC
 5651 ACCAG ATTGC CCTGA GGATG GAGGT TCTGG GCTGC GAGGC ACAGG ACCTC
 5701 TACGA CAAAA CTCAC ACATG CCCAC CGTGC CCAGC TCCAG AACTC CTGGG
 5751 CGGAC CGTCA GTCTT CCTCT TCCCC CCAAA ACCCA AGGAC ACCCT CATGG
 5801 CCTCC CGGAC CCCTG AGGTC ACATG CGTGG TGGTG GACGT GAGCC ACGAA
 5851 GACCC TGAGG TCAAG TTCAA CTGGT ACGTG GACGG CGTGG AGGTG CATAA
 5901 TGCCA AGACA AAGCC GCGGG AGGAG CAGTA CAACA GCACG TACCG TGTGG
 5951 TCAGC GTCCT CACCG TCCTG GCCCA GGACT GGCTG AATGG CAAGG AGTAC
 6001 AAGTG CAAGG TCTCC AACAA AGCCC TCCCA GCCCC CATCG AGAAA ACCAT
 6051 CTCCA AAGCC AAAGG GCAGC CCCGA GAACC ACAGG TGTAC ACCCT GCCCC
 6101 CATCC CGCGA TGAGC TGACC AAGAA CCAGG TCAGC CTGAC CTGCC TGGTC
 6151 AAAGG CTTCT ATCCC AGCGA CATCG CCGTG GAGTG GGAGA GCAAT GGGCA
 6201 GCCGG AGAAC AACTA CAAGA CCACG CCTCC CGTGT TGGAC TCCGA CGGCT
 6251 CCTTC TTCCT CTACA GCAAG CTCAC CGTGG ACAAG AGCAG GTGGC AGCAG
 6301 GGGAA CGTCT TCTCA TGCTC CGTGA TGCAT GAGGC TCTGC ACAAC GCCTA
 6351 CACGC AGAAG AGCCT CTCCC TGTCT CCGGG TAAAT GA

FVIII 263 protein sequence(IHH triple mutant) (SEQ ID NO: 157)

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQGAP TSESATPESG
 51 PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 101 TSTEPSEGS A PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS
 151 ESATPESGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL GELPVDARFP
 201 PRVPKSFPPN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
 251 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPF
 301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
 351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
 401 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHFTFLVRNH
 451 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
 501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
 551 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
 601 TDETFKTREA IQHESGILGP LLYGEVGDIT LIIFKNQASR PYNIYPHGIT
 651 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
 701 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
 751 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
 801 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
 851 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
 901 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
 951 SGPGSEPAT S GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
 1001 EGTSESATPE SGPGSEPAT S GSETPGTSES ATPESGPGSP AGSPTSTEEG
 1051 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
 1101 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
 1151 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
 1201 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD

1251 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPOFK
 1301 KVVVFQFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
 1351 PYSFYSSLIS YEEDQROGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
 1401 CKAWAYFSDV DLEKDVHSGI IGPLLVCHTN TLNPAHGRQV TVQEFALFFT
 1451 IFDETKSWYF TENMERNCR A PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
 1501 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
 1551 VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
 1601 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMI I
 1651 HGIKTQGARQ KFSSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
 1701 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
 1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
 1801 VDFQKTMKVT GVTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
 1851 VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
 1901 YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMASRTPEV TCVVVDVSHE
 1951 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL AQDWLNGKEY
 2001 KCKVSNKALP APIEKTISKA KGQPREPVY TLPPSRDEL T KQVSLTCLV
 2051 KGFYPSDIAV EWESNGQPEN NYKTTPPVL SDGSFFLYSK LTVDKSRWQQ
 2101 GNVFSCSVMH EALHNAYTQK SLSLSPGK*

FVIII 282 nucleotide sequence (SEQ ID NO: 158)

1 ATGCA AATAG AGCTC TCCAC CTGCT TCTTT CTGTG CCTTT TGC GA TTCTG
 51 CTTTA GTGCC ACCAG AAGAT ACTAC CTGGG TGCAG TGGAA CTGTC ATGGG
 101 ACTAT ATGCA AAGTG ATCTC GGTGA GCTGC CTGTG GACGC AAGAT TTCCT
 151 CCTAG AGTGC CAAAA TCTTT TCCAT TCAAC ACCTC AGTCG GTTAC AAAAA
 201 GACTC TGTTT GTAGA ATTCA CGGAT CACCT TTTCA ACATC GCTAA GCCAA
 251 GGCCA CCCTG GATGG GTCTG CTAGG TCCTA CCATC CAGGC TGAGG TTTAT
 301 GATAC AGTGG TCATT AACTT TAAGA ACATG GCTTC CCATC CTGTC AGTCT
 351 TCATG CTGTT GGTGT ATCCT ACTGG AAAGC TTCTG AGGGA GCTGA ATATG
 401 ATGAT CAGAC CAGTC AAAGG GAGAA AGAAG ATGAT AAAGT CTTC CTGGT
 451 GGAAG CCATA CATAT GTCTG GCAGG TCCTG AAAGA GAATG GTCCA ATGGC
 501 CTCTG ACCCA CTGTG CCTTA CCTAC TCATA TCTTT CTCAT GTGGA CTGG
 551 TAAAA GACTT GAATT CAGGC CTCAT TGGAG CCCTA CTAGT ATGTA GAGAA
 601 GGGAG TCTGG CCAAG GAAAA GACAC AGACC TTGCA CAAAT TTATA CTACT
 651 TTTTG CTGTA TTTGA TGAAG GGAAA AGTTG GCACT CAGAA ACAA GAACT
 701 CCTTG ATGCA GGATA GGGAT GCTGC ATCTG CTCGG GCCTG GCCTA AAATG
 751 CACAC AGTCA ATGGT TATGT AAACA GGTCT CTGCC AGGTC TGATT GGATG
 801 CCACA GGAAA TCAGT CTATT GGCAT GTGAT TGGAA TGGGC ACCAC TCCTG
 851 AAGTG CACTC AATAT TCCTC GAAGG TCACA CATTT CTTGT GAGGA ACCAT
 901 CGCCA GGCTA GCTTG GAAAT CTCGC CAATA ACTTT CCTTA CTGCT CAAAC
 951 ACTCT TGATG GACCT TGGAC AGTTT CTACT GTTTT GTCAT ATCTC TTCCC
 1001 ACCAA CATGA TGGCA TGGAA GCTTA TGTC AAGTA GACAG CTGTC CAGAG
 1051 GAACC CCAAC TACGA ATGAA AAATA ATGAA GAAGC GGAAG ACTAT GATGA
 1101 TGATC TTAAT GATTC TGAAA TGGAT GTGGT CAGGT TTGAT GATGA CAACT
 1151 CTCTT TCCTT TATCC AAATT CGCTC AGTTG CCAAG AAGCA TCCTA AAAT
 1201 TGGGT ACATT ACATT GCTGC TGAAG AGGAG GACTG GGACT ATGCT CCCTT
 1251 AGTCC TCGCC CCCGA TGACA GAAGT TATAA AAGTC AATAT TTGAA CAATG
 1301 GCCCT CAGCG GATTG GTAGG AAGTA CAAAA AAGTC CGATT TATGG CATAC
 1351 ACAGA TGAAA CCTTT AAGAC TCGTG AAGCT ATTCA GCATG AATCA GGAAT
 1401 CTTGG GACCT TTAAT TTATG GGGAA GTTGG AGACA CACTG TTGAT TATAT
 1451 TTAAG AATCA AGCAA GCAGA CCATA TAACA TCTAC CCTCA CGGAA TCACT
 1501 GATGT CCGTC CTTTG TATTC AAGGA GATTA CCAAA AGGTG TAAAA CATTT
 1551 GAAGG ATTTT CCAAT TCTGC CAGGA GAAAT ATTCA AATAT AAATG GACAG
 1601 TGACT GTAGA AGATG GGCCA ACTAA ATCAG ATCCT CGGTG CCTGA CCCGC
 1651 TATTA CTCTA GTTTC GTTAA TATGG AGAGA GATCT AGCTT CAGGA CTCAT
 1701 TGGCC CTCTC CTCAT CTGCT ACAA GAATC TGTA ATCAA AGAGG AAACC
 1751 AGATA ATGTC AGACA AGAGG AATGT CATCC TGTTT TCTGT ATTTG ATGAG
 1801 AACCG AAGCT GTTAC CTCAC AGAGA ATATA CAACG CTTTC TCCCC AATCC
 1851 AGCTG GAGTG CAGCT TGAGG ATCCA GAGTT CCAAG CCTCC AACAT CATGC

1901	ACAGC	ATCAA	TGGCT	ATGTT	TTTGA	TAGTT	TGCAG	TTGTC	AGTTT	GTTTG
1951	CATGA	GGTGG	CATAC	TGGTA	CATTC	TAAGC	ATTGG	AGCAC	AGACT	GACTT
2001	CCTTT	CTGTC	TTCTT	CTCTG	GATAT	ACCTT	CAAAC	ACAAA	ATGGT	CTATG
2051	AAGAC	ACACT	CACCC	TATTC	CCATT	CTCAG	GAGAA	ACTGT	CTTCA	TGTCG
2101	ATGGA	AAACC	CAGGT	CTATG	GATTC	TGGGG	TGCCA	CAACT	CAGAC	TTTCG
2151	GAACA	GAGGC	ATGAC	CGCCT	TACTG	AAGGT	TTCTA	GTTGT	GACAA	GAACA
2201	CTGGT	GATTA	TTACG	AGGAC	AGTTA	TGAAG	ATATT	TCAGC	ATACT	TGCTG
2251	AGTAA	AAACA	ATGCC	ATTGA	ACCAA	GAAGC	TTCTC	TCAAA	ACGGC	GCGCC
2301	AACAT	CAGAG	AGCGC	CACCC	CTGAA	AGTGG	TCCCG	GGAGC	GAGCC	AGCCA
2351	CATCT	GGGTC	GGAAA	CGCCA	GGCAC	AAGTG	AGTCT	GCAAC	TCCCG	AGTCC
2401	GGACC	TGGCT	CCGAG	CCTGC	CACTA	GCGGC	TCCGA	GACTC	CGGGA	ACTTC
2451	CGAGA	GCGCT	ACACC	AGAAA	GCGGA	CCCGG	AACCA	GTACC	GAACC	TAGCG
2501	AGGGC	TCTGC	TCCGG	GCAGC	CCAGC	CGGCT	CTCCT	ACATC	CACGG	AGGAG
2551	GGCAC	TTCCG	AATCC	GCCAC	CCCGG	AGTCA	GGGCC	AGGAT	CTGAA	CCCGC
2601	TACCT	CAGGC	AGTGA	GACGC	CAGGA	ACGAG	CGAGT	CCGCT	ACGAA	GGAGA
2651	GTGGG	CCAGG	GAGCC	CTGCT	GGATC	TCCTA	CGTCC	ACTGA	GGAAG	GGTCA
2701	CCAGC	GGGCT	CGCCC	ACCAG	CACTG	AAGAA	GGTGC	CTCGA	GCCCA	CCAGT
2751	CTTGA	AACGC	CATCA	AGCTG	AAATA	ACTCG	TACTA	CTCTT	CAGTC	AGATC
2801	AAGAG	GAAAT	CGATT	ATGAT	GATAC	CATAT	CAGTT	GAAAT	GAAGA	AGGAA
2851	GATTT	TGACA	TTTAT	GATGA	GGATG	AAAAT	CAGAG	CCCCC	GCAGC	TTTCA
2901	AAAGA	AAACA	CGACA	CTATT	TTATT	GCTGC	AGTGG	AGAGG	CTCTG	GGATT
2951	ATGGG	ATGAG	TAGCT	CCCCA	CATGT	TCTAA	GAAAC	AGGGC	TCAGA	GTGGC
3001	AGTGT	CCCTC	AGTTC	AAGAA	AGTTG	TTTTT	CAGGA	ATTTA	CTGAT	GGCTC
3051	CTTTA	CTCAG	CCCTT	ATACC	GTGGA	GAACT	AAATG	AACAT	TTGGG	ACTCC
3101	TGGGG	CCATA	TATAA	GAGCA	GAAAT	TGAAG	ATAAT	ATCAT	GGTAA	CTTTC
3151	AGAAA	TCAGG	CCTCT	CGTCC	CTATT	CCTTC	TATTC	TAGCC	TTATT	TCTTA
3201	TGAGG	AAGAT	CAGAG	GCAAG	GAGCA	GAAAC	TAGAA	AAAAC	TTTGT	CAAGC
3251	CTAAT	GAAAC	CAAAA	CTTAC	TTTTG	GAAAG	TGCAA	CATCA	TATGG	CACCC
3301	ACTAA	AGATG	AGTTT	GACTG	CAAAG	CCTGG	GCTTA	TTTCT	CTGAT	GTTGA
3351	CCTGG	AAAAA	GATGT	GCACT	CAGGC	CTGAT	TGGAC	CCCTT	CTGGT	CTGCC
3401	ACACT	AACAC	ACTGA	ACCTT	GCTCA	TGGGA	GACAA	GTGAC	AGTAC	AGGAA
3451	TTTGC	TCTGT	TTTTT	ACCAT	CTTTG	ATGAG	ACCAA	AAGCT	GGTAT	TTTCA
3501	TGAAA	ATATG	GAAAG	AAACT	GCAGG	GCTCC	CTGCA	ATATC	CAGAT	GGAAG
3551	ATCCC	ACTTT	TAAAG	AGAAAT	TATCG	CTTCC	ATGCA	ATCAA	TGGCT	ACATA
3601	ATGGA	TACAC	TACCT	GGCTT	AGTAA	TGGCT	CAGGA	TCAAA	GGATT	CGATG
3651	GTATC	TGCTC	AGCAT	GGGCA	GCAAT	GAAAA	CATCC	ATTCT	ATTCA	TTTCA
3701	GTGGA	CATGT	GTTCA	CTGTA	CGAAA	AAAAG	AGGAG	TATAA	AATGG	CACTG
3751	TACAA	TCTCT	ATCCA	GGTGT	TTTTG	AGACA	GTGGA	AATGT	TACCA	TCCAA
3801	AGCTG	GAATT	TGGCG	GGTGG	AATGC	CTTAT	TGGCG	AGCAT	CTACA	TGCTG
3851	GGATG	AGCAC	ACTTT	TTCTG	GTGTA	CAGCA	ATAAG	TGTCA	GACTC	CCCTG
3901	GGAAT	GGCTT	CTGGA	CACAT	TAGAG	ATTTT	CAGAT	TACAG	CTTCA	GGACA
3951	ATATG	GACAG	TGGGC	CCCAA	AGCTG	GCCAG	ACTTC	ATTAT	TCCGG	ATCAA
4001	TCAAT	GCCTG	GAGCA	CCAAG	GAGCC	CTTTT	CTTGG	ATCAA	GGTGG	ATCTG
4051	TTGGC	ACCAA	TGATT	ATTCA	CGGCA	TCAAG	ACCCA	GGGTG	CCCGT	CAGAA
4101	GTTCT	CCAGC	CTCTA	CATCT	CTCAG	TTTAT	CATCA	TGTAT	AGTCT	TGATG
4151	GGAAG	AAGTG	GCAGA	CTTAT	CGAGG	AAATT	CCACT	GGAAC	CTTAA	TGGTC
4201	TTCTT	TGGCA	ATGTG	GATTC	ATCTG	GGATA	AAACA	CAATA	TTTTT	AACCC
4251	TCCAA	TTATT	GCTCG	ATACA	TCCGT	TTGCA	CCCAA	CTCAT	TATAG	CATTC
4301	GCAGC	ACTCT	TCGCA	TGGAG	TTGAT	GGGCT	GTGAT	TTAAA	TAGTT	GCAGC
4351	ATGCC	ATTGG	GAATG	GAGAG	TAAAG	CAATA	TCAGA	TGCAC	AGATT	ACTGC
4401	TTCAT	CCTAC	TTTAC	CAATA	TGTTT	GCCAC	CTGGT	CTCCT	TCAAA	AGCTC
4451	GACTT	CACCT	CCAAG	GGAGG	AGTAA	TGCCT	GGAGA	CCTCA	GGTGA	ATAAT
4501	CCAAA	AGAGT	GGCTG	CAAGT	GGACT	TCCAG	AAGAC	AATGA	AAGTC	ACAGG
4551	AGTAA	CTACT	CAGGG	AGTAA	AATCT	CTGCT	TACCA	GCATG	TATGT	GAAGG
4601	AGTTC	CTCAT	CTCCA	GCAGT	CAAGA	TGGCC	ATCAG	TGGAC	TCTCT	TTTTT
4651	CAGAA	TGGCA	AAGTA	AAGGT	TTTTT	AGGGA	AATCA	AGACT	CCTTC	ACACC
4701	TGTGG	TGAAC	TCTCT	AGACC	CACCG	TTACT	GACTC	GCTAC	CTTCG	AATTC
4751	ACCCC	CAGAG	TTGGG	TGCAC	CAGAT	TGCCC	TGAGG	ATGGA	GGTTC	TGGGC
4801	TGCGA	GGCAC	AGGAC	CTCTA	CGACA	AAACT	CACAC	ATGCC	CACCG	TGCCC

```

4851 AGCTC CAGAA CTCCT GGGCG GACCG TCAGT CTTCC TCTTC CCCCC AAAAC
4901 CCAAG GACAC CCTCA TGATC TCCCG GACCC CTGAG GTCAC ATGCG TGGTG
4951 GTGGA CGTGA GCCAC GAAGA CCCTG AGGTC AAGTT CAACT GGTAC GTGGA
5001 CGGCG TGGAG GTGCA TAATG CCAAG ACAAA GCCGC GGGAG GAGCA GTACA
5051 ACAGC ACGTA CCGTG TGGTC AGCGT CCTCA CCGTC CTGCA CCAGG ACTGG
5101 CTGAA TGGCA AGGAG TACAA GTGCA AGGTC TCCAA CAAAG CCCTC CCAGC
5151 CCCCC TCGAG AAAAC CATCT CCAAA GCCAA AGGGC AGCCC CGAGA ACCAC
5201 AGGTG TACAC CCTGC CCCCC TCCCG GGATG AGCTG ACCAA GAACC AGGTC
5251 AGCCT GACCT GCCTG GTCAA AGGCT TCTAT CCCAG CGACA TCGCC GTGGA
5301 GTGGG AGAGC AATGG GCAGC CGGAG AACAA CTACA AGACC ACGCC TCCCG
5351 TGTTG GACTC CGACG GCTCC TTCTT CCTCT ACAGC AAGCT CACCG TGGAC
5401 AAGAG CAGGT GGCAG CAGGG GAACG TCTTC TCATG CTCCG TGATG CATGA
5451 GGCTC TGCAC AACCA CTACA CGCAG AAGAG CCTCT CCCTG TCTCC GGGTA
5501 AATGA

```

FVIII 282 protein sequence (SEQ ID NO: 159)

```

1 MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQSDL GELPVDARFP
51 PRVPKSFPPN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
451 TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIIYPHGIT
501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMF
701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYD YEDISAYLL
751 SKNNAIEPRS FSQNGAPTSE SATPESGPGS EPATSGSETP GTSESATPES
801 GPGSEPATSG SETPGTSESA TPESGPGTST EPSEGSAPGS PAGSPTSTEE
851 GTSESATPES GPGSEPATSG SETPGTSESA TPESGPGSPA GSPTSTEEGS
901 PAGSPTSTEE GASSPPVLKR HQAEITRRTL QSDQEEIDYD DTISVEMKKE
951 DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLNRNAQSG
1001 SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
1051 RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY FWKVQHMAP
1101 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1151 FALFFTIFDE TKSIFYTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI
1201 MDTLPGLVMA QDQIRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMA
1251 YNLYPGVFET VEMLPSKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
1301 GMASGHIRDF QITASGQYQG WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1351 LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
1401 FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1451 MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1501 PKEWLQVDFQ KTMKVTGVT TQGVKSLLTSM YVKEFLISS QDGHQWTLFF
1551 QNGKVKVFG QNDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
1601 CEAQDLYDKT HTPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCV
1651 VDVSHEDPEV KFNWYVDGVE VHNATKPRE EQYNSTYRVV SVLTVLHQDW
1701 LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
1751 SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGDS FFLYSKLTV
1801 KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

```

FVIII 283 nucleotide sequence (FVIII 169 with IHH triple mutation) (SEQ ID NO: 160)

1	ATGCA	AATAG	AGCTC	TCCAC	CTGCT	TCTTT	CTGTG	CCTTT	TGCGA	TTCTG
51	CTTTA	GTGCC	ACCAG	AAGAT	ACTAC	CTGGG	TGCAG	TGGAA	CTGTC	ATGGG
101	ACTAT	ATGCA	AAGTG	ATCTC	GGTGA	GCTGC	CTGTG	GACGC	AAGAT	TTCTT
151	CCTAG	AGTGC	CAAAA	TCTTT	TCCAT	TCAAC	ACCTC	AGTCG	TGTAC	AAAAA
201	GACTC	TGTTT	GTAGA	ATTCA	CGGAT	CACCT	TTTCA	ACATC	GCTAA	GCCAA
251	GGCCA	CCCTG	GATGG	GTCTG	CTAGG	TCCTA	CCATC	CAGGC	TGAGG	TTTAT
301	GATAC	AGTGG	TCATT	ACACT	TAAGA	ACATG	GCTTC	CCATC	CTGTC	AGTCT
351	TCATG	CTGTT	GGTGT	ATCCT	ACTGG	AAAGC	TTCTG	AGGGA	GCTGA	ATATG
401	ATGAT	CAGAC	CAGTC	AAAGG	GAGAA	AGAAG	ATGAT	AAAGT	CTTCC	CTGGT
451	GGAAG	CCATA	CATAT	GTCTG	GCAGG	TCCTG	AAAGA	GAATG	GTCCA	ATGGC
501	CTCTG	ACCCA	CTGTG	CCTTA	CCTAC	TCATA	TCTTT	CTCAT	GTGGA	CCTGG
551	TAAAA	GACTT	GAATT	CAGGC	CTCAT	TGGAG	CCCTA	CTAGT	ATGTA	GAGAA
601	GGGAG	TCTGG	CCAAG	GAAAA	GACAC	AGACC	TTGCA	CAAA	TTATA	CTACT
651	TTTTG	CTGTA	TTTGA	TGAAG	GGAAA	AGTTG	GCACT	CAGAA	ACAAA	GAAC
701	CCTTG	ATGCA	GGATA	GGGAT	CTGTC	ATCTG	CTCGG	GCCTG	GCCTA	AAATG
751	CACAC	AGTCA	ATGGT	TATGT	AAACA	GGTCT	CTGCC	AGGTC	TGATT	GGATG
801	CCACA	GGAAA	TCAGT	CTATT	GGCAT	GTGAT	TGGAA	TGGGC	ACCAC	TCCTG
851	AAGTG	CACTC	AATAT	TCCTC	GAAGG	TCACA	CATTT	CTTGT	GAGGA	ACCAT
901	CGCCA	GGCTA	GCTTG	GAAA	CTCGC	CAATA	ACTTT	CCTTA	CTGCT	CAAAC
951	ACTCT	TGATG	GACCT	TGGAC	AGTTT	CTACT	GTTTT	GTCAT	ATCTC	TTCCC
1001	ACCAA	CATGA	TGGCA	TGGAA	GCTTA	TGTCA	AAGTA	GACAG	CTGTC	CAGAG
1051	GAACC	CCAAC	TACGA	ATGAA	AAATA	ATGAA	GAAGC	GGAAG	ACTAT	GATGA
1101	TGATC	TTACT	GATTG	TGAAA	TGGAT	GTGGT	CAGGT	TTGAT	GATGA	CAACT
1151	CTCCT	TCCTT	TATCC	AAATT	CGCTC	AGTTG	CCAAG	AAGCA	TCCTA	AAACT
1201	TGGGT	ACATT	ACATT	GCTGC	TGAAG	AGGAG	GACTG	GGACT	ATGCT	CCCTT
1251	AGTCC	TCGCC	CCCGA	TGACA	GAAGT	TATAA	AAGTC	AATAT	TTGAA	CAATG
1301	GCCCT	CAGCG	GATTG	GTAGG	AAGTA	CAAAA	AAGTC	CGATT	TATGG	CATAC
1351	ACAGA	TGAAA	CCTTT	AAGAC	TCGTG	AAGCT	ATTCA	GCATG	AATCA	GGAAT
1401	CTTGG	GACCT	TTACT	TTATG	GGGAA	GTTGG	AGACA	CACTG	TTGAT	TATAT
1451	TTAAG	AATCA	AGCAA	GCAGA	CCATA	TAACA	TCTAC	CCTCA	CGGAA	TCACT
1501	GATGT	CCGTC	CTTTG	TATTC	AAGGA	GATTA	CCAAA	AGGTG	TAAAA	CATTT
1551	GAAGG	ATTTT	CCAAT	TCTGC	CAGGA	GAAAT	ATTCA	AATAT	AAATG	GACAG
1601	TGAGT	GTAGA	AGATG	GGCCA	ACTAA	ATCAG	ATCCT	CGGTG	CCTGA	CCCGC
1651	TATTA	CTCTA	GTTTC	GTTAA	TATGG	AGAGA	GATCT	AGCTT	CAGGA	CTCAT
1701	TGGCC	CTCTC	CTCAT	CTGCT	ACAAA	GAATC	TGTAG	ATCAA	AGAGG	AAACC
1751	AGATA	ATGTC	AGACA	AGAGG	AATGT	CATCC	TGTTT	TCTGT	ATTTG	ATGAG
1801	AACCG	AAGCT	GGTAC	CTCAC	AGAGA	ATATA	CAACG	CTTTC	TCCCC	AATCC
1851	AGCTG	GAGTG	CAGCT	TGAGG	ATCCA	GAGTT	CCAAG	CCTCC	AACAT	CATGC
1901	ACAGC	ATCAA	TGGCT	ATGTT	TTTGA	TAGTT	TGCAG	TTGTC	AGTTT	GTTTG
1951	CATGA	GGTGG	CATAC	TGGTA	CATTC	TAAGC	ATTGG	AGCAC	AGACT	GACTT
2001	CCTTT	CTGTC	TTCTT	CTCTG	GATAT	ACCTT	CAAAC	ACAAA	ATGGT	CTATG
2051	AAGAC	ACACT	CACCC	TATTC	CCATT	CTCAG	GAGAA	ACTGT	CTTCA	TGTCG
2101	ATGGA	AAACC	CAGGT	CTATG	GATTG	TGGGG	TGCCA	CAACT	CAGAC	TTTCG
2151	GAACA	GAGGC	ATGAC	CGCCT	TACTG	AAGGT	TTCTA	GTTGT	GACAA	GAACA
2201	CTGGT	GATTA	TTACG	AGGAC	AGTTA	TGAAG	ATATT	TCAGC	ATACT	TGCTG
2251	AGTAA	AAACA	ATGCC	ATTGA	ACCAA	GAAGC	TTCTC	TCAAA	ACGGC	GCGCC
2301	AGGTA	CCTCA	GAGTC	TGCTA	CCCCC	GAGTC	AGGGC	CAGGA	TCAGA	GCCAG
2351	CCACC	TCCGG	GTCTG	AGACA	CCCGG	GACTT	CCGAG	AGTGC	CACCC	CTGAG
2401	TCCGG	ACCCG	GGTCC	GAGCC	CGCCA	CTTCC	GGCTC	CGAAA	CTCCC	GGCAC
2451	AAGCG	AGAGC	GCTAC	CCCAG	AGTCA	GGACC	AGGAA	CATCT	ACAGA	GCCCT
2501	CTGAA	GGCTC	CGCTC	CAGGG	TCCCC	AGCCG	GCAGT	CCCAC	TAGCA	CCGAG
2551	GAGGG	AACCT	CTGAA	AGCGC	CACAC	CCGAA	TCAGG	GCCAG	GGTCT	GAGCC
2601	TGCTA	CCAGC	GGCAG	CGAGA	CACCA	GGCAC	CTCTG	AGTCC	GCCAC	ACCAG
2651	AGTCC	GGACC	CGGAT	CTCCC	GCTGG	GAGCC	CCACC	TCCAC	TGAGG	AGGGA
2701	TCTCC	TGCTG	GCTCT	CCAAC	ATCTA	CTGAG	GAAGG	TACCT	CAACC	GAGCC
2751	ATCCG	AGGGA	TCAGC	TCCCG	GCACC	TCAGA	GTCGG	CAACC	CCGGA	GTCTG
2801	GACCC	GGAAC	TTCCG	AAAGT	GCCAC	ACCAG	AGTCC	GGTCC	CGGGA	CTTCA
2851	GAATC	AGCAA	CACCC	GAGTC	CGGCC	CTGGG	TCTGA	ACCCG	CCACA	AGTGG
2901	TAGTG	AGACA	CCAGG	ATCAG	AACCT	GCTAC	CTCAG	GGTCA	GAGAC	ACCCG

2951 GATCT CCGGC AGGCT CACCA ACCTC CACTG AGGAG GGCAC CAGCA CAGAA
 3001 CCAAG CGAGG GCTCC GCACC CGGAA CAAGC ACTGA ACCCA GTGAG GGTTC
 3051 AGCAC CCGGC TCTGA GCCGG CCACA AGTGG CAGTG AGACA CCCGG CACTT
 3101 CAGAG AGTGC CACCC CCGAG AGTGG CCCAG GCACT AGTAC CGAGC CCTCT
 3151 GAAGG CAGTG CGCCA GCCTC GAGCC CACCA GTCTT GAAAC GCCAT CAAGC
 3201 TGAAA TAACT CGTAC TACTC TTCAG TCAGA TCAAG AGGAA ATCGA TTATG
 3251 ATGAT ACCAT ATCAG TTGAA ATGAA GAAGG AAGAT TTTGA CATTT ATGAT
 3301 GAGGA TGAAA ATCAG AGCCC CCGCA GCTTT CAAAA GAAAA CACGA CACTA
 3351 TTTTA TTGCT GCAGT GGAGA GGCTC TGGGA TTATG GGATG AGTAG CTCCC
 3401 CACAT GTTCT AAGAA ACAGG GCTCA GAGTG GCAGT GTCCC TCAGT TCAAG
 3451 AAAGT TGTTT TCCAG GAATT TACTG ATGGC TCCTT TACTC AGCCC TTATA
 3501 CCGTG GAGAA CTAAA TGAAC ATTTG GGACT CCTGG GGCCA TATAT AAGAG
 3551 CAGAA GTTGA AGATA ATATC ATGGT AACTT TCAGA AATCA GGCCT CTCGT
 3601 CCCTA TTCCT TCTAT TCTAG CCTTA TTTCT TATGA GGAAG ATCAG AGGCA
 3651 AGGAG CAGAA CCTAG AAAAA ACTTT GTCAA GCCTA ATGAA ACCAA AACTT
 3701 ACTTT TGGAA AGTGC AACAT CATAT GGCAC CCACT AAAGA TGAGT TTGAC
 3751 TGCAA AGCCT GGGCT TATTT CTCTG ATGTT GACCT GGAAA AAGAT GTGCA
 3801 CTCAG GCCTG ATTGG ACCCC TTCTG GTCTG CCACA CTAAC ACACT GAACC
 3851 CTGCT CATGG GAGAC AAGTG ACAGT ACAGG AATTT GCTCT GTTTT TCACC
 3901 ATCTT TGATG AGACC AAAAG CTGGT ACTTC ACTGA AAATA TGGAA AGAAA
 3951 CTGCA GGGCT CCCTG CAATA TCCAG ATGGA AGATC CCACT TTTAA AGAGA
 4001 ATTAT CGCTT CCATG CAATC AATGG CTACA TAATG GATAC ACTAC CTGGC
 4051 TTAGT AATGG CTCAG GATCA AAGGA TTCGA TGGTA TCTGC TCAGC ATGGG
 4101 CAGCA ATGAA AACAT CCATT CTATT CATTT CAGTG GACAT GTGTT CACTG
 4151 TACGA AAAAA AGAGG AGTAT AAAAT GGCAC TGTAC AATCT CTATC CAGGT
 4201 GTTTT TGAGA CAGTG GAAAT GTTAC CATCC AAAGC TGGAA TTTGG CGGGT
 4251 GGAAT GCCTT ATTGG CGAGC ATCTA CATGC TGGGA TGAGC ACACT TTTTC
 4301 TGGTG TACAG CAATA AGTGT CAGAC TCCCC TGGGA ATGGC TTCTG GACAC
 4351 ATTAG AGATT TTCAG ATTAC AGCTT CAGGA CAATA TGGAC AGTGG GCCCC
 4401 AAAGC TGGCC AGACT TCATT ATTCC GGATC AATCA ATGCC TGGAG CACCA
 4451 AGGAG CCCTT TTCTT GGATC AAGGT GGATC TGTTG GCACC AATGA TTATT
 4501 CACGG CATCA AGACC CAGGG TGCCC GTCAG AAGTT CTCCA GCCTC TACAT
 4551 CTCTC AGTTT ATCAT CATGT ATAGT CTTGA TGGGA AGAAG TGCCA GACTT
 4601 ATCGA GGAAA TTCCA CTGGA ACCTT AATGG TCTTC TTTGG CAATG TGGAT
 4651 TCATC TGGGA TAAAA CACAA TATTT TTAAC CCTCC AATTA TTGCT CGATA
 4701 CATCC GTTTG CACCC AACTC ATTAT AGCAT TCGCA GCACT CTTCG CATGG
 4751 AGTTG ATGGG CTGTG ATTTA AATAG TTGCA GCATG CCATT GGGAA TGGAG
 4801 AGTAA AGCAA TATCA GATGC ACAGA TTACT GCTTC ATCCT ACTTT ACCAA
 4851 TATGT TTGCC ACCTG GTCTC CTTCA AAAGC TCGAC TTCAC CTCCA AGGGA
 4901 GGAGT AATGC CTGGA GACCT CAGGT GAATA ATCCA AAAGA GTGGC TGCAA
 4951 GTGGA CTTCC AGAAG ACAAT GAAAG TCACA GGAGT AACTA CTCAG GGAGT
 5001 AAAAT CTCTG CTTAC CAGCA TGTAT GTGAA GGAGT TCCTC ATCTC CAGCA
 5051 GTCAA GATGG CCATC AGTGG ACTCT CTTTT TTCAG AATGG CAAAG TAAAG
 5101 GTTTT TCAGG GAAAT CAAGA CTCCCT TCACA CCTGT GGTGA ACTCT CTAGA
 5151 CCCAC CGTTA CTGAC TCGCT ACCTT CGAAT TCACC CCCAG AGTTG GGTGC
 5201 ACCAG ATTGC CCTGA GGATG GAGGT TCTGG GCTGC GAGGC ACAGG ACCTC
 5251 TACGA CAAAA CTCAC ACATG CCCAC CGTGC CCAGC TCCAG AACTC CTGGG
 5301 CGGAC CGTCA GTCTT CCTCT TCCCC CCAAA ACCCA AGGAC ACCCT CATGG
 5351 CCTCC CGGAC CCCTG AGGTG ACATG CGTGG TGGTG GACGT GAGCC ACGAA
 5401 GACCC TGAGG TCAAG TTCAA CTGGT ACGTG GACGG CGTGG AGGTG CATAA
 5451 TGCCA AGACA AAGCC GCGGG AGGAG CAGTA CAACA GCACG TACCG TGTGG
 5501 TCAGC GTCCT CACCG TCCTG GCCCA GGACT GGCTG AATGG CAAGG AGTAC
 5551 AAGTG CAAGG TCTCC AACAA AGCCC TCCCA GCCCC CATCG AGAAA ACCAT
 5601 CTCCA AAGCC AAAGG GCAGC CCCGA GAACC ACAGG TGTAC ACCCT GCCCC
 5651 CATCC CGGGA TGAGC TGACC AAGAA CCAGG TCAGC CTGAC CTGCC TGGTC
 5701 AAAGG CTTCT ATCCC AGCGA CATCG CCGTG GAGTG GGAGA GCAAT GGGCA
 5751 GCCGG AGAAC AACTA CAAGA CCACG CCTCC CGTGT TGGAC TCCGA CGGCT
 5801 CCTTC TTCCT CTACA GCAAG CTCAC CGTGG ACAAG AGCAG GTGGC AGCAG
 5851 GGGAA CGTCT TCTCA TGCTC CGTGA TGCAT GAGGC TCTGC ACAAC GCCTA

5901 CACGC AGAAG AGCCT CTCCC TGTCT CCGGG TAAAT GA

FVIII 283 protein sequence (FVIII 169 with IHH triple mutation) (SEQ ID NO: 161)

```

1   MQIELSTCFF LCLLRFCFSA TRRYYLGAVE LSWDYMQSDL GELPVDARFP
51  PRVPKSFPPN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
101 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
451 TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIIYPHGIT
501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVEDTLTLF PFSGETVFM
701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYED SYEDISAYLL
751 SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
801 SGPGSEPATS GSETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
851 EGTSESATPE SGPGSEPATS GSETPGTSES ATPESGPGSP AGSPTSTEEG
901 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
951 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
1001 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
1051 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
1101 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVQPQFK
1151 KVVVFQEFDTG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1201 PYSFYSSLIS YEEDQRQGA PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1251 CKAWAYFSDV DLEKDVHSGI IGPLLVCNTN TLNPAHGRQV TVQEFALFFT
1301 IFDETKSWYF TENMERN CRA PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1351 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
1401 VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
1451 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
1501 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
1551 SSGIKHNIFN PPIIARYIRL HPTHYSIRST LRMELMGCDL NSCSMPLGME
1601 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
1651 VDFQKTMKVT GVTQGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
1701 VFQGNQDSFT PVVNSLDPEL LTRYLRHPQ SWVHQIALRM EVLGCEAQDL
1751 YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMASRTPEV TCVVVDVSHE
1801 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL AQDWLNGKEY
1851 KCKVSNKALP APIEKTISKA KGQPREPQVY TLPPSRDELT KNQVSLTCLV
1901 KGFYPSDIAV EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ
1951 GNVFSCSVMH EALHNAYTQK SLSLSPGK*

```

pSYNFVIII 010 nucleotide sequence-(Dual chain FVIIIc) (SEQ ID NO: 162)

```

1   ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG
51  CTTTAGTGCC ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG
101 ACTATATGCA AAGTGATCTC GGTGAGCTGC CTGTGGACGC AAGATTTCCCT
151 CCTAGAGTGC CAAAATCTTT TCCATTCAAC ACCTCAGTCG TGTACAAAAA
201 GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC GCTAAGCCAA
251 GGCCACCCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT
301 GATACAGTGG TCATTACACT TAAGAACATG GCTTCCCATC CTGTCAGTCT
351 TCATGCTGTT GGTGTATCCT ACTGGAAAGC TTCTGAGGGA GCTGAATATG

```

401	ATGATCAGAC	CAGTCAAAGG	GAGAAAAGAAG	ATGATAAAGT	CTTCCCTGGT
451	GGAAGCCATA	CATATGTCTG	GCAGGTCCCTG	AAAGAGAATG	GTCCAATGGC
501	CTCTGACCCA	CTGTGCCTTA	CCTACTCATA	TCTTTCTCAT	GTGGACCTGG
551	TAAAAGACTT	GAATTCAGGC	CTCATTTGGAG	CCCTACTAGT	ATGTAGAGAA
601	GGGAGTCTGG	CCAAGGAAAA	GACACAGACC	TTGCACAAAT	TTATACTACT
651	TTTTGCTGTA	TTTGATGAAG	GGAAAAGTTG	GCACTCAGAA	ACAAAGAACT
701	CCTTGATGCA	GGATAGGGAT	GCTGCATCTG	CTCGGGCCTG	GCCTAAAATG
751	CACACAGTCA	ATGGTTATGT	AAACAGGTCT	CTGCCAGGTC	TGATTGGATG
801	CCACAGGAAA	TCAGTCTATT	GGCATGTGAT	TGGAATGGGC	ACCACTCCTG
851	AAGTGCACCT	AATATTCCTC	GAAGGTCACA	CATTTCTTGT	GAGGAACCAT
901	CGCCAGGCGT	CCTTGGAAT	CTCGCCAATA	ACTTTCCCTTA	CTGCTCAAAC
951	ACTCTTGATG	GACCTTGGAC	AGTTTCTACT	GTTTTGTCAT	ATCTCTTCCC
1001	ACCAACATGA	TGGCATGGAA	GCTTATGTCA	AAGTAGACAG	CTGTCCAGAG
1051	GAACCCCAAC	TACGAATGAA	AAATAATGAA	GAAGCGGAAG	ACTATGATGA
1101	TGATCTTACT	GATTCTGAAA	TGGATGTGGT	CAGGTTTGAT	GATGACAACT
1151	CTCCTTCCTT	TATCCAAATT	CGCTCAGTTG	CCAAGAAGCA	TCCTAAAACCT
1201	TGGGTACATT	ACATTGCTGC	TGAAGAGGAG	GACTGGGACT	ATGCTCCCTT
1251	AGTCCTCGCC	CCCGATGACA	GAAGTTATAA	AAGTCAATAT	TTGAACAATG
1301	GCCCTCAGCG	GATTGGTAGG	AAGTACAAAA	AAGTCCGATT	TATGGCATAAC
1351	ACAGATGAAA	CCTTTAAGAC	TCGTGAAGCT	ATTCAGCATG	AATCAGGAAT
1401	CTTGGGACCT	TTACTTTATG	GGGAAGTTGG	AGACACACTG	TTGATTATAT
1451	TTAAGAATCA	AGCAAGCAGA	CCATATAACA	TCTACCCTCA	CGGAATCACT
1501	GATGTCCGTC	CTTTGTATTC	AAGGAGATTA	CCAAAAGGTG	TAAAACATTT
1551	GAAGGATTTT	CCAATTCTGC	CAGGAGAAAT	ATTCAAATAT	AAATGGACAG
1601	TGACTGTAGA	AGATGGGCCA	ACTAAATCAG	ATCCTCGGTG	CCTGACCCGC
1651	TATTACTCTA	GTTTCGTTAA	TATGGAGAGA	GATCTAGCTT	CAGGACTCAT
1701	TGGCCCTCTC	CTCATCTGCT	ACAAAAGATC	TGTAGATCAA	AGAGGAAACC
1751	AGATAATGTC	AGACAAGAGG	AATGTCAATC	TGTTTTCTGT	ATTTGATGAG
1801	AACCGAAGCT	GGTACCTCAC	AGAGAATATA	CAACGCTTTC	TCCCCAATCC
1851	AGCTGGAGTG	CAGCTTGAGG	ATCCAGAGTT	CCAAGCCTCC	AACATCATGC
1901	ACAGCATCAA	TGGCTATGTT	TTTGATAGTT	TGCAGTTGTC	AGTTTGTTTG
1951	CATGAGGTGG	CATACTGGTA	CATTCTAAGC	ATTGGAGCAC	AGACTGACTT
2001	CCTTTCTGTC	TTCTTCTCTG	GATATACCTT	CAAACACAAA	ATGGTCTATG
2051	AAGACACACT	CACCTTATTC	CCATTCTCAG	GAGAAACTGT	CTTCATGTCG
2101	ATGGAAAAACC	CAGGTCTATG	GATTCTGGGG	TGCCACAACCT	CAGACTTTTCG
2151	GAACAGAGGC	ATGACCGCCT	TACTGAAGGT	TTCTAGTTGT	GACAAGAACA
2201	CTGGTGATTA	TTACGAGGAC	AGTTATGAAG	ATATTTTCAGC	ATACTTGCTG
2251	AGTAAAAACA	ATGCCATTGA	ACCAAGAAGC	TTCTCTCAAA	ACCCACCAGT
2301	CTTGAAACGC	CATCAACGGG	AAATAACTCG	TACTACTCTT	CAGTCAGATC
2351	AAGAGGAAAT	TGACTATGAT	GATACCATAT	CAGTTGAAAT	GAAGAAGGAA
2401	GATTTTGACA	TTTATGATGA	GGATGAAAAT	CAGAGCCCCC	GCAGCTTTCA
2451	AAAGAAAACA	CGACACTATT	TTATTGCTGC	AGTGGAGAGG	CTCTGGGATT
2501	ATGGGATGAG	TAGCTCCCCA	CATGTTCTAA	GAAACAGGGC	TCAGAGTGGC
2551	AGTGTCCCTC	AGTTCAAGAA	AGTTGTTTTT	CAGGAATTTA	CTGATGGCTC
2601	CTTTACTCAG	CCCTTATACC	GTGGAGAACT	AAATGAACAT	TTGGGACTCC
2651	TGGGGCCATA	TATAAGAGCA	GAAGTTGAAG	ATAATATCAT	GGTAACTTTC
2701	AGAAATCAGG	CCTCTCGTCC	CTATTCCTTC	TATTCTAGCC	TTATTTCTTA
2751	TGAGGAAGAT	CAGAGGCAAG	GAGCAGAACC	TAGAAAAAAC	TTTGTCAAGC
2801	CTAATGAAAC	CAAACTTAC	TTTTGGAAAG	TGCAACATCA	TATGGCACCC
2851	ACTAAAGATG	AGTTTGACTG	CAAAGCCTGG	GCTTATTTCT	CTGATGTTGA
2901	CCTGGAAAAA	GATGTGCACT	CAGGCCTGAT	TGGACCCCTT	CTGGTCTGCC
2951	ACACTAACAC	ACTGAACCTT	GCTCATGGGA	GACAAGTGAC	AGTACAGGAA
3001	TTTGCTCTGT	TTTTACCCAT	CTTTGATGAG	ACCAAAAAGCT	GGTACTTCAC
3051	TGAAAAATATG	GAAAGAAACT	GCAGGGCTCC	CTGCAATATC	CAGATGGAAG
3101	ATCCCACTTT	TAAAGAGAAAT	TATCGCTTCC	ATGCAATCAA	TGGCTACATA
3151	ATGGATACAC	TACCTGGCTT	AGTAATGGCT	CAGGATCAAA	GGATTCGATG
3201	GTATCTGCTC	AGCATGGGCA	GCAATGAAAA	CATCCATTCT	ATTCATTTCA
3251	GTGGACATGT	GTTCACTGTA	CGAAAAAAAG	AGGAGTATAA	AATGGCACTG
3301	TACAATCTCT	ATCCAGGTGT	TTTTGAGACA	GTGGAAATGT	TACCATCCAA

```

3351 AGCTGGAATT TGGCGGGTGG AATGCCTTAT TGGCGAGCAT CTACATGCTG
3401 GGATGAGCAC ACTTTTTCTG GTGTACAGCA ATAAGTGTC A GACTCCCCTG
3451 GGAATGGCTT CTGGACACAT TAGAGATTTT CAGATTACAG CTTTCAGGACA
3501 ATATGGACAG TGGGCCCCAA AGCTGGCCAG ACTTCATTAT TCCGGATCAA
3551 TCAATGCCTG GAGCACCAAG GAGCCCTTTT CTTGGATCAA GGTGGATCTG
3601 TTGGCACCAA TGATTATTCA CGGCATCAAG ACCCAGGGTG CCCGTGAGAA
3651 GTTCTCCAGC CTCTACATCT CTCAGTTTAT CATCATGTAT AGTCTTGATG
3701 GGAAGAAGTG GCAGACTTAT CGAGGAAAT CCCTGGAAC CTTAATGGTC
3751 TTCTTTGGCA ATGTGGATT C ATCTGGGATA AAACACAATA TTTTAAACCC
3801 TCCAATTATT GCTCGATACA TCCGTTTGCA CCCAACTCAT TATAGCATTC
3851 GCAGCACTCT TCGCATGGAG TTGATGGGCT GTGATTTAAA TAGTTGCAGC
3901 ATGCCATTGG GAATGGAGAG TAAAGCAATA TCAGATGCAC AGATTACTGC
3951 TTCATCCTAC TTTACCAATA TGTTTGCCAC CTGGTCTCCT TCAAAAGCTC
4001 GACTTCACCT CCAAGGGAGG AGTAATGCCT GGAGACCTCA GGTGAATAAT
4051 CCAAAAGAGT GGCTGCAAGT GGACTTCCAG AAGACAATGA AAGTACAGG
4101 AGTAACTACT CAGGGAGTAA AATCTCTGCT TACCAGCATG TATGTGAAGG
4151 AGTTCCTCAT CTCCAGCAGT CAAGATGGCC ATCAGTGGAC TCTCTTTTTT
4201 CAGAATGGCA AAGTAAAGGT TTTTCAGGGA AATCAAGACT CCTTCACACC
4251 TGTGGTGAAC TCTCTAGACC CACCGTTACT GACTCGCTAC CTTTCAATTC
4301 ACCCCCAGAG TTGGGTGCAC CAGATTGCCC TGAGGATGGA GGTCTGGGC
4351 TGCGAGGCAC AGGACCTCTA CGACAAAAC CACACATGCC CACCGTGCCC
4401 AGCTCCAGAA CTCCTGGGCG GACCGTCAGT CTTCTCTTTC CCCCCAAAC
4451 CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG
4501 GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT GGTACGTGGA
4551 CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA
4601 ACAGCACGTA CCGTGTGGTC AGCGTCTCA CCGTCTTGCA CCAGGACTGG
4651 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC
4701 CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCAC
4751 AGGTGTACAC CCTGCCCCCA TCCCGGGATG AGCTGACCAA GAACCAGGTC
4801 AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA
4851 GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG
4901 TGTTGGACTC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC
4951 AAGAGCAGGT GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA
5001 GGCTCTGCAC AACCCTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA
5051 AATGA

```

pSYNFVIII 010 protein sequence-(Dual chain FVIII Fc) (SEQ ID NO: 163)

```

1 MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQSDL GELPVDARFP
51 PRVPKSFPPN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
101 DTVVITLKNM ASHPVSLHAV GVSYWKA SEG AEYDDQTSQR EKEDDKVFP G
151 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
201 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
251 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
301 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
351 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKH PKT
401 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
451 TDETFKTREA IQHESGILGP LLYGEVGD TL LIIFKNQASR PYN IYPHGIT
501 DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
551 YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
601 NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
651 HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETV FMS
701 MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDY YED SYEDISAYLL
751 SKNNAIEPRS FSQNPPVLKR HQREITR TTL QSDQEEIDYD DTISVEMKKE
801 DFDIYEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLNRNAQSG
851 SVPGFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
901 RNQASRPYSF YSSLISYEED QRQGAEP RKN FVKPNETKTY FEKWQH HMAP
951 TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1001 FALFFTIFDE TKS WYFTENM ERNCRAPCNI QMEDPTFKEN YRFHAINGYI

```

1051	MDTLPGLVMA	QDQIRIRWYLL	SMGSNENIHS	IHFSGHVFTV	RKKEEYKMAL
1101	YNLYPGVFET	VEMLPSKAGI	WRVECLIGEH	LHAGMSTLFL	VYSNKCQTPL
1151	GMASGHIRDF	QITASGQYGQ	WAPKLARLHY	SGSINAWSTK	EPFSWIKVDL
1201	LAPMIIHGIIK	TQGARQKFSS	LYISQFIIMY	SLDGKKWQTY	RGNSTGTLMV
1251	FFGNVDSSGI	KHNIFNPPII	ARYIRLHPTH	YSIRSTLRME	LMGCDLNSCS
1301	MPLGMESKAI	SDAQITASSY	FTNMFATWSP	SKARLHLQGR	SNAWRPQVNN
1351	PKEWLQVDFQ	KTMKVTGVTT	QGVKSLLTSM	YVKEFLISSS	QDGHQWTLFF
1401	QNGKVKVFQ	NQDSFTPVVN	SLDPPLLTRY	LRIHPQSWVH	QIALRMEVLG
1451	CEAQDLYDKT	HTCPPCPAPE	LLGGPSVFLF	PPKPKDTLMI	SRTPEVTCVV
1501	VDVSHEDPEV	KFNWYVDGVE	VHNAKTKPRE	EQYNSTYRVV	SVLTVLHQDW
1551	LNGKEYKCKV	SNKALPAPIE	KTISKAKGQP	REPQVYTLPP	SRDELTKNQV
1601	SLTCLVKGFY	PSDIAVEWES	NGQPENNYKT	TPPVLDSDGS	FFLYSKLTV
1651	KSRWQQGNVF	SCSVMEALH	NHYTQKSLSL	SPGK*	

FVIII 195 protein sequence (dual chain FVIII₁₉₅ with two 144 AE XTENs at amino acid 1656 and 1900) (SEQ ID NO: 73)

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGAWE	LSWDYMQSDL	GELPVDARFP
51	PRVPKSFPPN	TSVVYKKTLE	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY
101	DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFP
151	GSHTYVWQVL	KENGPMASDP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE
201	GSLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM
251	HTVNGYVNR	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH
301	RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSCPE
351	EPQLRMKNNE	EAEDYDDDLT	DSEMDVVRFD	DDNSPSFIQI	RSVAKKHPKT
401	WVHYIAAE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
451	TDETFTKREA	IQHESGILGP	LLYGEVGD	LIIFKNQASR	PYNIYPHGIT
501	DVRPLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
551	YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
601	NRSWYLTE	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
651	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFMS
701	MENPGLWILG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYIED	SYEDISAYLL
751	SKNNAIEPRS	FSQNPPVLKR	HQREITRTTL	QGAPGTPGSG	TASSSPGASP
801	GTSTSTGSPGA	SPGTSTSTGSP	GASPGTSTSTG	SPGSSPSAST	GTGPGTPGSG
851	TASSSPGASP	GTSTSTGSPGA	SPGTSTSTGSP	GASPGTSTSTG	SPGSSPSAST
901	TGSPGSSTPS	GATGSPGASP	GTSTSTGSPAS	SSDQEEIDYD	DTISVEMKKE
951	DFDIYDEDEN	QSPRSFQKKT	RHYFIAAVER	LWDYGMSSSP	HVLRNRAQSG
1001	SVPQFKKVVF	QEFTDGSFTQ	PLYRGELNEH	LGLLGPYIRA	EVEDNIMVTF
1051	RNQASRPYSF	YSSLISYEED	QRQGAEPKRN	FVKPNETKTY	FWKVQHMAP
1101	TKDEFDCKAW	AYFSDVDLEK	DVHSGLIGPL	LVCHTNTLNP	AHGRQVTVQE
1151	FALFFTIFDE	TKSWYFTENM	ERNCRGAPTS	ESATPESGPG	SEPATSGSET
1201	PGTSESATPE	SGPGSEPATS	GSETPGTSES	ATPESGPGTS	TEPSEGSAPG
1251	TSESATPESG	PGSPAGSPTS	TEEGSPAGSP	TSTEEGSPAG	SPTSTEEGTS
1301	ESATPESGPG	TSTEPSEGS	PGASSAPCNI	QMEDPTFKEN	YRFHAINGYI
1351	MDTLPGLVMA	QDQIRIRWYLL	SMGSNENIHS	IHFSGHVFTV	RKKEEYKMAL
1401	YNLYPGVFET	VEMLPSKAGI	WRVECLIGEH	LHAGMSTLFL	VYSNKCQTPL
1451	GMASGHIRDF	QITASGQYGQ	WAPKLARLHY	SGSINAWSTK	EPFSWIKVDL
1501	LAPMIIHGIIK	TQGARQKFSS	LYISQFIIMY	SLDGKKWQTY	RGNSTGTLMV
1551	FFGNVDSSGI	KHNIFNPPII	ARYIRLHPTH	YSIRSTLRME	LMGCDLNSCS
1601	MPLGMESKAI	SDAQITASSY	FTNMFATWSP	SKARLHLQGR	SNAWRPQVNN
1651	PKEWLQVDFQ	KTMKVTGVTT	QGVKSLLTSM	YVKEFLISSS	QDGHQWTLFF
1701	QNGKVKVFQ	NQDSFTPVVN	SLDPPLLTRY	LRIHPQSWVH	QIALRMEVLG
1751	CEAQDLYDKT	HTCPPCPAPE	LLGGPSVFLF	PPKPKDTLMI	SRTPEVTCVV
1801	VDVSHEDPEV	KFNWYVDGVE	VHNAKTKPRE	EQYNSTYRVV	SVLTVLHQDW
1851	LNGKEYKCKV	SNKALPAPIE	KTISKAKGQP	REPQVYTLPP	SRDELTKNQV
1901	SLTCLVKGFY	PSDIAVEWES	NGQPENNYKT	TPPVLDSDGS	FFLYSKLTV
1951	KSRWQQGNVF	SCSVMEALH	NHYTQKSLSL	SPGK*	

pSYN-FVIII-173 mature Protein sequencing (SEQ ID NO: 72):

```

1   ATRRYYLGA V  ELSWDYMQSD  LGELPVDARF  PPRVPKSFPF  NTSVVYKCTL
51  FVEFTDHLFN  IAKPRPPWMG  LLGPTIQAEV  YDTVVTILKN  MASHPVSLHA
101 VGSYWKASE    GA EYDDQTSQ  REKEDDKVFP  GGSHTYVWQV  LKENGPMASD
151 PLCLTYSYLS   HVDLVKDLNS  GLIGALLVCR  EGSLAKEKTQ  TLHKFILLFA
201 VFDEGKSWHS   ETKNSLMQDR  DAASARAWPK  MHTVNGYVNR  SLPGLIGCHR
251 KSVYWHVIGM   GTTPEVHSIF  LEGHTFLVRN  HRQASLEISP  ITFLTAQTLL
301 MDLGQFLLFC   HISSHQHDGM  EAYVKVDSCP  EEPQLRMKNN  EEAEDYDDDL
351 TDSEMDVVRF   DDDNSPSFIQ  IRSVAKKHPK  TWVHYIAAEE  EDWDYAPLVL
401 APDDRSYKSQ   YLNNGPQRIG  RYKKVRFMA  YTDETFKTRE  AIQHEGILG
451 PLLYGEVGD T  LLIIFKNQAS  RPYNIYPHGI  TDVRPLYSR  LPKGVKHLKD
501 FPILPGEIFK   YKWTVTVEDG  PTKSDPRCLT  RYYSFVNME  RDLASGLIGP
551 LLICYKESVD   QRGNQIMSDK  RNVILFSVFD  ENRSWYLTEN  IQRFLPNPAG
601 VQLEDPEFQA   SNIMHSINGY  VFDSLQLSVC  LHEVAYWYIL  SIGAQTDFLS
651 VFFSGYTFFKH  KMYEDTLTL  FFFSGETVFM  SMENPGLWIL  GCHNSDFRNR
701 GMTALLKVSS   CDKNTGDYEE  DSYEDISAYL  LSKNNAIEPR  SFSQNGAPGT
751 SESATPESGP   GSEPATSGSE  TPGTSESATP  ESGPGSEPAT  SGSETPGTSE
801 SATPESGPGT   STEPSEGSAP  GSPAGSPTST  EEGTSESATP  ESGPGSEPAT
851 SGSETPGTSE   SATPESGPGS  PAGSPTSTEE  GSPAGSPTST  EEGTSTEPSE
901 GSAPGTSESA   TPESGPGTSE  SATPESGPGT  SESATPESGP  GSEPATSGSE
951 TPGSEPATSG   SETPGSPAGS  PTSTEEGTST  EPSEGSAPGT  STEPSEGSAP
1001 GSEPATSGSE   TPGTSESATP  ESGPGTSTEP  SEGSA PASSP  PVLKRHQREI
1051 TRTTLQSDQE   EIDYDDTISV  EMKKEDFDIY  DEDENQSPRS  FQKKTRHYFI
1101 AAVERLWDYG   MSSSPHVLRN  RAQSGSV PQF  KKVVFQETD  GSFTQPLYRG
1151 ELNEHLGLLG   PYIRAEVEDN  IMVTRFNQAS  RPYSFYSSLI  SYEEDQRQGA
1201 EPRKNFVKPN   ETKTYFWKVQ  HHMAPTKDEF  DCKAWAYFSD  VDLEKDVHSG
1251 LIGPLLVCHT   NTLNPAHGRQ  VTVQEFALFF  TIFDETKSWY  FTENMERNCR
1301 APCNIQMEDP   TFKENYRFHA  INGYIMDTLP  GLVMAQDQRI  RYLLSMGSN
1351 ENIHSIHFSG   HVFTVRKKEE  YKMALYNLYP  GVFE TVEMLP  SKAGIWRVEC
1401 LIGEHLHAGM   STLFLVYSNK  CQTPLGMASG  HIRDFQITAS  GQYQGWAPKL
1451 ARLHYSGSIN   AWSTKEPFSW  IKVDLLAPMI  IHGIKTQGAR  QKFSSLYISQ
1501 FIIMYSLDGK   KWQTYRGNST  GTLMVFFGNV  DSSGIKHNIF  NPPIIARYIR
1551 LHPTHYSIRS   TLRMELMGCD  LNSCSMPLGM  ESKAISDAQI  TASSYFTNMF
1601 ATWSPSKARL   HLQGRSNAWR  PQVNNPKEWL  QVDFQKTMKV  TGVTTQGVKS
1651 LLTSMYVKEF   LISSSQDGHQ  WTLFFQNGKV  KVFQGNQDSF  TPVNSLDPP
1701 LLTRYLRIHP   QSWVHQIALR  MEVLGCEAQD  LYDKTHTCPP  CPAPELLGGP
1751 SVFLFPKPK    DTLMISRTPE  VTCVVVDVSH  EDPEVKFNWY  VDGVEVHNAK
1801 TKPREEQYNS   TYRVVSVLTV  LHQDWLNGKE  YKCKVSNKAL  PAPIEKTISK
1851 AKGQPREPQV   YTLPPSRDEL  TKNQVSLTCL  VKGFYPSDIA  VEWESNGQPE
1901 NNYKTPPVVL   DSDGSFFLYS  KLTVDKSRWQ  QGNVFSCSVM  HEALHNHYTQ
1951 KSLSLSPGK

```

FVIII 196 protein sequence (dual chain FVIII Fc with three 144 AE XTENs at amino acid 26, 1656 and 1900) (SEQ ID NO: 74)

```

1   MQIELSTCFF  LCLLRFCFSA  TRRYYLGA VE  LSWDYMQSDL  GELPVGAPGS
51  SPSASTGTGP  GSSPSASTGT  GPGASPGTSS  TGSPGASPGT  SSTGSPGSST
101 PSGATGSPGS  SPSASTGTGP  GASPGTSSTG  SPGSSPSAST  GTGPGTPGSG
151 TASSSPGSST  PSGATGSPGS  STPSGATGSP  GASPGTSSTG  SPASSDARFP
201 PRVPKSFPFN  TSVVYKKTLE  VEFTDHLFNI  AKPRPPWMGL  LGPTIQAEVY
251 DTVVTILKNM  ASHPVSLHAV  GVSYWKASEG  AEYDDQTSQR  EKEDDKVFPG
301 GSHTYVWQVL  KENGPMASDP  LCLTYSYLSH  VDLVKDLNSG  LIGALLVCRE
351 GSLAKEKTQT  LHKFILLFAV  FDEGKSWHSE  TKNSLMQDRD  AASARAWPKM
401 HTVNGYVNR  LPGLIGCHRK  SVYWHVIGMG  TTPEVHSIFL  EGHTFLVRNH
451 RQASLEISPI  TFLTAQTLLM  DLGQFLLFCH  ISSHQHDGME  AYVKVDSCPE
501 EPQLRMKNNE  EAEDYDDDLT  DSEMDVVRFD  DDNSPSFIQI  RSVAKKHPKT
551 WVHYIAAEEE  DWDYAPLVLA  PDDRSYKSQY  LNNGPQRIGR  KYKKVRFMAY

```

601	TDETFKTTREA	IQHESGILGP	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
651	DVRPLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
701	YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
751	NRSWYL TENI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
801	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFMS
851	MENPGLWILG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYYED	SYEDISAYLL
901	SKNNAIEPRS	FSQNPPVLKR	HQREITRRTL	QGAPGTPGSG	TASSSPGASP
951	GTSTTGSPGA	SPGTSSTGSP	GASPGTSSTG	SPGSSPSAST	GTGPGTPGSG
1001	TASSSPGASP	GTSTTGSPGA	SPGTSSTGSP	GASPGTSSTG	SPGSSTPSGA
1051	TGSPGSSTPS	GATGSPGASP	GTSTTGSPAS	SSDQEEIDYD	DTISVEMKKE
1101	DFDIYDEDEN	QSPRSFQKKT	RHYFIAAVER	LWDYGMSSSP	HVLRNRAQSG
1151	SVPQFKKVVF	QEFTDGSFTQ	PLYRGELNEH	LGLLGPYIRA	EVEDNIMVTF
1201	RNQASRPYSF	YSSLISYEED	QRQGAEPKRN	FVKPNETKTY	FWKVQHMAP
1251	TKDEFDCKAW	AYFSDVDLEK	DVHSGLIGPL	LVCHTNTLNP	AHGRQVTVQE
1301	FALFFTIFDE	TKSWYFTENM	ERNCRGAPTS	ESATPESGPG	SEPATSGSET
1351	PGTSESATPE	SGPGSEPATS	GSETPGTSES	ATPESGPGTS	TEPSEGSAPG
1401	TSESATPESG	PGSPAGSPTS	TEEGSPAGSP	TSTEEGSPAG	SPTSTEEGTS
1451	ESATPESGPG	TSTEPSEGSA	PGASSAPCNI	QMEDPTFKEN	YRFHAINGYI
1501	MDTLPGLVMA	QDQIRRWYLL	SMGSNENIHS	IHFSGHVFTV	RKKEEYKMAL
1551	YNLYPGVFET	VEMLPSKAGI	WRVECLIGEH	LHAGMSTLFL	VYSNKCQTPPL
1601	GMASGHIRDF	QITASGQYGQ	WAPKLARLHY	SGSINAWSTK	EPFSWIKVDL
1651	LAPMIIHGKI	TQGARQKFSS	LYISQFIIMY	SLDGKKWQTY	RGNSTGTLMV
1701	FFGNVDSSGI	KHNIFNPPII	ARYIRLHPTH	YSIRSTLRME	LMGCDLNSCS
1751	MPLGMESKAI	SDAQITASSY	FTNMFATWSP	SKARLHLQGR	SNAWRPQVNN
1801	PKEWLQVDFQ	KTMKVTGVTT	QGVKSLTSM	YVKEFLISSS	QDGHQWTLFF
1851	QNGKVKVFQ	NQDSFTPVVN	SLDPPLLTRY	LRIHPQSWVH	QIALRMEVLG
1901	CEAQDLYDKT	HTCPPCPAPE	LLGGPSVFLF	PPKPKDTLMI	SRTPEVTCVV
1951	VDVSHEDPEV	KFNWYVDGVE	VHNAKTKPRE	EQYNSTYRVV	SVLTVLHQDW
2001	LNGKEYKCKV	SNKALPAPIE	KTISKAKGQP	REPQVYTLPP	SRDELTKNQV
2051	SLTCLVKGFY	PSDIAVEWES	NGQPENNYKT	TPPVLDSDGS	FFLYSKLTVD
2101	KSRWQQGNVF	SCSVMHEALH	NHYTQKSLSL	SPGK*	

FVIII 199 protein sequence (single chain FVIII Fc with three 144 AE XTENs at amino acid 1656 and 1900) (SEQ ID NO: 75)

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGAVE	LSWDYMQSDL	GELPVDARFP
51	PRVPKSFPPN	TSVVYKKTLE	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY
101	DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFP
151	GSHTYVWQVL	KENGPMASDP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE
201	GSLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM
251	HTVNGYVNRS	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH
301	RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSCPE
351	EPQLRMKNNE	EAEDYDDDLT	DSEMDVVRFD	DDNSPSFIQI	RSVAKKHPKT
401	WVHYIAAEEE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
451	TDETFKTTREA	IQHESGILGP	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
501	DVRPLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
551	YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
601	NRSWYL TENI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
651	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFMS
701	MENPGLWILG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYYED	SYEDISAYLL
751	SKNNAIEPRS	FSQNPPVLKR	HQAEITRRTL	QGAPGTPGSG	TASSSPGASP
801	GTSTTGSPGA	SPGTSSTGSP	GASPGTSSTG	SPGSSPSAST	GTGPGTPGSG
851	TASSSPGASP	GTSTTGSPGA	SPGTSSTGSP	GASPGTSSTG	SPGSSTPSGA
901	TGSPGSSTPS	GATGSPGASP	GTSTTGSPAS	SSDQEEIDYD	DTISVEMKKE
951	DFDIYDEDEN	QSPRSFQKKT	RHYFIAAVER	LWDYGMSSSP	HVLRNRAQSG
1001	SVPQFKKVVF	QEFTDGSFTQ	PLYRGELNEH	LGLLGPYIRA	EVEDNIMVTF
1051	RNQASRPYSF	YSSLISYEED	QRQGAEPKRN	FVKPNETKTY	FWKVQHMAP
1101	TKDEFDCKAW	AYFSDVDLEK	DVHSGLIGPL	LVCHTNTLNP	AHGRQVTVQE

1151	FALFFTIFDE	TKSWYFTENM	ERNCRGAPTS	ESATPESGPG	SEPATSGSET
1201	PGTSESATPE	SGPGSEPATS	GSETPGTSES	ATPESGPGTS	TEPSEGSAPG
1251	TSESATPESG	PGSPAGSPTS	TEEGSPAGSP	TSTEEGSPAG	SPTSTEEGTS
1301	ESATPESGPG	TSTEPSEGS	PGASSAPCNI	QMEDPTFKEN	YRFHAINGYI
1351	MDTLPGLVMA	QDQIRRWYLL	SMGSNENIHS	IHFSGHVFTV	RKKEEYKMAL
1401	YNLYPGVFET	VEMLPSKAGI	WRVECLIGEH	LHAGMSTLFL	VYSNKCQTPL
1451	GMASGHIRDF	QITASGQYGQ	WAPKLARLHY	SGSINAWSTK	EPFSWIKVDL
1501	LAPMIIHGK	TQGARKQFSS	LYISQFIIMY	SLDGKKWQTY	RGNSTGTLMV
1551	FFGNVDSSGI	KHNIFNPPII	ARYIRLHPTH	YSIRSTLRME	LMGCDLNSCS
1601	MPLGMESKAI	SDAQITASSY	FTNMFATWSP	SKARLHLQGR	SNAWRPQVNN
1651	PKEWLQVDFQ	KTMKVTGVTT	QGVKSLLTSM	YVKEFLISS	QDGHQWTLFF
1701	QNGKVKVFQ	NQDSFTPVVN	SLDPPLLTRY	LRIHPQSWVH	QIALRMEVLG
1751	CEAQDLYDKT	HTCPPCPAPE	LLGGPSVFLF	PPKPKDTLMI	SRTPEVTCV
1801	VDVSHEDPEV	KFNWYVDGVE	VHNAKTKPRE	EQYNSTYRVV	SVLTVLHQDW
1851	LNGKEYKCKV	SNKALPAPIE	KTISKAKGQP	REPQVYTLPP	SRDELTKNQV
1901	SLTCLVKGFY	PSDIAVEWES	NGQPENNYKT	TPPVLDSDGS	FFLYSKLTV
1951	KSRWQQGNVF	SCSVMHEALH	NHYTQKSLSL	SPGK*	

FVIII 201 protein sequence (single chain FVIII Fc with three 144 AE XTENs at amino acid 26, 1656 & 1900) (SEQ ID NO: 76)

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGAVE	LSWDYMQSDL	GELPVGAPGS
51	SPSASTGTGP	GSSPSASTGT	GPGASPGTSS	TGSPGASPGT	SSTGSPGSST
101	PSGATGSPGS	SPSASTGTGP	GASPGTSSTG	SPGSSPSAST	GTGPGTPGSG
151	TASSSPGSST	PSGATGSPGS	STPSGATGSP	GASPGTSSTG	SPASSDARFP
201	PRVPKSFPPN	TSVVYKKTLE	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY
251	DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFPG
301	GSHTYVWQVL	KENGPMASDP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE
351	GSLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM
401	HTVNGYVNR	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH
451	RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSCPE
501	EPQLRMKNNE	EAEDYDDDLT	DSEMDVVRFD	DDNSPSFIQI	RSVAKKHPKT
551	WVHYIAAEEE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
601	TDETFTKREA	IQHESGILGP	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
651	DVRPLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
701	YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
751	NRSWYLTEI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
801	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFM
851	MENPGLWILG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYYED	SYEDISAYLL
901	SKNNAIEPRS	FSQNPPVLKR	HQAEITRRTL	QGAPGTPGSG	TASSSPGASP
951	GTSTTGSPGA	SPGTSSTGSP	GASPGTSSTG	SPGSSPSAST	GTGPGTPGSG
1001	TASSSPGASP	GTSTTGSPGA	SPGTSSTGSP	GASPGTSSTG	SPGSSTPGSA
1051	TGSPGSSTPS	GATGSPGASP	GTSTTGSPAS	SSDQEEIDYD	DTISVEMKKE
1101	DFDIYDEDEN	QSPRSFQKKT	RHYFIAAVER	LWDYGMSSSP	HVLRNRAQSG
1151	SVPQFKKVVF	QEFTDGSFTQ	PLYRGELNEH	LGLLGPYIRA	EVEDNIMVTF
1201	RNQASRPYSF	YSSLISYEED	QRQGAEPKRN	FVKPNETKTY	FWKVQHMAP
1251	TKDEFDCKAW	AYFSDVDLEK	DVHSGLIGPL	LVCHTNTLNP	AHGRQVTVQE
1301	FALFFTIFDE	TKSWYFTENM	ERNCRGAPTS	ESATPESGPG	SEPATSGSET
1351	PGTSESATPE	SGPGSEPATS	GSETPGTSES	ATPESGPGTS	TEPSEGSAPG
1401	TSESATPESG	PGSPAGSPTS	TEEGSPAGSP	TSTEEGSPAG	SPTSTEEGTS
1451	ESATPESGPG	TSTEPSEGS	PGASSAPCNI	QMEDPTFKEN	YRFHAINGYI
1501	MDTLPGLVMA	QDQIRRWYLL	SMGSNENIHS	IHFSGHVFTV	RKKEEYKMAL
1551	YNLYPGVFET	VEMLPSKAGI	WRVECLIGEH	LHAGMSTLFL	VYSNKCQTPL
1601	GMASGHIRDF	QITASGQYGQ	WAPKLARLHY	SGSINAWSTK	EPFSWIKVDL
1651	LAPMIIHGK	TQGARKQFSS	LYISQFIIMY	SLDGKKWQTY	RGNSTGTLMV
1701	FFGNVDSSGI	KHNIFNPPII	ARYIRLHPTH	YSIRSTLRME	LMGCDLNSCS
1751	MPLGMESKAI	SDAQITASSY	FTNMFATWSP	SKARLHLQGR	SNAWRPQVNN
1801	PKEWLQVDFQ	KTMKVTGVTT	QGVKSLLTSM	YVKEFLISS	QDGHQWTLFF

1851	QNGKVKVFQG	NQDSFTPVVN	SLDPPLLTRY	LRIHPQSWVH	QIALRMEVLG
1901	CEAQDLYDKT	HTCPPCPAPE	LLGGPSVFLF	PPKPKDTLMI	SRTPEVTCVV
1951	VDVSHEDPEV	KFNWYVDGVE	VHNAKTKPRE	EQYNSTYRVV	SVLTVLHQDW
2001	LNGKEYKCKV	SNKALPAPIE	KTISKAKGQP	REPQVYTLPP	SRDELTKNQV
2051	SLTCLVKGFY	PSDIAVEWES	NGQPENNYKT	TPPVLDSDGS	FFLYSKLTVD
2101	KSRWQQGNVF	SCSVMHEALH	NHYTQKSLSL	SPGK*	

FVIII 203 protein sequence (single chain FVIII Fc with two AE XTENS; one 288AE XTEN in B-domain and one 144 AE XTEN at amino acid 1900) (SEQ ID NO: 77)

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGAVE	LSWDYMQSDL	GELPVDARFP
51	PRVPKSFPPN	TSVVYKKTLE	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY
101	DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFPG
151	GSHTYVWQVL	KENGPMASDP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE
201	GSLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM
251	HTVNGYVNRS	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH
301	RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSCPE
351	EPQLRMKNNE	EAEDYDDDLT	DSEMDVVRFD	DDNSPSFIQI	RSVAKKHPKT
401	WVHYIAAEEE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
451	TDETFKTTREA	IQHESGILGP	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
501	DVRPLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
551	YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
601	NRSWYLTENI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
651	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFMMS
701	MENPGLWLIG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYDYE	SYEDISAYLL
751	SKNNAIEPRS	FSQNGAPGTS	ESATPESGPG	SEPATSGSET	PGTSESATPE
801	SGPGSEPATS	GSETPGTSES	ATPESGPGTS	TEPSEGSAPG	SPAGSPTSTE
851	EGTSESATPE	SGPGSEPATS	GSETPGTSES	ATPESGPGSP	AGSPTSTEEG
901	SPAGSPTSTE	EGTSTEPSEG	SAPGTSESAT	PESGPGTSES	ATPESGPGTS
951	ESATPESGPG	SEPATSGSET	PGSEPATSGS	ETPGSPAGSP	TSTEEGTSTE
1001	PSEGSAPGTS	TEPSEGSAPG	SEPATSGSET	PGTSESATPE	SGPGTSTEPS
1051	EGSAPASSPP	VLKRHQAEIT	RTTLQSDQEE	IDYDDTISVE	MKKEDFDIYD
1101	EDENQSPRSF	QKKTRHYFIA	AVERLWDYGM	SSSPHVLNRN	AQSGSVPPQFK
1151	KVVVFQFTDG	SFTQPLYRGE	LNEHLGLLGP	YIRAEVEDNI	MVTFRNQASR
1201	PYSFYSSLIS	YEEDQRQGAE	PRKNFVKPNE	TKTYFWKVQH	HMAPTKDEFD
1251	CKAWAYFSDV	DLEKDVHSGL	IGPLLVCHTN	TLNPAHGRQV	TVQEFALFFT
1301	IFDETKSWYF	TENMERNCRG	APTSESATPE	SGPGSEPATS	GSETPGTSES
1351	ATPESGPGSE	PATSGSETPG	TSESATPESG	PGTSTEPSEG	SAPGTSESAT
1401	PESGPGSPAG	SPTSTEEGSP	AGSPTSTEEG	SPAGSPTSTE	EGTSESATPE
1451	SGPGTSTEPS	EGSAPGASSA	PCNIQMEDPT	FKENYRFHAI	NGYIMDTLPG
1501	LVMAQDQIR	WYLLSMGSNE	NIHSIHFSGH	VFTVRKKEEY	KMALYNLYPG
1551	VFETVEMLPS	KAGIWRVECL	IGEHLHAGMS	TLFLVYSNKC	QTPLGMASGH
1601	IRDFQITASG	QYGQWAPKLA	RLHYSGSINA	WSTKEPFSWI	KVDLLAPMII
1651	HGIKTQGARQ	KFSSLYISQF	IIMYSLDGKK	WQTYRGNSTG	TLMVFFGNVD
1701	SSGIKHNIFN	PPIIARYIRL	HPTHYSIRST	LRMELMGCDL	NSCSMPLGME
1751	SKAISDAQIT	ASSYFTNMFA	TWSPSKARLH	LQGRSNAWRP	QVNNPKEWLQ
1801	VDFQKTMKVT	GVTTQGVKSL	LTSMYVKEFL	ISSSQDGHQW	TLFFQNGKVK
1851	VFQGNQDSFT	PVVNSLDPPL	LTRYLRIHPQ	SWVHQIALRM	EVLGCEAQDL
1901	YDKTHTCPPC	PAPELLGGPS	VFLFPPKPKD	TLMISRTPEV	TCVVVDVSHE
1951	DPEVKFNWYV	DGVEVHNAKT	KPREEQYNST	YRVVSVLTVL	HQDWLNGKEY
2001	KCKVSNKALP	APIEKTISKA	KGQPREPQVY	TLPPSRDELT	KNQVSLTCLV
2051	KGFYPSDIAV	EWESNGQPEN	NYKTTPPVLD	SDGSFFLYSK	LTVDKSRWQQ
2101	GNVVFSCVMH	EALHNHYTQK	SLSLSPGK*		

FVIII 204 protein sequence (single chain FVIII Fc with two AE XTENS; one 288AE XTEN in B-domain and one 144 AE XTEN at amino acid 403) (SEQ ID NO: 78)

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGAVE	LSWDYMQSDL	GELPVDARFP
---	------------	------------	------------	------------	------------

```

51   PRVPKSFPPN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
101  DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
151  GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
201  GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
251  HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
301  RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
351  EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
401  WVHYIAAEEE DWDYAPLVLA PDGAPTSTEP SEGSAPGSPA GSPTSTEEGT
451  STEPSEGSAP GTSTEPSEGS APGTSESATP ESGPGTSTEP SEGSAPGTSE
501  SATPESGPGS EPATSGSETP GTSTEPSEGS APGTSTEPSE GSAPGTSESA
551  TPESGPGTSE SATPESGPGA SSDRSYKSQY LNNGPQRIGR KYKKVRFMAY
601  TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIIYPHGIT
651  DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
701  YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
751  NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
801  HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVEDTLTLF PFSGETVFM
851  MENPGLWLIG CHNSDFRNRG MTALLKVSSC DKNTGDYED SYEDISAYLL
901  SKNNAIEPRS FSQNGAPGTS ESATPESGPG SEPATSGSET PGTSESATPE
951  SGPGSEPAT SGETPGTSES ATPESGPGTS TEPSEGSAPG SPAGSPTSTE
1001 EGTSESATPE SGPGSEPAT SGETPGTSES ATPESGPGSP AGSPTSTEEG
1051 SPAGSPTSTE EGTSTEPSEG SAPGTSESAT PESGPGTSES ATPESGPGTS
1101 ESATPESGPG SEPATSGSET PGSEPATSGS ETPGSPAGSP TSTEEGTSTE
1151 PSEGSAPGTS TEPSEGSAPG SEPATSGSET PGTSESATPE SGPGTSTEPS
1201 EGSAPASSPP VLKRHQAEIT RTTLQSDQEE IDYDDTISVE MKKEDFDIYD
1251 EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR AQSGSVPQFK
1301 KVVVFQFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI MVTFRNQASR
1351 PYSFYSSLIS YEEDQRQGAE PRKNFVKPNE TKTYFWKVQH HMAPTKDEFD
1401 CKAWAYFSDV DLEKDVHSGI IGPLLVCNTN TLNPAHGRQV TVQEFALFFT
1451 IFDETKSWYF TENMERNCR PCNIQMEDPT FKENYRFHAI NGYIMDTLPG
1501 LVMAQDQIR WYLLSMGSNE NIHSIHFSGH VFTVRKKEEY KMALYNLYPG
1551 VFETVEMLPS KAGIWRVECL IGEHLHAGMS TLFLVYSNKC QTPLGMASGH
1601 IRDFQITASG QYGQWAPKLA RLHYSGSINA WSTKEPFSWI KVDLLAPMII
1651 HGIKTQGARQ KFSSLYISQF IIMYSLDGKK WQTYRGNSTG TLMVFFGNVD
1701 SSGIKHNIFN PPIIARYIRL HPHTYSIRST LRMELMGCDL NSCSMPLGME
1751 SKAISDAQIT ASSYFTNMFA TWSPSKARLH LQGRSNAWRP QVNNPKEWLQ
1801 VDFQKTMKVT GVTTOGVKSL LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK
1851 VFQGNQDSFT PVVNSLDPPL LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL
1901 YDKTHTCPPC PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE
1951 DPEVKFNWYV DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY
2001 KCKVSNKALP APIEKTISKA KGQPREPVY TLPPSRDELT KNQVSLTCLV
2051 KGFYPSDIAV EWESNGQPEN NYKTTPPVL SDGSFFLYSK LTVDKSRWQQ
2101 GNVFSCSVMH EALHNNHYTQK SLSLSPGK*

```

FVIII 205 protein sequence (single chain FVIIIc with two AE XTENS; one 288AE XTEN in B-domain and one 144 AE XTEN at amino acid 18) (SEQ ID NO: 79)

```

1   MQIELSTCFF LCLLRFCFSA TRRYLGAWE LSWDYMQGAP TSESATPESG
51  PGSEPATSGS ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
101 TSTEPSEGS SA PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS
151 ESATPESGPG SPAGSPTSTE EGSPAGSPTS TEEGASSSDL GELPVDARFP
201 PRVPKSFPPN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
251 DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
301 GSHTYVWQVL KENGPMASDP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
351 GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
401 HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
451 RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
501 EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
551 WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
601 TDETFKTREA IQHESGILGP LLYGEVGDTL LIIFKNQASR PYNIIYPHGIT

```

651	DVRPLYSRRL	PKG VKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
701	YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
751	NRSWYL TENI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
801	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETV FMS
851	MENPGLWILG	CHNSDFRNRG	MTALLKVSSC	DKNTGDY YED	SYEDISAYLL
901	SKNNAIEPRS	FSQNGAPGTS	ESATPESGPG	SEPATSGSET	PGTSESATPE
951	SGPGSEPAT S	GSETPGTSES	ATPESGPGTS	TEPSEGSAPG	SPAGSPTSTE
1001	EGTSESATPE	SGPGSEPAT S	GSETPGTSES	ATPESGPGSP	AGSPTSTEEG
1051	SPAGSPTSTE	EGTSTEPSEG	SAPGTSESAT	PESGPGTSES	ATPESGPGTS
1101	ESATPESGPG	SEPATSGSET	PGSEPATSGS	ETPGSPAGSP	TSTEEGTSTE
1151	PSEGSAPGTS	TEPSEGSAPG	SEPATSGSET	PGTSESATPE	SGPGTSTEPS
1201	EGSAPASSPP	VLKRHQAEIT	RTTLQSDQEE	IDYDDTISVE	MKKEDFDIYD
1251	EDENQSPRSF	QKKTRHYFIA	AVERLWDYGM	SSSPHVLNR	AQSGSV PQFK
1301	KVVVFQEF TDG	SFTQPLYRGE	LNEHLG L LGP	YIRAEVEDNI	MVTFRNQASR
1351	PYSFYSSLIS	YEEDQRQGAE	PRKNFVKPNE	TKTYFWKVQH	HMAPTKDEFD
1401	CKAWAYFSDV	DLEKDVHSGL	IGPLLVCHTN	TLNPAHGRQV	TVQEFALFFT
1451	IFDETKSWYF	TENMERN CRA	PCNIQMEDPT	FKENYRFHAI	NGYIMDTLPG
1501	LVMAQDQ RIR	WYLLSMGSNE	NIHSIHFSGH	VFTVRKKEEY	KMALYNLYPG
1551	VFETVEMLPS	KAGIWRVECL	IGEHLHAGMS	TLFLVYSNKC	QTPLGMA SGH
1601	IRDFQITASG	QYGQWAPKLA	RLHYSGSINA	WSTKEPFSWI	KVDLLAPMII
1651	HGIKTQGARQ	KFSSLYISQF	IIMYSLDGKK	WQTYRGNSTG	TLMVFFGNVD
1701	SSGIKHNI FN	PPIIARYIRL	HPTHYSIRST	LRMELMGCDL	NSCSMPLGME
1751	SKAISDAQIT	ASSYFTNMFA	TWSPSKARLH	LQGRSNAWRP	QVNNPKEWLQ
1801	VDFQKTMKVT	GVTTQGVKSL	LTSMYVKEFL	ISSSQDGHQW	TLFFQNGKVK
1851	VFQGNQDSFT	PVVNSLDPPL	LTRYLRHPQ	SWVHQIALRM	EVLGCEAQDL
1901	YDKTHTCPPC	PAPELLGGPS	VFLFPPKPKD	TLMISRTPEV	TCVVVDVSHE
1951	DPEVKFNWYV	DGVEVHNAKT	KPREEQYNST	YRVVSVLTVL	HQDWLNGKEY
2001	KCKVSNKALP	APIEKTISKA	KGQPREPQVY	TLPPSRDELT	KNQVSLTCLV
2051	KGFPSPDIAV	EWESNGQPEN	NYKTTPPVLD	SDGSFFLYSK	LTVDKSRWQQ
2101	GNVFSCSVMH	EALHNHYTQK	SLSLSPGK*		

pSYN FVIII 266 protein sequence (FVIII Fc with 42 AE-XTEN at amino acid 18 and 288 AE XTEN in B-domain) SEQ ID NO: 80)

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGA VE	LSWDYMQGAP	GSPAGSPTST
51	EEGTSESATP	ESGPGSEPAT	SGSETPASSS	DLGELPVDAR	FPPRPVKSFP
101	FNTSVVYKKT	LFVEFTDHLF	NIAKPRPPWM	GLLGPTIQAE	VYD TVVITLK
151	NMASHPVSLH	AVGVSYWKAS	EGAEYDDQTS	QREKEDDKVF	PGGSHTYVWQ
201	VLKENGPMAS	DPLCLTYSYL	SHVDLVKDLN	SGLIGALLVC	REGSLAKEKT
251	QTLHKFILLF	AVFDEGKSWH	SETKNLSMQD	RDAASARAWP	KMHTVNGYVN
301	RSLPGLIGCH	RKSVYWHVIG	MGTTPEVHSI	FLEGHTFLVR	NHRQASLEIS
351	PITFLTAQTL	LMDLGQFLLF	CHISSHQHDG	MEAYVKVDSC	PEEPQLRMKN
401	NEEAEDYDDD	LTDSEMDVVR	FDDDNPSFI	QIRSVAKKHP	KTWVHYIAAE
451	EEDWDYAPLV	LAPDDRSYKS	QYLNNGPQRI	GRKYKKVRFM	AYTDETFKTR
501	EAIQHESGIL	GPLLYGEVGD	TLLIIFKNQA	SRPYNIYPHG	ITDVRPLYSR
551	RLPKGVKHLK	DFPILPGEIF	KYKWTVTVED	GPTKSDPRCL	TRYYSFVN M
601	ERDLASGLIG	PLLCYKESV	DQRGNQIMSD	KRNVLFSVF	DENRSWYLTE
651	NIQRFLPNPA	GVQLEDPEFQ	ASNIMHSING	YVFDSLQLSV	CLHEVAYWYI
701	LSIGAQTDFL	SVFFSGYTFK	HKMVYEDTLT	LFPFSGETVF	MSMENPGLWI
751	LGCHNSDFRN	RGMTALLKVS	SCDKNTGDY Y	EDSYEDISAY	LLSKNNAIEP
801	RSFSQNGAPG	TSESATPESG	PGSEPATSGS	ETPGTSESAT	PESGPGSEPA
851	TSGSETPGTS	ESATPESGPG	TSTEPSEGSA	PGSPAGSPTS	TEEGTSESAT
901	PESGPGSEPA	TSGSETPGTS	ESATPESGPG	SPAGSPTSTE	EGSPAGSPTS
951	TEEGTSTEPS	EGSAPGTSES	ATPESGPGTS	ESATPESGPG	TSESATPESG
1001	PGSEPATSGS	ETPGSEPAT S	GSETPGSPAG	SPTSTEEGTS	TEPSEGSAPG
1051	TSTEPSEGSA	PGSEPATSGS	ETPGTSESAT	PESGPGTSTE	PSEGSAPASS
1101	PPVLKRHQAE	ITRTTLQSDQ	EEIDYDDTIS	VEMKKEDFDI	YDEDENQSPR

1151	SFQKKTRHYF	IAAVERLWDY	GMSSSPHVLR	NRAQSGSVPO	FKKVVFQEF
1201	DGSFTQPLYR	GELNEHLGLL	GPYIRAEVED	NIMVTFRNQA	SRPYSFYSSL
1251	ISYEEDQRQG	AEPRKNFVKP	NETKTYFWKV	QHHMAPTKDE	FDCKAWAYFS
1301	DVDLEKDVHS	GLIGPLLIVCH	TNTLNPAHGR	QVTVQEFALF	FTIFDETKSW
1351	YFTENMERN	RAPCNQMED	PTFKENYRFH	AINGYIMDTL	PGLVMAQDQR
1401	IRWYLLSMGS	NENIHSIHFS	GHVFTVRKKE	EYKMALYNLY	PGVFETVEM
1451	PSKAGIWRVE	CLIGEHLHAG	MSTLFLVYSN	KCQTPLGMAS	GHIRDFQITA
1501	SGQYQGWAPK	LARLHYSGSI	NAWSTKEPFS	WIKVDLLAPM	IIHGIKTQGA
1551	RQKFSSLYIS	QFIIMYSLDG	KKWQTYRGNS	TGTLMVFFGN	VDSSGIKHNI
1601	FNPPIIARYI	RLHPHYSIR	STLRMELMGC	DLNSCSMPLG	MESKAISDAQ
1651	ITASSYFTNM	FATWSPSKAR	LHLQGRSNAW	RPQVNNPKEW	LQVDFQKTMK
1701	VTGVTQGVK	SLLTSMYVKE	FLISSSQDGH	QWTLFFQNGK	VKVFQGNQDS
1751	FTPVVNSLDP	PLLTRYLRH	PQSWVHQIAL	RMEVLGCEAQ	DLYDKTHTCP
1801	PCPAPELLGG	PSVFLFPKP	KDTLMISRTP	EVTCTVVDVS	HEDPEVKFNW
1851	YVDGVEVHNA	KTKPREEQYN	STYRVSVLT	VLHQDWLNGK	EYKCKVSNKA
1901	LPAPIEKTIS	KAKGQPREPQ	VYTLPPSRDE	LTKNQVSLTC	LVKGFYPSDI
1951	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY	SKLTVDKSRW	QQGNVFSCSV
2001	MHEALHNHYT	QKSLSLSPGK	*		

pSYN FVIII 267 protein sequence (FVIII Fc with 72 AE-XTEN at amino acid 18 and 288 AE XTEN in B-domain) SEQ ID NO: 81)

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGA	VE	LSWDYMQGAP	TSESATPESG
51	PGSEPATSGS	ETPGTSESAT	PESGPGSEPA	TSGSETPGTS	ESATPESGPG	
101	TSTEPSEGS	A	PGASSSDLGE	LPVDARFP	PPR	VPKSFPFNTS
151	FTDHLFNI	AK	PRPPWMGLLG	PTIQAEVYDT	VVITLKNMAS	HPVSLHAVGV
201	SYWKASEG	A	YDDQTSQREK	EDDKVFP	GG	HTYVWQVLKE
251	LTYSYLSH	VD	LVKDLNSGLI	GALLVCREGS	LAKEKTQTLH	KFILLFAVFD
301	EGKSWHSE	TK	NSLMQDRDAA	SARAWPKMHT	VNGYVNRSLP	GLIGCHRSKV
351	YWHVIGMG	TT	PEVHSIFLEG	HTFLVRNHRQ	ASLEISPITF	LTAQTLLMDL
401	GQFLLFCH	IS	SHQHDGMEAY	VKVDSCPEEP	QLRMKNNEEA	EDYDDDLTDS
451	EMDVVRFD	DD	NSPSFIQIRS	VAKKHPKTWV	HYIAAEEEDW	DYAPLVLAPD
501	DRSYKSQY	LN	NGPQRIGRKY	KKVRFMAYTD	ETFKTREAIQ	HESGILGPLL
551	YGEVGDTL	LI	IFKNQASRPY	NIYPHGITDV	RPLYSRRLPK	GVKHLKDFPI
601	LPGEIFKY	KW	TVTVEDGPTK	SDPRCLTRY	Y	SSFVNMERDL
651	CYKESVDQ	RG	NQIMSDKRN	V	ILFSVFDENR	SWYLTENIQR
701	EDPEFQAS	NI	MHSINGYVFD	SLQLSVCLHE	VAYWYILSIG	AQTDFLSVFF
751	SGYTFKKH	KMV	YEDTLTLFPP	SGETVFMSME	NPGLWILGCH	NSDFRNRGMT
801	ALLKVS	CD	NTGDYYEDSY	EDISAYLLSK	NNAIEPR	SFS
851	ATPESGPG	SE	PATSGSETPG	TSESATPESG	PGSEPATSGS	ETPGTSESAT
901	PESGPGT	STE	PSEGSAPGSP	AGSPTSTEEG	TSESATPESG	PGSEPATSGS
951	ETPGTSES	AT	PESGPGSPAG	SPTSTEEGSP	AGSPTSTEEG	TSTEPSEGS
1001	PGTSESAT	PE	SGPGTSESAT	PESGPGTSES	ATPESGPGSE	PATSGSETPG
1051	SEPATSGSE	T	PGSPAGSPTS	TEEGTSTEPS	EGSAPGTSTE	PSEGSAPGSE
1101	PATSGSET	PG	TSESATPESG	PGTSTEPSEG	SAPASSPPVL	KRHQAEITRT
1151	TLQSDQEE	ID	YDDTISVEMK	KEDFDIYDED	ENQSPRSFQK	KTRHYFIAAV
1201	ERLWDYGM	SS	SPHVLRNRAQ	SGSVPQFKKV	VFQEF	TDGSF
1251	EHLGLLG	PYI	RAEVEDNIMV	TFRNQASRPY	SFYSSLISYE	EDQRQGAEP
1301	KNFVKPNE	TK	TYFWKVQH	HM	APTKDEFDCK	AWAYFSDVDL
1351	PLLVCHTN	TNTL	NPAHGRQVTV	QEFALFF	TIF	DETKSWYFTE
1401	NIQMEDPT	FK	ENYRFHAIN	G	YIMDTLPGLV	MAQDQIRIRWY
1451	HSIHFSGH	V	TVRKKEEYKM	ALYNLYPGV	F	ETVEMLP
1501	EHLHAGM	STL	FLVYSNKCQT	PLGMASGHIR	DFQITASGQY	GQWAPKLARL
1551	HYSGSINA	WS	TKEPFSWIKV	DLLAPMIIHG	IKTQGARQKF	SSLYISQFII
1601	MYSLDGKK	WQ	TYRGNSTGTL	MVFFGNVDSS	GIKHNI	FNPP
1651	THYSIRST	LR	MELMGCDLNS	CSMPLGMESK	AI	SDAQITAS
1701	SPSKARLH	LQ	GRSNAWRPQV	NNPKEWLQVD	FQKTMKVTGV	TTQGVKSLLT
1751	SMYVKEFL	IS	SSQDGHQWTL	FFQNGKVKVF	QGNQDSF	TPV

1801	RYLRIHPQSW	VHQIALRMEV	LGCEAQDLYD	KTHTCPPCPA	PELLGGPSVF
1851	LFPPKPKDTL	MISRTPEVTC	VVVDVSHEDP	EVKFNWYVDG	VEVHNAKTKP
1901	REEQYNSTYR	VVSVLTVLHQ	DWLNGKEYKC	KVSNKALPAP	IEKTISKAKG
1951	QPREPQVYTL	PPSRDELTKN	QVSLTCLVKG	FYPSDIAVEW	ESNGQPENNY
2001	KTTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN	VFSCSVMHEA	LHNHYTQKSL
2051	SLSPGK*				

pSYN FVIII 268 protein sequence (FVIII Fc with 144 AE-XTEN at amino acid 18) SEQ ID NO: 82)

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGAWE	LSWDYMQGAP	TSESATPESG
51	PGSEPATSGS	ETPGTSESAT	PESGPGSEPA	TSGSETPGTS	ESATPESGPG
101	TSTEPSEGS	PGSPAGSPTS	TEEGTSESAT	PESGPGSEPA	TSGSETPGTS
151	ESATPESGPG	SPAGSPTSTE	EGSPAGSPTS	TEEGASSSDL	GELPVDARFP
201	PRVPKSFPFN	TSVVYKKTLE	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY
251	DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFPF
301	GSHTYVWQVL	KENGPMASDP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE
351	GSLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM
401	HTVNGYVNRS	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH
451	RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSCPE
501	EPQLRMKNNE	EAEDYDDDLT	DSEMDVVRFD	DDNSPSFIQI	RSVAKKHPKT
551	WVHYIAAEIE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
601	TDETFTKTREA	IQHESGILGP	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
651	DVRPLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
701	YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
751	NRSWYLTENI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDSLQLSVCL
801	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFMS
851	MENPGLWILG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYED	SYEDISAYLL
901	SKNNAIEPRS	FSQNPPVLKR	HQAEITRRTL	QSDQEEIDYD	DTISVEMKKE
951	DFDIYDEDEN	QSPRSFQKKT	RHYFIAAVER	LWDYGMSSSP	HVLRNRAQSG
1001	SVPQFKKVVV	QEFTDGSFTQ	PLYRGELNEH	LGLLGPYIRA	EVEDNIMVTF
1051	RNQASRPYSF	YSSLISYEED	QRQGAEPKRN	FVKPNETKTY	FWKVQHMAP
1101	TKDEFDCKAW	AYFSDVDLEK	DVHSGLIGPL	LVCHTNTLNP	AHGRQVTVQE
1151	FALFFTIFDE	TKSWYFTENM	ERNCRAPCNI	QMEDPTFKEN	YRFHAINGYI
1201	MDTLPGLVMA	QDQIRIRWYLL	SMGSNENIHS	IHFSGHVFTV	RKKEEYKMAL
1251	YNLYPGVFET	VEMLPSKAGI	WRVECLIGEH	LHAGMSTLFL	VYSNKCQTPL
1301	GMASGHIRDF	QITASGQYGQ	WAPKLARLHY	SGSINAWSTK	EPFSWIKVDL
1351	LAPMIIHGK	TQGARQKFSS	LYISQFIIMY	SLDGKKWQTY	RGNSTGTLMV
1401	FFGNVDSSGI	KHNIFNPPII	ARYIRLHPTH	YSIRSTLRME	LMGCDLNSCS
1451	MPLGMESKAI	SDAQITASSY	FTNMFATWSP	SKARLHLQGR	SNAWRPQVNN
1501	PKEWLQVDFQ	KTMKVTGVTT	QGVKSLLTSM	YVKEFLISS	QDGHQWTLFF
1551	QNGKVKVFQ	NQDSFTPVVN	SLDPPLLTRY	LRIHPQSWVH	QIALRMEVLG
1601	CEAQDLYDKT	HTCPPCPAPE	LLGGPSVFLF	PPKPKDTLMI	SRTPEVTCVV
1651	VDVSHEDPEV	KFNWYVDGVE	VHNAKTKPRE	EQYNSTYRVV	SVLTVLHQDW
1701	LNGKEYKCKV	SNKALPAPIE	KTISKAKGQP	REPQVYTLPP	SRDELTKNQV
1751	SLTCLVKGFY	PSDIAVEWES	NGQPENNYKT	TPPVLDSDGS	FFLYSKLTV
1801	KSRWQQGNVF	SCSVMHEALH	NHYTQKSLSL	SPGK*	

pSYN FVIII 269 protein sequence (FVIII Fc with 72 AE-XTEN at amino acid 18) SEQ ID NO: 83)

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGAWE	LSWDYMQGAP	TSESATPESG
51	PGSEPATSGS	ETPGTSESAT	PESGPGSEPA	TSGSETPGTS	ESATPESGPG
101	TSTEPSEGS	PGASSSDLGE	LPVDARFPPR	VPKSFPPNTS	VVYKKTLFVE
151	FTDHLFNI	PRPPWMGLLG	PTIQAEVYDT	VVITLKNMAS	HPVSLHAVGV
201	SYWKASEGAE	YDDQTSQREK	EDDKVFPGGS	HTYVWQVLKE	NGPMASDPLC
251	LTYSYLSHVD	LVKDLNSGLI	GALLVCREGS	LAKEKTQTLH	KFILLFAVFD

```

301  EGKSWHSETK NSLMQDRDAA SARAWPKMHT VNGYVNRSLP GLIGCHRKSV
351  YWHVIGMGTT PEVHSIFLEG HTFLVRNHRQ ASLEISPITF LTAQTLLMDL
401  GQFLLFCHIS SHQHDGMEAY VKVDSCPEEP QLRMKNNEEA EDYDDDLTDS
451  EMDVVRFDDD NSPSFIQIRS VAKKHPKTWV HYIAAEEEDW DYAPLV LAPD
501  DRSYKSQYLN NGPQIRGRKY KKVRFMAYTD ETFKTREAIQ HESGILGPLL
551  YGEVGD TLLI IFKNQASRPY NIYPHGITDV RPLYSRRLPK GVKHLKDFPI
601  LPGEIFKYKW TVTVEDGPTK SDPRCLTRY Y SSFVNMERDL ASGLIGPLLI
651  CYKESVDQRG NQIMSDKRN V ILFSVFDENR SWYL TENIQR FL PNPAGVQL
701  EDPEFQASNI MHSINGYVFD SLQLSVCLHE VAYWYILSIG AQTD FLSVFF
751  SGYTFKH K MV YEDTLTLFPF SGETVFMSME NPGLWILGCH NSDFRNRGMT
801  ALLKVSSCDK NTGDY YEDSY EDISAYLLSK NNAIEPRSF S QNPPVLKRHQ
851  AEITRTTLQS DQEEIDYDDT ISVEMKKEDF DIYDE DENQS PRSFQKKTRH
901  YFIAAVERLW DYGMSSSPHV LRNRAQSGSV PQFKKVFQFE FTDG SFTQPL
951  YRGELNEHLG LLGPYIRAEV EDNIMVTFRN QASRPYSFYS SLISYEEDQR
1001 QGAEPKRN FV KPNETKTYFW KVQH HMAPTK DEFDC KAWAY FSDVDLEKDV
1051 HSGLIGPLLV CHTNTLNPAH GRQVT VQEFA LFFTIFDETK SWYFTENMER
1101 NCRAPCNIQM EDPTFKENYR FHAINGYIMD TLPGLVMAQD QRIRWYLLSM
1151 GSNENIHSIH FSGHVFTVRK KEEYKMALYN LYPGVFETVE MLPSKAGIWR
1201 VECLIGEHLH AGMSTLFLVY SNKCQTPLGM ASGHIRDFQI TASGQY GQWA
1251 PKLARLHYS G SINAWSTKEP FSWIKVDLLA PMIIHG IKTQ GARQKFSSLY
1301 ISQFIIMYSL DGKKWQTYRG NSTGTLMVFF GNVDS SGIKH NIFNPPIAR
1351 YIRLHPHYS IRSTLRMELM GCDLNSC SMP LGMESKAISD AQITASSYFT
1401 NMFATWSPSK ARLHLQGRSN AWRPQVNNPK EWLQVDFQKT MKVTGVTTQG
1451 VKSLLTSMYV KEFLISSSQD GHQWTLFFQN GKVKVFQGNQ DSFTPVVNSL
1501 DPPLLTRYLR IHPQSWVHQI ALRMEVLGCE AQDLYDKTHT CPPCPAPELL
1551 GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH
1601 NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYCKKVS N KALPAPIEKT
1651 ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG
1701 QPENNYK TTP PVLDS DGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH
1751 YTQKSLSLSP GK*

```

pSYNFVIII 271 protein sequence (FVIII Fc with 42 AE-XTEN at amino acid 18) SEQ ID NO: 84)

```

1  MQIELSTCFF LCLLRFCFSA TRRYYLGA VE LSWDYMQGAP GSPAGSPTST
51  EEGTSESATP ESGPGSEPAT SGSETPASSS DLGELPVDAR FPPRPVKSFP
101 FNTSVVYKKT LFVEFTDHLF NIAKPRPPWM GLLGPTIQAE VYDTVVITLK
151 NMASHPVSLH AVGVSYWKAS EGA EYDDQTS QREKEDDKVF PGGSHTYVWQ
201 VLKENGPMAS DPLCLTYSYL SHVDLVKDLN SGLIGALLVC REGSLAKEKT
251 QTLHKFILLF AVFDEGKSWH SETKNSLMQD RDAASARAWP KMHTVNGYVN
301 RSLPGLIGCH RKS VYWHVIG MGTTPEVHSI FLEGHTFLVR NHRQASLEIS
351 PITFLTAQTL LMDLGQFLLF CHISSHQHDG MEAYVKVDSC PEEPQLRMKN
401 NEEAEDYDDD LTDSEMDVVR FDDDNPSFI QIRSVAKKHP KTWVHYIAAE
451 EEDWDYAPLV LAPDDRSYKS QYLNNGPQRI GRKYKKVRFM AYTDETFKTR
501 EAIQHESGIL GPLLYGEVGD TLLIIFKNQA SRPYNIPHG ITDVRPLYSR
551 RLPKGVKHLK DFPILPGEIF KYKWTVTVED GPTKSDPRCL TRYYS SFVNM
601 ERDLASGLIG PLLICYKESV DQRGNQIMSD KRNVILFSVF DENRSWYLTE
651 NIQRFLPNPA GVQLEDPEFQ ASNIMHSING YVFD SLQLSV CLHEVAYWYI
701 LSIGAQTDFL SVFFSGYTFK HKMVYEDTLT LFPFSGETVF MSMENPGLWI
751 LGCHNSDFRN RGMTALLKVS SCDKNTGDY Y EDSYEDISAY LLSKNNAIEP
801 RSFSQNPPVL KRHQAEITRT TLQSDQEEID YDDTISVEMK KEDFDIYDED
851 ENQSPRSFQK KTRHYFIAAV ERLWDYGMSS SPHVLRNRAQ SGSPVQFKKV
901 VFQEFTDGSF TQPLYRGELN EHLG LLGPYI RAEVEDNIMV TFRNQASRPY
951 SFYSSLISYE EDQRQGAEP R KNFVKPNETK TYFWKVQH HMAPTKDEFDC
1001 AWAYFSDVDL EKDVHSGLIG PLLVCHTNTL NPAHGRQVTV QEFALFFTIF
1051 DETKSWYFTE NMERNCRAPC NIQMEDPTFK ENYRFHAING YIMDTLPGLV
1101 MAQDQRIRWY LLSMGSNENI HSIHFSGHVF TVRKKEEYKM ALYNLYPGVF
1151 ETVEMLPSKA GIWRVECLIG EHLHAGMSTL FLVYSNKCQT PLGMASGHIR

```

1201	DFQITASGQY	GQWAPKLARL	HYSGSINAW	TKEPFSWIKV	DLLAPMIH
1251	IKTQGARQKF	SSLYISQFII	MYSLDGKKWQ	TYRGNSTGTL	MVFFGNVDSS
1301	GIKHNIFNPP	IIARYIRLHP	THYSIRSTLR	MELMGCDLNS	CSMPLGMESK
1351	AISDAQITAS	SYFTNMFATW	SPSKARLHLQ	GRSNAWRPQV	NNPKEWLQVD
1401	FQKTMKVTGV	TTQGVKSLLT	SMYVKEFLIS	SSQDGHQWTL	FFQNGKVKVF
1451	QGNQDSFTPV	VNSLDPPLLT	RYLRIHPQSW	VHQIALRMEV	LGCEAQDLYD
1501	KTHTCPPCPA	PELLGGPSVF	LFPPKPKDTL	MISRTPEVTC	VVVDVSHEDP
1551	EVKFNWYVDG	VEVHNAKTKP	REEQYNSTYR	VVSVLTVLHQ	DWLNGKEYKC
1601	KVSNKALPAP	IEKTISKAKG	QPREPQVYTL	PPSRDELTKN	QVSLTCLVKG
1651	FYPSDIAVEW	ESNGQPENNY	KTPPVLDSD	GSFFLYSKLT	VDKSRWQQGN
1701	VFSCSVMHEA	LHNHYTQKSL	SLSPGK*		

pSYN FVIII protein sequence 272 (FVIII with 144 AE XTEN at amino acid 18 and 244 AE XTEN in B-domain- no Fc) SEQ ID NO: 85)

1	MQIELSTCFF	LCLLRFCFSA	TRRYYLGA	LSWDYMQGAP	TSESATPESG
51	PGSEPATSGS	ETPGTSESAT	PESGPGSEPA	TSGSETPGTS	ESATPESGPG
101	TSTEPSEGS	PGSPAGSPTS	TEEGTSESAT	PESGPGSEPA	TSGSETPGTS
151	ESATPESGPG	SPAGSPTSTE	EGSPAGSPTS	TEEGASSDL	GELPVDARFP
201	PRVPKSFPPN	TSVVYKKTLE	VEFTDHLFNI	AKPRPPWMGL	LGPTIQAEVY
251	DTVVITLKNM	ASHPVSLHAV	GVSYWKASEG	AEYDDQTSQR	EKEDDKVFP
301	GSHTYVWQVL	KENGPMASDP	LCLTYSYLSH	VDLVKDLNSG	LIGALLVCRE
351	GSLAKEKTQT	LHKFILLFAV	FDEGKSWHSE	TKNSLMQDRD	AASARAWPKM
401	HTVNGYVNRS	LPGLIGCHRK	SVYWHVIGMG	TTPEVHSIFL	EGHTFLVRNH
451	RQASLEISPI	TFLTAQTLLM	DLGQFLLFCH	ISSHQHDGME	AYVKVDSCPE
501	EPQLRMKNNE	EAEDYDDDLT	DSEMDVVRFD	DDNSPSFIQI	RSVAKKHPKT
551	WVHYIAAEEE	DWDYAPLVLA	PDDRSYKSQY	LNNGPQRIGR	KYKKVRFMAY
601	TDETFTKTREA	IQHESGILGP	LLYGEVGDTL	LIIFKNQASR	PYNIYPHGIT
651	DVRPLYSRRL	PKGVKHLKDF	PILPGEIFKY	KWTVTVEDGP	TKSDPRCLTR
701	YYSSFVNMER	DLASGLIGPL	LICYKESVDQ	RGNQIMSDKR	NVILFSVFDE
751	NRSWYLTENI	QRFLPNPAGV	QLEDPEFQAS	NIMHSINGYV	FDLSQLSVCL
801	HEVAYWYILS	IGAQTDFLSV	FFSGYTFKHK	MVYEDTLTLF	PFSGETVFM
851	MENPGLWLIG	CHNSDFRNRG	MTALLKVSSC	DKNTGDYIED	SYEDISAYLL
901	SKNNAIEPRS	FSQNGAPGTS	ESATPESGPG	SEPATSGSET	PGTSESATPE
951	SGPGSEPATS	GSETPGTSES	ATPESGPGTS	TEPSEGSAPG	SPAGSPTSTE
1001	EGTSESATPE	SGPGSEPATS	GSETPGTSES	ATPESGPGSP	AGSPTSTEEG
1051	SPAGSPTSTE	EGTSTEPSEG	SAPGTSESAT	PESGPGTSES	ATPESGPGTS
1101	ESATPESGPG	SEPATSGSET	PGSEPATSGS	ETPGSPAGSP	TSTEEGTSTE
1151	PSEGSAPGTS	TEPSEGSAPG	SEPATSGSET	PGTSESATPE	SGPGTSTEPS
1201	EGSAPASSPP	VLKRHQAEIT	RTTLQSDQEE	IDYDDTISVE	MKKEDFDIYD
1251	EDENQSPRSF	QKKTRHYFIA	AVERLWDYGM	SSSPHVLRNR	AQSGSVPPQFK
1301	KVVVFQEFDTG	SFTQPLYRGE	LNEHLGLLGP	YIRAEVEDNI	MVTFRNQASR
1351	PYSFYSSLIS	YEEDQRQGAE	PRKNFVKPNE	TKTYFWKVQH	HMAPTKDEFD
1401	CKAWAYFSDV	DLEKDVHSG	IGPLLVCHTN	TLNPAHGRQV	TVQEFALFFT
1451	IFDETKSWYF	TENMERNCR	PCNIQMEDPT	FKENYRFHAI	NGYIMDTLPG
1501	LVMAQDQIR	WYLLSMGSNE	NIHSIHFSGH	VFTVRKKEEY	KMALYNLYPG
1551	VFETVEMLPS	KAGIWRVECL	IGEHLHAGMS	TLFLVYSNKC	QTPLGMASGH
1601	IRDFQITASG	QYGQWAPKLA	RLHYSGSINA	WSTKEPFSWI	KVDLLAPMII
1651	HGIKTQGARQ	KFSSLYISQF	IIMYSLDGKK	WQTYRGNSTG	TLMVFFGNVD
1701	SSGIKHNIFN	PPIIARYIRL	HPTHYSIRST	LRMELMGCDL	NSCSMPLGME
1751	SKAISDAQIT	ASSYFTNMFA	TWSPSKARLH	LQGRSNAWRP	QVNNPKEWLQ
1801	VDFQKTMKVT	GVTTQGVKSL	LTSMYVKEFL	ISSSQDGHQW	TLFFQNGKVK
1851	VFQGNQDSFT	PVVNSLDPPL	LTRYLRIHPQ	SWVHQIALRM	EVLGCEAQDL
1901	Y*				

pSYN-FVIII-161 protein sequence (SEQ ID NO: 69) (FVIII sequence amino acid position 1-1457; underlined region represents Fc region; curvy underline represents cleavable linker in between first Fc and VWF fragment; double underlined region represents VWF fragment; bold region represents cleavable linker in between VWF fragment and Fc).

```

1      MQIELSTCFF LCLLRFCFSA TRRYYLGAWE LSWDYMQSDL GELPVDARFP
51     PRVPKSFPPN TSVVYKKTLE VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY
101    DTVVITLKNM ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
151    GSHTYVWQVL KENGPMASDP LCLTYSYLSE VDLVKDLNSG LIGALLVCRE
201    GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD AASARAWPKM
251    HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL EGHTFLVRNH
301    RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME AYVKVDSCPE
351    EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI RSVAKKHPKT
401    WVHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR KYKKVRFMAY
451    TDETFTKREA IQHESGILGP LLYGEVGDLE LIIFKNQASR PYNIYPHGIT
501    DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP TKSDPRCLTR
551    YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR NVILFSVFDE
601    NRSWYLTENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV FDSLQLSVCL
651    HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF PFSGETVFMS
701    MENPGLWLIG CHNSDFRNRG MTALLKVSSC DKNTGDYYED SYEDISAYLL
751    SKNNAIEPRS FSQNPVVLKR HQREITRRTL QSDQEEIDYD DTISVEMKKE
801    DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP HVLNRNAQSG
851    SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA EVEDNIMVTF
901    RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY FWKVQHMAP
951    TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP AHGRQVTVQE
1001   FALFFTIFDE TKSIFYTENM ERNCRAPCNI QMEDPTFKEN YRFHAIINGYI
1051   MDTLPLGLVMA QDQIRRWYLL SMGSNENIHS IHFSGHVFTV RKKEEYKMAL
1101   YNLYPGVFET VEMLPKAGI WRVECLIGEH LHAGMSTLFL VYSNKCQTPL
1151   GMASGHIRDF QITASGQYQG WAPKLARLHY SGSINAWSTK EPFSWIKVDL
1201   LAPMIIHGKI TQGARQKFSS LYISQFIIMY SLDGKKWQTY RGNSTGTLMV
1251   FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME LMGCDLNSCS
1301   MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR SNAWRPQVNN
1351   PKEWLQVDFQ KTMKVTGVT TQGVKSLLTSM YVKEFLISS QDGHQWTLFF
1401   QNGKVKVFGQ NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH QIALRMEVLG
1451   CEAQDLYDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV
1501   VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW
1551   LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP SRDELTKNQV
1601   SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSDGS FFLYSKLTVD
1651   KSRWQQGNVF SCSVMEALH NHYTQKSLSL SPGKRRRRSG GGGSGGGGSG
1701   GGGSGGGGSG GGGSGGGGSR KRRKRSLSR PPMVKLVCAP DNLRAEGLEC
1751   TKTCQNYDLE CMSMGCVSGC LCPPGMVRHE NRCVALERCP CFHQGKEYAP
1801   GETVKIGCNT CVCRDRKWC TDHVCDATCS TIGMAHYLTF DGLKYLFPGE
1851   CQYVLVQDYC GSNPGTFRIL VGNKGCSHPS VKCKKRVTL VEGGEIELFD
1901   GEVNVKRPMT DETHFEVVE GRYIILLGK ALSVVWDRHL SISVLKQTY
1951   QEKVCGLCGN FDGIQNNDLT SSNLQVEEDP VDFGNSWKVS SQCADTRKVP
2001   LDSSPATCHN NIMKQTMVDS SCRILTSDFV QDCNKLVDPE PYLDVCIYDT
2051   CSCESIGDCA AFCDTIAAYA HVCAQHGVV TWRTATLCPQ SCEERNLREN
2101   GYEAEWRYNS CAPACQVTCQ HPEPLACPVO CVEGCHAHCP PGKILDELLQ
2151   TCVDPEDCPV CEVAGRRFAS GKKVTLNPSD PEHCQICHCD VVNLTCEACQ
2201   EPISGTSESA TPESGPGSEP ATSGSETPGT SESATPESGP GSEPATSGSE
2251   TPGTSESATP ESGPGTSTEP SEGSAPGSPA GSPTSTEEGT SESATPESGP
2301   GSEPATSGSE TPGTSESATP ESGPGSPAGS PTSTEEGSPA GSPTSTEEGT
2351   STEPSEGSAP GTSESATPES GPSTSESATP ESGPGTSESA TPESGPGSEP
2401   ATSGSETPGS EPATSGSETP GSPAGSPTST EEGTSTEPSE GSAPGTSTEP
2451   SEGSAPGSEP ATSGSETPGT SESATPESGP GTSTEPSEGS APDSGGGGSG
2501   GGGSGGGGSG GGGSGGGGSL VPRGSGGDKT HTCPPCPAPE LLGGPSVFLF

```

2551 PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNATKPRE
 2601 EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP
 2651 REPQVYTLPP SRDELTKNOV SLTCLVKGFY PSDIAVEWES NGOPENNYKT
 2701 TFPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL
 2751 SPGK

pSYN-FVIII-170 protein sequence (SEQ ID NO: 71)

1 SLSCRPPMVK LVCPADNLRA EGLECTKTCQ NYDLECMSMG CVSGCLCPPG
 51 MVRHENRCVA LERCPCFHQG KEYAPGETVK IGCNTCVCRD RKWNCTDHVC
 101 DATCSTIGMA HYLTFDGLKY LFPGECQYVL VQDYCGSNPG TFRILVGNKG
 151 CSHPSVKCKK RVTILVEGGE IELFDGEVNV KRPMKDETHF EVVESGRYII
 201 LLLGKALSVM WDRHLSISVV LKQTYQEKVC GLCGNFDGIQ NNDLTSSNLQ
 251 VEEDPVDFGN SWKVSSQCAD TRKVPLDSSP ATCHNNIMKQ TMVDSSCRIL
 301 TSDVFQDCNK LVDPEPYLDV CIYDTCSCES IGDCAAFCDT IAAYAHVCAQ
 351 HGKVVWTRTA TLC PQSCEER NLRENGYEA E WRYN SCAPAC QVTCQHPEPL
 401 ACPVQCVEGC HAHCPPGKIL DELLQTCVDP EDCPVCEVAG RRFASGKKVT
 451 LNPDPEHCQ ICHCDVNLIT CEACQEPISG TSESATPESG PGSEPATSGS
 501 ETPGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG TSTEPSEGS
 551 PGSPAGSPTS TEEGTSESAT PESGPGSEPA TSGSETPGTS ESATPESGPG
 601 SPAGSPTS TEEGTSESAT TEEGTSTEPS EGSAPGTSES ATPESGPGTS
 651 ESATPESGPG TSESATPESG PGSEPATSGS ETPGSEPAT GSETPGSPAG
 701 SPTSTEEGTS TEPSEGSAPG TSTEPSEGS PGSEPATSGS ETPGTSESAT
 751 PESGPGTSTE PSEGSAPDSG GGGSGGGGSG GGGSGGGGSG GGGSLVPRGS
 801 GGASATRRYY LGAVELSWDY MQSDLGELPV DARFPPRPVK SFPFNTSVVY
 851 KKTFLVEFTD HLFNIAKPRP PWMGLLGPTI QAEVYDTVVI TLKNMASHPV
 901 SLHAVGVSYW KASEGA EYDD QTSQREKEDD KVFP GGSHTY VWQVLKENG P
 951 MASDPLCLTY SYLSHVDLVK DLNSGLIGAL LVCREGSLAK EKTQTLHKFI
 1001 LLFAVFDEGK SWHSETKNSL MQDRDAASAR AWPKMHTVNG YVNRSLPGLI
 1051 GCHRKSVYWH VIGMGTTPEV HSIFLEGHTF LVRNHRQASL EISPITFLTA
 1101 QTLMLDLGQF LLFCHISSHQ HDGMEAYVKV DSCPEEPQLR MKNNEEAEDY
 1151 DDDLTDSEMD VVRFDDDNSP SFIQIRSVAK KHPKTWVHYI AAEEDWDYA
 1201 PLVLAPDDRS YKSQYLNNGP QRIGRKYKKV RFMAYTDEF KTREAIQHE
 1251 GILGPLLYGE VGDTLLIIFK NQASRPYNIY PHGITDVRPL YSRRLPKGVK
 1301 HLKDFPILPG EIFKYKWTVT VEDGPTKSDP RCLTRYSSS VNMERDLASG
 1351 LIGPLLIC YK ESVDQRGNQI MSDKRNVILF SVFDENRSWY LTENIQRF LP
 1401 NPAGVQLED P EFQASNIMHS INGYVFD SLQ LSVCLHEVAY WYILSIGAQ T
 1451 DFLSVFFSGY TFKHKMVYED TLTLFPFSGE TVFMSMENPG LWILGCHNSD
 1501 FRNRGMTALL KVSSCDKNTG DYYEDSYEDI SAYLLSKNNA IEPRSF SQNP
 1551 PVLKRHQREI TRTTLQSDQE EIDYDDTISV EMKKEDFDIY DEDENQSPRS
 1601 FQKKTRHYFI AAVERLWDYG MSSSPHVL RN RAQSGSV PQF KKVVFQEF TD
 1651 GSFTQPLYRG ELNEHLGLLG PYIRAEVEDN IMVTFRNQAS RPYSFYSSLI
 1701 SYEEDQRQGA EPRKNFVKPN ETKTYFWKVQ HHMAPTKDEF DKAWAYFSD
 1751 VDLEKDVHSG LIGPLLVCHT NTLNPAHGRQ VTVQEFALFF TIFDETSWY
 1801 FTENMERNCR APCNIQMEDP TFKENYRFHA INGYIMDTLP GLVMAQDQRI
 1851 RWYLLSMGSN ENIHSIHFSG HVFTVRKKEE YKMALYNLYP GVFETVEMLP
 1901 SKAGIWRVEC LIGEHLHAGM STLFLVYSNK CQTPLGMASG HIRDFQITAS
 1951 GQYGQWAPKL ARLHYS GSIN AWSTKEPFSW IKVDLLAPMI IHGIKTQGAR
 2001 QKFSSLYISQ FIIMYS LDGK KWQTYRGNST GTLMVFFGNV DSSGIKH NIF
 2051 NPPIIARYIR LHPHTYSIRS TLRMELMGCD LNCSMPLGM ESKAISDAQI
 2101 TASSYFTNMF ATWSPSKARL HLQGRSNAWR PQVNNPKEWL QVDFQKTMKV
 2151 TGVTTQGVKS LLTSMYVKEF LISSSQDGHQ WTLFFQNGKV KVFQGNQDSF
 2201 TPVVNSLDPP LLTRYLRIHP QSWVHQIALR MEVLGCEAQD LY

**pSYN FVIII 310 nucleotide sequence (encoding FVIII with complete B-domain deletion
 except 2 amino acid residues and 288 AE-XTEN inserted after aa 742) (SEQ ID NO:170)**

1	ATGCAAATAG	AGCTCTCCAC	CTGCTTCTTT	CTGTGCCTTT	TGCGATTCTG
51	CTTTAGTGCC	ACCAGAAGAT	ACTACCTGGG	TGCAGTGGAA	CTGTCATGGG
101	ACTATATGCA	AAGTGATCTC	GGTGAGCTGC	CTGTGGACGC	AAGATTTCCCT
151	CCTAGAGTGC	CAAAATCTTT	TCCATTCAAC	ACCTCAGTCG	TGTACAAAAA
201	GACTCTGTTT	GTAGAATTCA	CGGATCACCT	TTTCAACATC	GCTAAGCCAA
251	GGCCACCCTG	GATGGGTCTG	CTAGGTCCTA	CCATCCAGGC	TGAGGTTTAT
301	GATACAGTGG	TCATTACACT	TAAGAACATG	GCTTCCCATC	CTGTCAGTCT
351	TCATGCTGTT	GGTGTATCCT	ACTGGAAAAG	TTCTGAGGGA	GCTGAATATG
401	ATGATCAGAC	CAGTCAAAGG	GAGAAAAGAG	ATGATAAAGT	CTTCCCTGGT
451	GGAAGCCATA	CATATGTCTG	GCAGGTCCTG	AAAGAGAATG	GTCCAATGGC
501	CTCTGACCCA	CTGTGCCTTA	CCTACTCATA	TCTTTCTCAT	GTGGACCTGG
551	TAAAAGACTT	GAATTCAGGC	CTCATTGGAG	CCCTACTAGT	ATGTAGAGAA
601	GGGAGTCTGG	CCAAGGAAAA	GACACAGACC	TTGCACAAAT	TTATACTACT
651	TTTTGCTGTA	TTTGATGAAG	GGAAAAGTTG	GCACTCAGAA	ACAAAGAACT
701	CCTTGATGCA	GGATAGGGAT	CTGCATCTG	CTCGGGCCTG	GCCATAAATG
751	CACACAGTCA	ATGGTTATGT	AAACAGGTCT	CTGCCAGGTC	TGATTGGATG
801	CCACAGGAAA	TCAGTCTATT	GGCATGTGAT	TGGAATGGGC	ACCACTCCTG
851	AAGTGCACCTC	AATATTCCCTC	GAAGGTCACA	CATTTCTTGT	GAGGAACCAT
901	CGCCAGGCGT	CCTTGGAAT	CTCGCCAATA	ACTTTCCCTTA	CTGCTCAAAC
951	ACTCTTGATG	GACCTTGGAC	AGTTTCTACT	GTTTTGTTCAT	ATCTCTTCCC
1001	ACCAACATGA	TGGCATGGAA	GCTTATGTCA	AAGTAGACAG	CTGTCCAGAG
1051	GAACCCCAAC	TACGAATGAA	AAATAATGAA	GAAGCGGAAG	ACTATGATGA
1101	TGATCTTACT	GATTCTGAAA	TGGATGTGGT	CAGGTTTGAT	GATGACAACT
1151	CTCCTTCCTT	TATCCAAATT	CGCTCAGTTG	CCAAGAAGCA	TCCTAAAACCT
1201	TGGGTACATT	ACATTGCTGC	TGAAGAGGAG	GACTGGGACT	ATGCTCCCTT
1251	AGTCCTCGCC	CCCAGTGACA	GAAGTTATAA	AAGTCAATAT	TTGAACAATG
1301	GCCCTCAGCG	GATTGGTAGG	AAGTACAAAA	AAGTCCGATT	TATGGCATAAC
1351	ACAGATGAAA	CCTTTAAGAC	TCGTGAAGCT	ATTCAGCATG	AATCAGGAAT
1401	CTTGGGACCT	TTACTTTATG	GGGAAGTTGG	AGACACACTG	TTGATTATAT
1451	TTAAGAATCA	AGCAAGCAGA	CCATATAACA	TCTACCCCTCA	CGGAATCACT
1501	GATGTCCGTC	CTTTGTATTC	AAGGAGATTA	CCAAAAGGTG	TAAAACATTT
1551	GAAGGATTTT	CCAATTCTGC	CAGGAGAAAT	ATTCAAAATAT	AAATGGACAG
1601	TGACTGTAGA	AGATGGGCCA	ACTAAATCAG	ATCCTCGGTG	CCTGACCCGC
1651	TATTACTCTA	GTTTCGTTAA	TATGGAGAGA	GATCTAGCTT	CAGGACTCAT
1701	TGGCCCTCTC	CTCATCTGCT	ACAAAAGAATC	TGTAGATCAA	AGAGGAAACC
1751	AGATAATGTC	AGACAAGAGG	AATGTTCATCC	TGTTTTCTGT	ATTTGATGAG
1801	AACCGAAGCT	GGTACCTCAC	AGAGAATATA	CAACGCTTTC	TCCCCAATCC
1851	AGCTGGAGTG	CAGCTTGAGG	ATCCAGAGTT	CCAAGCCTCC	AACATCATGC
1901	ACAGCATCAA	TGGCTATGTT	TTTGATAGTT	TGCAGTTGTC	AGTTTGTGTTG
1951	CATGAGGTGG	CATACTGGTA	CATTCTAAGC	ATTGGAGCAC	AGACTGACTT
2001	CCTTTCTGTC	TTCTTCTCTG	GATATACCTT	CAAACACAAA	ATGGTCTATG
2051	AAGACACACT	CACCCTATTC	CCATTCTCAG	GAGAAACTGT	CTTCATGTCTG
2101	ATGGAAAACC	CAGGTCTATG	GATTCTGGGG	TGCCACAACCT	CAGACTTTCG
2151	GAACAGAGGC	ATGACCGCCT	TACTGAAGGT	TTCTAGTTGT	GACAAGAACA
2201	CTGGTGATTA	TTACGAGGAC	AGTTATGAAG	ATATTTTCAGC	ATACTTGCTG
2251	AGTAAAAACA	ATGCCATTGA	ACCAAGAAGC	TTCCGTACCT	CAGAGTCTGC
2301	TACCCCCGAG	TCAGGGCCAG	GATCAGAGCC	AGCCACCTCC	GGGTCTGAGA
2351	CACCCGGGAC	TTCCGAGAGT	GCCACCCCTG	AGTCCGGACC	CGGGTCCGAG
2401	CCCGCCACTT	CCGGCTCCGA	AACTCCCGGC	ACAAGCGAGA	GCGCTACCCC
2451	AGAGTCAGGA	CCAGGAACAT	CTACAGAGCC	CTCTGAAGGC	CTCGTCCAG
2501	GGTCCCCAGC	CGGCAGTCCC	ACTAGCACCG	AGGAGGGAAC	CTCTGAAAAGC
2551	GCCACACCCG	AATCAGGGCC	AGGGTCTGAG	CCTGCTACCA	GCGGCAGCGA
2601	GACACCAGGC	ACCTCTGAGT	CCGCCACACC	AGAGTCCGGA	CCCGGATCTC
2651	CCGCTGGGAG	CCCCACCTCC	ACTGAGGAGG	GATCTCCTGC	TGGCTCTCCA
2701	ACATCTACTG	AGGAAGGTAC	CTCAACCGAG	CCATCCGAGG	GATCAGCTCC
2751	CGGCACCTCA	GAGTCGGCAA	CCCCGGAGTC	TGGACCCGGA	ACTTCCGAAA
2801	GTGCCACACC	AGAGTCCGGT	CCCGGGACTT	CAGAATCAGC	AACACCCGAG
2851	TCCGGCCCTG	GGTCTGAACC	CGCCACAAGT	GGTAGTGAGA	CACCAGGATC
2901	AGAACCTGCT	ACCTCAGGGT	CAGAGACACC	CGGATCTCCG	GCAGGCTCAC

2951	CAACCTCCAC	TGAGGAGGGC	ACCAGCACAG	AACCAAGCGA	GGGCTCCGCA
3001	CCCGGAACAA	GCACTGAACC	CAGTGAGGGT	TCAGCACCCG	GCTCTGAGCC
3051	GGCCACAAGT	GGCAGTGAGA	CACCCGGCAC	TTCAGAGAGT	GCCACCCCG
3101	AGAGTGCCCC	AGGCACTAGT	ACCGAGCCCT	CTGAAGGCAG	TGCGCCAGCC
3151	TCGAGCGAAA	TAACTCGTAC	TACTCTTCAG	TCAGATCAAG	AGGAAATCGA
3201	TTATGATGAT	ACCATATCAG	TTGAAATGAA	GAAGGAAGAT	TTTGACATTT
3251	ATGATGAGGA	TGAAAATCAG	AGCCCCCGCA	GCTTTCAAAA	GAAAACACGA
3301	CACTATTTTA	TTGCTGCAGT	GGAGAGGCTC	TGGGATTATG	GGATGAGTAG
3351	CTCCCCACAT	GTTCTAAGAA	ACAGGGCTCA	GAGTGGCAGT	GTCCCTCAGT
3401	TCAAGAAAGT	TGTTTTCCAG	GAATTTACTG	ATGGCTCCTT	TACTCAGCCC
3451	TTATACCGTG	GAGAACTAAA	TGAACATTTG	GGACTCCTGG	GGCCATATAT
3501	AAGAGCAGAA	GTTGAAGATA	ATATCATGGT	AACTTTCAGA	AATCAGGCCT
3551	CTCGTCCCTA	TTCTTCTAT	TCTAGCCTTA	TTTCTTATGA	GGAAGATCAG
3601	AGGCAAGGAG	CAGAACCTAG	AAAAAACTTT	GTCAAGCCTA	ATGAAACCAA
3651	AACTTACTTT	TGGAAAGTGC	AACATCATAT	GGCACCACCT	AAAGATGAGT
3701	TTGACTGCAA	AGCCTGGGCT	TATTTCTCTG	ATGTTGACCT	GGAAAAAGAT
3751	GTGCACTCAG	GCCTGATTGG	ACCCCTTCTG	GTCTGCCACA	CTAACACACT
3801	GAACCTTGCT	CATGGGAGAC	AAGTGACAGT	ACAGGAATTT	GCTCTGTTTT
3851	TCACCATCTT	TGATGAGACC	AAAAGCTGGT	ACTTCACTGA	AAATATGGAA
3901	AGAAACTGCA	GGGCTCCCTG	CAATATCCAG	ATGGAAGATC	CCACTTTTAA
3951	AGAGAATTAT	CGCTTCCATG	CAATCAATGG	CTACATAATG	GATACACTAC
4001	CTGGCTTAGT	AATGGCTCAG	GATCAAAGGA	TTCGATGGTA	TCTGCTCAGC
4051	ATGGGCAGCA	ATGAAAACAT	CCATTCTATT	CATTTTCAGT	GACATGTGTT
4101	CACTGTACGA	AAAAAAGAGG	AGTATAAAAT	GGCACTGTAC	AATCTCTATC
4151	CAGGTGTTTT	TGAGACAGTG	GAAATGTTAC	CATCCAAAGC	TGGAATTTGG
4201	CGGGTGGAAT	GCCTTATTGG	CGAGCATCTA	CATGCTGGGA	TGAGCACACT
4251	TTTTCTGGTG	TACAGCAATA	AGTGTGAGAC	TCCCCTGGGA	ATGGCTTCTG
4301	GACACATTAG	AGATTTTCAG	ATTACAGCTT	CAGGACAATA	TGGACAGTGG
4351	GCCCCAAAGC	TGGCCAGACT	TCATTATTCC	GGATCAATCA	ATGCCTGGAG
4401	CACCAAGGAG	CCCTTTTCTT	GGATCAAGGT	GGATCTGTTG	GCACCAATGA
4451	TTATTACCGG	CATCAAGACC	CAGGGTGCCC	GTCAGAAAGT	CTCCAGCCTC
4501	TACATCTCTC	AGTTTATCAT	CATGTATAGT	CTTGATGGGA	AGAAGTGGCA
4551	GACTTATCGA	GGAAATTCCA	CTGGAACCTT	AATGGTCTTC	TTTGGCAATG
4601	TGGATTATC	TGGGATAAAA	CACAATATTT	TTAACCCTCC	AATTATTGCT
4651	CGATACATCC	GTTTGACACC	AACTCATTAT	AGCATTTCGCA	GCACCTCTCG
4701	CATGGAGTTG	ATGGGCTGTG	ATTTAAATAG	TTGCAGCATG	CCATTGGGAA
4751	TGGAGAGTAA	AGCAATATCA	GATGCACAGA	TTACTGCTTC	ATCCTACTTT
4801	ACCAATATGT	TTGCCACCTG	GTCTCCTTCA	AAAGCTCGAC	TTACCTCCA
4851	AGGGAGGAGT	AATGCCTGGA	GACCTCAGGT	GAATAATCCA	AAAGAGTGGC
4901	TGCAAGTGGA	CTTCCAGAAG	ACAATGAAAG	TCACAGGAGT	AACTACTCAG
4951	GGAGTAAAT	CTCTGCTTAC	CAGCATGTAT	GTGAAGGAGT	TCCTCATCTC
5001	CAGCAGTCAA	GATGGCCATC	AGTGGACTCT	CTTTTTTTCAG	AATGGCAAAG
5051	TAAAGGTTTT	TCAGGGAAAT	CAAGACTCCT	TCACACCTGT	GGTGAACCTC
5101	CTAGACCCAC	CGTTACTGAC	TCGCTACCTT	CGAATTCACC	CCCAGAGTTG
5151	GGTGCAACCAG	ATTGCCCTGA	GGATGGAGGT	TCTGGGCTGC	GAGGCACAGG
5201	ACCTCTACGA	CAAAACTCAC	ACATGCCAC	CGTGCCACAGC	TCCAGAACTC
5251	CTGGGCGGAC	CGTCAGTCTT	CCTCTTCCCC	CCAAAACCCA	AGGACACCCT
5301	CATGATCTCC	CGGACCCCTG	AGGTCACATG	CGTGGTGGTG	GACGTGAGCC
5351	ACGAAGACCC	TGAGGTCAAG	TTCAACTGGT	ACGTGGACGG	CGTGGAGGTG
5401	CATAATGCCA	AGACAAAGCC	GCGGGAGGAG	CAGTACAACA	GCACGTACCG
5451	TGTGGTCAAGC	GTCTCACCAG	TCTTGACCCA	GGACTGGCTG	AATGGCAAGG
5501	AGTACAAGTG	CAAGGTCTCC	AACAAAGCCC	TCCCAGCCCC	CATCGAGAAA
5551	ACCATCTCCA	AAGCCAAAGG	GCAGCCCCGA	GAACCACAGG	TGTACACCCT
5601	GCCCCATCC	CGGGATGAGC	TGACCAAGAA	CCAGGTCAGC	CTGACCTGCC
5651	TGGTCAAAGG	CTTCTATCCC	AGCGACATCG	CCGTGGAGTG	GGAGAGCAAT
5701	GGGCAGCCGG	AGAACAATA	CAAGACCACG	CCTCCCGTGT	TGGACTCCGA
5751	CGGCTCCTTC	TTCTCTACA	GCAAGCTCAC	CGTGGACAAG	AGCAGGTGGC
5801	AGCAGGGGAA	CGTCTTCTCA	TGCTCCGTGA	TGCATGAGGC	TCTGCACAAC
5851	CACTACACGC	AGAAGAGCCT	CTCCCTGTCT	CCGGGTAAAT	GA

pSYN FVIII 310 protein sequence (FVIII with complete B-domain deletion except 2 amino acid residues and 288 AE-XTEN inserted after aa 742) (SEQ ID NO:171)

```

1   ATRRYYLGAVALSWDYMQSD LGELPVDARF PPRVPKSFPP NTSVVKKTL
51  FVEFTDHLFN IAKPRPPWMG LLGPTIQAEV YDTVVITLKN MASHPVSLHA
101 VGVSYWKASE GA EYDDQTSQ REKEDDKVFP GGSHTYVWQV LKENGPMASD
151 PLCLTYSYLS HVDLVKDLNS GLIGALLVCR EGSLAKEKTQ TLHKFILLFA
201 VFDEGKSWHS ETKNSLMQDR DAASARAWPK MHTVNGYVNR SLPGLIGCHR
251 KSVYWHVIGM GTTPEVHSIF LEGHTFLVRN HRQASLEISP ITFLTAQTLL
301 MDLGQFLLFC HISSHQHDGM EAYVKVDSCP EEPQLRMKNN EEAEDYDDDL
351 TDSEMDVVRF DDDNSPSFIQ IRSVAKKHPK TWVHYIAAEE EDWDYAPLVL
401 APDDRSYKSQ YLNNGPQRIG RKYKKVRFMA YTDETFKTR EAIQHESGILG
451 PLLYGEVGD TLLIIFKNQAS RPYNIYPHGI TDVRPLYSR LPKGVKHLKD
501 FPILPGEIFK YKWTVTVEDG PTKSDPRCLT RYYSSFVNME RDLASGLIGP
551 LLICYKESVD QRGNQIMSDK RNVILFSVFD ENRSWYLTEN IQRFLPNPAG
601 VQLEDPEFQA SNIMHSINGY VFDSLQLSVC LHEVAYWYIL SIGAQTDFLS
651 VFFSGYTFKH KMYEDTLTL FPFSGETVFM SMENPGLWIL GCHNSDFRNR
701 GMTALLKVSS CDKNTGDYEE DSYEDISAYL LSKNNAIEPR SFGTSESATP
751 ESGPGSEPAT SGSETPGTSE SATPESGPGS EPATSGSETP GTSESATPES
801 GPGTSTEPSE GSAPGSPAGS PTSTEEGTSE SATPESGPGS EPATSGSETP
851 GTSESATPES GPGSPAGSPT STEEGSPAGS PTSTEEGTST EPSEGSAPGT
901 SESATPESGP GTSESATPES GPGTSESATP ESGPGSEPAT SGSETPGSEP
951 ATSGSETPGS PAGSPTSTEE GTSTEPSEGS APGTSTEPSE GSAPGSEPAT
1001 SGSETPGTSE SATPESGPGT STEPSEGSAP ASSEITRTTL QSDQEEIDYD
1051 DTISVEMKKE DFDIYDEDEN QSPRSFQKKT RHYFIAAVER LWDYGMSSSP
1101 HVLNRNAQSG SVPQFKKVVF QEFTDGSFTQ PLYRGELNEH LGLLGPYIRA
1151 EVEDNIMVTF RNQASRPYSF YSSLISYEED QRQGAEPKRN FVKPNETKTY
1201 FWKVQHMAP TKDEFDCKAW AYFSDVDLEK DVHSGLIGPL LVCHTNTLNP
1251 AHGRQVTVQE FALFFTIFDE TKS WYFTENM ERNCRAPCNI QMEDPTFKEN
1301 YRFHAINGYI MDTLPGLVMA QDQIRWYLL SMGSNENIHS IHFSGHVFTV
1351 RKKEEYKMAL YNLYPGVFET VEMLPKAGI WRVECLIGEH LHAGMSTLFL
1401 VYSNKCQTPL GMASGHIRDF QITASGQYGQ WAPKLARLHY SGSINAWSTK
1451 EPFSWIKVDL LAPMIIHGK TQGARQKFSS LYISQFIIMY SLDGKKWQTY
1501 RGNSTGTLMV FFGNVDSSGI KHNIFNPPII ARYIRLHPTH YSIRSTLRME
1551 LMGCDLNSCS MPLGMESKAI SDAQITASSY FTNMFATWSP SKARLHLQGR
1601 SNAWRPQVNN PKEWLQVDFQ KTMKVTGVT TQGVKSLLTSM YVKEFLISS
1651 QDGHQWTLFF QNGKVKVFQG NQDSFTPVVN SLDPPLLTRY LRIHPQSWVH
1701 QIALRMEVLG CEAQDLYDKT HCCPPCPAPE LLGGPSVFLF PPKPKDTLMI
1751 SRTPEVTCVV VDVSHEDPEV KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV
1801 SVLTVLHQDW LNGKEYKCKV SNKALPAPIE KTISKAKGQP REPQVYTLPP
1851 SRDELTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPVLDSGDS
1901 FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGK*

```

pSYN FVIII 312 nucleotide sequence (encoding FVIII with complete B-domain deletion except 5 amino acid residues and 288 AE-XTEN inserted after aa 745- B5 version) (SEQ ID NO:172)

```

1   ATGCAAATAG AGCTCTCCAC CTGCTTCTTT CTGTGCCTTT TGCGATTCTG
51  CTTTAGTGCC ACCAGAAGAT ACTACCTGGG TGCAGTGGAA CTGTCATGGG
101 ACTATATGCA AAGTGATCTC GGTGAGCTGC CTGTGGACGC AAGATTTCCT
151 CCTAGAGTGC CAAAATCTTT TCCATTCAAC ACCTCAGTCG TGTACAAAAA
201 GACTCTGTTT GTAGAATTCA CGGATCACCT TTTCAACATC GCTAAGCCAA
251 GGCCACCCTG GATGGGTCTG CTAGGTCCTA CCATCCAGGC TGAGGTTTAT

```

301	GATACAGTGG	TCATTACACT	TAAGAACATG	GCTTCCCATC	CTGTCAGTCT
351	TCATGCTGTT	GGTGTATCCT	ACTGGAAAGC	TTCTGAGGGA	GCTGAATATG
401	ATGATCAGAC	CAGTCAAAGG	GAGAAAAGAAG	ATGATAAAGT	CTTCCCTGGT
451	GGAAGCCATA	CATATGTCTG	GCAGGTCCCTG	AAAGAGAATG	GTCCAATGGC
501	CTCTGACCCA	CTGTGCCTTA	CCTACTCATA	TCTTTCTCAT	GTGGACCTGG
551	TAAAAGACTT	GAATTCAGGC	CTCATTTGGAG	CCCTACTAGT	ATGTAGAGAA
601	GGGAGTCTGG	CCAAGGAAAA	GACACAGACC	TTGCACAAAT	TTATACTACT
651	TTTTGCTGTA	TTTGATGAAG	GGAAAAGTTG	GCACTCAGAA	ACAAAAGAACT
701	CCTTGATGCA	GGATAGGGAT	GCTGCATCTG	CTCGGGCCTG	GCCTAAAATG
751	CACACAGTCA	ATGGTTATGT	AAACAGGTCT	CTGCCAGGTC	TGATTGGATG
801	CCACAGGAAA	TCAGTCTATT	GGCATGTGAT	TGGAATGGGC	ACCACTCCTG
851	AAGTGCACCT	AATATTCCTC	GAAGGTCACA	CATTTCTTGT	GAGGAACCAT
901	CGCCAGGCGT	CCTTGGAAT	CTCGCCAATA	ACTTTCCCTTA	CTGCTCAAAC
951	ACTCTTGATG	GACCTTGGAC	AGTTTCTACT	GTTTTGTCTAT	ATCTCTTCCC
1001	ACCAACATGA	TGGCATGGAA	GCTTATGTCA	AAGTAGACAG	CTGTCAGAG
1051	GAACCCCAAC	TACGAATGAA	AAATAATGAA	GAAGCGGAAG	ACTATGATGA
1101	TGATCTTACT	GATTCTGAAA	TGGATGTGGT	CAGGTTTGAT	GATGACAACCT
1151	CTCCTTCCTT	TATCCAAATT	CGCTCAGTTG	CCAAGAAGCA	TCCTAAAACCT
1201	TGGGTACATT	ACATTGCTGC	TGAAGAGGAG	GACTGGGACT	ATGCTCCCTT
1251	AGTCCTCGCC	CCCAGTGACA	GAAGTTATAA	AAGTCAATAT	TTGAACAATG
1301	GCCCTCAGCG	GATTGGTAGG	AAGTACAAAA	AAGTCCGATT	TATGGCATACT
1351	ACAGATGAAA	CCTTTAAGAC	TCGTGAAGCT	ATTCAGCATG	AATCAGGAAT
1401	CTTGGGACCT	TTACTTTATG	GGGAAGTTGG	AGACACACTG	TTGATTATAT
1451	TTAAGAATCA	AGCAAGCAGA	CCATATAACA	TCTACCCTCA	CGGAATCACT
1501	GATGTCCGTC	CTTTGTATTTC	AAGGAGATTA	CCAAAAGGTG	TAAAACATTT
1551	GAAGGATTTT	CCAATTCTGC	CAGGAGAAAAT	ATTCAAATAT	AAATGGACAG
1601	TGACTGTAGA	AGATGGGCCA	ACTAAATCAG	ATCCTCGGTG	CCTGACCCGC
1651	TATTACTCTA	GTTTCGTTAA	TATGGAGAGA	GATCTAGCTT	CAGGACTCAT
1701	TGGCCCTCTC	CTCATCTGCT	ACAAAGAATC	TGTAGATCAA	AGAGGAAACC
1751	AGATAATGTC	AGACAAGAGG	AATGTCTATCC	TGTTTTCTGT	ATTTGATGAG
1801	AACCGAAGCT	GGTACCTCAC	AGAGAATATA	CAACGCTTTC	TCCCCAATCC
1851	AGCTGGAGTG	CAGCTTGAGG	ATCCAGAGTT	CCAAGCCTCC	AACATCATGG
1901	ACAGCATCAA	TGGCTATGTT	TTTGATAGTT	TGCAGTTGTC	AGTTTGTTTG
1951	CATGAGGTGG	CATACTGGTA	CATTCTAAGC	ATTGGAGCAC	AGATTGACTT
2001	CCTTTCTGTC	TTCTTCTCTG	GATATACCTT	CAAACACAAA	ATGGTCTATG
2051	AAGACACACT	CACCTTATTC	CCATTCTCAG	GAGAAACTGT	CTTCATGTCTG
2101	ATGGAAAACC	CAGGTCTATG	GATTCTGGGG	TGCCACAACCT	CAGACTTTTCG
2151	GAACAGAGGC	ATGACCGCCT	TACTGAAGGT	TTCTAGTTGT	GACAAGAACA
2201	CTGGTGATTA	TTACGAGGAC	AGTTATGAAG	ATATTTTCAGC	ATACTTGCTG
2251	AGTAAAAACA	ATGCCATTGA	ACCAAGAAGC	TTCTCTCAA	ACGGTACCTC
2301	AGAGTCTGCT	ACCCCGAGT	CAGGGCCAGG	ATCAGAGCCA	GCCACCTCCG
2351	GGTCTGAGAC	ACCCGGGACT	TCCGAGAGTG	CCACCCCTGA	GTCCGGACCC
2401	GGGTCCGAGC	CCGCCACTTC	CGGCTCCGAA	ACTCCCGGCA	CAAGCGAGAG
2451	CGCTACCCCA	GAGTCAGGAC	CAGGAACATC	TACAGAGCCC	TCTGAAGGCT
2501	CCGCTCCAGG	GTCCCCAGCC	GGCAGTCCCA	CTAGCACCGA	GGAGGGAACC
2551	TCTGAAAGCG	CCACACCCGA	ATCAGGGCCA	GGGTCTGAGC	CTGCTACCAG
2601	CGGCAGCGAG	ACACCAGGCA	CCTCTGAGTC	CGCCACACCA	GAGTCCGGAC
2651	CCGGATCTCC	CGCTGGGAGC	CCCACCTCCA	CTGAGGAGGG	ATCTCTTGCT
2701	GGCTCTCCAA	CATCTACTGA	GGAAGGTACC	TCAACCGAGC	CATCCGAGG
2751	ATCAGTCCCC	GGCACCTCAG	AGTCGGCAAC	CCCGGAGTCT	GGACCCGGAA
2801	CTTCCGAAAAG	TGCCACACCA	GAGTCCGGTC	CCGGGACTTC	AGAATCAGCA
2851	ACACCCGAGT	CCGGCCCTGG	GTCTGAACCC	GCCACAAGTG	GTAGTGAGAC
2901	ACCAGGATCA	GAACCTGCTA	CCTCAGGGTC	AGAGACACCC	GGATCTCCGG
2951	CAGGCTCACC	AACCTCCACT	GAGGAGGGCA	CCAGCACAGA	ACCAAGCGAG
3001	GGCTCCGCAC	CCGGAACAAG	CACTGAACCC	AGTGAGGGTT	CAGCACCCGG
3051	CTCTGAGCCG	GCCACAAGTG	GCAGTGAGAC	ACCCGGCACT	TCAGAGAGTG
3101	CCACCCCGA	GAGTGGCCCA	GGCACTAGTA	CCGAGCCCTC	TGAAGGCAGT
3151	GCGCCAGCCT	CGAGCGAAAT	AACTCGTACT	ACTCTTCAGT	CAGATCAAGA
3201	GGAAATCGAT	TATGATGATA	CCATATCAGT	TGAAATGAAG	AAGGAAGATT

```

3251   TTGACATTTA TGATGAGGAT GAAAATCAGA GCCCCCGCAG CTTTCAAAAG
3301   AAAACACGAC ACTATTTTAT TGCTGCAGTG GAGAGGCTCT GGGATTATGG
3351   GATGAGTAGC TCCCCACATG TTCTAAGAAA CAGGGCTCAG AGTGGCAGTG
3401   TCCCTCAGTT CAAGAAAGTT GTTTTCCAGG AATTTACTGA TGGCTCCTTT
3451   ACTCAGCCCT TATACCGTGG AGAACTAAAT GAACATTTGG GACTCCTGGG
3501   GCCATATATA AGAGCAGAAG TTGAAGATAA TATCATGGTA ACTTTTCAGAA
3551   ATCAGGCCTC TCGTCCCTAT TCCTTCTATT CTAGCCTTAT TTCTTATGAG
3601   GAAGATCAGA GGCAAGGAGC AGAACCTAGA AAAAACTTTG TCAAGCCTAA
3651   TGAAACCAAA ACTTACTTTT GGAAAGTGCA ACATCATATG GCACCCACTA
3701   AAGATGAGTT TGACTGCAAA GCCTGGGCTT ATTTCTCTGA TGTGACCTG
3751   GAAAAAGATG TGCACTCAGG CCTGATTGGA CCCCTTCTGG TCTGCCACAC
3801   TAACACACTG AACCCTGCTC ATGGGAGACA AGTGACAGTA CAGGAATTTG
3851   CTCTGTTTTT CACCATCTTT GATGAGACCA AAAGCTGGTA CTTCACTGAA
3901   AATATGGAAA GAAACTGCAG GGCTCCCTGC AATATCCAGA TGGAAGATCC
3951   CACTTTTAAA GAGAATTATC GCTTCCATGC AATCAATGGC TACATAATGG
4001   ATACACTACC TGGCTTAGTA ATGGCTCAGG ATCAAAGGAT TCGATGGTAT
4051   CTGCTCAGCA TGGGCAGCAA TGAAAACATC CATTCTATTC ATTTCACTGG
4101   ACATGTGTTT ACTGTACGAA AAAAAGAGGA GTATAAAATG GCACGTGTACA
4151   ATCTCTATCC AGGTGTTTTT GAGACAGTGG AAATGTTACC ATCCAAAGCT
4201   GGAATTTGGC GGGTGGAAATG CCTTATTGGC GAGCATCTAC ATGCTGGGAT
4251   GAGCACACTT TTTCTGGTGT ACAGCAATAA GTGTCAGACT CCCCTGGGAA
4301   TGGCTTCTGG ACACATTAGA GATTTTCAGA TTACAGCTTC AGGACAATAT
4351   GGACAGTGGG CCCCAAAGCT GGCCAGACTT CATTATTCCG GATCAATCAA
4401   TGCCTGGAGC ACCAAGGAGC CCTTTTCTTG GATCAAGGTG GATCTGTTGG
4451   CACCAATGAT TATTCACGGC ATCAAGACCC AGGGTGCCCG TCAGAAGTTC
4501   TCCAGCCTCT ACATCTCTCA GTTTATCATC ATGTATAGTC TTGATGGGAA
4551   GAAGTGGCAG ACTTATCGAG GAAATTCAC TGGAAACCTTA ATGGTCTTCT
4601   TTGGCAATGT GGATTCATCT GGGATAAAAC ACAATATTTT TAACCCCTCCA
4651   ATTATTGCTC GATACATCCG TTTGCACCCA ACTCATTATA GCATTGCGAG
4701   CACTCTTCGC ATGGAGTTGA TGGGCTGTGA TTTAAATAGT TGCAGCATGC
4751   CATTGGGAAT GGAGAGTAAA GCAATATCAG ATGCACAGAT TACTGCTTCA
4801   TCCTACTTTA CCAATATGTT TGCCACCTGG TCTCCTTCAA AAGCTCGACT
4851   TCACCTCCAA GGGAGGAGTA ATGCCTGGAG ACCTCAGGTG AATAATCCAA
4901   AAGAGTGGCT GCAAGTGGAC TTCCAGAAGA CAATGAAAGT CACAGGAGTA
4951   ACTACTCAGG GAGTAAAAATC TCTGCTTACC AGCATGTATG TGAAGGAGTT
5001   CCTCATCTCC AGCAGTCAAG ATGGCCATCA GTGGACTCTC TTTTTCAGAA
5051   ATGGCAAAGT AAAGGTTTTT CAGGGAAATC AAGACTCCTT CACACCTGTG
5101   GTGAACTCTC TAGACCCACC GTTACTGACT CGCTACCTTC GAATTCACCC
5151   CCAGAGTTGG GTGCACCAGA TTGCCCTGAG GATGGAGGTT CTGGGCTGCG
5201   AGGCACAGGA CCTCTACGAC AAAACTCACA CATGCCACC GTGCCAGCT
5251   CCAGAACTCC TGGGCGGACC GTCAGTCTTC CTCTTCCCCC CAAAACCCAA
5301   GGACACCCTC ATGATCTCCC GGACCCCTGA GGTACATGC GTGGTGGTGG
5351   ACGTGAGCCA CGAAGACCC T GAGGTCAAGT TCAACTGGTA CGTGGACGGC
5401   GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG
5451   CACGTACCGT GTGGTCAGCG TCCTCACCCT CCTGCACCAG GACTGGCTGA
5501   ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC
5551   ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG AACCACAGGT
5601   GTACACCCTG CCCCATCCC GGGATGAGCT GACCAAGAAC CAGGTCAGCC
5651   TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC CGTGGAGTGG
5701   GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTGT
5751   GGACTCCGAC GGCTCCTTCT TCCTCTACAG CAAGCTCACC GTGGACAAGA
5801   GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT
5851   CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC CGGGTAAATG

```

pSYN FVIII 312 protein sequence (FVIII with complete B-domain deletion except 5 amino acid residues and 288 AE-XTEN inserted after aa 745- B5 version) (SEQ ID NO:173)

```

1   ATRRYYLGA V ELSWDYMQSD LGELPVDARF PPRVPKSF PF NTSVYKKT L

```

51	FVEFTDHLFN	IAKPRPPWMG	LLGPTIQAEV	YDTVVITLKN	MASHPVSLHA
101	VGVSYWKASE	GAEYDDQTSQ	REKEDDKVFP	GGSHTYVWQV	LKENGPMASD
151	PLCLTYSYLS	HVDLVKDLNS	GLIGALLVCR	EGSLAKEKTQ	TLHKFILLFA
201	VFDEGKSWHS	ETKNSLMQDR	DAASARAWPK	MHTVNGYVNR	SLPGLIGCHR
251	KSVYWHVIGM	GTTPEVHSIF	LEGHTFLVRN	HRQASLEISP	ITFLTAQTLL
301	MDLGQFLLFC	HISSHQHDGM	EAYVKVDSCP	EEPQLRMKNN	EEAEDYDDDL
351	TDSEMDVVRF	DDDNSPSFIQ	IRSVAKKHPK	TWVHYIAAEE	EDWDYAPLVL
401	APDDRSYKSQ	YLNNGPQRIG	RKYKKVRFMA	YTDETFKTR	AIQHESGILG
451	PLLYGEVGD	LLIIFKNQAS	RPYNIYPHGI	TDVRPLYSRR	LPGVKVHLKD
501	FPILPGEIFK	YKWTVTVEDG	PTKSDPRCLT	RYYSSFVNME	RDLASGLIGP
551	LLICYKESVD	QRGNQIMSDK	RNVILFSVFD	ENRSWYL TEN	IQRFLPNPAG
601	VQLEDPEFQA	SNIMHSINGY	VFDSLQLSVC	LHEVAYWYIL	SIGAQTDFLS
651	VFFSGYTFKH	KMVYEDTLTL	FFFSGETVFM	SMENPGLWIL	GCHNSDFRNR
701	GMTALLKVSS	CDKNTGDYYE	DSYEDISAYL	LSKNNAI EPR	SFSQNGTSES
751	ATPESGPGSE	PATSGSETPG	TSESATPESG	PGSEPATSGS	PGSEPATSGS
801	PESGPGTSTE	PSEGSAPGSP	AGSPTSTEEG	TSESATPESG	PGSEPATSGS
851	ETPGTSESAT	PESGPGSPAG	SPTSTEEGSP	AGSPTSTEEG	TSTEPSEGSA
901	PGTSESATPE	SGPGTSESAT	PESGPGTSES	ATPESGPGSE	PATSGSETPG
951	SEPATSGSET	PGSPAGSPTS	TEEGTSTEPS	EGSAPGTSTE	PSEGSAPGSE
1001	PATSGSETPG	TSESATPESG	PGTSTEPSEG	SAPASSEITR	TTLQSDQEEI
1051	DYDDTISVEM	KKEDFDIYDE	DENQSPRSFQ	KKTRHYFIAA	VERLWDYGMS
1101	SSPHVLRNRA	QSGSVPQFKK	VVFQEF TDGS	FTQPLYRGEL	NEHLGLLGPY
1151	IRAEVEDNIM	VTFRNQASRP	YSFYSSLISY	EEDQRQGAEP	RKNFVKPNET
1201	KTYFWKVQHH	MAPTKDEFDC	KAWAYFSDVD	LEKDVHSGLI	GPLL VCHTNT
1251	LNPAHGRQVT	VQEFALFFTI	FDETKSWYFT	ENMERNCRAP	CNIQMEDPTF
1301	KENYRFHAIN	GYIMDTLPGL	VMAQDQIRIW	YLLSMGSNEN	IHSIHFSGHV
1351	FTVRKKEEYK	MALYNLYPGV	FETVEMLPSK	AGIWRVECLI	GEHLHAGMST
1401	LFLVYSNKCQ	TPLGMASGHI	RDFQITASGQ	YGQWAPKLAR	LHYSGSINAW
1451	STKEPFSWIK	VDLLAPMIIH	GIKTQGARQK	FSSLYISQFI	IMYSLDGKKW
1501	QTYRGNSTGT	LMVFFGNVDS	SGIKHNIFNP	PIIARYIRLH	PTHYSIRSTL
1551	RMELMGCDLN	SCSMPLGMES	KAISDAQITA	SSYFTNMFAT	WSPSKARLHL
1601	QGRSNAWRPQ	VNNPKEWLQV	DFQKTMKVTG	VTTQGVKSLL	TSMYVKEFLI
1651	SSSQDGHQWT	LFFQNGKVKV	FQGNQDSFTP	VVNSLDPPLL	TRYLRHPQS
1701	WVHQIALRME	VLGCEAQDLY	DKTHTCPPCP	APELLGGPSV	FLFPPKPKDT
1751	LMISRTPEVT	CVVVDVSHED	PEVKFNWYVD	GVEVHNAKTK	PREEQYNSTY
1801	RVVSVLTVLH	QDWLNGKEYK	CKVSNKALPA	PIEKTISKAK	GQPREPQVYT
1851	LPPSRDELTK	NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTTTPVLDL
1901	DGSFFLYSKL	TVDKSRWQQG	NVFSCSV MHE	ALHNHYTQKS	LSLSPGK*

pSYN VWF059 nucleotide sequence (encoding VWF D'D3-Fc with acidic region 2 (a2) thrombin site in the linker) (SEQ ID NO: 196)

1	ATGATTCTCTG	CCAGATTGTC	CGGGGTGCTG	CTTGCTCTGG	CCCTCATTTT
51	GCCAGGGACC	CTTTGTGCAG	AAGGAACTCG	CGGCAGGTCA	TCCACGGCCC
101	GATGCAGCCT	TTTCGGAAGT	GACTTCGTCA	ACACCTTTGA	TGGGAGCATG
151	TACAGCTTTG	CGGGATACTG	CAGTTACCTC	CTGGCAGGGG	GCTGCCAGAA
201	ACGCTCCTTC	TCGATTATTG	GGGACTTCCA	GAATGGCAAG	AGAGTGAGCC
251	TCTCCGTGTA	TCTTGGGGAA	TTTTTTGACA	TCCATTTGTT	TGTCAATGGT
301	ACCGTGACAC	AGGGGGACCA	AAGAGTCTCC	ATGCCCTATG	CCTCCAAAGG
351	GCTGTATCTA	GAAACTGAGG	CTGGGTACTA	CAAGCTGTCC	GGTGAGGCCT
401	ATGGCTTTGT	GGCCAGGATC	GATGGCAGCG	GCAACTTTCA	AGTCCTGCTG
451	TCAGACAGAT	ACTTCAACAA	GACCTGCGGG	CTGTGTGGCA	ACTTTAACAT
501	CTTTGCTGAA	GATGACTTTA	TGACCCAAGA	AGGGACCTTG	ACCTCGGACC
551	CTTATGACTT	TGCCAACTCA	TGGGCTCTGA	GCAGTGAGGA	ACAGTGGTGT
601	GAACAGGCAT	CTCCTCCACG	CAGCTCATGC	AACATCTCCT	CTGGGGAAAT
651	GCAGAAGGGC	CTGTGGGAGC	AGTGCCAGCT	TCTGAAGAGC	ACCTCGGTGT
701	TTGCCCCGCTG	CCACCCTCTG	GTGGACCCCG	AGCCTTTTGT	GGCCCTGTGT
751	GAGAAGACTT	TGTGTGAGTG	TGCTGGGGGG	CTGGAGTGCG	CCTGCCCTGC

801	CCTCCTGGAG	TACGCCCCGA	CCTGTGCCCCA	GGAGGGAATG	GTGCTGTACG
851	GCTGGACCGA	CCACAGCGCG	TGCAGCCCAG	TGTGCCCTGC	TGGTATGGAG
901	TATAGGCAGT	GTGTGTCCCC	TTGCGCCAGG	ACCTGCCAGA	GCCTGCACAT
951	CAATGAAATG	TGTCAGGAGC	GATGCGTGGA	TGGCTGCAGC	TGCCCTGAGG
1001	GACAGCTCCT	GGATGAAGGC	CTCTGCGTGG	AGAGCACCAG	GTGTCCCTGC
1051	GTGCATTCCG	GAAAGCGCTA	CCCTCCCGGC	ACCTCCCTCT	CTCGAGACTG
1101	CAACACCTGC	ATTTGCCGAA	ACAGCCAGTG	GATCTGCAGC	AATGAAGAAT
1151	GTCCAGGGGA	GTGCCTTGTC	ACTGGTCAAT	CCCAC TTCAA	GAGCTTTGAC
1201	AACAGATACT	TCACCTTCAG	TGGGATCTGC	CAGTACCTGC	TGGCCCGGGA
1251	TTGCCAGGAC	CACTCCTTCT	CCATTGTTCAT	TGAGACTGTC	CAGTGTGCTG
1301	ATGACCGCGA	CGCTGTGTGC	ACCCGCTCCG	TCACCGTCCG	GCTGCCTGGC
1351	CTGCACAACA	GCCTTGTGAA	ACTGAAGCAT	GGGGCAGGAG	TTGCCATGGA
1401	TGGCCAGGAC	ATCCAGCTCC	CCCTCCTGAA	AGGTGACCTC	CGCATCCAGC
1451	ATACAGTGAC	GGCCTCCGTG	CGCCTCAGCT	ACGGGGAGGA	CCTGCAGATG
1501	GACTGGGATG	GCCGCGGGAG	GCTGCTGGTG	AAGCTGTCCC	CCGTCTATGC
1551	CGGGAAGACC	TGCGGCCTGT	GTGGGAATTA	CAATGGCAAC	CAGGGCGACG
1601	ACTTCCTTAC	CCCCCTCTGGG	CTGGCGGAGC	CCCGGGTGGA	GGACTTCGGG
1651	AACGCTTGA	AGCTGCACGG	GGACTGCCAG	GACCTGCAGA	AGCAGCACAG
1701	CGATCCCTGC	GCCCTCAACC	CGCGCATGAC	CAGGTTCTCC	GAGGAGGCGT
1751	GCGCGGTCT	GACGTCCCCC	ACATTTCGAGG	CCTGCCATCG	TGCCGTCAGC
1801	CCGCTGCCCT	ACCTGCGGAA	CTGCCGCTAC	GACGTGTGCT	CCTGCTCGGA
1851	CGGCCGCGAG	TGCCTGTGCG	GCGCCCTGGC	CAGCTATGCC	GCGGCCTGCG
1901	CGGGGAGAGG	CGTGCGCGTC	GCGTGCGCGC	AGCCAGGCCG	CTGTGAGCTG
1951	AACTGCCCCA	AAGGCCAGGT	GTACCTGCAG	TGCGGGACCC	CCTGCAACCT
2001	GACCTGCCGC	TCTCTCTCTT	ACCCGGATGA	GGAATGCAAT	GAGGCCTGCC
2051	TGGAGGGCTG	CTTCTGCCCC	CCAGGGCTCT	ACATGGATGA	GAGGGGGGAC
2101	TGCGTGCCCA	AGGCCCAGTG	CCCCTGTTAC	TATGACGGTG	AGATCTTCCA
2151	GCCAGAAGAC	ATCTTCTCAG	ACCATCACAC	CATGTGCTAC	TGTGAGGATG
2201	GCTTCATGCA	CTGTACCATG	AGTGAGATCC	CCGGAAGCTT	GCTGCCTGAC
2251	GCTGTCTCTA	GCAGTCCCCC	GTCTCATCGC	AGCAAAAAGGA	GCCTATCCTG
2301	TCGGCCCCCC	ATGGTCAAGC	TGGTGTGTCC	CGCTGACAAC	CTGCGGGCTG
2351	AAGGGCTCGA	GTGTACCAAA	ACGTGCCAGA	ACTATGACCT	GGAGTGCAATG
2401	AGCATGGGCT	GTGTCTCTGG	CTGCCTCTGC	CCCCCGGGCA	TGGTCCGGCA
2451	TGAGAACAGA	TGTGTGGCCC	TGGAAAGGTG	TCCCTGCTTC	CATCAGGGCA
2501	AGGAGTATGC	CCCTGGAGAA	ACAGTGAAGA	TTGGCTGCAA	CAC TTGTGTC
2551	TGTCGGGACC	GGAAGTGGAA	CTGCACAGAC	CATGTGTGTG	ATGCCACGTG
2601	CTCCACGATC	GGCATGGCCC	ACTACCTCAC	CTTCGACGGG	CTCAAATACC
2651	TGTTCCCCCG	GGAGTGCCAG	TACGTTCTGG	TGCAGGATTA	CTGCGGCAGT
2701	AACCCTGGGA	CCTTTCGGAT	CCTAGTGGGG	AATAAGGGAT	GCAGCCACCC
2751	CTCAGTGAAA	TGCAAGAAAC	GGGTCACCAT	CCTGGTGGAG	GGAGGAGAGA
2801	TTGAGCTGTT	TGACGGGGAG	GTGAATGTGA	AGAGGCCCAT	GAAGGATGAG
2851	ACTCACTTTG	AGGTGGTGGA	GTCTGGCCGG	TACATCATT	TGCTGCTGGG
2901	CAAAGCCCTC	TCCGTGGTCT	GGGACCGCCA	CCTGAGCATC	TCCGTGGTCC
2951	TGAAGCAGAC	ATACCAGGAG	AAAGTGTGTG	GCCTGTGTGG	GAATTTTGAT
3001	GGCATCCAGA	ACAATGACCT	CACCAGCAGC	AACCTCCAAG	TGGAGGAAGA
3051	CCCTGTGGAC	TTTGGGAACT	CCTGGAAAAGT	GAGCTCGCAG	TGTGCTGACA
3101	CCAGAAAAGT	GCCTCTGGAC	TCATCCCCTG	CCACCTGCCA	TAACAACATC
3151	ATGAAGCAGA	CGATGGTGGA	TTCTCCTGT	AGAATCCTTA	CCAGTGACGT
3201	CTTCCAGGAC	TGCAACAAGC	TGGTGGACCC	CGAGCCATAT	CTGGATGTCT
3251	GCATTTACGA	CACCTGCTCC	TGTGAGTCCA	TTGGGGACTG	CGCCGCATT
3301	TGCGACACCA	TTGCTGCCTA	TGCCCACGTG	TGTGCCCAGC	ATGGCAAGGT
3351	GGTGACCTGG	AGGACGGCCA	CATTGTGCCC	CCAGAGCTGC	GAGGAGAGGA
3401	ATCTCCGGGA	GAACGGGTAT	GAGGCTGAGT	GGCGCTATAA	CAGCTGTGCA
3451	CCTGCCTGTC	AAGTCACGTG	TCAGCACCCCT	GAGCCACTGG	CCTGCCCTGT
3501	GCAGTGTGTG	GAGGGCTGCC	ATGCCCCTG	CCCTCCAGGG	AAAATCCTGG
3551	ATGAGCTTTT	GCAGACCTGC	GTTGACCCTG	AAGACTGTCC	AGTGTGTGAG
3601	GTGGCTGGCC	GGCGTTTTGC	CTCAGGAAAG	AAAGTCACCT	TGAATCCAG
3651	TGACCCTGAG	CACTGCCAGA	TTTGCCACTG	TGATGTTGTC	AACCTCACCT
3701	GTGAAGCCTG	CCAGGAGCCG	ATATCGGGCG	CGCCAACATC	AGAGAGCGCC

```

3751  ACCCCTGAAA GTGGTCCCGG GAGCGAGCCA GCCACATCTG GGTCGGAAAC
3801  GCCAGGCACA AGTGAGTCTG CAACTCCCGA GTCCGGACCT GGCTCCGAGC
3851  CTGCCACTAG CGGCTCCGAG ACTCCGGGAA CTTCCGAGAG CGCTACACCA
3901  GAAAGCGGAC CCGGAACCAG TACCGAACCT AGCGAGGGCT CTGCTCCGGG
3951  CAGCCCAGCC GGCTCTCCTA CATCCACGGA GGAGGGCACT TCCGAATCCG
4001  CCACCCCGGA GTCAGGGCCA GGATCTGAAC CCGCTACCTC AGGCAGTGAG
4051  ACGCCAGGAA CGAGCGAGTC CGCTACACCG GAGAGTGGGC CAGGGAGCCC
4101  TGCTGGATCT CCTACGTCCA CTGAGGAAGG GTCACCAGCG GGCTCGCCCA
4151  CCAGCACTGA AGAAGGTGCC TCGATATCTG ACAAGAACAC TGGTGATTAT
4201  TACGAGGACA GTTATGAAGA TATTTAGCA TACTTGCTGA GTAAAAACAA
4251  TGCCATTGAA CCAAGAAGCT TCTCTGACAA AACTCACACA TGCCCACCGT
4301  GCCCAGCTCC AGAACTCCTG GGCGGACCGT CAGTCTTCCT CTTCCCCCCA
4351  AAACCCAAGG ACACCCTCAT GATCTCCCGG ACCCTGAGG TCACATGCGT
4401  GGTGGTGGAC GTGAGCCACG AAGACCCTGA GGTCAAGTTC AACTGGTACG
4451  TGGACGGCGT GGAGGTGCAT AATGCCAAGA CAAAGCCGCG GGAGGAGCAG
4501  TACAACAGCA CGTACCGTGT GGTACGCGTC CTCACCGTCC TGCACCAGGA
4551  CTGGCTGAAT GGCAAGGAGT ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC
4601  CAGCCCCCAT CGAGAAAAACC ATCTCCAAAG CCAAAGGGCA GCCCCGAGAA
4651  CCACAGGTGT ACACCCTGCC CCCATCCCGG GATGAGCTGA CCAAGAACCA
4701  GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTATCCCAGC GACATCGCCG
4751  TGGAGTGGGA GAGCAATGGG CAGCCGGAGA ACAACTACAA GACCACGCCT
4801  CCCGTGTTGG ACTCCGACGG CTCTTCTTTC CTCTACAGCA AGCTACCGT
4851  GGACAAGAGC AGGTGGCAGC AGGGGAACGT CTTCTCATGC TCCGTGATGC
4901  ATGAGGCTCT GCACAACCAC TACACGCAGA AGAGCCTCTC CCTGTCTCCG
4951  GGTAAATGA

```

pSYN VWF059 protein sequence (VWF D'D3-Fc with a2 region of FVIII thrombin site in the linker) - bold underlined area shows a2 region (SEQ ID NO: 197)

```

1    MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFVNTFDGSM
51   YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVLSLVYLGE FFDIHLFVNG
101  TVTQGDQRV S MPYASKGLYL ETEAGYYKLS GEAYGFVARI DGSGNFQVLL
151  SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
201  ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
251  EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWDHSA CSPVCPAGME
301  YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTPCPC
351  VHS GKRYPPG TSLSRDCNTC ICRNSQWICS NEECPGEC LV TGQSHFKSFD
401  NRYFTFSGIC QYLLARDCQD HSFSIVIETV QCADDRDAVC TRSVTVRLPG
451  LHNSLVKLKH GAGVAMDGQD IQLPLLKGD L RIQHTVTASV RLSYGEDLQM
501  DWDGRGRLLV KLSVPYAGKT CGLCGNYNGN QGDDFLTPSG LAEPRVEDFG
551  NAWKLHGDCQ DLQKQHS DPC ALNPRMTRFS EEACAVLTSP TFEACHRAVS
601  PLPYLRNCRY DVCSCSDGRE CLCGALASYA AACAGRGVRV AWREPGRC EL
651  NCPKQGVYLQ CGTPCNLTCR SLSYPDEECN EACLEGCFCP PGLYMDERGD
701  CVPKAQCPCY YDGEIFQPED IFSDHHTMCY CEDGFMHCTM SGVPGSLLPD
751  AVLSSPLSHR SKRSLSCRPP MVKLVC PADN LRAEGLECTK TCQNYDLECM
801  SMGCVSGCLC PPGMVRHENR CVALERCPCF HQGKEYAPGE TVKIGCNTCV
851  CRDRKWNCTD HVCDATCSTI GMAHYLTFDG LKYLFPGECQ YVLVQDYCGS
901  NPGTFRILVG NKGCSHPSVK CKKRVTILVE GGEIELFDGE VNVKRPMKDE
951  THFEVVESGR YIILLGKAL SVVWDRHLSI SVVLKQTYQE KVCGLCGNFD
1001 GIQNNDLTSS NLQVEEDPVD FGNSWKVSSQ CADTRKVPLD SSPATCHNNI
1051 MKQTMVDSSC RILTSDVFQD CNKLVDPEPY LDVCIYDTCS CESIGDCAAF
1101 CDTIAAYAHV CAQH GKVV TW RTATLCPQSC EERNLRENGY EAEWRYN SCA
1151 PACQVTCQHP EPLACPVQCV EGCHAHCPPG KILDELLQTC VDPEDCPVCE
1201 VAGRRFASGK KVT LNPSDPE HCQICHCDVV NLTCEACQEP ISGAPTSESA
1251 TPESGPGSEP ATSGSETPGT SESATPESGP GSEPATSGSE TPGTSESATP
1301 ESGPGTSTEP SEGSA PGSPA GSPTSTEEGT SESATPESGP GSEPATSGSE
1351 TPGTSESATP ESGPGSPAGS PTSTEEGSPA GSPTSTEEGA SIS DKNTGDY
1401 YEDSYEDISA YLLSKNNAIE PRSFSDKTHT CPPCPAPELL GGP SVFLFPF
1451 KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ

```

```

1501   YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKQPRE
1551   PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTFP
1601   PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP
1651   GK*

```

pSYN VWF062 nucleotide sequence (encoding VWF D'D3-Fc with no thrombin site in the linker) (SEQ ID NO: 198)

```

1   ATGATTCTCTG CCAGATTTCG CGGGGTGCTG CTTGCTCTGG CCCTCATTTT
51  GCCAGGGACC CTTTGTGCAG AAGGAACTCG CGGCAGGTCA TCCACGGCCC
101 GATGCAGCCT TTTCGGAAGT GACTTCGTCA ACACCTTTGA TGGGAGCATG
151 TACAGCTTTG CGGGATACTG CAGTTACCTC CTGGCAGGGG GCTGCCAGAA
201 ACGCTCCTTC TCGATTATTG GGGACTTCCA GAATGGCAAG AGAGTGAGCC
251 TCTCCGTGTA TCTTGGGGAA TTTTGTGACA TCCATTTGTT TGTCAATGGT
301 ACCGTGACAC AGGGGGACCA AAGAGTCTCC ATGCCCTATG CCTCCAAAGG
351 GCTGTATCTA GAAACTGAGG CTGGGTACTA CAAGCTGTCC GGTGAGGCCCT
401 ATGGCTTTGT GGCCAGGATC GATGGCAGCG GCAACTTTCA AGTCCTGCTG
451 TCAGACAGAT ACTTCAACAA GACCTGCGGG CTGTGTGGCA ACTTTAACAT
501 CTTTGTGTA GATGACTTTA TGACCAAGA AGGGACCTTG ACCTCGGACC
551 CTTATGACTT TGCCAACCTA TGGGCTCTGA GCAGTGGAGA ACAGTGGTGT
601 GAACGGGCAT CTCTCCAG CAGCTCATGC AACATCTCCT CTGGGGAAAT
651 GCAGAAGGGC CTGTGGGAGC AGTGCCAGCT TCTGAAGAGC ACCTCGGTGT
701 TTGCCCCTG CCACCCCTCTG GTGGACCCCG AGCCTTTTGT GGCCCTGTGT
751 GAGAAGACTT TGTGTGAGTG TGCTGGGGGG CTGGAGTGC GCTGCCCTGC
801 CCTCCTGGAG TACGCCCAGA CCTGTGCCCA GGAGGGAATG GTGCTGTACG
851 GCTGGACCGA CCACAGCGCG TGCAGCCCAG TGTGCCCTGC TGGTATGGAG
901 TATAGGCAGT GTGTGTCCCC TTGCGCCAGG ACCTGCCAGA GCCTGCACAT
951 CAATGAAATG TGTCAGGAGC GATGCGTGGA TGGCTGCAGC TGCCCTGAGG
1001 GACAGCTCCT GGATGAAGGC CTCTGCGTGG AGAGCACCGA GTGTCCCTGC
1051 GTGCATTCCG GAAAGCGCTA CCCTCCCGGC ACCTCCCTCT CTCGAGACTG
1101 CAACACCTGC ATTTGCCGAA ACAGCCAGTG GATCTGCAGC AATGAAGAAT
1151 GTCCAGGGGA GTGCCTTGTC ACTGGTCAAT CCCACTTCAA GAGCTTTGAC
1201 AACAGATACT TCACCTTCAG TGGGATCTGC CAGTACCTGC TGGCCCGGGA
1251 TTGCCAGGAC CACTCCTTCT CCATTGTCAT TGAGACTGTC CAGTGTGCTG
1301 ATGACCGCGA CGCTGTGTGC ACCCGCTCCG TCACCGTCCG GCTGCCCTGGC
1351 CTGCACAACA GCCTTGTGAA ACTGAAGCAT GGGGCAGGAG TTGCCATGGA
1401 TGGCCAGGAC ATCCAGCTCC CCCTCCTGAA AGGTGACCTC CGCATCCAGC
1451 ATACAGTGAC GGCTCCGTG CGCTCAGCT ACGGGGAGGA CCTGCAGATG
1501 GACTGGGATG GCCGCGGGAG GCTGCTGGTG AAGCTGTCCC CCGTCTATGC
1551 CGGGAAGACC TGCGGCCTGT GTGGGAATTA CAATGGCAAC CAGGGCGACG
1601 ACTTCCTTAC CCCCTCTGGG CTGGCGGAGC CCCGGGTGGA GGACTTCGGG
1651 AACGCCTGGA AGCTGCACGG GGACTGCCAG GACCTGCAGA AGCAGCACAG
1701 CGATCCCTGC GCCCTCAACC CGCGCATGAC CAGGTTCTCC GAGGAGGCGT
1751 GCGCGGTCTT GACGTCCCC ACATTGAGG CCTGCCATCG TGCCGTGAGC
1801 CCGCTGCCCT ACCTGCGGAA CTGCCGCTAC GACGTGTGCT CCTGCTCGGA
1851 CGGCCGCGAG TGCTGTGCG GCGCCCTGGC CAGCTATGCC GCGGCCTGCG
1901 CGGGGAGAGG CGTGCGCGTC GCGTGGCGCG AGCCAGGCCG CTGTGAGCTG
1951 AACTGCCCCA AAGGCCAGGT GTACCTGCAG TGCGGGACCC CCTGCAACCT
2001 GACCTGCCGC TCTCTCTCTT ACCCGGATGA GGAATGCAAT GAGGCCTGCC
2051 TGGAGGGCTG CTTCTGCCCC CCAGGGCTCT ACATGGATGA GAGGGGGGAC
2101 TGCGTGCCCA AGGCCAGTG CCCCTGTTAC TATGACGGTG AGATCTTCCA
2151 GCCAGAAGAC ATCTTCTCAG ACCATCACAC CATGTGCTAC TGTGAGGATG
2201 GCTTCATGCA CTGTACCATG AGTGGAGTCC CCGGAAGCTT GCTGCCCTGAC
2251 GCTGTCCCTA GCAGTCCCTT GTCTCATCGC AGCAAAAGGA GCCTATCCTG
2301 TCGGCCCCC ATGGTCAAGT TGGTGTGTCC CGCTGACAAC CTGCGGGCTG
2351 AAGGGCTCGA GTGTACCAAA ACGTGCCAGA ACTATGACCT GGAGTGCATG
2401 AGCATGGGCT GTGTCTCTGG CTGCCTCTGC CCCCCGGGCA TGGTCCGGCA
2451 TGAGAACAGA TGTGTGGCCC TGGAAAGGTG TCCCTGCTTC CATCAGGGCA
2501 AGGAGTATGC CCCTGGAGAA ACAGTGAAGA TTGGCTGCAA CACTTGTGTC

```

```

2551  TGTCGGGACC GGAAGTGGAA CTGCACAGAC CATGTGTGTG ATGCCACGTG
2601  CTCCACGATC GGCATGGCCC ACTACCTCAC CTTGACGGG CTCAAATACC
2651  TGTTCCCCGG GGAGTGCCAG TACGTTCTGG TGCAGGATTA CTGCGGCAGT
2701  AACCTTGGGA CCTTTCGGAT CCTAGTGGGG AATAAGGGAT GCAGCCACCC
2751  CTCAGTGAAA TGCAAGAAAC GGGTCACCAT CCTGGTGGAG GGAGGAGAGA
2801  TTGAGCTGTT TGACGGGGAG GTGAATGTGA AGAGGCCCAT GAAGGATGAG
2851  ACTCACTTTG AGGTGGTGGA GTCTGGCCGG TACATCATTC TGCTGCTGGG
2901  CAAAGCCCTC TCCGTGGTCT GGGACCGCCA CCTGAGCATC TCCGTGGTCC
2951  TGAAGCAGAC ATACCAGGAG AAAGTGTGTG GCCTGTGTGG GAATTTTGAT
3001  GGCATCCAGA ACAATGACCT CACCAGCAGC AACCTCCAAG TGGAGGAAGA
3051  CCCTGTGGAC TTTGGGAACT CCTGGAAAAGT GAGCTCGCAG TGTGCTGACA
3101  CCAGAAAAGT GCCTCTGGAC TCATCCCCTG CCACCTGCCA TAACAACATC
3151  ATGAAGCAGA CGATGGTGGA TTCCTCCTGT AGAATCCTTA CCAGTGACGT
3201  CTTCCAGGAC TGCAACAAGC TGGTGGACCC CGAGCCATAT CTGGATGTCT
3251  GCATTTACGA CACCTGCTCC TGTGAGTCCA TTGGGGACTG CGCCGCATTG
3301  TGCGACACCA TTGCTGCCCTA TGCCACCGTG TGTGCCAGC ATGGCAAGGT
3351  GGTGACCTGG AGGACGGCCA CATTGTGCCC CCAGAGCTGC GAGGAGAGGA
3401  ATCTCCGGGA GAACGGGTAT GAGGCTGAGT GGCCTATAA CAGCTGTGCA
3451  CCTGCCTGTC AAGTCACGTG TCAGCACCCCT GAGCCACTGG CCTGCCCTGT
3501  GCAGTGTGTG GAGGGCTGCC ATGCCCACTG CCCTCCAGGG AAAATCCTGG
3551  ATGAGCTTTT GCAGACCTGC GTTGACCCTG AAGACTGTCC AGTGTGTGAG
3601  GTGGCTGGCC GCGTTTTGTC CTCAGGAAAG AAAGTCACCT TGAATCCAG
3651  TGACCCTGAG CACTGCCAGA TTTGCCACTG TGATGTTGTC AACCTCACCT
3701  GTGAAGCCTG CCAGGAGCCG ATATCGGGCG CGCCAACATC AGAGAGCGCC
3751  ACCCCTGAAA GTGGTCCCGG GAGCGAGCCA GCCACATCTG GGTCGGAAC
3801  GCCAGGCACA AGTGAGTCTG CAACTCCCGA GTCCGGACCT GGCTCCGAGC
3851  CTGCCACTAG CGGCTCCGAG ACTCCGGGAA CTTCCGAGAG CGCTACACCA
3901  GAAAGCGGAC CCGGAACCAG TACCGAACCT AGCGAGGGCT CTGCTCCGGG
3951  CAGCCCAGCC GGCTCTCCTA CATCCACGGA GGAGGGCACT TCCGAATCCG
4001  CCACCCCGGA GTCAGGGCCA GGATCTGAAC CCGCTACCTC AGGCAGTGAG
4051  ACGCCAGGAA CGAGCGAGTC CGCTACACCG GAGAGTGGGC CAGGGAGCCC
4101  TGCTGGATCT CCTACGTCCA CTGAGGAAGG GTCACCAGCG GGTCTGCCCA
4151  CCAGACTGA AGAAGGTGCC TCGAGCGACA AAACCTACAC ATGCCCACCG
4201  TGCCAGCTC CAGAACTCCT GGGCGGACCG TCAGTCTTCC TCTTCCCCC
4251  AAAACCCAAG GACACCTCA TGATCTCCCG GACCCCTGAG GTCACATGCG
4301  TGGTGGTGGA CGTGAGCCAC GAAGACCTG AGGTCAAGTT CAACTGGTAC
4351  GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAAGCCGC GGGAGGAGCA
4401  GTACAACAGC ACGTACCGTG TGGTCAGCGT CCTCACCGTC CTGCACCAGG
4451  ACTGGCTGAA TGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGCCCTC
4501  CCAGCCCCCA TCGAGAAAAC CATCTCCAAA GCCAAAGGGC AGCCCCGAGA
4551  ACCACAGGTG TACACCTGC CCCATCCCG GGATGAGCTG ACCAAGAACC
4601  AGGTCAGCCT GACCTGCCTG GTCAAAGGCT TCTATCCCAG CGACATCGCC
4651  GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AACAACCTACA AGACCACGCC
4701  TCCCGTGTG GACTCCGACG GCTCCTTCTT CCTCTACAGC AAGCTCACCG
4751  TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
4801  CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
4851  GGGTAAATGA

```

pSYN VWF062 protein sequence (VWF D'D3-Fc with no thrombin site in the linker) (SEQ ID NO: 199)

```

1      MIPARFAGVL LALALILPGT LCAEGTRGRS STARCSLFGS DFNFTFDGSM
51     YSFAGYCSYL LAGGCQKRSF SIIGDFQNGK RVSLSVYLGE FFDIHLFVNG
101    TVTQGDQQRVS MPYASKGLYL ETEAGYYKLS GEAYGFVARI DSGSNFQVLL
151    SDRYFNKTCG LCGNFNIFAE DDFMTQEGTL TSDPYDFANS WALSSGEQWC
201    ERASPPSSSC NISSGEMQKG LWEQCQLLKS TSVFARCHPL VDPEPFVALC
251    EKTLCECAGG LECACPALLE YARTCAQEGM VLYGWTDHSA CSPVCPAGME
301    YRQCVSPCAR TCQSLHINEM CQERCVDGCS CPEGQLLDEG LCVESTECPC

```

```

351  VHSGKRYPPG  TSLSRDCNTC  ICRNSQWICS  NEECPGECLV  TGQSHFKSFD
401  NRYFTFSGIC  QYLLARDCQD  HSFSIVIETV  QCADDRDAVC  TRSVTVRLPG
451  LHNSLVKLKH  GAGVAMDQD  IQLPLLKGD  RIQHTVTASV  RLSYGEDLQM
501  DWDGRGRLLV  KLSVPYAGKT  CGLCGNYNGN  QGDDFLTSPG  LAEPRVEDFG
551  NAWKLHGDQC  DLQKHSDPC  ALNPRMTRFS  EEACAVLTSP  TFEACHRAVS
601  PLPYLRNCRY  DVCSCSDGRE  CLCGALASYA  AACAGRGVRV  AWREPGRCEL
651  NCPKGQVYLQ  CGTPCNLTCR  SLSYPDEECN  EACLEGCFCP  PGLYMDERGD
701  CVPKAQCPCY  YDGEIFQPED  IFSDHHTMCY  CEDGFMHCTM  SGVPGSLLPD
751  AVLSSPLSHR  SKRSLSCRPP  MVKLVCPADN  LRAEGLECTK  TCQNYDLECM
801  SMGCVSGCLC  PPGMVRHENR  CVALERCPCF  HQGKEYAPGE  TVKIGCNTCV
851  CRDRKWNCTD  HVCDATCSTI  GMAHYLTFDG  LKYLFPGECQ  YVLVQDYCGS
901  NPGTFRILVG  NKGCSHPSVK  CKKRVTLIVE  GGEIELFDGE  VNVKRPMKDE
951  THFEVVESGR  YIILLGKAL  SVVWDRHLSI  SVVLKQTYQE  KVCGLCGNFD
1001  GIQNNDLTSS  NLQVEEDPVD  FGNSWKVSSQ  CADTRKVPLD  SSPATCHNNI
1051  MKQTMVHSSC  RILTSDFVQD  CNKLVDPEPY  LDVCIYDTCSE  SIGDCAAF
1101  CDTIAAYAHV  CAQHKGKVVW  RTATLCPQSC  EERNLRENGY  EAEWRYNSCA
1151  PACQVTCQHP  EPLACPVQCV  EGCHAHCPPG  KILDELLQTC  VDPEDCPVCE
1201  VAGRRFASGK  KVTLNPSDPE  HCQICHCDVV  NLTCEACQEP  ISGAPTESA
1251  TPESGPGSEP  ATSGSETPGT  SESATPESGP  GSEPATSGSE  TPGTSESATP
1301  ESGPGTSTEP  SEGSAPGSPA  GSPTSTEEGT  SESATPESGP  GSEPATSGSE
1351  TPGTSESATP  ESGPGSPAGS  PTSTEEGSPA  GSPTSTEEGA  SSDKTHTCPP
1401  CPAPELLGGP  SVFLFPKPK  DTLMISRTPE  VTCVVVDVSH  EDPEVKFNWY
1451  VDGVEVHNAK  TKPREEQYNS  TYRVVSVLTV  LHQDWLNGKE  YKCKVSNKAL
1501  PAPIEKTISK  AKGQPREPQV  YTLPPSRDEL  TKNQVSLTCL  VKGFYPSDIA
1551  VEWESNGQPE  NNYKTTPPVL  DSDGSFFLYS  KLTVDKSRWQ  QGNVFSCSVM
1601  HEALHNHYTQ  KSLSLSPGK*

```

pSYN VWF073 nucleotide sequence- (encoding VWFD1D2D'D3- 144 AE XTEN- FVIII truncated a2 thrombin site-Fc) (SEQ ID NO:174)

```

1    ATGATTCTCTG  CCAGATTTCG  CGGGGTGCTG  CTTGCTCTGG  CCCTCATTTT
51   GCCAGGGACC  CTTTGTGCAG  AAGGAACTCG  CGGCAGGTCA  TCCACGGCCC
101  GATGCAGCCT  TTTCGGAAGT  GACTTCGTCA  ACACCTTTGA  TGGGAGCATG
151  TACAGCTTTG  CGGGATACTG  CAGTTACCTC  CTGGCAGGGG  GCTGCCAGAA
201  ACGCTCCTTC  TCGATTATTG  GGGACTTCCA  GAATGGCAAG  AGAGTGAGCC
251  TCTCCGTGTA  TCTTGGGGAA  TTTTGTGACA  TCCATTTGTT  TGTCAATGGT
301  ACCGTGACAC  AGGGGGACCA  AAGAGTCTCC  ATGCCCTATG  CCTCCAAAGG
351  GCTGTATCTA  GAAACTGAGG  CTGGGTACTA  CAAGCTGTCC  GGTGAGGCCT
401  ATGGCTTTGT  GGCCAGGATC  GATGGCAGCG  GCAACTTTCA  AGTCCTGCTG
451  TCAGACAGAT  ACTTCAACAA  GACCTGCGGG  CTGTGTGGCA  ACTTTAACAT
501  CTTTGTCTGAA  GATGACTTTA  TGACCCAAGA  AGGGACCTTG  ACCTCGGACC
551  CTTATGACTT  TGCCAACTCA  TGGGCTCTGA  GCAGTGGAGA  ACAGTGGTGT
601  GAACGGGCAT  CTCTCCCAAG  CAGCTCATGC  AACATCTCCT  CTGGGGAAAT
651  GCAGAAGGGC  CTGTGGGAGC  AGTGCCAGCT  TCTGAAGAGC  ACCTCGGTGT
701  TTGCCCCTG  CCACCCCTCT  GTGGACCCCG  AGCCTTTTGT  GGCCCTGTGT
751  GAGAAGACTT  TGTGTGAGTG  TGCTGGGGGG  CTGGAGTGCG  CCTGCCCTGC
801  CCTCCTGGAG  TACGCCCGGA  CCTGTGCCCA  GGAGGGAATG  GTGCTGTACG
851  GCTGGACCGA  CCACAGCGCG  TGCAGCCCAG  TGTGCCCTGC  TGGTATGGAG
901  TATAGGCAGT  GTGTGTCCCC  TTGCGCCAGG  ACCTGCCAGA  GCCTGCACAT
951  CAATGAAATG  TGTCAGGAGC  GATGCGTGGA  TGGCTGCAGC  TGCCCTGAGG
1001  GACAGCTCCT  GGATGAAGGC  CTCTGCGTGG  AGAGCACCGA  GTGTCCCTGC
1051  GTGCATTCCG  GAAAGCGCTA  CCCTCCCGGC  ACCTCCCTCT  CTCGAGACTG
1101  CAACACCTGC  ATTTGCCGAA  ACAGCCAGTG  GATCTGCAGC  AATGAAGAAT
1151  GTCCAGGGGA  GTGCCTTGTC  ACTGGTCAAT  CCCACTTCAA  GAGCTTTGAC
1201  AACAGATACT  TCACCTTCAG  TGGGATCTGC  CAGTACCTGC  TGGCCCGGGA
1251  TTGCCAGGAC  CACTCCTTCT  CCATTGTCAT  TGAGACTGTC  CAGTGTGCTG
1301  ATGACCGCGA  CGCTGTGTGC  ACCCGCTCCG  TCACCGTCCG  GCTGCCCTGG

```

1351	CTGCACAACA	GCCTTGTGAA	ACTGAAGCAT	GGGGCAGGAG	TTGCCATGGA
1401	TGGCCAGGAC	ATCCAGCTCC	CCCTCCTGAA	AGGTGACCTC	CGCATCCAGC
1451	ATACAGTGAC	GGCCTCCGTG	CGCCTCAGCT	ACGGGGAGGA	CCTGCAGATG
1501	GACTGGGATG	GCCGCGGGAG	GCTGCTGGTG	AAGCTGTCCC	CCGTCTATGC
1551	CGGGAAGACC	TGCGGCCCTG	GTGGGAATTA	CAATGGCAAC	CAGGGCGACG
1601	ACTTCCTTAC	CCCCCTCTGG	CTGGCGGAGC	CCCGGGTGGG	GGACTTCGGG
1651	AACGCCTGGA	AGCTGCACGG	GGACTGCCAG	GACCTGCAGA	AGCAGCACAG
1701	CGATCCCTGC	GCCCTCAACC	CGCGCATGAC	CAGGTTCTCC	GAGGAGGCGT
1751	GCGCGGTCC	GACGTCCCCC	ACATTTCGAG	CCTGCCATCG	TGCCGTCAGC
1801	CCGCTGCCCT	ACCTGCGGAA	CTGCCGCTAC	GACGTGTGCT	CCTGCTCGGA
1851	CGGCCGCGAG	TGCCTGTGCG	GCGCCCTGGC	CAGCTATGCC	GCGGCCTGCG
1901	CGGGGAGAGG	CGTGCGCGTC	GCGTGGCGCG	AGCCAGGCCG	CTGTGAGCTG
1951	AACTGCCCCA	AAGGCCAGGT	GTACCTGCAG	TGCGGGACCC	CCTGCAACCT
2001	GACCTGCCGC	TCTCTCTCTT	ACCCGGATGA	GGAATGCAAT	GAGGCCCTGCC
2051	TGGAGGGCTG	CTTCTGCCCC	CCAGGGCTCT	ACATGGATGA	GAGGGGGGAC
2101	TGCGTGCCCA	AGGCCCAGTG	CCCCGTGTAC	TATGACGGTG	AGATCTTCCA
2151	GCCAGAAGAC	ATCTTCTCAG	ACCATCACAC	CATGTGCTAC	TGTGAGGATG
2201	GCTTCATGCA	CTGTACCATG	AGTGGAGTCC	CCGGAAGCTT	GCTGCCTGAC
2251	GCTGTCTCTA	GCAGTCCCCC	GTCTCATCGC	AGCAAAAGGA	GCCTATCCTG
2301	TCGGCCCCCC	ATGGTCAAGC	TGGTGTGTCC	CGCTGACAAC	CTGCGGGCTG
2351	AAGGGCTCGA	GTGTACCAA	ACGTGCCAGA	ACTATGACCT	GGAGTGCATG
2401	AGCATGGGCT	GTGTCTCTGG	CTGCCTCTGC	CCCCCGGGCA	TGGTCCGGCA
2451	TGAGAACAGA	TGTGTGGCCC	TGAAAAGGTG	TCCCTGCTTC	CATCAGGGCA
2501	AGGAGTATGC	CCCTGGAGAA	ACAGTGAAGA	TTGGCTGCAA	CACTTGTGTC
2551	TGTCGGGACC	GGAAGTGGAA	CTGCACAGAC	CATGTGTGTG	ATGCCACGTG
2601	CTCCACGATC	GGCATGGCCC	ACTACCTCAC	CTTCGACGGG	CTCAAATACC
2651	TGTTCCCCCG	GGAGTGCCAG	TACGTTCTGG	TGCAGGATTA	CTGCGGCAGT
2701	AACCCTGGGA	CCTTTCGGAT	CCTAGTGGGG	AATAAGGGAT	GCAGCCACCC
2751	CTCAGTGA	TGCAAGAAAC	GGGTACCAT	CCTGGTGGAG	GGAGGAGAGA
2801	TTGAGCTGTT	TGACGGGGAG	GTGAATGTGA	AGAGGCCCAT	GAAGGATGAG
2851	ACTCACTTTG	AGGTGGTGGA	GTCTGGCCCG	TACATCATTC	TGCTGTCTGG
2901	CAAAGCCCTC	TCCGTGGTCT	GGGACGCCCA	CCTGAGCATC	TCCGTGGTCC
2951	TGAAGCAGAC	ATACCAGGAG	AAAGTGTGTG	GCCTGTGTGG	GAATTTTGAT
3001	GGCATCCAGA	ACAATGACCT	CACCAGCAGC	AACCTCCAAG	TGGAGGAAGA
3051	CCCTGTGGAC	TTTGGGAACT	CCTGGAAAAGT	GAGCTCGCAG	TGTGCTGACA
3101	CCAGAAAAGT	GCCTCTGGAC	TCATCCCCCTG	CCACCTGCCA	TAACAACATC
3151	ATGAAGCAGA	CGATGGTGGA	TTCTCTCCTGT	AGAATCCTTA	CCAGTGACGT
3201	CTTCCAGGAC	TGCAACAAGC	TGGTGGACCC	CGAGCCATAT	CTGGATGTCT
3251	GCATTTACGA	CACCTGCTCC	TGTGAGTCCA	TTGGGGACTG	CGCCGCATTC
3301	TGCGACACCA	TTGCTGCCTA	TGCCCACGTG	TGTGCCCAGC	ATGGCAAGGT
3351	GGTGACCTGG	AGGACGGCCA	CATTGTGCCC	CCAGAGCTGC	GAGGAGAGGA
3401	ATCTCCGGGA	GAACGGGTAT	GAGGCTGAGT	GGCGCTATAA	CAGCTGTGCA
3451	CCTGCCTGTC	AAGTCACGTG	TCAGCACCCCT	GAGCCACTGG	CCTGCCCTGT
3501	GCAGTGTGTG	GAGGGCTGCC	ATGCCCCACTG	CCCTCCAGGG	AAAATCCTGG
3551	ATGAGCTTTT	GCAGACCTGC	GTTGACCCTG	AAGACTGTCC	AGTGTGTGAG
3601	GTGGCTGGCC	GGCGTTTTGC	CTCAGGAAAG	AAAGTCACCT	TGAATCCAG
3651	TGACCCTGAG	CACTGCCAGA	TTTGCCACTG	TGATGTTGTC	AACCTCACCT
3701	GTGAAGCCTG	CCAGGAGCCG	GGCGCGCCAA	CATCAGAGAG	CGCCACCCCT
3751	GAAAGTGGTC	CCGGGAGCGA	GCCAGCCACA	TCTGGGTCGG	AAACGCCAGG
3801	CACAAGTGAG	TCTGCAACTC	CCGAGTCCGG	ACCTGGCTCC	GAGCCTGCCA
3851	CTAGCGGCTC	CGAGACTCCG	GGAACCTCCG	AGAGCGCTAC	ACCAGAAAGC
3901	GGACCCGGAA	CCAGTACCGA	ACCTAGCGAG	GGCTCTGCTC	CGGGCAGCCC
3951	AGCCGGCTCT	CCTACATCCA	CGGAGGAGGG	CACTTCCGAA	TCCGCCACCC
4001	CGGAGTCAGG	GCCAGGATCT	GAACCCGCTA	CCTCAGGCAG	TGAGACGCCA
4051	GGAACGAGCG	AGTCCGCTAC	ACCGGAGAGT	GGGCCAGGGA	GCCCTGCTGG
4101	ATCTCCTACG	TCCACTGAGG	AAGGGTCACC	AGCGGGCTCG	CCCACCAGCA
4151	CTGAAGAAGG	TGCCTCGAGC	GGCGGTGGAG	GATCCGGTGG	CGGGGGATCC
4201	GGTGGCGGGG	GATCCGGTGG	CGGGGGATCC	GGTGGCGGGG	GATCCGGTGG
4251	CGGGGGATCC	ATTGAACCAA	GAAGCTTCTC	TGGCAGCGGA	GGCGACAAAA

4301	CTCACACATG	CCCACCGTGC	CCAGCTCCAG	AACTCCTGGG	CGGACCGTCA
4351	GTCTTCCTCT	TCCCCCAAAA	ACCCAAGGAC	ACCCTCATGA	TCTCCCGGAC
4401	CCCTGAGGTC	ACATGCGTGG	TGGTGGACGT	GAGCCACGAA	GACCCTGAGG
4451	TCAAGTTCAA	CTGGTACGTG	GACGGCGTGG	AGGTGCATAA	TGCCAAGACA
4501	AAGCCGCGGG	AGGAGCAGTA	CAACAGCACG	TACCGTGTGG	TCAGCGTCCT
4551	CACCGTCCTG	CACCAGGACT	GGCTGAATGG	CAAGGAGTAC	AAGTGAAGG
4601	TCTCCAACAA	AGCCCTCCCA	GCCCCCATCG	AGAAAACCAT	CTCCAAAGCC
4651	AAAGGGCAGC	CCCAGAAACC	ACAGGTGTAC	ACCCTGCCCC	CATCCCGGGA
4701	TGAGCTGACC	AAGAACCAGG	TCAGCCTGAC	CTGCCTGGTC	AAAGGCTTCT
4751	ATCCCAGCGA	CATCGCCGTG	GAGTGGGAGA	GCAATGGGCA	GCCGGAGAAC
4801	AACTACAAGA	CCACGCCTCC	CGTGTGGGAC	TCCGACGGCT	CCTTCTTCCT
4851	CTACAGCAAG	CTCACCCTGG	ACAAGAGCAG	GTGGCAGCAG	GGGAACGTCT
4901	TCTCATGCTC	CGTGATGCAT	GAGGCTCTGC	ACAACCACTA	CACGCAGAAG
4951	AGCCTCTCCC	TGTCTCCGGG	TAAATGA		

pSYN VWF073 protein sequence- (VWFD1D2D'D3- 144 AE XTEN- truncated a2 thrombin site-Fc) (SEQ ID NO:175)

1	MIPARFAGVL	LALALILPGT	LCAEGTRGRS	STARCSLFGS	DFVNTFDGSM
51	YSFAGYCSYL	LAGGCQKRSE	SIIGDFQNGK	RVSLSVYLGE	FFDIHLFVNG
101	TVTQGDQRV	MPYASKGLYL	ETEAGYYKLS	GEAYGFVARI	DGSGNFQVLL
151	SDRYFNKTCG	LCGNFNIFAE	DDFMTQEGTL	TSDPYDFANS	WALSSGEQWC
201	ERASPPSSSC	NISSGEMQKG	LWEQCQLLKS	TSVFARCHPL	VDPEPFVALC
251	EKTLCECAGG	LECACPALLE	YARTCAQEGM	VLYGWTDHSA	CSPVCPAGME
301	YRQCVSPCAR	TCQSLHINEM	CQERCVDGCS	CPEGQLLDEG	LCVESTPCPC
351	VHSGKRYPPG	TSLSRDCNTC	ICRNSQWICS	NEECPEGCLV	TGQSHFKSFD
401	NRYFTFSGIC	QYLLARDCQD	HSFSIVIETV	QCADDRDAVC	TRSVTVRLPG
451	LHNSLVKLKH	GAGVAMDGQD	IQLPLLKGD	RIQHTVTASV	RLSYGEDLQM
501	DWDGRGRLLV	KLSPVYAGKT	CGLCGNYNGN	QGDDFLTPSG	LAEPREDFG
551	NAWKLHGDCQ	DLQKQHS DPC	ALNPRMTRFS	EEACAVLTSP	TFEACHRAVS
601	PLPYLRNCRY	DVCSCSDGRE	CLCGALASYA	AACAGRGVRV	AWREPGRCCL
651	NCPKGQVYLQ	CGTPCNLTCT	SLSYPDEECN	EACLEGCFCP	PGLYMDERGD
701	CVPKACQPCY	YDGEIFQPED	IFSDHHTMCY	CEDGFMHCTM	SGVPGSLLPD
751	AVLSSPLSHR	SKRSLSCRPP	MVKLVCPADN	LRAEGLECTK	TCQNYDLECM
801	SMGCVSGCLC	PPGMVRHENR	CVALERCPCF	HQKEYAPGE	TVKIGCNTCV
851	CRDRKWNCTD	HVC DATCSTI	GMAHYLTFDG	LKYLFPGECQ	YVLVQDYCGS
901	NPGTFRILVG	NKGCSHPSVK	CKKRVTILVE	GGEIELFDGE	VNVKRPMKDE
951	THFEVVESEGR	YIILLGKAL	SVVWDRHLSI	SVVLKQTYQE	KVCGLCGNFD
1001	GIQNNDLTSS	NLQVEEDPVD	FGNSWKVSSQ	CADTRKVPLD	SSPATCHNNI
1051	MKQTMVDSSC	RILTSDVFQD	CNKLVDPPEY	LDVCIYDTCS	CESIGDCAAF
1101	CDTIAAYAHV	CAQHGVVVTW	RTATLCPQSC	EERNLRENGY	EAEWRYNSCA
1151	PACQVTCQHP	EPLACPVQCV	EGCHAHCPPG	KILDELLQTC	VPEDPCPVCE
1201	VAGRRFASGK	KVTLNPSDPE	HCQICHCDVV	NLTCEACQEP	GAPTSESATP
1251	ESGPGSEPAT	SGSETPGTSE	SATPESGPGS	EPATSGSETP	GTSESATPES
1301	GPGTSTEPSE	GSAPGSPAGS	PTSTEEGTSE	SATPESGPGS	EPATSGSETP
1351	GTSESATPES	GPGSPAGSPT	STEEGSPAGS	PTSTEEGASS	GGGSGGGGGS
1401	GGGSGGGGGS	GGGSGGGGGS	IEPRSFSGSG	GDKTHTCPPC	PAPELLGGPS
1451	VFLFPKPKD	TLMISRTPEV	TCVVVDVSHE	DPEVKFNWYV	DGVEVHNAKT
1501	KPREEQYNST	YRVVSVLTVL	HQDWLNGKEY	KCKVSNKALP	APIEKTISKA
1551	KGQPREPVY	TLPPSRDEL	KNQVSLTCLV	KGFYPSDIAV	EWESNGQPEN
1601	NYKTTPVLD	SDGSFFLYSK	LTVDKSRWQQ	GNVFSCSVMH	EALHNHYTQK
1651	SLSLSPGK*				

- [0365] 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.
- [0366] 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. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.
- [0367] All patents and publications cited herein are incorporated by reference herein in their entirety.

WHAT IS CLAIMED IS:

1. A chimeric protein comprising (i) a first polypeptide which comprises a Factor VIII ("FVIII") protein fused to a first immunoglobulin ("Ig") constant region or a portion thereof and (ii) a second polypeptide which comprises a von Willebrand Factor ("VWF") protein comprising a D' domain and a D3 domain of VWF fused to a second Ig constant region or a portion thereof by an XTEN sequence in-between, wherein the XTEN sequence contains less than 288 amino acid residues and wherein the first polypeptide is linked to or associated with the second polypeptide.
2. The chimeric protein of claim 1, wherein the XTEN sequence in the second polypeptide consists of an amino acid sequence having a length of between 12 amino acids and 287 amino acids.
3. The chimeric protein of claim 1 or 2, wherein the chimeric protein exhibits a longer half-life compared to a corresponding fusion protein comprising the first polypeptide and the second polypeptide wherein the second polypeptide of the fusion protein comprises an XTEN sequence containing at least 288 amino acids.
4. The chimeric protein of claim 3, wherein the XTEN sequence containing at least 288 amino acids is AE288.
5. The chimeric protein of claim 4, wherein AE288 is SEQ ID NO: 8.
6. The chimeric protein of any one of claims 1 to 5, wherein the XTEN sequence of the second polypeptide contains about 36, about 42, about 72, or about 144 amino acids.
7. The chimeric protein of claim 6, wherein the XTEN sequence of the second polypeptide is selected from AE42, AE72, AE144, AG42, AG72, or AG144.
8. The chimeric protein of claim 7, wherein the XTEN sequence of the second polypeptide is selected from SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; SEQ ID NO: 63.
9. The chimeric protein of any one of claims 1 to 8, wherein the first polypeptide further comprises a second XTEN sequence which links the FVIII protein with the first Ig constant region or a portion thereof.
10. The chimeric protein of claim 9, wherein the first polypeptide comprises a third XTEN sequence which is inserted at one or more insertion sites within the FVIII protein.

11. The chimeric protein of any one of claims 1 to 8, wherein the first polypeptide further comprises a second XTEN sequence which is inserted at one or more insertion sites within the FVIII protein.
12. The chimeric protein of claim 11, wherein the first polypeptide comprises a third XTEN sequence which links the FVIII protein with the first Ig constant region or a portion thereof.
13. The chimeric protein of any one of claims 9 to 12, wherein the second XTEN sequence, the third XTEN sequence, or the second and third XTEN sequences are each independently selected from AE42, AE72, AE864, AE576, AE288, AE144, AG864, AG576, AG288, and AG144.
14. The chimeric protein of claim 13, wherein the second XTEN sequence, the third XTEN sequence, or the second and third XTEN sequences are each independently selected from SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 17; SEQ ID NO: 54; SEQ ID NO: 19; SEQ ID NO: 16; SEQ ID NO: 18; SEQ ID NO: 15; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63.
15. The chimeric protein of claim 13 or 14, wherein the second XTEN sequence, the third XTEN sequence, or both the second and third XTEN sequences are each independently AE288 or AG288.
16. The chimeric protein of any one of claims 1 to 15, wherein the XTEN sequence in the second polypeptide is fused to the second Ig constant region or a portion thereof by a linker.
17. The chimeric protein of claim 16, wherein the linker is a cleavable linker.
18. The chimeric protein of claim 17, wherein the linker is cleavable by a protease selected from factor XIa, factor XIIa, kallikrein, factor VIIa, factor IXa, factor Xa, factor IIa (thrombin), Elastase-2, Granzyme-B, TEV, Enterokinase, Protease 3C, Sortase A, MMP-12, MMP-13, MMP-17, and MMP-20.
19. The chimeric protein of claim 18, wherein the linker is cleavable by factor IIa (thrombin).
20. The chimeric protein of claim 17, wherein the linker comprises one or more cleavage sites comprising an amino acid sequence selected from RRRR (SEQ ID NO: 102), RKRRKR (SEQ ID NO: 103), RRRRS (SEQ ID NO: 104), TQSFNDFTR (SEQ ID NO: 2), SVSQTSLKTR (SEQ ID NO: 3), DFLAEGGGVR (SEQ ID NO: 4), TTKIKPR (SEQ ID NO: 5), LVPRG (SEQ ID NO: 6), ALRPR (SEQ ID NO: 7), KLTRAET (SEQ ID NO: 121), DFTRVVG (SEQ ID NO: 122), TMTRIVGG (SEQ ID NO: 123), SPFRSTGG (SEQ ID NO: 124), LQVRIVGG (SEQ ID NO: 125), PLGRIVGG (SEQ ID NO: 126), IEGRTVGG (SEQ ID NO: 127), LTPRSLLV (SEQ ID NO: 128), LGPVSGVP (SEQ ID NO: 129), VAGDSLEE (SEQ ID NO: 130), GPAGLGGA (SEQ ID NO: 131), GPAGLRGA (SEQ ID NO: 132), and GPAGLRGA (SEQ ID NO: 133).

NO: 132), APLGLRLR (SEQ ID NO: 133), PALPLVAQ (SEQ ID NO: 134), ENLYFQG (SEQ ID NO: 135), DDDKIVGG (SEQ ID NO: 136), LEVLFQGP (SEQ ID NO: 137), and LPKTGSES (SEQ ID NO: 138).

21. The chimeric protein of claim 17, wherein the linker comprises TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 146).

22. The chimeric protein of claim 20, wherein the cleavage sites comprise an amino acid sequence of LVPRG (SEQ ID NO: 6).

23. The chimeric protein of any one of claims 1 to 22, wherein the first Ig constant region or a portion thereof comprises a first Fc region and/or the second Ig constant region or a portion thereof comprises a second Fc region.

24. The chimeric protein of claim 23, wherein the first Ig constant region or a portion thereof and the second Ig constant region or a portion thereof extend the half-life of the chimeric protein.

25. The chimeric protein of any one of claims 1 to 24, wherein the first polypeptide and the second polypeptide is fused by a linker.

26. The chimeric protein of claim 25, wherein the first polypeptide and the second polypeptide is fused by a processable linker.

27. The chimeric protein of any one of claims 1 to 24, wherein the first Ig constant region or a portion thereof is associated with the second Ig constant region or a portion thereof.

28. The chimeric protein of claim 27, wherein the first Ig constant region or a portion thereof is associated with the second Ig constant region or a portion thereof by a covalent bond.

29. The chimeric protein of claim 28, wherein the covalent bond is a disulfide bond.

30. A chimeric protein comprising each of the following formulae (a)-(hh):

(a) FVIII-F1:F2-L2-X-L1-V;

(b) FVIII-F1:V-L1-X-L2-F2;

(c) F1-FVIII:F2-L2-X-L1-V;

(d) F1-FVIII:V-L1-X-L2-F2;

- (e) FVIII-X2-F1:F2-L2-X1-L1-V;
- (f) FVIII-X2-F1:V-L1-X1-L2-F2;
- (g) FVIII(X2)-F1:F2-L2-X1-L1-V;
- (h) FVIII(X2)-F1:V-L1-X1-L2-F2;
- (i) F1-X2-F1:F2-L2-X1-L1-V;
- (j) F1-X2-F1:V-L1-X1-L2-F2;
- (k) V-L1-X-L2-F2-L3-FVIII-L4-F1;
- (l) V-L1-X-L2-F2-L3-F1-L4-FVIII;
- (m) F1-L4-FVIII-L3-F2-L2-X-L1-V;
- (n) FVIII-L4-F1-L3-F2-L2-X-L1-V;
- (o) FVIII-L4-F1-L3-V-L1-X-L2-F2;
- (p) FVIII-L4-F1-L3-F2-L2-X-L1-V;
- (q) F2-L2-X-L1-V-L3-F1-L4-FVIII;
- (r) F2-L2-X-L1-V-L3-FVIII-L4-F1;
- (s) V-L1-X1-L2-F2-L3-FVIII(X2)-L4-F1;
- (t) V-L1-X1-L2-F2-L3-F1-L4-FVIII(X2);
- (u) F1-L4-FVIII(X2)-L3-F2-L2-X1-L1-V;
- (v) F-L4-FVIII(X2)-L3-V-L1-X1-L2-F2;
- (w) FVIII(X2)-L4-F1-L3-V-L1-X1-L2-F2;
- (x) FVIII(X2)-L4-F1-L3-F2-L2-X1-L1-V;
- (y) F2-L2-X1-L1-V-L3-F1-L4-FVIII(X2);
- (z) F2-L2-X1-L1-V-L3-FVIII(X2)-L4-F1;

- (aa) V-L1-X2-L2-F2-L3-FVIII-L4-X2-L5-F1;
- (bb) V-L1-X2-L2-F2-L3-F1-L5-X2-L4-FVIII;
- (cc) F1-L5-X2-L4-FVIII-L3-F2-L2-X2-L1-V;
- (dd) F1-L5-X2-L4-FVIII-L3-V-L1-X2-L2-F2;
- (ee) FVIII-L5-X2-L4-F2-L3-V-L1-X1-L2-F1;
- (ff) FVIII-L5-X2-L4-F2-L3-F1-L2-X1-L1-V;
- (gg) F1-L2-X1-L1-V-L3-F2-L4-X2-L5-FVIII; or
- (hh) F1-L2-X1-L1-V-L3-FVIII-L5-X2-L4-F2;

wherein V is a VWF protein, which comprises a D' domain and a D3 domain,

X or X1 is a first XTEN sequence that contains less than 288 amino acids,

X2 is a second XTEN sequence,

FVIII comprises a FVIII protein,

FVIII(X2) comprises a FVIII protein having a second XTEN sequence inserted in one or more insertion sites within the FVIII protein,

F1 is a first Ig constant region or a portion thereof,

F2 is a second Ig constant region or a portion thereof,

L1, L2, L3, L4, or L5 is an optional linker,

(-) is a peptide bond; and

(:) is a covalent bond or a non-covalent bond.

31. The chimeric protein of claim 30, wherein the X or X1 consists of an amino acid sequence in length between 12 amino acids and 287 amino acids.

32. The chimeric protein of claim 30 or 31, which exhibits a longer half-life compared to a corresponding chimeric protein comprising the formula except that the X or X1 is AE288.

33. The chimeric protein of claim 32, wherein AE288 is SEQ ID NO: 8.
34. The chimeric protein of any one of claims 30 to 33 wherein the X or X1 in the formula contains about 36, about 42, about 72, or about 144 amino acids.
35. The chimeric protein of claim 34, wherein the X or X1 in the formula is selected from AE42, AE72, AE144, AG42, AG72, or AG144.
36. The chimeric protein of claim 34, wherein the X or X1 in the formula is selected from SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63.
37. The chimeric protein of any one of claims 30 to 36, wherein the X2 comprises an amino acid sequence having a length of at least about 36 amino acids, at least about 42 amino acids, at least about 144 amino acids, at least about 288 amino acids, at least about 576 amino acids, or at least about 864 amino acids.
38. The chimeric protein of claim 37, wherein the X2 is selected from AE42, AE72, AE864, AE576, AE288, AE144, AG864, AG576, AG288, and AG144.
39. The chimeric protein of claim 37, wherein the X2 is selected from SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 17; SEQ ID NO: 54; SEQ ID NO: 19; SEQ ID NO: 16; SEQ ID NO: 18; SEQ ID NO: 15; SEQ ID NO: 55; SEQ ID NO: 56; SEQ ID NO: 57; SEQ ID NO: 58; SEQ ID NO: 59; SEQ ID NO: 14; SEQ ID NO: 60; SEQ ID NO: 61; SEQ ID NO: 62; or SEQ ID NO: 63.
40. The chimeric protein of claim 37, wherein the X2 is AE288 or AG288.
41. The chimeric protein of any one of claims 30 to 40, wherein the chimeric protein comprising X or X1 and/or X2 exhibits a longer half-life compared to the chimeric protein not comprising X or X1 and/or X2.
42. The chimeric protein of any one of claims 30 to 41, wherein the L1 and/or L2 is a cleavable linker.
43. The chimeric protein of any one of claims 30 to 41, wherein the L4 and/or L5 is a cleavable linker.
44. The chimeric protein of claim 42 or 43, wherein the linker is cleavable by a protease selected from factor XIa, factor XIIa, kallikrein, factor VIIa, factor IXa, factor Xa, factor IIa (thrombin), Elastase-2, Granzyme-B, TEV, Enterokinase, Protease 3C, Sortase A, MMP-12, MMP-13, MMP-17, and MMP-20.

45. The chimeric protein of claim 42 or 43, wherein the linker is cleavable by factor IIa (thrombin).
46. The chimeric protein of claim 42 or 43, wherein the linker comprises one or more cleavage sites comprising an amino acid sequence selected from RRRR (SEQ ID NO: 102), RKRRKR (SEQ ID NO: 103), RRRRS (SEQ ID NO: 104), TQSFNDFTR (SEQ ID NO: 1), SVSQTSLKTR (SEQ ID NO: 3), DFLAEGGGVR (SEQ ID NO: 4), TTKIKPR (SEQ ID NO: 5), LVPRG (SEQ ID NO: 6), ALRPR (SEQ ID NO: 7), KLTRAET (SEQ ID NO: 121), DFTRVVG (SEQ ID NO: 122), TMTRIVGG (SEQ ID NO: 123), SPFRSTGG (SEQ ID NO: 124), LQVRIVGG (SEQ ID NO: 125), PLGRIVGG (SEQ ID NO: 126), IEGRTVGG (SEQ ID NO: 127), LTPRSLLV (SEQ ID NO: 128), LGPVSGVP (SEQ ID NO: 129), VAGDSLEE (SEQ ID NO: 130), GPAGLGGA (SEQ ID NO: 131), GPAGLRGA (SEQ ID NO: 132), APLGLRLR (SEQ ID NO: 133), PALPLVAQ (SEQ ID NO: 134), ENLYFQG (SEQ ID NO: 135), DDDKIVGG (SEQ ID NO: 136), LEVLFGQP (SEQ ID NO: 137), and LPKTGSES (SEQ ID NO: 138).
47. The chimeric protein of claim 42 or 43, wherein the linker comprises TLDPRSFLLRNPNDKYEPFWEDEEK (SEQ ID NO: 146).
48. The chimeric protein of claim 42 or 43, wherein the linker comprises an amino acid sequence of LVPRG (SEQ ID NO: 6).
49. The chimeric protein of claim 42 or 43, wherein the linker comprises an a1 region of FVIII, an a2 region of FVIII, an a3 region of FVIII, or any combination thereof.
50. The chimeric protein of claim 49, wherein the a2 region of FVIII comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to ISDKNTGDYYEDSYEDISAYLLSKNNAIEPRSF (SEQ ID NO: 106).
51. The chimeric protein of claim 49, wherein the a1 region comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to ISMKNNEEAEDYDDDLTDSEMDVVRFDNNSPSFIQIRSV (SEQ ID NO: 107).
52. The chimeric protein of claim 49, wherein the a3 region comprises an amino acid sequence at least about 80%, about 85%, about 90%, about 95%, or 100% identical to ISEITRTTLQSDQEEIDYDDTISVEMKKEDFDIYDEDENQSPRSFQ (SEQ ID NO: 108).
53. The chimeric protein of any one of claims 30 to 52, wherein the F1 comprises a first Fc region and/or the F2 comprises a second Fc region.

54. The chimeric protein of any one of claims 30 to 53, wherein the chimeric protein comprising the F1 and the F2 exhibits a longer half-life compared to the chimeric protein not comprising the F1 and the F2.
55. The chimeric protein of any one of claims 30 to 54, wherein the L3 is a processable linker.
56. The chimeric protein of any one of claims 1 to 55, wherein the VWF protein is associated with the FVIII protein by a non-covalent bond.
57. The chimeric protein of any one of claims 1 to 56, wherein the half-life of the chimeric protein is extended compared to a FVIII protein without the VWF protein and/or the XTEN sequence or compared to wild type FVIII.
58. The chimeric protein of claim 57, wherein the half-life of the chimeric protein is extended 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 a FVIII protein without the VWF protein or the XTEN sequence or than wild type FVIII.
59. The chimeric protein of claim 57, wherein the half-life of the chimeric protein is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours.
60. The chimeric protein of claim 57, wherein the half-life of the chimeric protein is about 40 hours in Hema mice.
61. The chimeric protein of any one of claims 1 to 60, wherein the VWF protein does not bind substantially to a VWF clearance receptor.
62. The chimeric protein of any one of claims 1 to 61, wherein the VWF protein is capable of protecting the FVIII protein from one or more protease cleavages, protecting the FVIII protein from activation, stabilizing the heavy chain and/or the light chain of the FVIII protein, or preventing clearance of the FVIII protein by one or more scavenger receptors.

63. The chimeric protein of any one of claims 1 to 62, wherein the VWF protein inhibits or prevents endogenous VWF from binding to the FVIII protein by shielding or blocking a VWF binding site on the FVIII protein.
64. The chimeric protein of claim 63, wherein the VWF binding site is located in the A3 domain or the C2 domain of the FVIII protein or both the A3 domain and the C2 domain.
65. The chimeric protein of claim 63, wherein the VWF binding site comprises the amino acid sequence corresponding to amino acids 1669 to 1689 and 2303 to 2332 of SEQ ID NO: 65.
66. The chimeric protein of any one of claims 1 to 65, wherein the first Ig constant region or a portion thereof and the second Ig constant region or a portion thereof are identical or different.
67. The chimeric protein of any one of claims 1 to 66, wherein the FVIII protein is linked to and/or inserted with at least two XTEN sequences, at least three XTEN sequences, at least four XTEN sequences, at least five XTEN sequences, or at least six XTEN sequences.
68. The chimeric protein of any one of claims 1 to 67, wherein the FVIII protein comprises one or more domains of FVIII selected from an A1 domain, a1 acidic region, an A2 domain, a2 acidic region, a B domain, an A3 domain, a3 acidic region, a C1 domain, a C2 domain, one or more fragments thereof, and any combinations thereof.
69. The chimeric protein of any one of claims 10 to 68, wherein the one or more insertion sites in the FVIII protein is located within one or more domains of the FVIII protein selected from the A1 domain, the a1 acidic region, the A2 domain, the a2 acidic region, the A3 domain, the B domain, the C1 domain, the C2 domain, and any combinations thereof or between one or more domains of the FVIII protein selected from the group consisting of the A1 domain and a1 acidic region, the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, the A3 domain and C1 domain, the C1 domain and C2 domain, and any combinations thereof or between two domains of the FVIII protein selected from the A1 domain and a1 acidic region, the a1 acidic region and A2 domain, the A2 domain and a2 acidic region, the a2 acidic region and B domain, the B domain and A3 domain, the A3 domain and C1 domain, the C1 domain and C2 domain, and any combinations thereof.
70. The chimeric protein of any one of claims 10 to 68, wherein the one or more insertion sites in the FVIII protein are one or more amino acids selected from the group consisting of the amino acid residues in Table 7, Table 8, Table 9 and Table 10.

71. The chimeric protein of claim 70, wherein the insertion sites in the FVIII protein are located immediately downstream of amino acid 745 corresponding to the mature FVIII protein (SEQ ID NO: 65).
72. The chimeric protein of any one of claims 10 to 68, wherein the insertion sites in the FVIII protein are located immediately downstream of residue 1656 and residue 1900 corresponding to the mature FVIII protein (SEQ ID NO: 65).
73. The chimeric protein of any one of claims 10 to 68, wherein the insertion sites in the FVIII protein are immediately downstream of residues 26, 1656, and 1900 corresponding to the mature FVIII protein (SEQ ID NO: 65).
74. The chimeric protein of any one of claims 10 to 68, wherein the insertion sites in the FVIII protein are immediately downstream of residues 403 and 745 corresponding to the mature FVIII protein (SEQ ID NO: 65).
75. The chimeric protein of any one of claims 10 to 68, wherein the insertion sites in the FVIII protein are immediately downstream of residues 745 and 1900 corresponding to the mature FVIII protein (SEQ ID NO: 65).
76. The chimeric protein of any one of claims 10 to 68, wherein the insertion sites in the FVIII protein are immediately downstream of residues 18 and 745 corresponding to the mature FVIII protein (SEQ ID NO: 65).
77. The chimeric protein of any one of claims 1 to 76, wherein the FVIII protein is a dual chain FVIII isoform.
78. The chimeric protein of any one of claims 1 to 76, wherein the FVIII protein is a single chain FVIII isoform.
79. The chimeric protein of any one of claims 1 to 78, wherein the FVIII protein comprises B domain or a portion thereof.
80. The chimeric protein of claim 79, wherein the FVIII protein is SQ B domain deleted FVIII.
81. The chimeric protein of claim 78 or 79, wherein the single chain FVIII isoform contains at least one amino acid substitution at a residue corresponding to residue 1648, residue 1645, or both residues corresponding to the full-length mature Factor VIII polypeptide (SEQ ID NO: 65) or residue 754, residue 751, or both residues of SQ BDD Factor VIII (SEQ ID NO: 67).

82. The chimeric protein of claim 81, wherein the amino acid substitution is an amino acid other than arginine.
83. The chimeric protein of claim 77, wherein the dual chain FVIII isoform comprises a first chain comprising a heavy chain of FVIII and a second chain comprising a light chain of FVIII, wherein the heavy chain and the light chain are associated with each other by a metal bond.
84. The chimeric protein of any one of claims 1 to 83, wherein the D' domain comprises an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 866 of SEQ ID NO: 21.
85. The chimeric protein of any one of claims 1 to 84, wherein the D3 domain comprises an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 867 to 1240 of SEQ ID NO: 21.
86. The chimeric protein of any one of claims 1 to 85, wherein the VWF protein is a monomer.
87. The chimeric protein of any one of claims 1 to 86, which comprises at least two VWF proteins, at least three VWF proteins, at least four VWF proteins, at least five VWF proteins, or at least six VWF proteins.
88. The chimeric protein of any one of claims 1 to 87, wherein the VWF protein comprises an amino acid sequence at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 764 to 1240 of SEQ ID NO: 21.
89. The chimeric protein of claim 88, wherein the VWF protein consists essentially of or consists of amino acids 764 to 1240 of SEQ ID NO: 21.
90. The chimeric protein of any one of claims 1 to 89, wherein the VWF protein contains at least one amino acid substitution at a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 of SEQ ID NO: 21.
91. The chimeric protein of any one of claims 1 to 89, wherein the VWF protein contains an amino acid other than cysteine substituted for a residue corresponding to residue 1099, residue 1142, or both residues 1099 and 1142 of SEQ ID NO: 21.
92. The chimeric protein of any one of claims 1 to 91, wherein the VWF protein further comprises the D1 domain, the D2 domain, or the D1 and D2 domains of VWF.

93. The chimeric protein of any one of claims 1 to 92, wherein the VWF protein further comprises a VWF domain selected from the A1 domain, the A2 domain, the A3 domain, the D4 domain, the B1 domain, the B2 domain, the B3 domain, the C1 domain, the C2 domain, the CK domain, one or more fragments thereof, and any combinations thereof.
94. The chimeric protein of any one of claims 1 to 92, wherein the VWF protein consists essentially of or consists of: (1) the D' and D3 domains of VWF or fragments thereof; (2) the D1, D', and D3 domains of VWF or fragments thereof; (3) the D2, D', and D3 domains of VWF or fragments thereof; (4) the D1, D2, D', and D3 domains of VWF or fragments thereof; or (5) the D1, D2, D', D3, and A1 domains of VWF or fragments thereof.
95. The chimeric protein of any one of claims 1 to 94, wherein the VWF protein further comprises a signal peptide of VWF or FVIII which is operably linked to the VWF protein.
96. The chimeric protein of any one of claims 16 to 95, wherein one or more of the linkers have a length of at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1200, 1400, 1600, 1800, or 2000 amino acid residues.
97. The chimeric protein of any one of claims 16 to 95, wherein one or more of the linkers have a length of about 1 to about 2000 amino acid residues.
98. The chimeric protein of claim 96 or 97, wherein one or more of the linkers comprise a gly/ser peptide.
99. The chimeric protein of claim 98, wherein the gly/ser peptide has a formula of $(\text{Gly}_4\text{Ser})_n$ (SEQ ID NO: 94) or $\text{S}(\text{Gly}_4\text{Ser})_n$ (SEQ ID NO: 164), wherein n is a positive integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.
100. The chimeric protein of claim 99, wherein the $(\text{Gly}_4\text{Ser})_n$ linker is $(\text{Gly}_4\text{Ser})_3$ (SEQ ID NO: 100) or $(\text{Gly}_4\text{Ser})_4$ (SEQ ID NO: 165).
101. The chimeric protein of any one of claims 16 to 100, wherein the linker comprises 20 amino acids, 35 amino acids, 48 amino acids, 73 amino acids, or 95 amino acids.
102. The chimeric protein of any one of claims 17 to 29 and 42 to 101, wherein the cleavable linker is SGGGGSGGGGSGGGGSGGGGSGGGGSLVPRGSGG (SEQ ID NO: 166).

103. The chimeric protein of any one of claims 1 to 102, which is polysialylated, pegylated, or hesylated.

104. The chimeric protein of any one of claims 1 to 20, wherein the first polypeptide comprises at least about 80%, 90%, 95%, 99%, or 100% identical to FVIII161 (SEQ ID NO: 69), FVIII169 (SEQ ID NO: 70), FVIII173 (SEQ ID NO: 72), FVIII195 (SEQ ID NO: 73), FVIII196 (SEQ ID NO: 74), FVIII199 (SEQ ID NO: 75), FVIII201 (SEQ ID NO: 76), FVIII203 (SEQ ID NO: 77), FVIII204 (SEQ ID NO: 78), FVIII205 (SEQ ID NO: 79), FVIII266 (SEQ ID NO: 80), FVIII267 (SEQ ID NO: 81), FVIII268 (SEQ ID NO: 82), FVIII269 (SEQ ID NO: 83), FVIII271 (SEQ ID NO: 84), FVIII272 (SEQ ID NO: 85), or FVIII282 (SEQ ID NO: 159), and the second polypeptide comprises at least about 80%, 90%, 95%, 99%, or 100% identical to VWF057 (SEQ ID NO: 152).

105. The chimeric protein of claim 104, wherein the first polypeptide comprises FVIII169 (SEQ ID NO: 70) and the second polypeptide comprises VWF057 (SEQ ID NO: 152).

106. The chimeric protein of any one of claims 1 to 105, wherein the chimeric protein is efficacious in preventing and/or stopping bleeding from a subject in need thereof.

107. A polynucleotide or a set of polynucleotides encoding the chimeric protein of any one of claims 1 to 106.

108. The polynucleotide of claim 107, further comprising a polynucleotide chain, which encodes PC5 or PC7.

109. A vector comprising the polynucleotide of claim 107 or 108 and one or more promoter operably linked to the polynucleotide or the set of polynucleotides.

110. The vector of claim 109, further comprising an additional vector, which comprises a polynucleotide chain encoding PC5 or PC7.

111. A host cell comprising the polynucleotide of claim 107 or 108 or the vector of claim 109 or 110.

112. The host cell of claim 111, which is a mammalian cell.

113. The host cell of claim 112, wherein the mammalian cell is selected from HEK293 cell, CHO cell, and BHK cell.

114. A pharmaceutical composition comprising the chimeric protein of any one of claims 1 to 106, the polynucleotide of claim 107 or 108, the vector of claim 109 or 110, or the host cell of any one of claims 111 to 113, and a pharmaceutically acceptable carrier.

115. The composition of claim 114, wherein the FVIII protein has extended half-life compared to wild type FVIII protein.

116. The composition of claim 114 or 115, wherein the half-life of the chimeric protein is extended 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 wild type FVIII.

117. The composition of claim 114 or 115 wherein the half-life of the chimeric protein is at least about 17 hours, at least about 18 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about 25 hours, at least about 26 hours, at least about 27 hours, at least about 28 hours, at least about 29 hours, at least about 30 hours, at least about 31 hours, at least about 32 hours, at least about 33 hours, at least about 34 hours, at least about 35 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, or at least about 108 hours.

118. The composition of claim 114 or 115, wherein the half-life of the chimeric protein is about 40 hours in HemA mice.

119. The composition of any one of claims 114 to 118, which is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration.

120. The composition of claim 119, wherein the parenteral administration is intravenous or subcutaneous administration.

121. The composition of any one of claims 114 to 120, which is used to treat a bleeding disease or condition in a subject in need thereof.

122. The composition of claim 121, wherein the bleeding disease or condition is selected from the group consisting of a bleeding coagulation disorder, 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 and any combinations thereof.

123. The composition of claim 121 or 122, wherein the subject is scheduled to undergo a surgery.

124. The composition of any one of claims 114 to 123 wherein the treatment is prophylactic or on-demand.
125. A method of extending or increasing half-life of a chimeric protein, wherein the method comprises adding an effective amount of the chimeric protein of any one of claims 1 to 106, the polynucleotide of claim 107 or 108, the vector of claim 109 or 110, the host cell of any one of claims 111 to 113, or the composition of any one of claims 114 to 124 to a subject in need thereof, wherein the VWF protein, the XTEN sequence, the first Ig constant region or a portion thereof, and the second Ig constant region or a portion thereof increase the half-life of the chimeric protein.
126. A method of treating a bleeding disease or disorder in a subject in need thereof comprising administering an effective amount of the chimeric protein of any one of claims 1 to 106, the polynucleotide of claim 107 or 108, the vector of claim 109 or 110, the host cell of any one of claims 111 to 113, or the composition of any one of claims 114 to 124, wherein the bleeding disease or disorder is selected from the group consisting of a bleeding coagulation disorder, 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, and bleeding in the iliopsoas sheath.
127. The method of claim 125 or 126, wherein the subject is an animal.
128. The method of claim 127, wherein the animal is a human.
129. The method of claim 127 or 128, wherein the subject is suffering from hemophilia A.
130. The method of any one of claims 126 to 129, wherein the treatment is prophylactic or on-demand.
131. The method of any one of claims 126 to 130, wherein the effective amount is 0.1 $\mu\text{g/kg}$ to 500 mg/kg.
132. The method of any one of claims 125 to 131, wherein the chimeric protein of any one of claims 1 to 106, the polynucleotide of claim 107 or 108, the vector of claim 109 or 110, the host cell of any one of claims 111 to 113, or the composition of any one of claims 114 to 124 is administered by a route selected from the group consisting of topical administration, intraocular administration, parenteral administration, intrathecal administration, subdural administration and oral administration.

133. The method of claim 132, wherein the parenteral administration is selected from the group consisting of intravenous administration, subcutaneous administration, intramuscular administration, and intradermal administration,

134. A method of making a chimeric protein, comprising transfecting one or more host cell with the polynucleotide of claim 107 or 108 or the vector of claim 109 or 110 and expressing the chimeric protein in the host cell.

135. The method of claim 134, which further comprises isolating the chimeric protein.

136. The method of any one of claims 124 to 135, wherein the chimeric protein is efficacious in stopping and/or preventing bleeding in the subject.

137. The chimeric protein of claim 19, wherein the linker comprises an $\alpha 2$ region of FVIII or a fragment thereof.

138. The chimeric protein of claim 137, wherein the linker comprises an amino acid sequence at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to DKNTGDYYEDSYEDISAYLLSKNNAIEPRSFS (SEQ ID NO: 88).

139. The chimeric protein of claim 19, wherein the linker comprises the amino acid sequence IEPR (SEQ ID NO: 200) or IEPRSFS (SEQ ID NO: 194).

140. The chimeric protein of claim 138 or 139, wherein the linker is not the full length $\alpha 2$ region of FVIII.

141. The chimeric protein of claim 42 or 43, wherein the linker comprises a fragment of an $\alpha 2$ region of FVIII.

142. The chimeric protein of claim 141, wherein the linker comprises an amino acid sequence selected from SEQ ID NOs: 88, 139, 140, 141, 142, 143, 144, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, and 200.

143. The chimeric protein of claim 142, wherein the linker comprises the amino acid sequence IEPR (SEQ ID NO: 200) or IEPRSFS (SEQ ID NO: 194).

144. The chimeric protein of any one of claims 141 to 143, wherein the linker is not the full length $\alpha 2$ region of FVIII.

145. A chimeric protein comprising:

- (i) a first polypeptide which comprises a Factor VIII ("FVIII") protein fused to a first immunoglobulin ("Ig") constant region or a portion thereof, wherein the FVIII protein comprises a 288-amino acid long XTEN sequence; and
- (ii) a second polypeptide which comprises a von Willebrand Factor ("VWF") protein comprising a D' domain and a D3 domain of VWF fused to a second Ig constant region or a portion thereof by a 144-amino acid XTEN sequence in-between;

wherein the first polypeptide is linked to or associated with the second polypeptide through the first and second Ig constant regions.

146. The chimeric protein of claim 145, further comprising a cleavable linker between the 144-amino acid XTEN sequence and the second Ig constant region.

147. The chimeric protein of claim 146, wherein the cleavable linker is cleavable by thrombin.

148. The chimeric protein of claim 146 or 147, wherein the cleavable linker has an amino acid sequence comprising IEPR (SEQ ID NO: 200) or IEPRSFS (SEQ ID NO: 194).

149. The chimeric protein of claim 146 or 147, wherein the cleavable linker has an amino acid sequence comprising IEPR (SEQ ID NO: 200) or IEPRSFS (SEQ ID NO: 194), and wherein the linker is not the full length $\alpha 2$ region of FVIII.