Provided are modified FIX polypeptides. The modified FIX polypeptides provided have improved procoagulant therapeutic properties compared to an unmodified FIX polypeptide. For example, among the modified FIX polypeptides provided herein are those that exhibit increased coagulant activity, increased catalytic activity, increased resistance to AT-III, heparin and/or the AT-III/heparin complex, and/or improved pharmacokinetic properties, such as i) decreased clearance, ii) altered (e.g. increased or decreased) volume of distribution, iii) enhanced *in vivo* recovery, iv) enhanced total protein exposure *in vivo* (i.e., AUC), v) increased serum half-life (α-, β-, and/or γ-phase), and/or vi) increased mean resonance time (MRT). In some examples, the improved pharmacokinetic properties are a result of increased glycosylation and/or decreased binding to the low-density lipoprotein receptor-related protein (LRP). Also provided are nucleic acids encoding the modified FIX polypeptides and methods of using the modified FIX polypeptides, such as for treatment of bleeding disorders.

In one aspect, the is provided a modified Factor IX (FIX) polypeptide, comprising the amino acid replacement T343R or T343K in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide, wherein:

corresponding amino acid residues are identified by alignment of the unmodified FIX polypeptide with the polypeptide of SEQ ID NO:3;

the unmodified FIX polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 and 325 or an active form thereof or a FIX polypeptide having at least 95% sequence identity to the polypeptide that comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 and 325;

the modified FIX polypeptide does not contain the modifications F342I/T343R/Y345T;

the modified FIX polypeptide exhibits at least one FIX activity or property that is improved compared to the unmodified FIX polypeptide that does not contain the modification; and

the activity or property is increased procoagulant activity compared to an active form of a polypeptide of any of SEQ ID NOS: 2, 3, 20 and 325.

In one aspect, there is provided nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FIX 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 FIX polypeptide, comprising:

culturing a cell or cell culture as described herein under conditions whereby the modified FIX polypeptide is expressed; and

optionally isolating the FIX polypeptide.

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

In one aspect, there is provided a modified FIX polypeptide as described herein for use for treatment of a disease or condition that is treated by administration of FIX.

In one aspect, there is provided a use of a modified FIX polypeptide oas described herein for formulation of a medicament for treatment of a disease or condition that is treated by administration of FIX.

Among the modified FIX polypeptides provided herein are those containing two amino acid replacements in unmodified FIX polypeptide, wherein the first amino acid replacement is at a position corresponding to a position selected from among 53, 61, 64, 85, 103, 104, 105, 106, 108, 155, 158, 159, 167, 169, 172, 179, 202, 203, 204, 205, 228, 239, 241, 243, 247, 249, 251, 257, 259, 260, 262, 265, 284, 293, 312, 314, 315, 316, 317, 318, 319, 321, 333, 338, 343, 346, 345, 392, 394, 400, 403, 410, 412 and 413 in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, and the second amino acid replacement is at a position corresponding to a position selected from among 5, 53, 61, 64, 85, 155, 158, 159, 167, 239, 260, 284, 293, 312, 318, 333, 338, 346, 400, 403, 410, 412 and 413 in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3.

In some examples, the first or the second amino acid replacement is replacement with an amino acid residue selected from among alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); cysteine (Cys, C); glutamic acid (Glu, E); glutamine (Gln, Q); glycine (Gly, G); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y); and valine (Val, V), providing the replacing

CLAIMS:

1. A modified Factor IX (FIX) polypeptide, comprising the amino acid replacement T343R or T343K in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide, wherein:

corresponding amino acid residues are identified by alignment of the unmodified FIX polypeptide with the polypeptide of SEQ ID NO:3;

the unmodified FIX polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 and 325 or an active form thereof or a FIX polypeptide having at least 95% sequence identity to the polypeptide that comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 and 325;

the modified FIX polypeptide does not contain the modifications F342I/T343R/Y345T;

the modified FIX polypeptide exhibits at least one FIX activity or property that is improved compared to the unmodified FIX polypeptide that does not contain the modification; and

the activity or property is increased procoagulant activity compared to an active form of a polypeptide of any of SEQ ID NOS: 2, 3, 20 and 325.

2. The modified FIX polypeptide of claim 1, comprising the amino acid replacement T343R.

3. The modified FIX polypeptide of claim 1, comprising the amino acid replacements R338E/T343R.

4. The modified FIX polypeptide of any of claims 1-3, further comprising an amino acid replacement is selected from among replacements corresponding to R318Y, R318E, R318F or R318W with reference to a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3.

5. The modified FIX polypeptide of any of claims 1-4, comprising an amino acid replacement at residue E410 or at an amino acid residue corresponding to 410 in an unmodified FIX polypeptide, wherein the replacement amino acid is N or S.

6. The modified FIX polypeptide of claim 5, wherein the amino acid replacement is N.

7. The modified FIX polypeptide of any of claims 1-6, comprising an amino acid replacement at residue 403 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3 or at amino acid residues corresponding to residue 403 in an unmodified FIX polypeptide, wherein the replacement is E.

8. The modified FIX polypeptide of claim 1, comprising the amino acid replacements R318Y, R338E, T343R, R403E and E410N in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3 or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

9. The modified FIX polypeptide of any of claims 1-8, wherein the unmodified polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 and 325.

10. The modified FIX polypeptide of any of claims 1-9, further comprising the amino acid replacement Y155F or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

11. The modified FIX polypeptide claim 1, further comprising the amino acid replacement Y155F or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

12. The modified FIX polypeptide of any of claims 1-11, comprising an amino acid replacement at an amino acid residue selected from among D203, F205 and K228, or at an amino acid residue corresponding to amino acid residue D203, F205 or K228 in an unmodified FIX polypeptide.

13. The modified FIX polypeptide of claim 12, comprising an amino acid replacement selected from among D203N, F205T and K228N, or the same amino acid replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

14. The modified FIX polypeptide of any of claims 1-13 that comprises an amino acid replacement selected from among A103N, D104N, N105S, K106S, K228N, I251S, N260S and N346D, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

15. The modified FIX polypeptide of any of claims 1-14, comprising an amino acid replacement at an amino acid residue selected from among K247, N249, R403 and E410, or at an amino acid residue corresponding to amino acid residue K247, N249, R403 or E410 in an unmodified FIX polypeptide.

16. The modified FIX polypeptide of claim 15, comprising an amino acid replacement selected from among K247N, N249S, R403E and E410N or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

17. The modified FIX polypeptide of any of claims 1-16, comprising one or more modifications that introduces and/or eliminates one or more glycosylation sites compared to the unmodified FIX polypeptide.

18. The modified FIX polypeptide of claim 17, wherein the modification introduces one or more glycosylation sites.

19. The modified FIX polypeptide of any of claims1-18, wherein the improved property is increased procoagulant activity compared with the unmodified FIX polypeptide.

20. The modified FIX polypeptide of any of claims1-19, wherein the unmodified FIX polypeptide consists of the sequence of amino acids set forth in SEQ ID NO:2, 3, 20 or 325.

21. The modified FIX polypeptide of claim 1, comprising amino acid replacements selected from among R338E/T343R/E410N; Y155F/R338E/T343R/E410N; Y155F/K247N/N249S/T343R/E410N; Y155F/T343R/R403E/E410N; K247N/N249S/T343R/R403E/E410N; Y155F/K247N/N249S/T343R/R403E/E410N; K247N/N249S/R338E/T343R/E410N; Y155F/K247N/N249S/R338E/T343R/E410N; K247N/N249S/R338E/T343R/R403E; Y155F/K247N/N249S/R338E/T343R/R403E; Y155F/K247N/N249S/R338E/T343R; K247N/N249S/R338E/T343R/R403E/E410N; Y155F/K247N/N249S/R338E/T343R/R403E/E410N; Y155F/K247N/N249S/T343R/R403E; Y155F/I251S/R338E/T343R/R403E; Y155F/R338E/T343R/R403E/E410S; R338E/T343R/R403E; Y155F/R338E/T343R/R403E; Y155F/R338E/T343R/R403E/E410N; R338E/T343R/R403E/E410N and R338E/T343R.

22. The modified FIX polypeptide of claim 21, comprising amino acid replacements selected from among K247N/N249S/R338E/T343R/R403E/E410N, R338E/T343R/E410N and R338E/T343R.

23. A modified FIX polypeptide of claim 1, comprising a sequence of amino acids set forth in any of SEQ ID NOS: 209, 215, 268, 332, 335, 351, 352, 361-363, 365, 367, 370, 371, 377, 387-392, 396, 399, 401 and 402.

24 The modified FIX polypeptide of claim 23, wherein the sequence of amino acid residues consists of the sequence set forth in any of SEQ ID NOS: 209, 215, 268, 332, 335, 351, 352, 361-363, 365, 367, 370, 371, 377, 387-392, 396, 399, 401 and 402.

25. The modified FIX polypeptide of any of claims 1-24 that is a mature polypeptide.

26. The modified FIX polypeptide of any of claims 1-24 that is a two-chain polypeptide.

27. The modified FIX polypeptide of any of claims 1-24 that is a single-chain polypeptide.

28. The modified FIX polypeptide of any of claims 1-27 that is activated.

29. The modified FIX polypeptide of any of claims 1-28 that is hyperglycosylated.

30. The modified FIX polypeptide of any of claims 1-28, wherein only the primary sequence is modified.

31. The modified FIX polypeptide of any of claims 1-28 that comprises a chemical modification or a post-translational modification.

32. The modified FIX polypeptide of claim 31, wherein the modified FIX polypeptide is glycosylated, carboxylated, hydroxylated, sulfated, phosphorylated, albuminated, or conjugated to a polyethylene glycol (PEG) moiety.

33. The modified FIX polypeptide of any of claims 1-32 that contains only 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 modifications.

34. A nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FIX polypeptide of any of claims 1-33.

35. A vector, comprising the nucleic acid molecule of claim 34.

36. The vector of claim 35, wherein the vector is a prokaryotic vector, viral vector, or a eukaryotic vector.

37. The vector of claim 35, wherein the vector is a mammalian vector.

38. The vector of claim 35, that is a viral vector is selected from among an adenovirus, an adeno-associated-virus, a retrovirus, a herpes virus, a lentivirus, a poxvirus, and a cytomegalovirus.

39. An isolated cell or cell culture, comprising the vector of any of claims 35-38.

40. The cell or cell culture of claim 39, that is a eukaryotic cell.

41. The cell or cell culture of claim 40, wherein the eukaryotic cell is a mammalian cell.

42. A method of producing a modified FIX polypeptide, comprising:

culturing a cell or cell culture of any of claims 39-41 under conditions whereby the modified FIX polypeptide is expressed; and

optionally isolating the FIX polypeptide.

43. A pharmaceutical composition, comprising a modified FIX polypeptide of any of claims 1-33 in a pharmaceutically acceptable vehicle.

44. The pharmaceutical composition of claim 43 that is formulated for local, systemic, or topical administration.

45. The pharmaceutical composition of claim 43 that is formulated for oral, nasal, pulmonary, buccal, transdermal, subcutaneous, intraduodenal, enteral, parenteral, intravenous, or intramuscular administration.

46. The pharmaceutical composition of any of claims 43-45 that is formulated for controlled-release.

47. The pharmaceutical composition of any of claims 43-46 that is formulated for single-dosage administration.

48 A modified FIX polypeptide of any of claims1-33 for use for treatment of a disease or condition that is treated by administration of FIX.

49. Use of a modified FIX polypeptide of any of claims 1-33 for formulation of a medicament for treatment of a disease or condition that is treated by administration of FIX.

50. The modified FIX polypeptide of claim 48 or use of claim 49, wherein the FIX is active FIX (FIXa).

51. The modified FIX polypeptide or use of any of claims 48-50, wherein the disease or condition to be treated is selected from among blood coagulation disorders, hematologic disorders, hemorrhagic disorders, hemophilias, and bleeding disorders.

52. The modified FIX polypeptide or use of any of claims 48-51, wherein the disease or disorder is hemophilia B.

CLAIMS

1. A modified FIX polypeptide, comprising an amino acid replacement in an unmodified FIX polypeptide, wherein the amino acid replacement is selected from among replacement of tyrosine (Y) at amino acid residue R318 (R318Y), R318E, R318F, R318W, R318D, R318I, R318K, R318L, R318M, R318S, R318V, S61A, S61C, S61D, S61E, S61F, S61G, S61I, S61K, S61L, S61P, S61R, S61V, S61W, S61Y, D64A, D64C, D64F, D64H, D64I, D64L, D64M, D64P, D64R, D64S, D64T, D64W, Y155F, Y155L, N157D, N157E, N157F, N157I, N157K, N157L, N157M, N157R, N157V, N157W, N157Y, S158A, S158D, S158E, S158F, S158G, S158I, S158K, S158L, S158M, S158R, S158V, S158W, S158Y, N167D, N167Q, N167E, N167F, N167G, N167H, N167I, N167K, N167L, N167M, N167P, N167R, N167V, N167W, N167Y, T169A, T169D, T169E, T169F, T169G, T169I, T169K, T169L, T169M, T169P, T169R, T169S, T169V, T169W, T169Y, T172A, T172D, T172E, T172F, T172G, T172I, T172K, T172L, T172M, T172P, T172R, T172S, T172V, T172W, T172Y, D203M, D203Y, D203F, D203H, D203I, D203K, D203L, D203R, D203V, D203W, A204M, A204Y, A204F, A204I, A204W, E239S, E239R, E239K, E239D, E239F, E239I, E239L, E239M, E239T, E239V, E239W, E239Y, H257F, H257E, H257D, H257I, H257K, H257L, H257M, H257Q, H257R, H257V, H257W, R312Y, R312L, R312C, R312D, R312E, R312F, R312I, R312K, R312M, R312P, R312S, R312T, R312V, R312W, K316M, K316D, K316F, K316H, K316I, K316L, K316R, K316V, K316W, K316Y, F342I, F342D, F342E, F342K, F342L, F342M, F342S, F342T, F342V, F342W, F342Y, T343R, T343E, T343D, T343F, T343I, T343K, T343L, T343M, T343S, T343V, T343W, T343Y, N346Y, N346E, N346F, N346H, N346I, N346K, N346L, N346M, N346Q, N346R, N346V, N346W,K400E, K400C, K400D, K400F, K400G, K400L, K400M, K400P, K400S, K400T, K400V, K400Y, R403D, R403F, R403I, R403K, R403L, R403M, R403S, R403V, R403Y, E410D, E410S, E410A, E410F, E410G, E410I, E410K, E410L, E410M, E410P, E410R, E410T, E410V, E410W, E410Y, T412A, T412V, T412C, T412D, T412E, T412F, T412G, T412I, T412M, T412P, T412W and T412Y in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or the same replacement at a corresponding amino acid residue in an unmodified FIX

polypeptide, wherein corresponding amino acid residues are identified by alignment of the unmodified FIX polypeptide with the polypeptide of SEQ ID NO:3; and

provided that the modified FIX polypeptide does not contain the modifications F342I/T343R/Y345T.

2. The modified FIX polypeptide of claim 1, comprising an amino acid replacement selected from among amino acid replacements R318Y, R318E, R318F, R318W, S61A, D64A, Y155F, Y155L, N157D, S158A, S158D, S158E, N167D, N167Q, T169A, T172A, D203M, D203Y, A204M, A204Y, E239S, E239R, E239K, H257F, H257E, R312Y, R312L, K316M, F342I, T343R, T343E, T343D, N346Y, K400E, R403D, E410D, E410S, E410A, T412A and T412V, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

3. The modified FIX polypeptide of claim 1 or claim 2 that comprises an amino acid replacement at R318 or at a residue corresponding to 318, wherein the amino acid replacement is selected from among Y, E, F and W.

4. The modified FIX polypeptide of claim 3 that comprises an amino acid replacement R318Y.

5. The modified FIX polypeptide of claim 3 or claim 4 that further comprises an amino acid replacement at R338 or at a residue corresponding to 338 in an unmodified FIX polypeptide.

6. The modified FIX polypeptide of any of claims 3-5 that comprises an amino acid replacement R338D or R338E or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

7. The modified FIX polypeptide of any of claims 3-6 that comprises an amino acid replacement R338E.

8. The modified FIX polypeptide of any of claims 3-6 that comprises amino acid replacements at residues R318 and R338 or at amino acid residues corresponding to amino acid residues R318 and R338 in an unmodified FIX polypeptide.

9. The modified FIX polypeptide of any of claims 3-8 comprising the amino acid replacements R318Y/R338E or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

10. The modified FIX polypeptide of claim 3 or claim 4 that further comprises an amino acid replacement at T343 or at an amino acid residue corresponding to amino acid residue T343 in an unmodified FIX polypeptide.

11. The modified FIX polypeptide of any of claims 1-10 that comprises an amino acid replacement T343R, T343E or T343D or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

12. The modified FIX polypeptide of any of claims 1-11 that comprises an amino acid replacement T343R.

13. The modified FIX polypeptide of any of claims 3-12 that comprises amino acid replacements at residues R318 and T343 or at amino acid residues corresponding to amino acid residues R318 andT343 in an unmodified FIX polypeptide.

14. The modified FIX polypeptide of any of claims 3-13 comprising the amino acid replacements R318Y/T343R or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

15. The modified FIX polypeptide of any of claims 3-14, further comprising an amino acid replacement at E410 or at an amino acid residue corresponding to amino acid residue E410 in an unmodified FIX polypeptide.

16. The modified FIX polypeptide of any of claims 3-15, comprising an amino acid replacement at residue E410 or at an amino acid residue corresponding to 410 in an unmodified FIX polypeptide that is N or S.

17. The modified FIX polypeptide of any of claims 3-16 that comprises amino acid replacements R318Y/E410N or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

18. The modified FIX polypeptide of any of claims 1-17 that comprises an amino acid replacement R318Y and an amino acid replacement at an amino acid residue selected from among residues 338, 343, 403 and 410 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3 or at amino acid residues corresponding to residues 338, 343, 403 or 410 in an unmodified FIX polypeptide.

19. The modified FIX polypeptide of any of claims 1-18, comprising an amino acid replacement selected from among R338E, T343R, R403E and E410N in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3 or the same

replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

20. The modified FIX polypeptide of any of claims 1-19 that comprises amino acid replacements selected from among replacements R318Y/R338E/R403E/E410N, R318Y/R338E/T343R/R403E/E410N, R318Y/R338E/T343R/E410N, Y155F/R318Y/R338E/T343R/R403E, Y155F/K228N/K247N/N249S/R318Y/R338E/T343R/R403E/E410N, Y155F/K247N/N249S/R318Y/R338E/T343R/R403E, K247N/N249S/R318Y/R338E/T343R/R403E, R318Y/R338E/T343R, Y155F/K247N/N249S/R318Y/R338E/T343R, K228N/R318Y/R338E/T343R/R403E/E410N, K228N/K247N/N249S/R318Y/R338E/T343R/R403E, R318Y/R338E/T343R/R403E/E410S, Y155F/K247N/N249S/R318Y/R338E, K247N/N249S/R318Y/R338E/T343R, R318Y/T343R/E410N, Y155F/R318Y/R338E/R403E, R318Y/R338E/R403E, R318Y/R338E/E410N, K228N/R318Y/E410N, R318Y/R403E/E410N, D203N/F205T/R318Y/E410N, A103N/N105S/R318Y/R338E/R403E/E410N, D104N/K106S/R318Y/R338E/R403E/E410N, K228N/R318Y/R338E/R403E/E410N, I251S/R318Y/R338E/R403E/E410N, D104N/K106S/I251S/R318Y/R338E/R403E/E410N, D104N/K106S/R318Y/R338E/E410N, I251S/R318Y/E410N/R338E, D104N/K106S/I251S/R318Y/R338E/E410N, A103N/N105S/K247N/N249S/R318Y/R338E/R403E/E410N, D104N/K106S/K247N/N249S/R318Y/R338E/R403E/E410N, K228N/K247N/N249S/R318Y/R338E/R403E/E410N, A103N/N105S/Y155F/R318Y/R338E/R403E/E410N, D104N/K106S/Y155F/R318Y/R338E/R403E/E410N, Y155F/K228N/R318Y/R338E/R403E/E410N, Y155F/I251S/R318Y/R338E/R403E/E410N, K247N/N249S/R318Y/R338E/R403E/E410N, Y155F/R318Y/R338E/R403E/E410N, K247N/N249S/R318Y/R338E/E410N, Y155F/R318Y/R338E/E410N, Y155F/K247N/N249S/R318Y/R338E/E410N,

R318Y/R338E/R403E/E410S, R318Y/R338E/R403E/E410N/T412V,
R318Y/R338E/R403E/E410N/T412A, R318Y/R338E/R403E/T412A,
R318Y/R338E/E410S, R318Y/R338E/T412A, R318Y/R338E/E410N/T412V,
D85N/K228N/R318Y/R338E/R403E/E410N, N260S/R318Y/R338E/R403E/E410N,
R318Y/R338E/N346D/R403E/E410N,
Y155F/R318Y/R338E/N346D/R403E/E410N,
K247N/N249S/N260S/R318Y/R338E/R403E/E410N,
D104N/K106S/N260S/R318Y/R338E/R403E/E410N,
Y155F/N260S/R318Y/R338E/R403E/E410N,
Y155F/R318Y/R338E/T343R/R403E/E410N,
D104N/K106S/Y155F/K247N/N249S/R318Y/R338E/R403E/E410N,
D104N/K106S/K228N/K247N/N249S/R318Y/R338E/R403E/E410N,
Y155F/K228N/K247N/N249S/R318Y/R338E/R403E/E410N,
Y155F/K247N/N249S/N260S/R318Y/R338E/R403E/E410N,
D104N/K106S/R318Y/R338E/T343R/R403E/E410N,
R318Y/R338E/N346Y/R403E/E410N,
R318Y/R338E/T343R/N346Y/R403E/E410N,
R318Y/R338E/T343R/N346D/R403E/E410N,
R318Y/R338E/Y345A/R403E/E410N,
K228N/I251S/R318Y/R338E/R403E/E410N,
R318Y/R338E/Y345A/N346D/R403E/E410N,
Y155F/K247N/N249S/R318Y/R338E/R403E,
K247N/N249S/R318Y/R338E/R403E,
Y155F/K247N/N249S/R318Y/R403E/E410N,
K247N/N249S/R318Y/R403E/E410N, R318Y/R338E/T343R/R403E,
Y155F/R318Y/R338E/T343R/E410N, R318Y/T343R/R403E/E410N,
Y155F/R318Y/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
Y155F/K228N/I251S/R318Y/R338E/R403E/E410N,
N260S/R318Y/R338E/T343R/R403E/E410N,
Y155F/N260S/R318Y/R338E/T343R/R403E/E410N,

K228N/K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/R403E, Y155F/K247N/N249S/R318Y/E410N,
K247N/N249S/R318Y/R338E/T343R/E410N,
Y155F/K247N/N249S/R318Y/R338E/T343R/E410N,
Y155F/K247N/N249S/R318Y/T343R/R403E/E410N,
K247N/N249S/R318Y/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/T343R/R403E,
K247N/N249S/R318Y/T343R/R403E,
Y155F/K247N/N249S/R318Y/T343R/E410N,
K247N/N249S/R318Y/T343R/E410N, Y155F/R318Y/R338E/T343R,
Y155F/R318Y/T343R/R403E, Y155F/R318Y/T343R/E410N,
K228N/K247N/N249S/R318Y/R338E/T343R/E410N,
Y155F/K247N/N249S/R318Y/T343R,
Y155F/K247N/N249S/R318Y/R338E/R403E/E410N,
K228N/K247N/N249S/R318Y/T343R/R403E/E410N and
Y155F/R318Y/R403E/E410N, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

21. The modified FIX polypeptide of any of claims 1-20 that comprises amino acid replacements R318Y/R338E/R403E/E410N,
R318Y/R338E/T343R/R403E/E410N, R318Y/R338E/T343R/E410N,
Y155F/R318Y/R338E/T343R/R403E,
Y155F/K228N/K247N/N249S/R318Y/R338E/T343R/R403E/E410N,
Y155F/K247N/N249S/R318Y/R338E/T343R/R403E,
K247N/N249S/R318Y/R338E/T343R/R403E, R318Y/R338E/T343R,
Y155F/K247N/N249S/R318Y/R338E/T343R,
K228N/R318Y/R338E/T343R/R403E/E410N,
K228N/K247N/N249S/R318Y/R338E/T343R/R403E,
R318Y/R338E/T343R/R403E/E410S, Y155F/K247N/N249S/R318Y/R338E,
K247N/N249S/R318Y/R338E/T343R, R318Y/T343R/E410N or
Y155F/R318Y/R338E/R403E.

22. The modified FIX polypeptide of any of claims 1-21 that comprises amino acid replacements R318Y/R338E/R403E/E410N,

R318Y/R338E/T343R/R403E/E410N or R318Y/R338E/T343R/E410N.

23. The modified FIX polypeptide of claim 3 or 4, comprising an amino acid replacement at an amino acid residue selected from among D203, F205 and K228, or at an amino acid residue corresponding to amino acid residue D203, F205 or K228 in an unmodified FIX polypeptide.

24. The modified FIX polypeptide of any of claims 1-23, comprising an amino acid replacement selected from among D203N, F205T and K228N, or the same amino acid replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

25. The modified FIX polypeptide of any of claims 1-24, comprising the amino acid replacement D203N/F205T/R318Yor K228N/R318Y, or the same replacements at a corresponding amino acid residue in an unmodified FIX polypeptide.

26. The modified FIX polypeptide of any of claims 1-25 that comprises an amino acid replacement at amino acid position Y155 or at a residue corresponding to 155 that is selected from F or L.

27. The modified FIX polypeptide of any of claims 1-26 that comprises the amino acid replacement Y155F or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

28. The modified FIX polypeptide of claim 26 or claim 27 that further comprises an amino acid replacement at residue 103, 104, 105, 106, 228, 251, 260 or 346 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or at an amino acid residue corresponding to 103, 104, 105, 106, 228, 251, 260 or 346 in an unmodified FIX polypeptide.

29. The modified FIX polypeptide of any of claims 1-28 that comprises an amino acid replacement selected from among A103N, D104N, N105S, K106S, K228N, I251S, N260S and N346D, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

30. The modified FIX polypeptide of any of claims 1-29 that comprises amino acid replacements selected from among Y155F/K228N, Y155F/I251S, D104N/K106S/Y155F/K228N, Y155F/N346D, Y155F/N260S, Y155F/N260S/N346D, D104N/K106S/Y155F/N260S,

D104N/K106S/Y155F/K228N, A103N/N105S/Y155F and D104N/K106S/Y155F, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

31. The modified FIX polypeptide of claim 26 or claim 27 that further comprises an amino acid replacement at a residue selected from among 338, 403 and 410 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or at an amino acid residue corresponding to 338, 403 or 410 in an unmodified FIX polypeptide.

32. The modified FIX polypeptide of any of claims 1-31 that comprises an amino acid replacement selected from among R338E, R403E and E410N, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

33. The modified FIX polypeptide of any of claims 1-32 that comprises amino acid replacements selected from among Y155F/R338E/T343R/R403E/E410N, Y155F/R338E/T343R/R403E, Y155F/R338E/T343R/R403E/E410S, Y155F/N260S/R338E/T343R/R403E, Y155F/T343R/R403E/E410N, Y155F/R338E/T343R/E410N and Y155F/R338E/R403E/E410N.

34. The modified FIX polypeptide of any of any of claims 1-33 that comprises the amino acid replacement Y155F and amino acid replacements at amino acid positions 247 and/or 249 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3.

35. The modified FIX polypeptide of any of claims 1-34 that comprises the amino acid replacement Y155F/K247N/N249S.

36. The modified FIX polypeptide of any of claims 1-35 that comprises amino acid replacements Y155F/K247N/N249S/R338E/R403E/E410N, D104N/K106S/Y155F/K228N/K247N/N249S, D104N/K106S/Y155F/K247N/N249S, Y155F/K228N/K247N/N249S, Y155F/K247N/N249S/N260S, D104N/K106S/Y155F/K247N/N249S/N260S, D104N/K106S/Y155F/K247N/N249S, Y155F/K247N/N249S/T343R/R403E, Y155F/K247N/N249S/R338E/T343R/R403E/E410N, Y155F/K247N/N249S/R338E/R403E, Y155F/K247N/N249S/R338E/T343R,

Y155F/K247N/N249S/R338E/E410N,
Y155F/K247N/N249S/R338E/T343R/R403E,
Y155F/K247N/N249S/R338E/T343R/E410N,
Y155F/K247N/N249S/T343R/R403E/E410N, Y155F/K247N/N249S/T343R/E410N and Y155F/K247N/N249S/R403E/E410N, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

37. The modified FIX polypeptide of any of claims 1-36 that comprises amino acid replacements Y155F/K247N/N249S/R338E/R403E/E410N.

38. The modified FIX polypeptide of any of claims 1-37 that comprises the amino acid replacement at T343 or at a residue corresponding to 343 that is selected from among R, E and D.

39. The modified FIX polypeptide of claim 38 that comprises an amino acid replacement T343R or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

40. The modified FIX polypeptide of claim 38 or claim 39 that comprises an amino acid replacement at an amino acid residue selected from among R338, Y345 and N346, or at the corresponding amino acid residue in an unmodified FIX polypeptide.

41. The modified FIX polypeptide of any of claims 1-40, comprising an amino acid replacement R338E, Y345T, N346Y or N346D, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

42. The modified FIX polypeptide of any of claims 1-41, comprising amino acid replacements T343R/Y345T, T343R/N346D, T343R/N346Y or R338E/T343R, or the same replacements at a corresponding amino acid residue in an unmodified FIX polypeptide.

43. The modified FIX polypeptide of any of claims 1-42 that comprises amino acid replacements K247N/N249S/R338E/T343R/R403E/E410N, R338E/T343R/E410N, Y155F/I251S/R338E/T343R/R403E, R338E/T343R/R403E/E410N, R338E/T343R/R403E, K247N/N249S/R338E/T343R/R403E or K247N/N249S/R338E/T343R/E410N.

44. The modified FIX polypeptide of any of claims 1-43 that comprises amino acid replacements K247N/N249S/R338E/T343R/R403E/E410N,

R338E/T343R/E410N or Y155F/I251S/R338E/T343R/R403E.

45. The modified FIX polypeptide of claim 38 or claim 39, comprising an amino acid replacement at an amino acid residue selected from among K247, N249, R403 and E410, or at an amino acid residue corresponding to amino acid residue K247, N249, R403 or E410 in an unmodified FIX polypeptide.

46. The modified FIX polypeptide of any of claims 1-45, comprising an amino acid replacement selected from among K247N, N249S, R403E and E410N or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

47. The modified FIX polypeptide of any of claims 1-46, comprising the amino acid replacement K247N/N249S/T343R/R403E/E410N.

48. The modified FIX polypeptide of any of claims 1-47, comprising amino acid replacements at one or more positions corresponding to 1, 5, 53, 61, 64, 85, 103, 104, 105, 106, 108, 148, 155, 157, 158, 159, 167, 169, 172, 179, 202, 202, 203, 204, 205, 228, 239, 241, 243, 247, 249, 251, 257, 259, 260, 262, 265, 284, 293, 312, 314, 315, 316, 317, 318, 319, 320, 321, 333, 338, 343, 345, 346, 392, 394, 400, 403, 410, 412 and 413 of a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or to a corresponding position in an unmodified FIX polypeptide.

49. The modified FIX polypeptide of any of claims 1-48, comprising an amino acid replacement selected from among Y1N, K5A, S53A, S61A, D64A, D64N, D85N, A103N, D104N, N105S, N105T, K106N, K106S, K106T, V108S, V108T, T148A, Y155F, Y155H, N157D, N157Q, S158A, S158D, S158E, T159A, N167D, N167Q, T169A, T172A, T179A, V202M, V202Y, D203M, D203Y, D203N, A204M, A204Y, F205S, F205T, K228N, E239N, E239S, E239A, E239R, E239K, T241N, H243S, H243T, K247N, N249S, N249T, I251S, H257F, H257Y, H257E, H257S, N260S, A262S, A262T, Y284N, K293E, K293A, R312Q, R312A, R312Y, R312L, F314N, H315S, K316S, K316T, K316M, G317N, R318E, R318Y, R318N, R318A, S319N, A320S, L321N, L321S, L321T, R333A, R333E, R338A, R338E, F342I, T343R, T343E, T343Q, Y345A, Y345T, N346D, N346T, K392N, K394S, K394T, K400A, K400E, R403A, R403E, E410Q, E410S, E410N, E410A, E410D, T412V, T412A and K413N, or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

50. The modified FIX polypeptide of any of claims 1-49, comprising amino acid replacements selected from replacements R318E/R403E, E410N/T412A or E410N/T412V or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

51. A modified Factor IX (FIX) polypeptide, comprising two amino acid replacements in unmodified FIX polypeptide, wherein:

the first amino acid replacement is at an amino acid residue selected from among 53, 61, 64, 85, 103, 104, 105, 106, 108, 155, 158, 159, 167, 169, 172, 179, 202, 203, 204, 205, 228, 239, 241, 243, 247, 249, 251, 257, 259, 260, 262, 265, 284, 293, 312, 314, 315, 316, 317, 318, 319, 321, 333, 338, 343, 345, 346, 392, 394, 400, 403, 410, 412 and 413 in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3; or a corresponding amino acid residue in an unmodified FIX polypeptide, wherein corresponding amino acid residues are identified by alignment of the unmodified FIX polypeptide with the polypeptide of SEQ ID NO:3; and

the second amino acid replacement is at an amino acid residue selected from among 5, 53, 61, 64, 85, 155, 158, 159, 167, 239, 260, 284, 293, 312, 318, 333, 338, 346, 400, 403, 410, 412 and 413 in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3, or a corresponding amino acid residue in an unmodified FIX polypeptide, wherein corresponding amino acid residues are identified by alignment of the unmodified FIX polypeptide with the polypeptide of SEQ ID NO:3.

52. The modified FIX polypeptide of claim 51, wherein the first amino acid replacement is replacement with an amino acid residue selected from among alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); cysteine (Cys, C); glutamic acid (Glu, E); glutamine (Gln, Q); glycine (Gly, G); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y); and valine (Val, V); wherein

the replacing amino acid is not the same as the amino acid it replaces.

53. The modified FIX polypeptide claim 51 or claim 52, wherein the first amino acid replacement is replacement with an amino acid residue selected from among alanine (Ala, A); asparagine (Asn, N); aspartic acid (Asp, D), glutamic acid (Glu, E); glutamine (Gln, Q); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L);

lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); serine (Ser, S); threonine (Thr, T); tyrosine (Tyr, Y); and valine (Val, V), or a conservative amino acid replacement.

54. The modified FIX polypeptide of any of claims 51-53, wherein the first amino acid replacement is selected from among S53A, S61A, D64A, D64N, D85N, A103N, D104N, N105S, K106S, K106N, V108S, Y155F, Y155H, Y155Q, S158A, S158D, S158E, T159A, N167D, N167Q, T169A, T172A, T179A, V202M, V202Y, D203M, D203Y, A204M, A204Y, K228N, E239A, E239N, E239S, E239R, E239K, T241N, H243S, K247N, N249S, I251S, H257F, H257E, H257F, H257Y, H257S, Y259S, N260S, A262S, K265T, Y284N, K293E, K293A, R312Q, R312A, R312Y, R312L, F314N, H315S, K316S, K316N, K316A, K316E, K316S, K316M, G317N, R318A, R318E, R318Y, R318N, S319N, A320S, L321S, K321N, R333A, R333E, R333S, R338A, R338E, R338L, F342I, T343R, T343E, T343Q, Y345A, Y345T, N346D, N346Y, K392N, K394S, K400A, K400E, R403A, R403E, E410Q, E410N, E410D, E410S, E410A, T412A, T412V and K413N, or a conservative amino acid replacement or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

55. The modified FIX polypeptide of any of claims 51-52, wherein the second amino acid replacement is replacement with an amino acid residue selected from among alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); cysteine (Cys, C); glutamic acid (Glu, E); glutamine (Gln, Q); glycine (Gly, G); histidine (His, H); isoleucine (Ile, I); leucine (Leu, L); lysine (Lys, K); methionine (Met, M); phenylalanine (Phe, F); proline (Pro, P); serine (Ser, S); threonine (Thr, T); tryptophan (Trp, W); tyrosine (Tyr, Y); and valine (Val, V); wherein

the replacing amino acid is not the same as the amino acid it replaces.

56. The modified FIX polypeptide of any of claims 51-55, wherein the second amino acid replacement is replacement with an amino acid residue selected from among alanine (Ala, A); arginine (Arg, R); asparagine (Asn, N); aspartic acid (Asp, D); glutamic acid (Glu, E); glutamine (Gln, Q); histidine (His, H); leucine (Leu, L); lysine (Lys, K); phenylalanine (Phe, F); serine (Ser, S); threonine (Thr, T); tyrosine (Tyr, Y); and valine (Val, V), or a conservative amino acid replacement.

57. The modified FIX polypeptide of any of claims 51-56, wherein the second amino acid replacement is selected from among K5A, S53A, S61A, D64A, D64N, D85N, Y155F, Y155H, Y155Q, S158A, S158D, S158E, T159A, N167D, N167Q, E239A, E239N, E239S, E239R, E239K, N260S, Y284N, K293E, K293A, R312Q, R312A, R312Y, R312L, R318A, R318E, R318Y, R318N, R333A, R333E, R333S, R338A, R338E, R338L, N346D, N346Y, K400A, K400E, R403A, R403E, E410Q, E410N, E410D, E410S, E410A, T412A, T412V and K413N, or a conservative amino acid replacement or the same replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

58. The modified FIX polypeptide of any of claims 51-57, wherein the first amino acid replacement is at a position selected from among 318, 155, 247, 249, 338, 403 and 410 or at a corresponding amino acid residue in an unmodified FIX polypeptide.

59. The modified FIX polypeptide of any of claims 51-58, wherein the first amino acid replacement is selected from among R318Y, Y155F, K247N, N249S, R338E, R403E and E410N or is the same amino acid replacement at a corresponding amino acid residue in an unmodified FIX polypeptide.

60. The modified FIX polypeptide of any of claims 51-59, wherein the second amino acid replacement is at a position selected from among 338, 155, 247, 249, 318, 403 and 410, or is at a corresponding amino acid residue in an unmodified FIX polypeptide.

61. The modified FIX polypeptide of any of claims 51-60, wherein the second amino acid replacement is selected from among R338E, Y155F, K247N, N249S, R318Y, R403E and E410N or is the same replacement at a corresponding amino acid residue.

62. The modified FIX polypeptide of any of claims 51-61, comprising amino acid replacements selected from among amino acid replacements K400E/R403E, D85N/K228N, D85N/I251S, K400A/R403A, R338A/R403A, R338E/R403E, K293A/R403A, K293E/R403E, R318A/R403A, R338E/E410N, K228N/E410N, K228N/R338E, K228N/R338A and R403E/E410N, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

63. The modified FIX polypeptide of any of claims 51-62, comprising a further amino acid replacement at a position selected from among 53, 61, 64, 85, 103, 104, 105, 106, 108, 155, 158, 159, 167, 169, 172, 179, 202, 203, 204, 205, 228, 239, 241, 243, 247, 249, 251, 257, 259, 260, 262, 265, 284, 293, 312, 314, 315, 316, 317, 318, 319, 321, 333, 338, 343, 346, 345, 392, 394, 400, 403, 410, 412 and 413 in a mature FIX polypeptide having a sequence set forth in SEQ ID NO:3.

64. The modified FIX polypeptide of claim 62 or claim 63, wherein a further amino acid replacement corresponds to an amino acid replacement selected from among Y1N, Y5A, S53A, S61A, D64A, D64N, D85N, A103N, D104N, N105S, K106S, K106N, V108S, Y155F, Y155H, Y155Q, S158A, S158D, S158E, T159A, N167D, N167Q, T169A, T172A, T179A, V202M, V202Y, D203M, D203Y, A204M, A204Y, K228N, E239A, E239N, E239S, E239R, E239K, T241N, H243S, K247N, N249S, I251S, H257F, H257E, H257Y, H257S, Y259S, N260S, A262S, K265T, Y284N, K293E, K293A, R312Q, R312A, R312Y, R312L, F314N, H315S, K316S, K316N, K316A, K316E, K316S, K316M, G317N, R318A, R318E, R318Y, R318N, S319N, A320S, L321S, R333A, R333E, R333S, R338A, R338E, R338L, F342I, T343R, T343E, T343Q, Y345A, Y345T, N346D, N346Y, K392N, K394S, K400A, K400E, R403A, R403E, E410Q, E410N, E410D, E410S, E410A, T412A, T412V and K413N, or the same replacements at corresponding amino acid residues in an unmodified FIX polypeptide.

65. The modified FIX polypeptide of any of claims 51-64, comprising amino acid replacements selected from among amino acid replacements corresponding to replacements D85N/D203N/F205T, D85N/D104N/K106S/I251S, K293A/R338A/R403A, K293E/R338E/R403E, R338E/R403E/E410N, D203N/F205T/K228N, D203N/F205T/E410N, D203N/F205T/R338E, D203N/F205T/R338A, D203N/F205T/R338E/R403E, K228N/R338E/R403E, K247N/N249S/N260S, D104N/K106S/N260S, D104N/K106S/K228N/K247N/N249S, A103N/N105S/K228N, D104N/K106S/K228N, A103N/N105S/I251S, D104N/K106S/I251S, A103N/N105S/K247N/N249S, D104N/K106S/K247N/N249S, K228N/K247N/N249S, D104N/K106S/N260S, Y259F/K265T/Y345T, D104N/K106S/K247N/N249S/N260S and K247N/N249S/R338E/R403E/E410N.

66. The modified FIX polypeptide of any of claims 1-65, comprising one or more modifications that introduces and/or eliminates one or more glycosylation sites compared to the unmodified FIX polypeptide.

67. The modified FIX polypeptide of claim 66, wherein the glycosylation sites are selected from among, N-, O- and S-glycosylation sites.

68. The modified FIX polypeptide of claim 66 or claim 67, wherein 1, 2, 3, 4, 5, 6, 7, 8 or more glycosylation sites are introduced.

69. The modified FIX polypeptide of any of claims 1-66 that exhibits increased resistance to antithrombin III and/or heparin compared with the unmodified FIX polypeptide.

70. The modified FIX polypeptide of claim 69, wherein the modified FIX polypeptide exhibits at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more increased resistance to antithrombin III and/or heparin compared with the unmodified FIX polypeptide.

71. The modified FIX polypeptide of any of claims 1-70 that exhibits increased catalytic activity compared with the unmodified FIX polypeptide.

72. The modified FIX polypeptide of claim 71, wherein the increased catalytic activity is in the presence of FVIIIa.

73. The modified FIX polypeptide of claim 72, wherein the increased catalytic activity is in the absence of FVIIIa.

74. The modified FIX polypeptide of any of claims 69-73, wherein catalytic activity of the modified FIX polypeptide is at least about or is 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more greater than the catalytic of an unmodified FIX polypeptide.

75. The modified FIX polypeptide of any of claims 1-74 that exhibits improved pharmacokinetic properties compared with the unmodified FIX polypeptide.

76. The modified FIX polypeptide of claim 75, wherein the improved pharmacokinetic properties are selected from among one or more of decreased clearance, altered volume of distribution, increased *in vivo* recovery, increased total

modified FIX polypeptide exposure *in vivo*, increased serum half-life, and increased mean resonance time (MRT) compared to the unmodified FIX polypeptide.

77. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property increased serum half-life, and the serum half life is α, β, or γ phase.

78. The modified FIX polypeptide of claim 76 or claim 77, wherein the improved pharmacokinetic property is decreased clearance, and clearance of the modified FIX polypeptide is decreased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of the clearance of an unmodified FIX polypeptide.

79. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property is altered volume of distribution, and the volume of distribution is increased or decreased.

80. The modified FIX polypeptide of claim 79, wherein the altered volume of distribution is decreased volume of distribution, and the volume of distribution of the modified FIX polypeptide is decreased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of the volume of distribution of an unmodified FIX polypeptide.

81. The modified FIX polypeptide of claim 79, wherein the altered volume of distribution is increased volume of distribution, and the volume of distribution of the modified FIX polypeptide is increased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more of the volume of distribution of an unmodified FIX polypeptide.

82. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property is increased *in vivo* recovery, and the *in vivo* recovery of the modified FIX polypeptide is increased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more

of the *in vivo* recovery of an unmodified FIX polypeptide.

83. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property is increased total modified FIX polypeptide exposure *in vivo*, and the total modified FIX polypeptide exposure *in vivo* is increased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more of the total exposure *in vivo* of an unmodified FIX polypeptide.

84. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property is increased serum half-life, and the serum half-life is increased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more of the serum half-life of an unmodified FIX polypeptide.

85. The modified FIX polypeptide of claim 76, wherein the improved pharmacokinetic property is increased MRT, and the MRT is increased by at least or about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more of the MRT *in vivo* of an unmodified FIX polypeptide.

86. The modified FIX polypeptide of any of claims 1-85 that exhibits increased procoagulant activity compared with the unmodified FIX polypeptide.

87. The modified FIX polypeptide of claim 86, wherein the procoagulant activity of the modified FIX polypeptide is at least about or is 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500% or more greater than the procoagulant activity of an unmodified FIX polypeptide.

88. The modified FIX polypeptide of any of claims 1-87, wherein the unmodified FIX polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 2, 3, 20 or 325, or a sequence having at least 60% sequence identity with the FIX polypeptide set forth in any of SEQ ID NOS: 2, 3, 20 or 325, or a sequence that is an active fragment of a FIX polypeptide set forth in any SEQ ID NOS: 2, 3, 20 or 325.

89. The modified FIX polypeptide of any of claims 1-88, wherein the unmodified FIX polypeptides comprises a sequence having at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with the FIX polypeptide set forth in any of SEQ ID NOS: 2, 3, 20 or 325.

90. The modified FIX polypeptide of any of claims 1-89, wherein the unmodified FIX polypeptide has a sequence of amino acids set forth in SEQ ID NO:2, 3, 20 or 325.

91. A modified FIX polypeptide, comprising a sequence of amino acids set forth in any of SEQ ID NOS: 76, 84-87, 90-91, 95-97, 100, 102, 104-105, 113, 121-122, 125-126, 129-134, 138, 146-173, 175-176, 178-185, 188, 193-194, 196-198, 200, 202-206, 208-210, 212, 215-272, 326-41 and 413-417, or a sequence of amino acids that exhibits at least 60% amino acid sequence identity with a sequence of amino acids set forth in any of SEQ ID NOS: 76, 84-87, 90-91, 95-97, 100, 102, 104-105, 113, 121-122, 125-126, 129-134, 138, 146-173, 175-176, 178-185, 188, 193-194, 196-198, 200, 202-206, 208-210, 212, 215-272, 326-41 and 413-417.

92. The modified FIX polypeptide of claim 91, comprising a sequence of amino acids that has at least or about 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to the modified FIX polypeptide set forth in any of SEQ ID NOS: 76, 84-87, 90-91, 95-97, 100, 102, 104-105, 113, 121-122, 125-126, 129-134, 138, 146-173, 175-176, 178-185, 188, 193-194, 196-198, 200, 202-206, 208-210, 212, 215-272, 326-41 and 413-417.

93. The modified FIX polypeptide of claim 91, wherein the modified FIX polypeptide comprises a sequence of amino acids set forth in any of SEQ ID NOS: 76, 84-87, 90-91, 95-97, 100, 102, 104-105, 113, 121-122, 125-126, 129-134, 138, 146-173, 175-176, 178-185, 188, 193-194, 196-198, 200, 202-206, 208-210, 212, 215-272, 326-41 and 413-417.

94. The modified FIX polypeptide of any of claims 1-93 that is a human polypeptide.

95. The modified FIX polypeptide of any of claims 1-94 that is a non-human polypeptide.

96. The modified FIX polypeptide of any of claims 1-95 that is a mature polypeptide.

97. The modified FIX polypeptide of any of claims 1-96 that is a two-chain polypeptide.

98. The modified FIX polypeptide of any of claims 1-97 that is a single-chain polypeptide.

99. The modified FIX polypeptide of any of claims 1-98 that is active or activated.

100. The modified FIX polypeptide of claim 99, wherein activation is effected by proteolytic cleavage by Factor XI (FXIa) or FVIIa.

101. The modified FIX polypeptide of claim 99, wherein activiation is effected by proteolytic cleavage by the Tissue Factor/Factor VIIa complex.

102. The modified FIX polypeptide of any of claims 1-101, wherein only the primary sequence is modified.

103. The modified FIX polypeptide of any of claims 1-101, further comprising a chemical modification or a post-translational modification.

104. The modified FIX polypeptide of claim 103, wherein the modified FIX polypeptide is glycosylated, carboxylated, hydroxylated, sulfated, phosphorylated, albuminated, or conjugated to a polyethylene glycol (PEG) moiety.

105. The modified FIX polypeptide of any of claims 1-104 that is a chimeric or fusion protein.

106. The modified FIX polypeptide of any of claims 1-105, comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more modifications.

107. The modified FIX polypeptide of any of claims 1-106, comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50 or 60 or more modifications, so long as the polypeptide exhibits at least one FIX activity of the unmodified FIX polypeptide.

108. The modified FIX polypeptide of claim 103, wherein the modified FIX polypeptide exhibits at least about or 1%, 2%, 3%, 4%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, 200%, 300%, 400%, 500%, or more of an activity of the unmodified FIX polypeptide.

109. The modified FIX polypeptide of claim 107 or claim 108, wherein the one or more activities are selected from among Factor VIIIa binding, Factor X binding, phospholipid binding, and/or coagulant activity of a FIX polypeptide.

110. The modified FIX polypeptide of any of claims 107-109, wherein the

activity is increased compared to the unmodified FIX polypeptide.

111. The modified FIX polypeptide of any of claims 107-109, wherein the activity is decreased compared to the unmodified FIX polypeptide.

112. The modified FIX polypeptide of any of claims 107-111, wherein the activity is measured *in vitro*, *ex vivo* or *in vivo*.

113. A nucleic acid molecule, comprising a sequence of nucleotides encoding a modified FIX polypeptide of any of claims 1-112.

114. A vector, comprising the nucleic acid molecule of claim 113.

115. The vector of claim 114, wherein the vector is a prokaryotic vector, viral vector, or a eukaryotic vector.

116. The vector of claim 114 or claim 115, wherein the vector is a mammalian vector.

117. The vector of claim 114 or claim 115, 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.

118. A cell, comprising the vector of any of claims 110-113.

119. The cell of claim 118, that is a eukaryotic cell.

120. The cell of claim 119, wherein the eukaryotic cell is a mammalian cell.

121. The cell of claim 120, wherein the mammalian cell is selected from among baby hamster kidney cells (BHK-21) or 293 cells or CHO cells.

122. The cell of any of claims claim 118-121, wherein the cell expresses the modified FIX polypeptide.

123. A modified FIX polypeptide that is produced by the cell of any of claims 118-122.

124. A pharmaceutical composition, comprising a modified FIX polypeptide of any of claims 1-112 and claim 123, in a pharmaceutically acceptable vehicle.

125. The pharmaceutical composition of claim 124 that is formulated for local, systemic, or topical administration.

126. The pharmaceutical composition of claim 124 that is formulated for oral, nasal, pulmonary, buccal, transdermal, subcutaneous, intraduodenal, enteral,

parenteral, intravenous, or intramuscular administration.

127. The pharmaceutical composition of any of claims 124-126 that is formulated for controlled-release.

128. The pharmaceutical composition of any of claims 124-127 that is formulated for single-dosage administration.

129. A method, comprising treating a subject by administering the pharmaceutical composition of any of claims 124-128, wherein the subject has a disease or condition that is treated by administration of FIX or a procoagulant.

130. The method of claim 129, wherein the disease or condition is treated by administration of active FIX (FIXa).

131. The method of claim 129 or claim 130, wherein treatment with the pharmaceutical composition ameliorates or alleviates the symptoms associated with the disease or condition.

132. The method of any of claims 129-131, further comprising monitoring the subject for changes in the symptoms associated with disease or condition that is treated by administration of FIX or a procoagulant.

133. The method of any of claims 129-132, wherein the disease or condition to be treated is selected from among blood coagulation disorders, hematologic disorders, hemorrhagic disorders, hemophilias, and bleeding disorders.

134. The method of claim 133, wherein the hemophilia is hemophilia B.

135. The method of any of claims 129-134, further comprising administering one or more additional coagulation factors.

136. The method of claim 135, wherein the one or more additional coagulation factors are selected from among plasma purified or recombinant coagulation factors, procoagulants, such as vitamin K, vitamin K derivative and protein C inhibitors, plasma, platelets, red blood cells and corticosteroids.

137. An article of manufacture, comprising packaging material and the pharmaceutical composition of any of claims 124-128 contained within the packaging material.

138. The article of manufacture of claim 133, wherein:

the modified FIX polypeptide is effective for treatment of a disease treatable by administration of FIX or a procoagulant, and the packaging material includes a

label that indicates that the modified FIX polypeptide is used for treatment of a disease treatable by administration of FIX or a procoagulant.

139. A kit, comprising the pharmaceutical composition of any of claims 124-128, a device for administration of the composition and, optionally, instructions for administration.

140. A modified FIX polypeptide of any of claims 1-112 and claim 123 for use in treating a disease or condition that is treated by administration of FIX or a pro-coagulant.

141. Use of a pharmaceutical composition of any of claims 124-128 in the preparation of a medicament for treatment of a disease or condition that is treated by administration of FIX or a pro-coagulant.

142. The modified FIX polypeptide of claim 140 or the use of claim 141, wherein the disease or condition is treated by administration of active FIX (FIXa).

143. The modified FIX polypeptide of claim 140 or the use of claim 141, wherein treatment ameliorates or alleviates the symptoms associated with the disease or condition.

144. The modified FIX polypeptide of any of claims 140 or 142 or 143, or the use of any of claims 141-143, further comprising monitoring the subject for changes in the symptoms associated with disease or condition that is treated by administration of FIX or a procoagulant.

145. The modified FIX polypeptide of any of claims 140 or 142-144, or the use of any of claims 141-144, wherein the disease or condition to be treated is selected from among blood coagulation disorders, hematologic disorders, hemorrhagic disorders, hemophilias, and bleeding disorders.

146. The modified FIX polypeptide of any of claims 140 or 142-145, or the use of any of claims 141-145, wherein the hemophilia is hemophilia B.

147. The modified FIX polypeptide of any of claims 140 or 142-146, or the use of any of claims 141-146, further comprising administering one or more additional coagulation factors.

148. The modified FIX polypeptide of use of claim 147, wherein the one or more additional coagulation factors are selected from among plasma purified or recombinant coagulation factors, procoagulants, such as vitamin K, vitamin K

derivative and protein C inhibitors, plasma, platelets, red blood cells and corticosteroids.

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