with FX are based on the therapeutic use of non-activated zymogen because it is considered to be a safer approach. There is a need for improved or alternative FX therapeutics.

SUMMARY

Provided herein are modified or variant Factor X (FX) polypeptides that exhibit altered properties or activities compared to an unmodified FX polypeptide such as increased cofactor (FVa) dependence, increased half-life, increased resistance to inhibitors (e.g. antithrombin III) and/or altered glycosylation. The modified FX polypeptides provided herein can exhibit some or all of the above properties or activities. The modified FX polypeptides can be mature, zymogen, active or activated or catalytically active forms thereof.

In one aspect, there is provided an isolated modified Factor X (FX) polypeptide, comprising an amino acid replacement at a position corresponding to position 196 in an unmodified FX polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein:

corresponding amino acid positions are identified by alignment of the unmodified FX polypeptide with the polypeptide whose set forth in SEQ ID NO:134;

the replacement is with a neutral polar amino acid residue that is serine (S) or threonine (T);

the unmodified FX polypeptide has the sequence of amino acids set forth in SEQ ID NO:134, or is the zymogen, active or catalytically active form thereof, or has a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:134 or the zymogen, active or catalytically active form thereof, respectively;

the active FX (FXa) form of the modified polypeptide exhibits increased co-factor dependence compared to the FXa form of the FX polypeptide not containing the amino acid replacement; and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

In one aspect, there is provided a modified active Factor X (FXa) polypeptide, comprising an amino acid replacement at a position 196 and at position 332 in an

unmodified FXa polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein:

the replacement at position 196 is serine (S), and the replacement at 332 alanine (A), aspartic acid (D), glutamic acid (E), serine (S) or glycine (G);

the modified FXa polypeptide contains only up to seven amino acid replacements;

the unmodified FXa comprises a light chain consisting of the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain consisting of the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134;

the modified FXa exhibits at least 50-fold increased FVa cofactor dependence compared to the unmodified FXa polypeptide not containing the amino acid replacements; and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

In one aspect, there is provided a nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FX polypeptide as described herein.

In one aspect, there is provided a vector, comprising the nucleic acid molecule as described herein.

In one aspect, there is provided an isolated cell or cell culture, comprising the vector as described herein.

In one aspect, there is provided a method of producing a modified FX polypeptide as described herein, comprising culturing a cell of a cell of any of claims 30-33, under conditions whereby the modified FX polypeptide is produced.

In one aspect, there is provided a pharmaceutical composition, comprising a modified FX polypeptide as described herein, a nucleic acid molecule as described herein in a pharmaceutically acceptable vehicle.

In one aspect, there is provided method of generating a modified active Factor X (FXa) polypeptide, comprising:

a) providing a modified FX polypeptide as described herein, wherein the modified FX polypeptide is a single chain zymogen;

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide;

or, comprising:

a) providing a composition comprising a modified FX zymogen selected from among a zymogen comprising:

a light chain having the sequence of amino acids 1-139 set forth in SEQ ID NO:134, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 1-139 of any of SEQ ID NOS:134 and that contains the amino acid replacement(s) selected from S and T at position 196; and

a heavy chain having the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265 and that contains amino acid replacement(s) compared to an unmodified FX polypeptide; and

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide.

In another aspect, there is provided a composition, comprising a modified FX polypeptide as described herein for use for treating a bleeding disorder, wherein:

the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

In another aspect, there is provided a of a modified FX polypeptide as described herein in the preparation of a medicament for treatment of a bleeding disorder, wherein:

the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

Claims:

1. An isolated modified active Factor X (FXa) polypeptide, comprising an amino acid replacement at a position corresponding to position 196 in an unmodified FXa polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein:

corresponding amino acid positions are identified by alignment of the unmodified FX polypeptide with the polypeptide set forth in SEQ ID NO:134;

the replacement is with a neutral polar amino acid residue that is serine (S) or threonine (T);

the unmodified FXa comprises a sequence of amino acids that exhibits at least 75% sequence identity to the FXa comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134, or a catalytically active form thereof;

the modified FXa exhibits at least 50-fold increased FVa cofactor dependence compared to the unmodified FXa polypeptide not containing the amino acid replacement at the position corresponding to position 196; and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

2. The isolated FXa polypeptide of claim 1 or claim 2, wherein the modified FX polypeptide comprises an amino acid replacement with S at a position corresponding to position 196 with reference to amino acid position set forth in SEQ ID NO:134, or the same replacement at a corresponding amino acid residue in the unmodified FX polypeptide that does not comprise S at the position corresponding to 196.

3. The isolated modified FXa polypeptide of claim 1 or claim 2 , comprising a further amino acid replacement(s) in the light chain or in the protease domain of the heavy chain, wherein:

the further amino acid replacement(s) confers altered glycosylation, increased resistance to an inhibitor, increased co-factor dependence, increased half-life and/or increased catalytic activity; and

is at a position corresponding to a position selected from among 211, 214, 216, 218, 219, 273, 276, 306, 326, 332, 338, 420 and 424, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134.

4. The isolated FXa polypeptide of claim 3, wherein:

the replacement confers increased resistance to an inhibitor; and

the inhibitor is a tissue factor pathway inhibitor (TFPI) or is anti-thrombin III (ATIII).

5. The isolated modified FXa polypeptide of claim 7, wherein the inhibitor is anti-thrombin III (ATIII).

6. The isolated modified FXa polypeptide of any of claims 1-5, comprising only one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty amino acid replacement(s) compared to the unmodified FXa polypeptide of the same form.

7. The isolated modified FXa polypeptide of any of claims 3-6, wherein the further amino acid replacement is selected from among amino acid replacement(s) with: S at a position corresponding to position 211; D at a position corresponding to position 214; A at a position corresponding to position 214; S at a position corresponding to position 214; R at a position corresponding to position 216; K at a position corresponding to position 216; A at a position corresponding to position 216; S at a position corresponding to position 216; R at a position corresponding to position 218; K at a position corresponding to position 218; A at a position corresponding to position 218; H at a position corresponding to position 219; A at a position corresponding to position 273; E at a position corresponding to position 273; A at a position corresponding to position 276; E at a position corresponding to position 276; E at a position corresponding to position 306; S at a position corresponding to position 326; T at a position corresponding to position 326; V at a position corresponding to position 326; Q at a position corresponding to position 326; N at a position corresponding to position 326; M at a position corresponding to position 326; K at a position corresponding to position 326; Y at a position corresponding to position 326; E at a position corresponding to position 326; D at a position corresponding to position 326; A at a position corresponding to position 332; D at a position corresponding to position 332; E at a position corresponding to position 332; S at a position corresponding to position 332; G at a position corresponding to position 332; A at a position corresponding to position 338; S at a position corresponding to position 338; N at a position corresponding to position 338; R at a position corresponding to position 338; V at a position corresponding to position 338; Y at a position corresponding to position 338; M at a position corresponding to position 338; A at a position corresponding to position 420; E at a position corresponding to position 420; A at a position corresponding to position 424; and E at a position corresponding to position 424, with reference to amino acid positions set forth in SEQ ID NO:134, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

8. The isolated modified FXa polypeptide of any of claims 1-7, comprising an amino acid replacement(s) selected from among amino acid replacement with A, D, E, S or G at a position corresponding to 332 in the unmodified FXa polypeptide.

9. The isolated modified FXa polypeptide of any of claims 1-8, comprising amino acid replacement(s) with S at a position corresponding to position 196 and A at a position corresponding to position 332, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134 or the same amino acid replacement at a corresponding amino acid residue in the unmodified FXa polypeptide.

10. The isolated modified FXa polypeptide of any of claims 1-9, comprising a further amino acid replacement(s) that introduce a non-native glycosylation site.

11. The isolated modified FXa polypeptide of any of claims 1-10, wherein the unmodified FXa polypeptide is selected from among:

a) an active FX (FXa) polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134; or

b) a catalytically active form of a).

12. The isolated modified FXa polypeptide of any of claims 1-11, wherein the unmodified polypeptide contains a heavy and light chain and consists of the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134.

13. The isolated modified FXa polypeptide of any of claims 1-10, comprising a sequence of amino acids selected from among:

a) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265); and

b) a catalytically active form of a) that includes the amino acid replacement(s) and exhibits catalytic activity.

14. A modified Factor X (FX) polypeptide, comprising at least two amino acid replacement(s) in an unmodified FX polypeptide, wherein:

the unmodified FX polypeptide has the sequence of amino acids set forth in SEQ ID NO:134, or is the zymogen, active or catalytically active form thereof, or has a sequence of amino acids that has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:134 or the zymogen, active or catalytically active form thereof, respectively;

the modified FX polypeptide comprises:

(i) an amino acid replacement at a position corresponding to position 196 in an unmodified FX polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein the amino acid replacement is with a neutral polar amino acid selected from Serine (S) or Threonine (T); and

(ii) a further amino acid replacement that confers one or more of increased co-factor dependence, increased resistance to inhibitors, altered glycosylation, increased half-life or increased catalytic activity, wherein:

the further amino acid replacement is not replacement with Isoleucine (I), Alanine (A), Serine (S) or Threonine (T) at a position corresponding to position 195 with reference to amino acid positions set forth in SEQ ID NO:134, or the same replacement at a corresponding amino acid residue in the unmodified FX polypeptide; and

the active FX (FXa) form of the modified polypeptide exhibits increased co-factor dependence compared to the FXa form of the FX polypeptide not containing the amino acid replacement; and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa; and

15. The modified FX polypeptide of claim 14, wherein the modified FX polypeptide comprises an amino acid replacement with S at a position corresponding to position 196 based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, or the same amino acid replacement at a corresponding amino acid residue in the unmodified FX polypeptide.

16. The modified FX polypeptide of claim 14 or claim 15, wherein the active FXa form of the modified polypeptide exhibits increased co-factor dependence and the

active FX (FXa) form of the modified polypeptide exhibits at least 50-fold increased FVa cofactor dependence compared to the FXa polypeptide that is the same as the modified FX polypeptide, but not containing the amino acid replacement(s).

17. The modified FX polypeptide of any of claims 14-16, wherein the active FXa form of the modified FX polypeptide exhibits increased resistance to an inhibitor and the inhibitor is selected from among tissue factor pathway inhibitor (TFPI) or is anti-thrombin III (ATIII).

18. The modified FX polypeptide of claim 17, wherein the inhibitor is anti-thrombin III (ATIII).

19. The modified FX polypeptide of any of claims 14-18, comprising only two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty amino acid replacement(s) compared to the unmodified polypeptide of the same form.

20. The modified FX polypeptide of any of claims 24-32, comprising an amino acid replacement(s) at an amino acid position corresponding to a position selected from among 211, 214, 216, 218, 219, 273, 276, 306, 326, 332, 338, 420 and 424, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein corresponding amino acid positions are identified by alignment of the unmodified FX polypeptide with the polypeptide set forth in SEQ ID NO:134.

21. The modified FX polypeptide of claim 33, comprising an amino acid replacement(s) selected from among amino acid replacement(s) with:

S at a position corresponding to position 211; D at a position corresponding to position 214; A at a position corresponding to position 214; S at a position corresponding to position 214; R at a position corresponding to position 216; K at a position corresponding to position 216; A at a position corresponding to position 216; S at a position corresponding to position 216; R at a position corresponding to position 218; K at a position corresponding to position 218; A at a position corresponding to position 218; H at a position corresponding to position 219; A at a position corresponding to position 273; E at a position corresponding to position 273; A at a position corresponding to position 276; E at a position corresponding to position 276; E at a position corresponding to position 306; S at a position corresponding to position 326; T at a position corresponding to position 326; V at a position corresponding to position 326; Q at a position corresponding to position 326; N at a position corresponding to position 326; M at a position corresponding to position 326; K at a position corresponding to position 326; Y at a position corresponding to position 326; E at a position corresponding to position 326; D at a position corresponding to position 326; A at a position corresponding to position 332; D at a position corresponding to position 332; E at a position corresponding to position 332; S at a position corresponding to position 332; G at a position corresponding to position 332; A at a position corresponding to position 338; S at a position corresponding to position 338; N at a position corresponding to position 338; R at a position corresponding to position 338; V at a position corresponding to position 338; Y at a position corresponding to position 338; M at a position corresponding to position 338; A at a position corresponding to position 420; E at a position corresponding to position 420; A at a position corresponding to position 424; and E at a position

corresponding to position 424, with reference to amino acid positions set forth in SEQ ID NO:134, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

22. The modified FX polypeptide of any of claims 21-34, comprising an amino acid replacement(s) selected from among amino acid replacement(s) with S at a position corresponding to position 196, and A, E, D, G or S at a position corresponding to position 332, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134 or the same amino acid replacement at a corresponding amino acid residue in the unmodified FX polypeptide, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

23. The modified FX polypeptide of any of claims 1-22 comprising amino acid replacements with S at a position corresponding to position 196 and A at a position corresponding to position 332, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134 or the same amino acid replacement at a corresponding amino acid residue in the unmodified FX polypeptide, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

24. The modified FX polypeptide of any of claims 14-23, comprising a further amino acid replacement(s) that introduces a non-native glycosylation site.

25. The modified FX polypeptide of any of claims 14-24, wherein the unmodified FX polypeptide is selected from among:

a) a zymogen FX polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of SEQ ID NO:134, or a sequence of amino acids that exhibits at least 75% sequence identity to the zymogen FX polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of SEQ ID NO:134;

b) an active FX (FXa) polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134, or a sequence of amino acids that exhibits at least 75% sequence identity to the FXa comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134; and

c) a catalytically active form of b).

26. The modified FX polypeptide of any of claims 1-25 that comprises the sequence of amino acids of the polypeptide of any of SEQ ID NOs: 92-96 and 224-228.

27. The modified FX polypeptide of any of claims 21-41, comprising a sequence of amino acids selected from among:

a) a sequence of amino acids set forth in any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids set forth in any of SEQ ID NOS:

137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s);

b) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s);

c) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s); and

d) a catalytically active form of c) that includes the modification(s) and exhibits catalytic activity.

28. The modified FX polypeptide of any of claims 1-27, comprising an amino acid replacement selected from among L at a position corresponding to position 195 with reference to amino acid positions set forth in SEQ ID NO:134, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

29. The modified FX polypeptide of any of claims 1-28, wherein the unmodified polypeptide is a human polypeptide.

30. The modified FX polypeptide of any of claims 1-2973, wherein the unmodified polypeptide is a non-human polypeptide.

31. The modified FX polypeptide of any of claims 14-30 that is a zymogen polypeptide.

32. The modified FX polypeptide of any of claims 14-30 that is a two-chain polypeptide.

33. The modified FX polypeptide of any of claims 14-30 that is a single-chain polypeptide.

34. The modified FX polypeptide of any of claims 21-74 that is active or activated.

35. The modified FX polypeptide of claim 34, wherein activation is effected by proteolytic cleavage by TF/FVIIa or FVIIIa/FIXa tenase complexes or Russell's Viper Venom FX Activator.

36. The modified FX polypeptide of any of claims 1-35, wherein only the primary sequence is modified.

37. The modified FX polypeptide of any of claims 1-36,comprising a chemical modification or a post-translational modification.

38. The modified FX polypeptide of claim 37, wherein the modified FX polypeptide is glycosylated, carboxylated, hydroxylated, sulfated, phosphorylated, albuminated or conjugated to a polyethylene glycol (PEG) moiety.

39. The modified FX polypeptide of any of claims 1-38 that is a chimeric or fusion protein.

40. The modified FX polypeptide of claim 39, comprising the sequence of amino acid residues set forth in any of SEQ ID NOs.:505-509.

41. A nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FX polypeptide of any of claims 1-40.

42. A vector, comprising the nucleic acid molecule of claim 41.

43. The vector of claim 42, wherein the vector is a prokaryotic vector, viral vector, or a eukaryotic vector.

44. The vector of claim 42 or claim 43, wherein the vector is a mammalian vector.

45. The vector of claim 43 or claim 44, wherein the viral vector is selected from among an adenovirus, an adeno-associated-virus, a retrovirus, a herpes virus, a lentivirus, a poxvirus, and a cytomegalovirus.

46. An isolated cell, comprising the vector of any of claims 42-45.

47. The cell of claim 46, that is a eukaryotic cell.

48. The cell of claim 47 wherein the eukaryotic cell is a mammalian cell.

49. The cell of claim 48, wherein the mammalian cell is selected from among baby hamster kidney cells (BHK-21) or 293 cells or CHO cells.

50. A method of producing a modified FX polypeptide of any of claims 1-40, comprising culturing a cell of a cell of any of claims 46-49, under conditions whereby the modified FX polypeptide is produced.

51. The method of claim 50, further comprising isolating the modified FX polypeptide.

52. A pharmaceutical composition, comprising a modified FX polypeptide of any of claims 1-40, a nucleic acid molecule of claim 41 or a vector of any of claims 42-45 in a pharmaceutically acceptable vehicle.

53. The pharmaceutical composition of claim 52 that is formulated for local, systemic or topical administration.

54. The pharmaceutical composition of claim 52 or claim 53 that is formulated for oral, nasal, pulmonary buccal, transdermal, subcutaneous, intraduodenal, enteral, parenteral, intravenous, or intramuscular administration.

55. The pharmaceutical composition of any of claims 52-54 that is formulated for controlled- release.

56. The pharmaceutical composition of any of claims 52-55 that is formulated for single-dosage administration.

57. A method of increasing the cofactor dependence of an active Factor Xa polypeptide, comprising introducing an amino acid replacement in a Factor X (FX) polypeptide at an amino acid position corresponding to position 196 in an unmodified FX polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, whereby the active FX (FXa) form of the modified polypeptide exhibits increased cofactor dependence.

58. The method of claim 57, wherein the unmodified FX polypeptide has the sequence of amino acids set forth in SEQ ID NO:134, or is the zymogen, active or catalytically active form thereof, or has a sequence of amino acids that has at least 75% sequence identity to SEQ ID NO:134 or the zymogen, active or catalytically active form thereof, respectively.

59. The method of claim 57 or claim 58, wherein the amino replacement is replacement to a neutral polar amino acid selected from among asparagine (N), glutamine (Q), serine (S), threonine (T), tryptophan (W), cysteine (C) and tyrosine (Y).

60. A method of generating a modified active Factor X (FXa) polypeptide, comprising:

a) providing a composition comprising a modified FX zymogen of claim 31; and

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide.

61. A method of generating a modified active Factor X (FXa) polypeptide, comprising:

a) providing a composition comprising a modified FX zymogen selected from among a zymogen comprising:

a light chain having the sequence of amino acids 1-139 set forth in SEQ ID NO:134, or a sequence of amino acids that exhibits at least 75% sequence identity to the sequence of amino acids 1-139 of any of SEQ ID NOS:134 and that contains the amino acid replacement(s) selected from S and T at position 196; and

a heavy chain having the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265, or a sequence of amino acids that exhibits at least 75% sequence identity to the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265 and that contains amino acid replacement(s) compared to an unmodified FX polypeptide; and

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide.

62. The method of claim 60 or claim 61, wherein activation cleavage is effected *in vivo* or *in vitro*.

63. The method of any of claims 60-62, wherein activation cleavage is effected by an activator selected from among TF/FVIIa or FVIIIa/FIXa tenase complexes or Russell's Viper Venom FX Activator.

64. The method of any of claims 60-631, further comprising removing the activator from the composition.

65. The method of any of claims 60-64, further comprising removing the activation peptide from the composition.

66. The method of any of claims 60-65, wherein the FX zymogen is produced by expression of a nucleic acid encoding a polypeptide set forth in any of SEQ ID NOS: 4-133 or 136-265 and 417-546, or a nucleic acid encoding a polypeptide having a sequence of amino acids that has at least 75% sequence identity to any of SEQ ID NOS: 4-133 or 136-265 and 417-546 and that contains an amino acid replacement(s) compared to an unmodified FX polypeptide.

67. A method of treating a disease or condition that is treating a bleeding disorder, comprising administering to a subject a modified FX polypeptide of any of claims 1-40 or a pharmaceutical composition of any of claims 52-56.

68. The method of claim 67, wherein the bleeding disorder is a congenital bleeding disorder or an acquired bleeding disorder.

69. The method of claim 67 or claim 68, wherein the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

70. The method of any of claims 67-69, wherein bleeding disorder is due to a deficiency of a coagulation factor or due to the presence of acquired inhibitors to a coagulation factor, wherein the coagulation factor is selected from among factor VII, factor IX, factor X, factor XI, factor V, factor XII, factor II and von Willebrand factor.

71. The method of any of claims 67-70, wherein the bleeding disorder is due to a deficiency of a coagulation factor and the coagulation factor is selected from among factor VII, factor VIII, factor IX, factor XI.

72. The method of any of claims 67-71, wherein the bleeding disorder is selected from among hemophilia A, hemophilia B or hemophilia C.

73. The method of any of claims 67-70, wherein the bleeding disorder is due to a deficiency of a coagulation factor, wherein the disorder is a familial multiple coagulation factor deficiency (FMFD).

74. The method of any of claims 67-70, wherein the bleeding disorder is due to the presence of acquired inhibitors to a coagulation factor, wherein the coagulation factor is selected from among factor VII, factor VIII, factor IX, factor X, factor XI, and factor XII.

75. The method of claim 74, wherein the acquired inhibitors are autoantibodies.

76. The method of claim 74 or claim 122, wherein the bleeding disorder is acquired hemophilia.

77. The method of any of claims 67-69, wherein the bleeding disorder is a hereditary platelet disorder selected from among Chediak-Higashi syndrome, Hermansky-Pudlak syndromes, thromboxane A2 dysfunction, Glanzmann's thrombasthenia, and Bernard-Soulier syndrome.

78. The method of claim 67 or claim 68, wherein the disorder results form anticoagulant therapy with a vitamin-K antagonist, wherein the vitamin-K antagonist is selected from among heparin, pentasaccharide, warfarin, small molecule antithrombotics and FXa inhibitors.

79. The method of any of claims 67-69, wherein the disorder is selected from among thrombosis, thrombocytopenia, stroke and coagulapathy.

80. The method of any of claims 67-69, wherein the disorder results from a trauma, surgery or wound.

81. The method of claim 80, wherein the bleeding is manifested as acute haemarthroses, chronic haemophilic arthropathy, haematomas, haematuria, central nervous system bleedings, gastrointestinal bleedings, or cerebral haemorrhage.

82. The method of claim 80, wherein the bleeding is due to dental extraction.

83. The method of claim 80, wherein the surgery is heart surgery, angioplasty, lung surgery, abdominal surgery, spinal surgery, brain surgery, vascular surgery, dental surgery, or organ transplant surgery.

84. The method of claim 83, wherein the transplant surgery is selected from among transplantation of bone marrow, heart, lung, pancreas, and liver.

85. A modified FX polypeptide of any of claims 1-40 for use in treating a bleeding disorder.

86. Use of a modified FX polypeptide of any of claims 1-40 in the preparation of a medicament for treatment of a bleeding disorder.

87. The modified FX polypeptide of claim 85 or use of claim 86, wherein the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s).

88. The modified FX polypeptide or use of any of claims 85-87, wherein the bleeding disorder is a congenital bleeding disorder or an acquired bleeding disorder.

89. The modified FX polypeptide or use of any of claims 85-87, wherein the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

90. The modified FX polypeptide or use of claim 89, wherein bleeding disorder is due to a deficiency of a coagulation factor or due to the presence of acquired inhibitors to a coagulation factor, wherein the coagulation factor is selected from among factor VII, factor IX, factor X, factor XI, factor V, factor XII, factor II and von Willebrand factor.

91. The modified FX polypeptide or use of claim 90, wherein the bleeding disorder is due to a deficiency of a coagulation factor and the coagulation factor is selected from among factor VII, factor VIII, factor IX, factor XI.

92. The modified FX polypeptide or use of any of claim 85-91, wherein the bleeding disorder is selected from among hemophilia A, hemophilia B or hemophilia C.

93. The modified FX polypeptide or use of any of claims 85-89, wherein the bleeding disorder is due to a deficiency of a coagulation factor, wherein the disorder is a familial multiple coagulation factor deficiency (FMFD).

94. The modified FX polypeptide or use of any of claims 85-89, wherein the bleeding disorder is due to the presence of acquired inhibitors to a coagulation factor, wherein the coagulation factor is selected from among factor VII, factor VIII, factor IX, factor X, factor XI, and factor XII.

95. The modified FX polypeptide or use of claim 94, wherein the acquired inhibitors are autoantibodies.

96. The modified FX polypeptide or use of claim 84 or claim 95, wherein the bleeding disorder is acquired hemophilia.

97. The modified FX polypeptide or use of any of claims 85-89, wherein the bleeding disorder is a hereditary platelet disorder selected from among Chediak-Higashi syndrome, Hermansky-Pudlak syndromes, thromboxane A2 dysfunction, Glanzmann's thrombasthenia, and Bernard-Soulier syndrome.

98. The modified FX polypeptide or use of any of claims 85-89, wherein the disorder results from anticoagulant therapy with a vitamin-K antagonist, wherein the vitamin-K antagonist is selected from among heparin, pentasaccharide, warfarin, small molecule antithrombotics and FXa inhibitors.

99. The modified FX polypeptide or use of any of claims 85-89, wherein the disorder is selected from among thrombosis, thrombocytopenia, stroke and coagulapathy.

100. The modified FX polypeptide or use of any of claims 85-89, wherein the disorder results from a trauma, surgery or wound.

101. The modified FX polypeptide or use of claim 151, wherein the bleeding is manifested as acute haemarthroses, chronic haemophilic arthropathy, haematomas, haematuria, central nervous system bleedings, gastrointestinal bleedings, or cerebral haemorrhage.

102. The modified FX polypeptide or use of claim 100, wherein the bleeding is due to dental extraction.

103. The modified FX polypeptide or use of claim 100, wherein the surgery is heart surgery, angioplasty, lung surgery, abdominal surgery, spinal surgery, brain surgery, vascular surgery, dental surgery, or organ transplant surgery.

104. The modified FX polypeptide or use of claim 103, wherein the transplant surgery is selected from among transplantation of bone marrow, heart, lung, pancreas, and liver.

CLAIMS:

1. An isolated modified Factor X (FX) polypeptide, comprising an amino acid replacement at a position corresponding to position 196 in an unmodified FX polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein:

corresponding amino acid positions are identified by alignment of the unmodified FX polypeptide with the polypeptide whose set forth in SEQ ID NO:134;

the replacement is with a neutral polar amino acid residue that is serine (S) or threonine (T);

the unmodified FX polypeptide has the sequence of amino acids set forth in SEQ ID NO:134, or is the zymogen, active or catalytically active form thereof, or has a sequence of amino acids that has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:134 or the zymogen, active or catalytically active form thereof, respectively;

the active FX (FXa) form of the modified polypeptide exhibits increased co-factor dependence compared to the FXa form of the FX polypeptide not containing the amino acid replacement: and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

2. The isolated modified FX polypeptide of claim 1 that is a FXa polypeptide, the unmodified FXa comprises a sequence of amino acids that exhibits at least 85% sequence identity to the FXa comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134, or a catalytically active form thereof.

3. The isolated FX polypeptide of claim 1 or claim 2, wherein the modified FX polypeptide comprises an amino acid replacement with S at a position corresponding to position 196 with reference to amino acid position set forth in SEQ ID NO:134, or the same replacement at a corresponding amino acid residue in the unmodified FX polypeptide that does not comprise S at the position corresponding to 196.

4. The isolated modified FX polypeptide of any of claims 1-3, comprising a further amino acid replacement(s) in the light chain or in the protease domain of the heavy chain, wherein:

the further amino acid replacement(s) confers altered glycosylation, increased resistance to an inhibitor, increased co-factor dependence, increased half-life and/or increased catalytic activity; and

is at a position corresponding to a position selected from among 211, 214, 216, 218, 219, 273, 276, 306, 326, 332, 338, 420 and 424, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134.

5. The isolated FX polypeptide of claim 4 , wherein:

the replacement confers increased resistance to an inhibitor; and

the inhibitor is a tissue factor pathway inhibitor (TFPI) or is anti-thrombin III (ATIII).

6. The isolated modified FX polypeptide of claim 5, wherein the inhibitor is anti-thrombin III (ATIII).

7. The isolated modified FX polypeptide of any of claims 1-5, comprising only one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty amino acid replacement(s) compared to the unmodified FXa polypeptide of the same form.

8. The isolated modified FX polypeptide of any of claims 1-6, comprising a further amino acid replacement selected from among amino acid replacement with:

S at a position corresponding to position 211; D at a position corresponding to position 214; A at a position corresponding to position 214; S at a position corresponding to position 214; R at a position corresponding to position 216; K at a position corresponding to position 216; A at a position corresponding to position 216; S at a position corresponding to position 216; R at a position corresponding to position 218; K at a position corresponding to position 218; A at a position corresponding to position 218; H at a position corresponding to position 219; A at a position corresponding to position 273; E at a position corresponding to position 273; A at a position corresponding to position 276; E at a position corresponding to position 276; E at a position corresponding to position 306; S at a position corresponding to position 326; T at a position corresponding to position 326; V at a position corresponding to position 326; Q at a position corresponding to position 326; N at a position corresponding to position 326; M at a position corresponding to position 326; K at a position corresponding to position 326; Y at a position corresponding to position 326; E at a position corresponding to position 326; D at a position corresponding to position 326; A at a position corresponding to position 332; D at a position corresponding to position 332; E at a position corresponding to position 332; S at a position corresponding to position 332; G at a position corresponding to position 332; A at a position

corresponding to position 338; S at a position corresponding to position 338; N at a position corresponding to position 338; R at a position corresponding to position 338; V at a position corresponding to position 338; Y at a position corresponding to position 338; M at a position corresponding to position 338; A at a position corresponding to position 420; E at a position corresponding to position 420; A at a position corresponding to position 424; and E at a position corresponding to position 424, with reference to amino acid positions set forth in SEQ ID NO:134, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

9. The isolated modified FX polypeptide of any of claims 1-8, comprising an amino acid replacement with S or T at a position corresponding to position 196, and an amino acid replacement selected from among amino acid replacement with A, D, E, S or G at a position corresponding to 332 in the unmodified FX polypeptide.

10. The isolated modified FX polypeptide of any of claims 1-9, comprising amino acid replacements with S at a position corresponding to position 196 and A at a position corresponding to position 332, based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134 or the same amino acid replacement at a corresponding amino acid residue in the unmodified FXa polypeptide.

11. The modified FX of any of claims 1-10, comprising the amino acid replacements corresponding to 196S and 332A, wherein the modified FX is a factor FXa.

12. The isolated modified FX polypeptide of any of claims 1-11, comprising a further amino acid replacement(s) that introduce a non-native glycosylation site.

13. The isolated modified FX polypeptide of any of claims 1-11, wherein the unmodified FX polypeptide is selected from among:

a) an active FX (FXa) polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134; or

b) a catalytically active form of a).

14. The isolated modified FX polypeptide of any of claims 1-12, wherein the unmodified polypeptide contains a heavy and light chain and consists of the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134.

15. The isolated modified FX polypeptide of any of claims 1-12, comprising a sequence of amino acids selected from among:

a) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265); and

b) a catalytically active form of a) that includes the amino acid replacement(s) and exhibits catalytic activity.

16. A modified active Factor X (FXa) polypeptide, comprising an amino acid replacement at a position 196 and at position 332 in an unmodified FXa polypeptide based on mature numbering with reference to amino acid positions set forth in SEQ ID NO:134, wherein:

the replacement at position 196 is serine (S), and the replacement at 332 alanine (A), aspartic acid (D), glutamic acid (E), serine (S) or glycine (G);

the modified FXa polypeptide contains only up to seven amino acid replacements;

the unmodified FXa comprises a light chain consisting of the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain consisting of the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134;

the modified FXa exhibits at least 50-fold increased FVa cofactor dependence compared to the unmodified FXa polypeptide not containing the amino acid replacements; and

co-factor dependence is the ratio of catalytic activity of the modified FXa in the presence of Factor Va (FVa) compared to in the absence of FVa.

17. The modified FXa polypeptide of claim 16, wherein the replacement at position 332 is A.

18. The modified FX polypeptide of any of claims 1-15, wherein the unmodified FX polypeptide is selected from among:

a) a zymogen FX polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of SEQ ID NO:134, or a sequence of amino acids that exhibits at least 85% sequence identity to the zymogen FX polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of SEQ ID NO:134;

b) an active FX (FXa) polypeptide comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the

sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134, or a sequence of amino acids that exhibits at least 75% sequence identity to the FXa comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 of SEQ ID NO:134, and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of SEQ ID NO:134; and

c) a catalytically active form of b).

19. The modified FX polypeptide of any of claims 1-16 that comprises the sequence of amino acids of the polypeptide of any of SEQ ID NOs: 92-96 and 224-228.

20. The modified FX polypeptide of any of claims 1-16, comprising a sequence of amino acids selected from among:

a) a sequence of amino acids set forth in any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids set forth in any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s);

b) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 143-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s);

c) a sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265, or a sequence of amino acids that exhibits at least 75% sequence identity to any of the sequence of amino acids comprising a light chain comprising the sequence of amino acids set forth as residues 1-139 and a heavy chain comprising the sequence of amino acids set forth as residues 195-448 of any of SEQ ID NOS: 137-157, 169, 205-208, 214-243 and 246-265 and that contains the amino acid replacement(s); and

d) a catalytically active form of c) that includes the modification(s) and exhibits catalytic activity.

21. The modified FX polypeptide of any of claims 1-20 comprising an amino acid replacement corresponding to 195L with reference to amino acid positions set forth in SEQ ID

NO:134, or the same replacement(s) at a corresponding amino acid residue in the unmodified FX polypeptide.

22. The modified FX polypeptide of any of claims 1-21, wherein the unmodified polypeptide is a human polypeptide.

23. The modified FX polypeptide of any of claims 1-22 that is selected from among a single chain zymogen polypeptide, a two-chain polypeptide and a single-chain polypeptide.

24. The modified FX polypeptide of any of claims 1-23 that is a FXa activated polypeptide.

25. The modified FX polypeptide of any of claims 1-24, wherein only the primary sequence is modified.

26. The modified FX polypeptide of any of claims 1-25 , wherein the modified FX polypeptide is glycosylated, carboxylated, hydroxylated, sulfated, phosphorylated, albuminated or conjugated to a polyethylene glycol (PEG) moiety.

27. The modified FX polypeptide of any claims 1-26 that is a chimeric or fusion protein.

28. The modified FX polypeptide of claim 27, comprising sequence of acid residues set forth in any of SEQ ID NOs.:505-509.

29. A nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FX polypeptide of any of claims 1-20.

30. A vector, comprising the nucleic acid molecule of claim 26.

31. The vector of claim 30, wherein the vector is a prokaryotic vector, viral vector, or a eukaryotic vector.

32. The vector of claim 30 that is a viral vector s selected from among an adenovirus, an adeno-associated-virus, a retrovirus, a herpes virus, a lentivirus, a poxvirus, and a cytomegalovirus vector.

33. An isolated cell or cell culture, comprising the vector of any of claims 30-32.

34. The cell or cell culture of claim 33, that is a eukaryotic cell or comprises eukaryotic cells..

35. The cell or cell culture of claim 34, wherein the eukaryotic cell is a mammalian cell.

36. The cell or cell culture of claim 35, wherein the mammalian cell is selected from among baby hamster kidney cells (BHK-21) or 293 cells or CHO cells.

37. A method of producing a modified FX polypeptide of any of claims 1-25, comprising culturing a cell of a cell of any of claims 30-33, under conditions whereby the modified FX

polypeptide is produced.

38. The method of claim 37, further comprising isolating the modified FX polypeptide.

39. A pharmaceutical composition, comprising a modified FX polypeptide of any of claims 1-28, a nucleic acid molecule of claim 29 or a vector of any of claims 30-32 in a pharmaceutically acceptable vehicle.

40. A method of generating a modified active Factor X (FXa) polypeptide, comprising:

a) providing a modified FX polypeptide of claim 23, wherein the modified FX polypeptide is a single chain zymogen;

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide;

or, comprising:

a) providing a composition comprising a modified FX zymogen selected from among a zymogen comprising:

a light chain having the sequence of amino acids 1-139 set forth in SEQ ID NO:134, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 1-139 of any of SEQ ID NOS:134 and that contains the amino acid replacement(s) selected from S and T at position 196; and

a heavy chain having the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 143-448 of any of SEQ ID NOS:136-265 and that contains amino acid replacement(s) compared to an unmodified FX polypeptide; and

b) removing the activation peptide from the polypeptide by activation cleavage of the zymogen, thereby generating a modified FXa polypeptide.

41. The method of claim 40, wherein activation cleavage is effected *in vivo* or *in vitro*.

42. The method of claim 40 or claim 41, further comprising removing the activation peptide from the composition.

43. The method of any of claims 40-42, wherein the FX polypeptide comprises the an amino acid replacement corresponding to 196S.

44. The method of any of claims 40-43, wherein the FX zymogen is produced by expression of a nucleic acid encoding a polypeptide set forth in any of SEQ ID NOS: 4-133 or 136-265 and 417-546, or a nucleic acid encoding a polypeptide having a sequence of amino acids that has

at least 95% sequence identity to any of SEQ ID NOS: 4-133 or 136-265 and 417-546 and that contains an amino acid replacement(s) compared to an unmodified FX polypeptide.

45. A composition, comprising a modified FX polypeptide of any of claims 1-28 for use for treating a bleeding disorder, wherein:

the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

46. Use of a modified FX polypeptide of any of claims 1-28 in the preparation of a medicament for treatment of a bleeding disorder, wherein:

the modified FX polypeptide is a zymogen, FXa or catalytically active form thereof that contains the amino acid replacement(s); and

the bleeding disorder is selected from among a disorder due to a deficiency of a coagulation factor, a disorder due to the presence of acquired inhibitors to a coagulation factor, a hematologic disorder, a hemorrhagic disorder, Von Willebrands' disease, a disorder that results from anticoagulant therapy with a vitamin-K antagonist, hereditary platelet disorders, vitamin K epoxide reductase C1 deficiency, gamma-carboxylase deficiency, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stoke, coagulopathy, disseminated intravascular coagulation (DIC), Bernard Soulier syndrome, Glanzman thromblastemia and storage pool deficiency.

47. The modified FX polypeptide of claim 45, wherein the bleeding disorder is a hemophilia.

48. The use of claim 46, wherein the bleeding disorder is a hemophilia.