CLAIMS

1. An *in vitro* 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 non-glycosylated CTP-modified hGH polypeptide; and

   (b) expressing the non-glycosylated CTP-modified hGH polypeptide in a host cell, wherein the expressing comprises glycosylation of the CTP units of the CTP-modified hGH polypeptide to provide a glycosylated 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 each of the glycosylated CTP units incrementally increases the hydrodynamic size or hydrodynamic volume of the hGH polypeptide by 28 to 40 kilodaltons (kDa).

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

3. The method of claim 2, wherein the O-glycosylation is attachment of a GalNAc attachment to a serine (Ser) residue or threonine (Thr) residue in the protein chain of the non-glycosylated 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, or O-mannosylation.

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

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

7. The method of any one of claims 1 to 6, wherein the CTP-modified hGH polypeptide is present within a biological sample, and wherein

(a) the retention time of the glycosylated CTP-modified hGH polypeptide within the biological sample is greater than the retention time of an unmodified hGH polypeptide;

(b) the bioactivity of the glycosylated CTP-modified hGH polypeptide within the biological sample is greater than the bioactivity of an unmodified hGH polypeptide; or

(c) both (a) and (b).

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 each of the glycosylated CTP units incrementally increases the hydrodynamic size or hydrodynamic volume of the hGH polypeptide by 28 to 29 kDa.

15. The method of any one of claims 1 to 14, wherein each of the glycosylated CTP units incrementally increases the hydrodynamic size or hydrodynamic volume of the hGH polypeptide by 28.9 kDa.

16. The method of any one of claims 1 to 15, wherein each of the glycosylated CTP units incrementally increases the molecular weight of the hGH polypeptide by 5.4 kDa.