CLAIMS

What is claimed is:

1. A method of increasing the hydrodynamic size or hydrodynamic volume of a polypeptide of interest or a fragment thereof, the method comprising attaching one to ten chorionic gonadotrophin carboxy terminal peptides (CTPs) to said polypeptide or a fragment thereof, wherein attaching said one to ten CTPs to said polypeptide or fragment thereof results in increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof by about 28-53 kDa per each CTP attached, thereby increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof.
2. The method of claim 1, wherein said one to ten CTPs are attached to the N-terminus of said polypeptide.
3. The method of claim 1, wherein said one to ten CTPs are attached to the C-terminus of said polypeptide.
4. The method of claim 1, wherein two or more CTPs of said one to ten CTPs are attached to the N-terminus and two or more CTPs of said one to ten CTPs are attached to the C-terminus of said polypeptide.
5. The method of claim 1, wherein one CTP is attached to the N-terminus of said polypeptide and two CTPs are attached to the C-terminus of said polypeptide.
6. The method of claim 1, wherein two CTPs are attached to the N-terminus of said polypeptide and two CTPs are attached to the C-terminus of said polypeptide.
7. The method of any one of claims 1 to 6, wherein said CTP is or said CTPs are glycosylated.
8. The method of claim 7, wherein said glycosylation is O-glycosylation.
9. The method of claim 8, wherein said O-glycosylation is a GalNAc attachment to serine (Ser) or threonine (Thr) in the protein chain by an α-glycosidic linkage.
10. The method of claim 8, wherein said O-glycosylation is core 1 glycosylation, O-fucosylation, O-mannosylation, or O-glycosylation.
11. The method of any one of claims 8 to 10, wherein said O-glycosylation is followed by the addition of one to sixty galactose molecules.

12. The method of any one of claims 8 to 11, wherein said O-glycosylation is followed by the addition of one to 120 sialic acid molecules.
13. The method of any one of claims 1 to 12, wherein attaching one glycosylated CTP contributes from about 28.0 to about 53.0 kDa to said polypeptide or fragment thereof.
14. The method of any one of claims 1 to 6, wherein said CTP is not glycosylated.
15. The method of any one of claims 1 to 14, wherein said polypeptide or fragment thereof is erythropoietin (EPO), human growth hormore (hGH), apolipoprotein-A1 (APO-A1), a coagulation factor, or oxyntomodulin (OXM).
16. The method of claim 15, wherein said coagulation factor is Factor IX (FIX) or Factor VIIa (FVIIa).
17. The method of any one of claims 1 to 16, wherein increasing said hydrodynamic volume increases the retention time of said protein in a biological sample.
18. The method of claim 17, wherein said biological sample is blood, cerebro-spinal fluid (CSF), lymph, or sera.
19. The method of any one of claims 1 to 18, wherein increasing said hydrodynamic volume increases the bioavailability of said protein.
20. The method of any one of claims 1 to 19, wherein increasing said hydrodynamic volume increases the bioactivity of said protein.
21. The method of any one of claims 1 to 20, wherein increasing said hydrodynamic volume reduces the administration frequency of said polypeptide or fragment thereof.
22. The method of any one of claims 1 to 21, wherein increasing said hydrodynamic volume extends the area under the curve (AUC) of said polypeptide or fragment thereof.
23. The method of any one of claims 1 to 22, wherein the amino acid sequence of at least one of said one to ten CTPs is selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.
24. The method of any one of claims 1 to 23, wherein at least one of said one to ten CTPs is truncated.
25. The method of any one of claims 1 to 24, wherein at least one of said one to ten CTPs is attached to said polypeptide via a linker.
26. The method of claim 25, wherein said linker is a peptide bond.

27. A method of increasing the apparent molecular weight of a polypeptide or a fragment thereof, the method comprising the step of increasing the hydrodynamic volume according to any one of claims 1 to 26.

28. A method of increasing the serum half-life of a polypeptide or a fragment thereof by the method comprising increasing the hydrodynamic volume of said polypeptide or fragment thereof, according to any one of claims 1 to 26.

29. A method of increasing the hydrodynamic size or hydrodynamic volume of a polypeptide or a fragment thereof, the method comprising attaching between one to ten chorionic gonadotrophin carboxy terminal peptides (CTPs) to said polypeptide or fragment thereof, wherein attaching said one to ten CTPs to said polypeptide or fragment thereof results in increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof by an amount that is dependent on the particular polypeptide or fragment thereof to which the CTP is attached, thereby increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof.

30. The method of claim 29, wherein said one to ten CTPs are attached to the N-terminus of said polypeptide.

31. The method of claim 29, wherein said one to ten CTPs are attached to the C-terminus of said polypeptide.

32. The method of claim 29, wherein one or more of said one to ten CTPs are attached to the N-terminus and one or more of said one to ten CTPs are attached to the C-terminus of said polypeptide.

33. The method of claim 29, wherein one CTP is attached to the N-terminus of said polypeptide and two CTPs are attached to the C-terminus of said polypeptide.

34. The method of claim 29, wherein two CTPs are attached to the N-terminus of said polypeptide and two CTPs are attached to the C-terminus of said polypeptide.

35. The method of any one of claims 29 to 34, wherein said CTP is or said CTPs are glycosylated.

36. The method of claim 35, wherein said glycosylation is O-glycosylation.

37. The method of claim 36, wherein said O-glycosylation is a GalNAc attachment to serine (Ser) or threonine (Thr) in the protein chain by an α-glycosidic linkage.

38. The method of claim 36, wherein said O-glycosylation is core 1 glycosylation, O-fucosylation, O-mannosylation, or O-glycosylation.
39. The method of any one of claims 36 to 38, wherein said O-glycosylation is followed by the addition of one to sixty galactose molecules.
40. The method of any one of claims 36 to 39, wherein said O-glycosylation is followed by the addition of one to 120 sialic acid molecules.
41. The method of any one of claims 29 to 34, wherein said CTP is not glycosylated.
42. The method of any one of claims 29-41, wherein said polypeptide is erythropoietin (EPO), human growth hormore (hGH), apolipoprotein-A1 (APO-A1), Factor IX (FIX), Factor VIIa (FVIIa) or Oxyntomodulin (OXM).
43. The method of claim 42, wherein one CTP contributes about 31 kDa to said EPO.
44. The method of claim 42, wherein one CTP contributes about 29 kDa to said hGH.
45. The method of claim 42, wherein one CTP contributes at least about 39 kDa to said APO-A1.
46. The method of claim 42, wherein one CTP contributes about 51 kDa to said FIX.
47. The method of claim 42, wherein one CTP contributes about 47 kDa to said FVIIa.
48. The method of claim 42, wherein one CTP contributes about 16 kDa to said EPO.
49. The method of claim 42, wherein one CTP contributes about 8 kDa to said hGH.
50. The method of claim 42, wherein one CTP contributes at least about 21 kDa to said APO-A1.
51. The method of claim 42, wherein one CTP contributes about 20 kDa to said FIX.
52. The method of claim 42, wherein one CTP contributes about 20 kDa to said FVIIa.
53. The method of any one of claims 29 to 52, wherein the amino acid sequence of at least one of said one to ten CTPs is selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.
54. The method of any one of claims 29 to 53, wherein at least one of said one to ten CTPs is truncated.
55. The method of any one of claims 29 to 54, wherein at least one of said one to ten CTPs is attached to said polypeptide via a linker.
56. The method of claim 55, wherein said linker is a peptide bond.
57. The method of any one of claims 29 to 56, wherein increasing said hydrodynamic volume increases the retention time of said protein in a biological sample.

58. The method of claim 57, wherein said biological sample is blood, cerebro-spinal fluid (CSF), lymph, or sera.
59. The method of any one of claims 29 to 58, wherein increasing said hydrodynamic volume increases the bioavailability of said protein.
60. The method of any one of claims 29 to 59, wherein increasing said hydrodynamic volume increases the bioactivity of said protein.
61. The method of any one of claims 29 to 60, wherein increasing said hydrodynamic volume reduces the administration frequency of said polypeptide or fragment thereof.
62. The method of any one of claims 29 to 61, wherein increasing said hydrodynamic volume extends the area under the curve (AUC) of said polypeptide or fragment thereof.
63. A method of increasing the apparent molecular weight of a polypeptide or a fragment thereof, the method comprising the step of increasing the hydrodynamic volume according to any one of claims 29 to 62.
64. A method of increasing the serum half-life of a polypeptide or a fragment thereof by the method comprising increasing the hydrodynamic volume of said polypeptide or fragment thereof any one of claims 29 to 63.

Claims

1. A method of increasing the hydrodynamic size or hydrodynamic volume of a polypeptide of interest or a fragment thereof, the method comprising attaching one to ten chorionic gonadotrophin carboxy terminal peptides (CTPs) to said polypeptide or a fragment thereof, wherein attaching said one to ten CTPs to said polypeptide or fragment thereof results in increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof by about 28-53 kDa per each CTP attached, thereby increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof.
2. The method of claim 1, wherein said one to ten CTPs are attached to the N-terminus of said polypeptide, or wherein said one to ten CTPs are attached to the C-terminus of said polypeptide, or wherein two or more CTPs of said one to ten CTPs are attached to the N-terminus and two or more CTPs of said one to ten CTPs are attached to the C-terminus of said polypeptide, or wherein one CTP is attached to the N-terminus of said polypeptide and two CTPs are attached to the C-terminus of said polypeptide, or wherein two CTPs are attached to the N-terminus of said polypeptide and two CTPs are attached to the C-terminus of said polypeptide.
3. The method of any one of claims 1 to 2 wherein said CTP is or said CTPs are glycosylated.
4. The method of claim 3, wherein said glycosylation is O-glycosylation.
5. The method of claim 4, wherein said O-glycosylation is a GalNAc attachment to serine (Ser) or threonine (Thr) in the protein chain by an α-glycosidic linkage.
6. The method of claim 5, wherein said O-glycosylation is core 1 glycosylation, O-fucosylation, O-mannosylation, or O-glycosylation.
7. The method of any one of claims 4 to 6, wherein said O-glycosylation is followed by the addition of one to sixty galactose molecules, or wherein said O-glycosylation is followed by the addition of one to 120 sialic acid molecules, or a combination thereof.
8. The method of any one of claims 1 to 7, wherein attaching one glycosylated CTP contributes from about 28.0 to about 53.0 kDa to said polypeptide or fragment thereof.
9. The method of any one of claims 1 to 2, wherein said CTP is not glycosylated.
10. The method of any one of claims 1 to 9, wherein said polypeptide or fragment thereof is erythropoietin (EPO), human growth hormone (hGH), apolipoprotein-A1 (APO-A1), Factor IX (FIX), Factor VII (FVII), Factor VIIa (FVIIa), or oxyntomodulin (OXM).
11. The method of any one of claims 1 to 10, wherein the amino acid sequence of at least one of said one to ten CTPs is selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

12. The method of any one of claims 1 to 11, wherein at least one of said one to ten CTPs is truncated.
13. The method of any one of claims 1 to 12, wherein at least one of said one to ten CTPs is attached to said polypeptide via a linker.
14. The method of claim 13, wherein said linker is a peptide bond.
15. The method of any one of claims 1-14, wherein said polypeptide or fragment thereof is human growth hormone (hGH) and wherein the amino acid sequence of the CTP-modified human growth hormone (hGH) polypeptide comprises amino acids 27-301 set forth in SEQ ID NO: 11 or a sequence at least 95% homologous thereto.
16. The method of any one of claims 1-14, wherein said polypeptide or fragment thereof is Factor VII or Factor VIIa and the amino acid sequence of the CTP-modified Factor VII or Factor VIIa coagulation factor polypeptide comprises amino acids 39-528 set forth in SEQ ID NO: 52 or a sequence at least 95% homologous thereto.
17. The method of any one of claims 1-14, wherein said polypeptide or fragment thereof is Factor IX and the amino acid sequence of the CTP-modified Factor IX coagulation factor polypeptide comprises amino acids 47-545 set forth in SEQ ID NO: 61 or a sequence at least 95% homologous thereto.
18. The method of any one of claims 1-14, wherein said polypeptide or fragment thereof is erythropoietin (EPO) and the amino acid sequence of the CTP-modified erythropoietin (EPO) polypeptide comprises amino acids 48-277 set forth in SEQ ID NO: 21 or a sequence at least 95% homologous thereto.
19. The method of any one of claims 1-14, wherein said polypeptide or fragment thereof is oxyntomodulin (OXM) and the amino acid sequence of said oxyntomodulin (OXM) polypeptide comprises SEQ ID NO: 18 or a sequence at least 95% homologous thereto.
20. The method of any one of claims 1-14, wherein said polypeptide or fragment thereof is human growth hormone (hGH) and the amino acid sequence of the CTP-modified human growth hormone (hGH) polypeptide comprises SEQ ID NO: 11 or a sequence at least 95% homologous thereto.
21. The method of any one of claims 1-14, wherein said polypeptide or fragment thereof is Factor VII or Factor VIIa coagulation factor and the amino acid sequence of the CTP-modified Factor VII or Factor VIIa coagulation factor polypeptide comprises SEQ ID NO: 52 or a sequence at least 95% homologous thereto.
22. The method of any one of claims 1-14, wherein said polypeptide or fragment thereof is Factor IX coagulation factor and the amino acid sequence of the CTP-modified Factor IX coagulation factor polypeptide comprises SEQ ID NO: 61 or a sequence at least 95% homologous thereto.

23. The method of any one of claims 1-14, wherein said polypeptide or fragment thereof is erythropoietin (EPO) and the amino acid sequence of the CTP-modified erythropoietin (EPO) polypeptide comprises SEQ ID NO: 21 or a sequence at least 95% homologous thereto.
24. The method of any one of claims 1-14 or 20-23, wherein said polypeptide comprises a signal peptide.
25. The method of any one of claims 1 to 24, wherein increasing said hydrodynamic volume increases the retention time of said polypeptide in a biological sample or increases the bioavailability of said polypeptide, or increases the bioactivity or said polypeptide, or reduces the administration frequency of said polypeptide, or reduces the administration frequency of said polypeptide, or extends the area under the curve (AUC) of said polypeptide, or a combination thereof.
26. The method of claim 25, wherein said biological sample is blood, cerebro-spinal fluid (CSF), lymph, or sera.
27. A method of increasing the apparent molecular weight of a polypeptide or a fragment thereof, the method comprising the step of increasing the hydrodynamic volume according to any one of claims 1 to 26.
28. A method of increasing the serum half-life of a polypeptide or a fragment thereof by the method comprising increasing the hydrodynamic volume of said polypeptide or fragment thereof, according to any one of claims 1 to 27.
29. A method of increasing the hydrodynamic size or hydrodynamic volume of a polypeptide or a fragment thereof, the method comprising attaching between one to ten chorionic gonadotrophin carboxy terminal peptides (CTPs) to said polypeptide or fragment thereof, wherein attaching said one to ten CTPs to said polypeptide or fragment thereof results in increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof by an amount that is dependent on the particular polypeptide or fragment thereof to which the CTP is attached, thereby increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof.
30. The method of claim 29, wherein said one to ten CTPs are attached to the N-terminus of said polypeptide or wherein said one to ten CTPs are attached to the C-terminus of said polypeptide, or wherein one or more of said one to ten CTPs are attached to the N-terminus and one or more of said one to ten CTPs are attached to the C-terminus of said polypeptide, or wherein one CTP is attached to the N-terminus of said polypeptide and two CTPs are attached to the C-terminus of said polypeptide, or wherein two CTPs are attached to the N-terminus of said polypeptide and two CTPs are attached to the C-terminus of said polypeptide.
31. The method of any one of claims 29 to 30, wherein said CTP is or said CTPs are glycosylated.

32. The method of claim 31, wherein said glycosylation is O-glycosylation.
33. The method of claim 32, wherein said O-glycosylation is a GalNAc attachment to serine (Ser) or threonine (Thr) in the protein chain by an α-glycosidic linkage, or wherein said O-glycosylation is core 1 glycosylation, O-fucosylation, O-mannosylation, or O-glycosylation.
34. The method of any one of claims 32 to 33, wherein said O-glycosylation is followed by the addition of one to sixty galactose molecules, or wherein said O-glycosylation is followed by the addition of one to 120 sialic acid molecules, or a combination thereof.
35. The method of any one of claims 29 to 30, wherein said CTP is not glycosylated.
36. The method of any one of claims 29-35, wherein said polypeptide is erythropoietin (EPO), human growth hormore (hGH), apolipoprotein-A1 (APO-A1), Factor IX (FIX), Factor VII (FVII), Factor VIIa (FVIIa), or Oxyntomodulin (OXM).
37. The method of claim 36, wherein one CTP contributes about 31 kDa to said EPO.
38. The method of claim 36, wherein one CTP contributes about 29 kDa to said hGH.
39. The method of claim 36, wherein one CTP contributes at least about 39 kDa to said APO-A1.
40. The method of claim 36, wherein one CTP contributes about 51 kDa to said FIX.
41. The method of claim 36, wherein one CTP contributes about 47 kDa to said FVIIa.
42. The method of claim 36, wherein one CTP contributes about 16 kDa to said EPO.
43. The method of claim 36, wherein one CTP contributes about 8 kDa to said hGH.
44. The method of claim 36, wherein one CTP contributes at least about 21 kDa to said APO-A1.
45. The method of claim 36, wherein one CTP contributes about 20 kDa to said FIX.
46. The method of claim 36, wherein one CTP contributes about 20 kDa to said FVIIa.
47. The method of any one of claims 32 to 46, wherein the amino acid sequence of at least one of said one to ten CTPs is selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.
48. The method of any one of claims 29 to 47, wherein at least one of said one to ten CTPs is truncated.
49. The method of any one of claims 29 to 48, wherein at least one of said one to ten CTPs is attached to said polypeptide via a linker.
50. The method of claim 49, wherein said linker is a peptide bond.
51. The method of any one of claims 29-36, 38, 43 or 47-50, wherein said polypeptide or fragment thereof is human growth hormone (hGH) and the amino acid sequence of the CTP-modified human growth hormone (hGH) polypeptide comprises amino acids 27-301 set forth in SEQ ID NO: 11 or a sequence at least 95% homologous thereto.
52. The method of any one of claims 29-36, 41, 46 or 47-50, wherein said polypeptide or fragment thereof is Factor VII or Factor VIIa coagulation factor and the amino acid sequence

of the CTP-modified Factor VII or Factor VIIa coagulation factor polypeptide comprises amino acids 39-528 set forth in SEQ ID NO: 52 or a sequence at least 95% homologous thereto.

53. The method of any one of claims 29-36, 40, 45 or 47-50, wherein said polypeptide or fragment thereof is Factor IX coagulation factor and the amino acid sequence of the CTP-modified Factor IX coagulation factor polypeptide comprises amino acids 47-545 set forth in SEQ ID NO: 61 or a sequence at least 95% homologous thereto.
54. The method of any one of claims 29-36, 37, 42 or 47-50, wherein said polypeptide or fragment thereof is erythropoietin (EPO) and the amino acid sequence of the CTP-modified erythropoietin (EPO) polypeptide comprises amino acids 48-277 set forth in SEQ ID NO: 21 or a sequence at least 95% homologous thereto.
55. The method of any one of claims 29-36, or 47-50, wherein said polypeptide or fragment thereof is oxyntomodulin (OXM) and the amino acid sequence of said oxyntomodulin (OXM) polypeptide comprises SEQ ID NO: 18 or a sequence at least 95% homologous thereto.
56. The method of any one of claims 29-36, 38, 43, 47-50, wherein said polypeptide or fragment thereof is human growth hormone (hGH) and the amino acid sequence of said CTP-modified human growth hormone (hGH) polypeptide comprises SEQ ID NO: 11 or a sequence at least 95% homologous thereto.
57. The method of claim 56, wherein said polypeptide or fragment thereof is Factor VII or Factor VIIa coagulation factor and the amino acid sequence of said CTP-modified Factor VII or Factor VIIa coagulation factor polypeptide comprises SEQ ID NO: 52 or a sequence at least 95% homologous thereto.
58. The method of claim 56, wherein said polypeptide or fragment thereof is Factor IX coagulation factor and the amino acid sequence of said CTP-modified Factor IX coagulation factor polypeptide comprises SEQ ID NO: 61 or a sequence at least 95% homologous thereto.
59. The method of claim 56, wherein said polypeptide or fragment thereof is erythropoietin (EPO) and the amino acid sequence of the CTP-modified erythropoietin (EPO) polypeptide comprises SEQ ID NO: 21 or a sequence at least 95% homologous thereto.
60. The method of any one of claims 29-50 or 56-59, wherein said polypeptide comprises a signal peptide.
61. The method of any one of claims 29 to 60, wherein increasing said hydrodynamic volume increases the retention time of said protein in a biological sample.
62. The method of claim 61, wherein said biological sample is blood, cerebro-spinal fluid (CSF), lymph, or sera.

63. The method of any one of claims 29 to 62, wherein increasing said hydrodynamic volume increases the bioavailability of said polypeptide, or increases the bioactivity of said polypeptide, or reduces the administration frequency of said polypeptide, or extends the area under the curve (AUC) of said polypeptide, or a combination thereof.
64. A method of increasing the apparent molecular weight of a polypeptide or a fragment thereof, the method comprising the step of increasing the hydrodynamic volume according to any one of claims 29 to 63.
65. A method of increasing the serum half-life of a polypeptide or a fragment thereof by the method comprising increasing the hydrodynamic volume of said polypeptide or fragment thereof according to any one of claims 29 to 64.