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 human growth hormone (hGH) polypeptide, the method comprising:

   (a) attaching one chorionic gonadotropin carboxy terminal peptide (CTP) unit to the amino terminus of the hGH polypeptide and two CTP units in tandem to the carboxy terminus of the hGH polypeptide to generate a CTP-modified hGH polypeptide; and

   (b) expressing the CTP-modified hGH polypeptide in a host cell, wherein the expressing comprises glycosylation of the CTP units of the CTP-modified hGH polypeptide,

   wherein the amino acid sequence of the CTP-modified hGH polypeptide is set forth in amino acids 27 to 301 of SEQ ID NO:11, and

   wherein the glycosylated CTP units incrementally increase the hydrodynamic size or hydrodynamic volume of the hGH polypeptide by about 28 to 40 kilodaltons (kDa) per each glycosylated CTP unit.

2. The method of claim 1, wherein the glycosylation is O-glycosylation.

3. The method of claim 2, wherein the O-glycosylation is a GalNAc attachment to serine (Ser) or threonine (Thr) in the protein chain of the CTP-modified hGH polypeptide by an α-glycosidic linkage.

4. The method of claim 2, wherein the O-glycosylation is core 1 glycosylation, O-fucosylation, O-mannosylation, or O-glycosylation.

5. The method of any one of claims 2 to 4, wherein the O-glycosylation is followed by the addition of one to sixty galactose molecules to the CTP-modified hGH polypeptide.

6. The method of any one of claims 2 to 5, wherein the O-glycosylation is followed by the addition of one to 120 sialic acid molecules to the CTP-modified hGH polypeptide.

7. The method of any one of claims 1 to 6, wherein the increasing the hydrodynamic size or hydrodynamic volume

   (a) increases the retention time of the hGH polypeptide in a biological sample;

   (b) increases the bioavailability of the hGH polypeptide;

(c) increases the bioactivity of the hGH polypeptide;

(d) reduces the administration frequency of the hGH polypeptide;

(e) extends the area under the curve (AUC) of the hGH polypeptide; or

(f) any combination thereof.

8. The method of claim 7, wherein the biological sample is blood, cerebro-spinal fluid (CSF), lymph, or sera.

9. The method of any one of claims 1 to 8, wherein the amino acid sequence of at least one of the CTP units is selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

10. The method of any one of claims 1 to 9, wherein at least one of the CTP units is attached to the hGH polypeptide via a linker.

11. The method of claim 10, wherein the linker is a peptide bond.

12. The method of any one of claims 1 to 11, wherein the CTP-modified hGH polypeptide is encoded by the nucleotide sequence set forth in nucleotides 89 to 913 of SEQ ID NO: 16.

13. The method of any one of claims 1 to 12, wherein the host cell is a Chinese Hamster Ovary (CHO) cell.

14. The method of any one of claims 1 to 13, wherein any one of the CTP units contributes about 29 kDa to the hydrodynamic size or hydrodynamic volume of the CTP-modified hGH polypeptide.