METHOD OF INCREASING THE HYDRODYNAMIC VOLUME OF POLYPEPTIDES BY ATTACHING TO GONADOTROPHIN CARBOXY TERMINAL PEPTIDES

FIELD OF INVENTION

[0001] This invention is directed to the use of a chorionic gonadotrophin carboxy terminal peptide (CTP) for increasing the hydrodynamic volume of a polypeptide or a fragment thereof.

BACKGROUND OF THE INVENTION

[0002] Biotechnology products cover an increased proportion of all therapeutic drugs, including monoclonal antibodies, vaccines, growth factors, hormones, cytokines, coagulation factors, fusion proteins, enzymes and other proteins. Other than monoclonal antibodies and vaccines, many on this list possess a molecular mass below 50 kDa and a short terminal half - life that is in the range of minutes to hours.

[0003] The efficacy of protein therapeutics is strongly determined by their pharmacokinetic properties, including their plasma half-lives, which influence distribution and excretion. Although a small size facilitates tissue penetration, these molecules are often rapidly cleared from circulation. Thus, to maintain a therapeutically effective concentration over a prolonged period of time, infusions or frequent administrations are performed, or the drug is applied loco - regional or subcutaneously utilizing a slow adsorption into the blood stream. These limitations of small size protein drugs have led to the development and implementation of half-life extension strategies to prolong circulation of these recombinant antibodies in the blood and thus improve administration and pharmacokinetic as well as pharmacodynamic properties.

[0004] The present invention employs such a strategy in order to increase the hydrodynamic size or volume of proteins of interest or fragments thereof, including peptides, by a particular factor and thereby improve administration, pharmacokinetics as well as pharmacodynamic properties of the same. This increase in hydrodynamic volume is achieved by making use of a peptide-based technology for extending serum half-life of proteins and peptides. This technology is based on using a natural peptide, the C-terminal peptide (CTP) of the beta chain of human chorionic gonadotropin (hCG), which provides hCG with the required longevity to

maintain pregnancy. The beta chain of luteinizing hormone (LH), a fertility hormone that triggers ovulation, is almost identical to hCG but does not include the CTP. As a result, LH has a significantly shorter half-life in blood. Attaching a predetermined number of CTPs to a protein or peptide of interest increases the hydrodynamic volume of the same by a specific factor and results in improved properties that include an enhanced serum half-life and potency of the protein or peptide of interest.

SUMMARY OF THE INVENTION

[0005] In one embodiment, the invention relates to 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 peptide (CTP) to said polypeptide or a fragment thereof, wherein attaching said one to ten CTP peptides 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 of hydrodynamic volume of said polypeptide or fragment thereof.

[0006] In another embodiment, the invention relates to 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 peptide (CTP) said polypeptide or fragment thereof, wherein attaching said one to ten CTP 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 to, thereby increasing the hydrodynamic size of hydrodynamic volume of said polypeptide or fragment thereof.

[0007] Other features and advantages of the present invention will become apparent from the following detailed description examples and figures. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, the inventions of which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0009] **Figure 1** shows an SDS-PAGE analysis of six different purified CTP-modified proteins and their corresponding native proteins. **1.** CTP-hGH-CTP-CTP (MOD-4023), **2.** Biotropin (rhGH), **3.** Size marker, **4.** CTP-EPO-CTP-CTP, **5.** CTP-CTP-EPO, **6.** CTP-CTP-EPO-CTP-CTP, **7.** EPREX® (rEPO), **8.** Size marker, **9.** APO-A1, **10.** Size marker, **11.** Apo-CTP, **12.** Apo-CTP-CTP, **13.** Size marker.

[00010] **Figure 2** shows an SDS-PAGE analysis of five different purified CTP-modified proteins and their corresponding native proteins. **1.** FIX –CTP-CTP-CTP **2.** size marker **3.** FIX-CTP-CTP-CTP- CTP **4.** FIX –CTP-CTP-CTP-CTP-CTP **5.** Mononine® (rFIX) **6.** size marker **7.** FVIIa –CTP-CTP-CTP **8.** FVIIa –CTP-CTP-CTP-CTP-CTP **9.** size marker.

[00011] **Figure 3** shows increment of molecular weight (kDa) of one copy of CTP of both non-glycosylated (A) and glycosylated (B) CTP-modified proteins as was measured by MALDI-TOF.

[00012] **Figure 4** shows increment of hydrodynamic size of glycosylated CTP-modified proteins compared to their corresponding native proteins, measured by SEC-HPLC. (A) exhibits the total increment of hydrodynamic size, while (B) exhibits the calculated increment per one copy of glycosylated CTP.

[00013] **Figure 5** shows increment of hydrodynamic size of non-glycosylated CTP-modified proteins compared to their corresponding native proteins, measured by SEC-HPLC column. (A) exhibits the total increment of hydrodynamic size, while (B) exhibits the calculated increment per one copy of non-glycosylated CTP.

DETAILED DESCRIPTION OF THE INVENTION

[00014] In one embodiment, provided herein is a method of increasing the hydrodynamic volume or hydrodynamic size of a polypeptide of interest or fragment thereof, the method comprising the step of fusing the polypeptide or fragment thereof to at least one chorionic gonadotropin C-terminal peptide (CTP) on either the N-terminus or C-terminus of the polypeptide or fragment thereof.

[00015] In one embodiment, the terms “protein” and “polypeptide” are used interchangeably herein. In another embodiment, the terms "polypeptide of interest or fragment thereof", or "protein of interest or fragment thereof" encompass native polypeptides (either degradation products, synthetically synthesized polypeptides or recombinant polypeptides) and peptidomimetics (typically, synthetically synthesized polypeptides), as well as peptoids and semipeptoids which are polypeptide analogs, which have, in another embodiment, modifications rendering the modified polypeptides provided herein even more stable while in a body or more capable of penetrating into cells. Moreover, the terms include peptides of interest as well. In another embodiment, at least one CTP peptide provided herein is attached to the polypeptides of interest or fragments thereof, or peptides of interest provided herein. In another embodiment, the term “fragment thereof” when in reference to a protein or polypeptide encompasses truncated versions of the protein or polypeptide of interest, including peptides of interest.

[00016] In another embodiment, the term “fragment thereof” of a protein or polypeptide refers to a functional fragment (e.g., a fragment that has biological activity as the parent polypeptide does or enhanced activity as compared to the parent polypeptide). Examples of fragments thereof can include variants of the polypeptide, or peptides derived from the parent polypeptide. Hence, it is to be understood that the terms “fragment thereof” of a protein or polypeptide, and the term “peptide” can be used interchangeably herein.

[00017] In one embodiment, provided herein is a method of increasing the hydrodynamic size or volume of a polypeptide of interest or a fragment thereof by at least about 28 kDa the method comprising the step of fusing the polypeptide or fragment thereof to at least one chorionic gonadotropin C-terminal peptide (CTP) on either the N-terminus or the C-terminus of the polypeptide or fragment thereof.

[00018] In one embodiment, provided herein is 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 peptide (CTP) to said polypeptide or a fragment thereof, wherein attaching said one to ten CTP peptides to said polypeptide or fragment thereof results in increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof. In another embodiment, the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof is increased by about 28-53 kDa per each glycosylated CTP attached to said polypeptide or fragment thereof, thereby increasing the hydrodynamic size of hydrodynamic volume of said polypeptide or fragment thereof. In another embodiment, the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof is increased by about 8.0-22 kDa per each non-glycosylated CTPattached to said polypeptide or fragment thereof, thereby increasing the hydrodynamic size of hydrodynamic volume of said polypeptide or fragment thereof. In another embodiment, the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof is increased by about 8.1-21.6 kDa per each non-glycosylated CTPattached to said polypeptide or fragment thereof, thereby increasing the hydrodynamic size of hydrodynamic volume of said polypeptide or fragment thereof.

[00019] In another embodiment, provided herein is 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 peptide (CTP) said polypeptide or fragment thereof, wherein attaching said one to ten CTP 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 to, thereby increasing the hydrodynamic size of hydrodynamic volume of said polypeptide or fragment thereof.

[00020] In one embodiment, said one to ten CTPs are attached to the N-terminus of said polypeptide. In another embodiment, said one to ten CTPs are attached to the C-terminus of said polypeptide. In another embodiment, said one to ten CTPs are attached to both, the N-terminus and the C-terminus of said polypeptide. In another embodiment, one CTP is attached to the N-terminus of said polypeptide and two CTPs are attached to said C-terminus of said polypeptide. In another embodiment, two CTPs are attached to the N-terminus of said polypeptide and two CTPs are attached to said C-terminus of said polypeptide.

[00021] In one embodiment, the terms “hydrodynamic size” or “hydrodynamic volume” are used interchangeably herein and each refers to the apparent size of a molecule (e.g., a protein

molecule) based on the diffusion of the molecule through an aqueous solution. The diffusion, or motion of a protein through solution can be processed to derive an apparent size of the protein, where the size is given by the "stokes radius" or "hydrodynamic radius" of the protein particle. The "hydrodynamic size" of a protein depends on both mass and shape (conformation), such that two proteins having the same molecular mass may have differing hydrodynamic sizes based on the overall conformation of the protein.

[00022] In another embodiment, the type of glycosylation is O-glycosylation. In another embodiment, the type of O-glycosylation is the GalNAc attachment to serine (Ser) or threonine (Thr) in the protein chain by an a-glycosidic linkage. In another embodiment, the type of O-glycosylation is the N-acetylglycosamine (GlcNac) attachement to Ser or Thr residues in the protein chain. In another embodiment, the type of O-glycosylation is O-fucosylation, O-mannosylation, core 1 O-glycosylation, core 2 O-glycosylation or O-glucosylation. In another embodiment, the O-glycosylation is mucin-type O-glycosylation. In another embodiment, the O-glycosylation comprises O-linked glycans attached to the hydroxy oxygen of serine, threonine, tyrosine, hydroxylysine, or hydroxyproline side-chains. In another embodiment, the O-glycosylation is followed by the addition of galactose and/or sialic acid, where in other embodiments at least one molecule of galactose is added, and/or at least one molecule of sialic acid is added to the protein of interest following O-glycosylation. In another embodiment, about 1 to 3 galactose molecules are added. In another embodiment, about 1 to 3 sialic acid molecules are added. In another embodiment, about 1 to 5 galactose molecules are added. In another embodiment, about 1 to 5 sialic acid molecules are added. In another embodiment, about 1 to 10 galactose molecules are added. In another embodiment, about 1 to 20 galactose molecules are added. In another embodiment, about 21 to 30 galactose molecules are added. In another embodiment, about 31 to 40 galactose molecules are added. I In another embodiment, about 41 to 50 galactose molecules are added. In another embodiment, about 51 to 60 galactose molecules are added. In another embodiment, about 61 to 70 galactose molecules are added. In another embodiment, about 1 to 10 sialic acid molecules are added. In another embodiment, 2 sialic acid molecules are added per each galactose molecule added. In another embodiment, about 1 to 5 galactose molecules are added per each CTP. In another embodiment, about 1 to 10 sialic acid molecules are added per each CTP. In another embodiment, about 1 to 60 galactose molecules are added and about 1 to 120 sialic acid molecules are added in total per each CTP-modified polypeptide or fragment

thereof. In one embodiment, one to six galactose molecules are added per each CTP. In one embodiment, one to 12 sialic acid molecules are added per each CTP. In another embodiment, one to six galactose molecules and one to 12 sialic acid molecules are added per each CTP.

[00023] In another embodiment, the type of glycosylation provided herein is N-glycosylation. In another embodiment, N-linked glycans are attached to a nitrogen of asparagine or arginine side-chains. The N-linked amino acid consensus sequence is Asn-any Amino acid- Ser or Thr, where any amino acid cannot be proline.

[00024] In another embodiment, provided herein is a method of increasing the hydrodynamic size or hydrodynamic volume of a polypeptide or a fragment thereof, the method comprising attaching at least one non-glycosylated chorionic gonadotrophin carboxy terminal peptide (CTP) to the N-terminus or C-terminus of the polypeptide of interest or fragment thereof, wherein attaching at least one CTP to the polypeptide or fragment thereof results in increasing the hydrodynamic size or hydrodynamic volume of the polypeptide or fragment thereof by an amount that is dependent on the particular polypeptide or fragment thereof to which the CTP is attached to, thereby increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof.

[00025] In one embodiment, glycosylated CTP increase the hydrodynamic volume of a protein to which it is attached or fused to. In another embodiment, non-glycosylated CTP increase the hydrodynamic volume of a protein to which it is attached or fused to.

[00026] In one embodiment, CTP modified proteins that contain glycans in the native portion of the protein contribute a higher increment to the hydrodynamic volume of one copy of glycosylated CTP, for instance, Example 3/Table 5 herein demonstrate that FIX and FVIIa-CTP modified proteins that contain glycans in the native portion of the protein contribute a higher increment to the hydrodynamic volume of one copy of glycosylated CTP.

[00027] It will be appreciated by the skilled artisan, when guided by the present invention's specification, that combinations of glycosylated and non-glycosylated CTPs may be employed for use to increase the hydrodynamic size or volume of polypeptides or fragments thereof provided herein. Such manipulations can be carried out in order to increase the hydrodynamic volume of the polypeptides or fragments thereof to an optimal or desired level. In one embodiment, such optimal or desired level of increase in the hydrodynamic volume is associated with an enhanced retention time in a subject, a low clearance rate from the subject, and an enhanced biological activity of the polypeptide of interest or fragment thereof. In one

embodiment, 1 to 5 glycosylated CTPs and 1 to 5 non-glycosylated CTPs are concurrently attached to a polypeptide or fragment thereof provided herein. In another embodiment, the glycosylated or non-glycosylated CTP peptides are tandemly attached on either the N- or C-terminus or are randomly attached to both N- and C-termini. It will also be appreciated by the skilled artisan that additional combinations of glycosylated and non-glycosylated CTP peptides may be used and are hence encompassed by the present invention.

[00028] In one embodiment, the term "attached" and grammatical variants thereof refers to binding of one protein, polypeptide or peptide to another protein, polypeptide or peptide. In another embodiment, such binding refers to the binding of a protein, polypeptide or peptide of interest to at least one CTP peptide provided herein. In another embodiment, such binding refers to the binding of a protein, polypeptide or peptide of interest to one to ten CTP peptides provided herein. Such binding can be accomplished through numerous means which include but are not limited to covalent binding, hydrogen binding, ionic binding, metallic binding, polar covalent binding, non-covalent binding (van der waals interactions, hydrophobic interactions, hydrogen bonding, etc.), binding through the use of linkers, and the like.

[00029] In one embodiment, provided herein is a method of increasing the hydrodynamic size or hydrodynamic volume of a polypeptide of interest or a fragment thereof, the method comprising attaching at least one non-glycosylated chorionic gonadotrophin carboxy terminal peptide (CTP) to the N-terminus or C-terminus of the polypeptide or fragment thereof, wherein attaching at least one CTP to said polypeptide or fragment thereof results in increasing the hydrodynamic size or hydrodynamic volume of the polypeptide or fragment thereof by an amount that is dependent on the particular polypeptide or fragment thereof to which the CTP is attached to, thereby increasing the hydrodynamic size or hydrodynamic volume of said polypeptide or fragment thereof.

[00030] In another embodiment, the CTP provided herein is deglycosylated using methods known in the art that include, but are not limited to, enzyme-based deglycosylation.

[00031] It will be appreciated by the skilled artisan thatthe terms "non-glycosylation" and "deglycosylation" and grammatical variants thereof are used interchangeably herein.

[00032] In another embodiment, provided herein is a method of increasing the *in-vivo* biological activity, increasing the serum half-life, increasing the bioavailability, increasing the potency, or extending the area under the curve (AUC), etc., as further provided herein, of a polypeptide of interest or fragment thereof, the method comprising the step of fusing at least

one glycosylated CTP peptide to the polypeptide or fragment thereof, wherein fusing the glycosylated CTP peptide to the polypeptide or fragment thereof results in increasing the hydrodynamic volume of the polypeptide or fragment thereof by at least about 28 kDa as compared to the hydrodynamic volume of an unmodified polypeptide or fragment thereof.

[00033] In another embodiment, the CTP-modified polypeptide has lower *in-vitro* biological activity but this lower activity is compensated by an extended half-life. In another embodiment, the CTP-modified polypeptide has an increased *in-vitro* biological activity.

[00034] In another embodiment, increasing the hydrodynamic volume of the polypeptide or fragment thereof provided herein reduces the administration frequency of the polypeptide or fragment thereof. In another embodiment, increasing the hydrodynamic volume of the polypeptide or fragment thereof also increases the apparent molecular weight of the polypeptide or fragment thereof.

[00035] In one embodiment, the apparent molecular weight is determined using methods well known in the art, including, but not limited to, size-exclusion chromatography (SEC), dynamic light scattering methods (DLS), sedimentation velocity, sedimentation equilibrium centrifugation and spectrophotometric detection. In another embodiment, the theoretical molecular weight is determined using a proteomic software available in the art. Such softwares include but are not limited to, Expasy portal, ProteoIQ, Scaffold 3, and the like. In another embodiment, the actual molecular weight is determined using methods well known in the art, including, but not limited to MALDI-TOF.

[00036] In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide of interest or fragment thereof by at least 28 kDa. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 1– 14 kDa, the method comprising attaching to the polypeptide or fragment thereof at least one CTP. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 15 – 27 kDa, the method comprising attaching to the polypeptide or fragment thereof at least one CTP. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 28 – 40.0 kDa, the method comprising attaching to the polypeptide or fragment thereof at least one CTP. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 28 – 55.0 kDa, the method comprising

attaching to the polypeptide or fragment thereof at least one CTP. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 28 – 70.0 kDa, the method comprising attaching to the polypeptide or fragment thereof at least one CTP. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 28 – 80.0 kDa, the method comprising attaching to the polypeptide or fragment thereof at least one CTP. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 28 – 90.0 kDa, the method comprising attaching to the polypeptide or fragment thereof at least one CTP. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 28 – 100.0 kDa, the method comprising attaching to the polypeptide or fragment thereof at least one CTP. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 41.0-50 kDa, the method comprising attaching to the polypeptide or fragment thereof at least one CTP peptide. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 51.0-60 kDa, the method comprising attaching to the polypeptide or fragment thereof at least one CTP peptide. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by about 61.0-70 kDa, the method comprising attaching to the polypeptide or fragment thereof at least one CTP peptide. In another embodiment, the CTP is glycosylated. In another embodiment, the CTP is a non-glycosylated CTP.

[00037] In one embodiment, the methods provided herein unexpectedly demonstrate that subsequent additions of glycosylated CTP peptides to a polypeptide of interest or a fragment thereof linearly contribute about the same apparent molecular weight as a prior attachment of a CTP peptide to the polypeptide of interest or a fragment thereof (see Table 4).

[00038] In another embodiment, at least one glycosylated CTP contributes about 28 to 40 kDa per each glycosylated CTP to the protein of interest. In another embodiment, at least one glycosylated CTP contributes about 28 to 55 kDa per each glycosylated CTP to the protein of interest, regardless of the number of CTP attached.

[00039] In one embodiment, the provided herein is a method of increasing the apparent moleculear weight of a polypeptide of interest or fragment thereof by a factor of about 2 over

the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching one to ten CTP peptide to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by a factor of about 3-5 over the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching at least one CTP peptide to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by a factor of about 6-10 over the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching one to ten CTP peptides to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by a factor of about 11-20 over the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching one to ten CTP peptides to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by a factor of about 21-30 over the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching one to ten CTP peptides to said polypeptide or fragment thereof, the method comprising the step of attaching at least one CTP peptide to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by a factor of about 31-40 over the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching one to ten CTP peptides to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by a factor of about 41-50 over the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching at least one CTP peptides to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by a factor of about 51-60 the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching at one to ten CTP peptides to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by a factor of about 61-70 over the theoretical molecular

weight of the polypeptide or fragment thereof, the method comprising the step of attaching one to ten CTP peptides to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof a factor of about 71-80 over the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching one to ten CTP peptides to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by a factor of about 81-90 over the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching one to ten CTP peptides to said polypeptide or fragment thereof. In another embodiment, provided herein is a method of increasing the apparent molecular weight of a polypeptide or fragment thereof by a factor of about 91-100 over the theoretical molecular weight of the polypeptide or fragment thereof, the method comprising the step of attaching one to ten CTP peptides to said polypeptide or fragment thereof. In another embodiment, the at least one CTP peptide is attached to said polypeptide or fragment thereof. In another embodiment, one to five CTP peptides are attached to said polypeptide or fragment thereof. In another embodiment, one to fifteen CTP peptides are attached to said polypeptide or fragment thereof. In another embodiment, one to twenty CTP peptides are attached to said polypeptide or fragment thereof. In another embodiment, the at least one CTP peptide is glycosylated. In another embodiment, at least one CTP peptide is a non-glycosylated CTP peptide.

[00040] In another embodiment, the method of increasing the biological activity, serum half-life, bioavailability, potency, etc., of a polypeptide of interest or fragment thereof or a peptide of interest, comprises increasing the total hydrodynamic volume of the polypeptide of interest or fragment thereof by about 28 kDa, as compared to an unmodified polypeptide or fragment thereof, by attaching to said polypeptide or fragment thereof a glycosylated CTP. In another embodiment, the method of increasing the biological activity, serum half-life, bioavailability, etc., of a polypeptide of interest or fragment thereof comprises increasing the total hydrodynamic volume of the polypeptide or fragments thereof by about 1-10 kDa, 11-20 kDa, 21-30 kDa, 31-40 kDa, 41-50 kDa, 51-60 kDa, 61-70 kDa, 71-80 kDa, 81-90 kDa, 91-100 kDa, 100-150 kDa, 151-200 kDa, 201-400 kDa, 401-1000 kDa, or 1001-5000 kDa as compared to an unmodified polypeptide of interest or fragment thereof, by attaching to said polypeptide or fragment thereof a CTP provided herein.

[00041] In another embodiment, in the methods provided herein at least one CTP is glycosylated. In another embodiment, in the methods provided herein at least one CTP contributes to the hydrodynamic volume about 28.3 to 38.7 kDa per each CTP to the polypeptide or fragment thereof also provided herein. In another embodiment, the glycosylated CTP contributes 28.3 to 38.7 kDa per each CTP regardless of which polypeptide or fragment thereof it is bound to (see Example 3 herein). In another embodiment, the glycosylated CTP contributes 28.3 to 38.7 kDa per each CTP regardless of the number of CTP peptides attached to the polypeptide (see Example 3 herein). In another embodiment, the contribution of at least one CTP is at least 20 kDa. In another embodiment, the contribution of at least one CTP is at least about 20 kDa to 27.9 kDa. In another embodiment, the contribution of one CTP is at least 28 kDa. In another embodiment, the contribution of one CTP is about 28 kDa to 40 kDa. In another embodiment, the contribution of one CTP is about 41 kDa to 50 kDa. In another embodiment, the contribution of one CTP is about 51 kDa to 60 kDa.

[00042] In another embodiment, the method of increasing the serum half-life of or enhancing the potency of the polypeptide of interest or fragment thereof comprises increasing the hydrodynamic volume of the polypeptide or fragment thereof by about 84 kDa as compared to an unmodified polypeptide or fragment thereof. In another embodiment, increasing the hydrodynamic volume by about 84 kDa corresponds to the polypeptide or fragment thereof being modified by attaching it to 3 glycosylated CTP peptides, according to the methods provided herein. In another embodiment, out of the 3 glycosylated CTP peptides attached to the polypeptide or fragment thereof provided herein, one glycosylated CTP peptide is attached to the N-terminus of the polypeptide or fragment thereof, while 2 glycosylated CTP peptides are tandemly attached to the C-terminus of the polypeptide or fragment thereof.

[00043] In one embodiment, the method of increasing the serum half-life of or the biological activity of the polypeptide of interest or fragment thereof, comprises increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 84 – 159 kDa as compared to an unmodified polypeptide or fragment thereof. In another embodiment, increasing the hydrodynamic size or volume by about 56-110 kDa is achieved by attaching 2 glycosylated CTP peptides to the polypeptide of interest or fragment thereof. In another embodiment, increasing the hydrodynamic size or volume by about 84-162 kDa is achieved

by attaching 3 glycosylated CTP peptides to the polypeptide of interest or fragment thereof. In another embodiment, increasing the hydrodynamic size or volume by about 112-230 kDa is achieved by attaching 4 glycosylated CTP peptides to the polypeptide of interest or fragment thereof. In another embodiment, increasing the hydrodynamic size or volume by about 140-280 kDa is achieved by attaching 5 glycosylated CTP peptides to the polypeptide of interest or fragment thereof. In another embodiment, increasing the hydrodynamic size or volume by about 168-330 kDa is achieved by attaching 6 glycosylated CTP peptides to the polypeptide of interest or fragment thereof. In another embodiment, increasing the hydrodynamic size or volume by about 196-390 kDa is achieved by attaching 7 glycosylated CTP peptides to the polypeptide of interest or fragment thereof. In another embodiment, increasing the hydrodynamic size or volume by about 224-425 kDa is achieved by attaching 8 glycosylated CTP peptides to the polypeptide of interest or fragment thereof. In another embodiment, increasing the hydrodynamic size or volume by about 252-480 kDa is achieved by attaching 9 glycosylated CTP peptides to the polypeptide of interest or fragment thereof. In another embodiment, increasing the hydrodynamic size or volume by about 280-330 kDa is achieved by attaching 10 glycosylated CTP peptides to the polypeptide of interest or fragment thereof.

[00044] In one embodiment, the methods provided herein also comprise attaching a non-glycosylated CTP to a polypeptide of interest or fragment thereof. It is to be understood that various manners of modifying a polyptide of interest or fragment thereof, with a glycosylated and/or non-glycosylated CTP can be carried out. In one embodiment, a polypeptide of interest or a fragment thereof is modified with at least one glycosylated CTP alone, or at least one non-glycosylated CTP alone. In another embodiment, a polypeptide of interest or a fragment thereof is modified with at least one glycosylated CTP and at least one non-glycosylated CTP. In another embodiment, truncated versions of both, glycosylated and/or non-glycosylated CTPs are employed for use in the methods provided herein.

[00045] In another embodiment, provided herein is a method of increasing the biological activity, serum half-life, bioavailability, potency, etc., of a polypeptide of interest or fragment thereof by increasing the hydrodynamic volume of the polypeptide of interest or fragment thereof by a specific amount, the method comprising attaching at least one chorionic gonadotrophin carboxy terminal peptide (CTP) to the N-terminus or C-terminus of the polypeptide, wherein attaching at least one non-glycosylated CTP peptide to the polypeptide of interest or fragment thereof results in increasing the hydrodynamic size or hydrodynamic

volume of the polypeptide or fragment thereof, as compared to an unmodified form of the polypeptide or fragment thereof, and wherein the specific amount is dependent on the polypeptide of interest or fragment thereof to which the non-glycosylated CTP is attached to. In another embodiment when at least one non-glycosylated CTP is attached to the polypeptide of interest or fragment thereof, the polypeptide of interest to which the non-glycosylated CTP is attached to dictates the amount of increase in the apparent molecular weight or hydrodynamic volume. In another embodiment, when one non-glycosylated CTP is attached to hGH, the non-glycosylated CTP contributes about 8 kDa to the hydrodynamic size or hydrodynamic volume of human growth hormone (hGH). In another embodiment, when one non-glycosylated CTP is attached to erythropoeitin (EPO), the non-glycosylated CTP contributes about 16 kDa to the hydrodynamic size or hydrodynamic volume of EPO. In another embodiment, when one non-glycosylated CTP is attached to apolipoprotein-A1 (APO-A1), the non-glycosylated CTP contributes about 21 kDa to the hydrodynamic size or hydrodynamic volume of APO-A1. In another embodiment, when one non-glycosylated CTP is attached to Factor IX (FIX), the non-glycosylated CTP contributes about 20 kDa to the hydrodynamic size or hydrodynamic volume of FIX. In another embodiment, when one non-glycosylated CTP is attached to Factor VIIa (FVIIa), the non-glycosylated CTP contributes about 20 kDa to the hydrodynamic size or hydrodynamic volume of FIX.

[00046] In one embodiment, the method of increasing the hydrodynamic volume of a polypeptide of interest or fragment thereof provided herein enhances the polypeptide's or fragment thereof's bioavailability.

[00047] In another embodiment, the method of increasing the hydrodynamic volume of a polypeptide of interest or fragment thereof provided herein reduces the dosing frequency of the polypeptide of interest or fragment thereof.

[00048] In one embodiment, the polypeptide that is modified by the methods provided herein is a cytokine, a monoclonal antibody, a growth factor, a hormone, a cytokine, a coagulation factor, an enzyme and the like.

[00049] In another embodiment, the polypeptide to which at least one CTP peptide is attached is erythropoietin (EPO), human growth hormone (hGH), apolipoprotein A1 (APO-A1), Factor IIa (FVIIa), Factor IX (FIX) or oxyntomodulin (OXM).

[00050] In one embodiment, at least one non-glycosylated CTP contributes about 16 kDa to EPO. In another embodiment, at least one non-glycosylated CTP contributes about 16 kDA to

the apparent molecular weight of EPO, when attached to the EPO. In another embodiment, at least one non-glycosylated CTP contributes about 8 kDa to hGH. In another embodiment, at least one non-glycosylated CTP contributes about 8 kDA to the apparent molecular weight of hGH, when attached to the hGH. In another embodiment, at least one non-glycosylated CTP contributes about 21 kDa to the APO-A1. In another embodiment, at least one non-glycosylated CTP contributes about 21 kDA to the apparent molecular weight of APO-A1, when attached to the APO-A1.

[00051] In one embodiment, the non-glycosylated CTP contributes a different hydrodynamic volume to each polypeptide to which the non-glycosylated CTP is linked with. In another embodiment, this difference depends on the polypeptide or fragment thereof to which the non-glycosylated CTP is bound to (see Example 3 herein). In another embodiment, the non-glycosylated CTP unexpectedly contributes the same hydrodynamic size per each CTP on each particular polypeptide regardless of the number of non-glycosylated CTP peptides attached to the polypeptide (see Example 3 herein).

[00052] In one embodiment, provided herein is a method of increasing the serum half-life of, or the biological activity of hGH, the method comprising the step of increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 28-40 kDa as compared to an unmodified hGH. In another embodiment, increasing the hydrodynamic size or volume by about 56-80 kDa is achieved by attaching about 2 glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 84-120 kDa is achieved by attaching about 3 glycosylated CTP peptides to the hGH. In another embodiment, increasing the hydrodynamic size or volume by about 112-160 kDa is achieved by attaching about 4 glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 140-200 kDa is achieved by attaching about 5 glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 168-240 kDa is achieved by attaching about 6 glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 196-280 kDa is achieved by attaching about 7 glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 224-320 kDa is achieved by attaching about 8 glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 252-360 kDa is achieved by attaching about 9 glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size

or volume by about 280-400 kDa is achieved by attaching about 10 glycosylated CTP peptides to hGH. In another embodiment, one to ten truncated or partial CTP peptides are attached to the polypeptide of interest or fragment thereof.

[00053] In one embodiment, provided herein is a method of increasing the serum half-life of or the biological activity of EPO, the method comprising the step of increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 28-40 kDa as compared to an unmodified EPO. In another embodiment, increasing the hydrodynamic size or volume by about 56-80 kDa is achieved by attaching 2 glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 84-120 kDa is achieved by attaching 3 glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 112-160 kDa is achieved by attaching 4 glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 140-200 kDa is achieved by attaching 5 glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 168-240 kDa is achieved by attaching 6 glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 196-280 kDa is achieved by attaching 7 glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 224-320 kDa is achieved by attaching 8 glycosylated CTP peptides EPO. In another embodiment, increasing the hydrodynamic size or volume by about 252-360 kDa is achieved by attaching 9 glycosylated CTP peptides EPO. In another embodiment, increasing the hydrodynamic size or volume by about 280-400 kDa is achieved by attaching 10 glycosylated CTP peptides EPO.

[00054] In one embodiment, provided herein is a method of increasing the serum half-life of or the biological activity of APO-A1, the method comprising the step of increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 28-40 kDa as compared to an unmodified APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 56-80 kDa is achieved by attaching 2 glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 84-120 kDa is achieved by attaching 3 glycosylated CTP peptides to the APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 112-160 kDa is achieved by attaching 4 glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 140-200 kDa is achieved by attaching 5 glycosylated

CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 168-240 kDa is achieved by attaching 6 glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 196-280 kDa is achieved by attaching 7 glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 224-320 kDa is achieved by attaching 8 glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 252-360 kDa is achieved by attaching 9 glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 280-400 kDa is achieved by attaching 10 glycosylated CTP peptides to APO-A1.

[00055] In one embodiment, provided herein is a method of increasing the serum half-life of,or the biological activity of FIX, the method comprising the step of increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 48-53 kDa as compared to an unmodified FIX. In another embodiment, increasing the hydrodynamic size or volume by about 96-106 kDa is achieved by attaching 2 glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 144-159 kDa is achieved by attaching 3 glycosylated CTP peptides to the FIX. In another embodiment, increasing the hydrodynamic size or volume by about 192-212 kDa is achieved by attaching 4 glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 240-265 kDa is achieved by attaching 5 glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 288-318 kDa is achieved by attaching 6 glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 336-371 kDa is achieved by attaching 7 glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 384-424 kDa is achieved by attaching 8 glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 432-530 kDa is achieved by attaching 9 glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 480-530 kDa is achieved by attaching 10 glycosylated CTP peptides to FIX.

[00056] In one embodiment, provided herein is a method of increasing the serum half-life of,or the biological activity of FVIIa, the method comprising the step of increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 43-50 kDa as

compared to an unmodified FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 86-100 kDa is achieved by attaching 2 glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 129-150 kDa is achieved by attaching 3 glycosylated CTP peptides to the FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 172-200 kDa is achieved by attaching 4 glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 215-250 kDa is achieved by attaching 5 glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 258-300 kDa is achieved by attaching 6 glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 301-350 kDa is achieved by attaching 7 glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 344-400 kDa is achieved by attaching 8 glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 387-450 kDa is achieved by attaching 9 glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 430-500 kDa is achieved by attaching 10 glycosylated CTP peptides to FVIIA.

[00057] In one embodiment, provided herein is a method of increasing the serum half-life of, or the biological activity of hGH, the method comprising the step of increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 8 kDa as compared to an unmodified hGH. In another embodiment, increasing the hydrodynamic size or volume by about 16 kDa is achieved by attaching 2 non-glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 24 kDa is achieved by attaching 3 non-glycosylated CTP peptides to the hGH. In another embodiment, increasing the hydrodynamic size or volume by about 32 kDa is achieved by attaching 4 non-glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 40 kDa is achieved by attaching 5 non-glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 48 kDa is achieved by attaching 6 non-glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 56 kDa is achieved by attaching 7 non-glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 64 kDa is achieved by attaching 8 non-glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size

or volume by about 72 kDa is achieved by attaching 9 non-glycosylated CTP peptides to hGH. In another embodiment, increasing the hydrodynamic size or volume by about 80 kDa is achieved by attaching 10 non-glycosylated CTP peptides to hGH.

[00058] In one embodiment, provided herein is a method of increasing the serum half-life of or the biological activity of EPO, the method comprising the step of increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 16 kDa as compared to an unmodified EPO. In another embodiment, increasing the hydrodynamic size or volume by about 32 kDa is achieved by attaching 2 non-glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 48 kDa is achieved by attaching 3 non-glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 16 kDa is achieved by attaching 4 non-glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 80 kDa is achieved by attaching 5 non-glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 96 kDa is achieved by attaching 6 non-glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 112 kDa is achieved by attaching 7 non-glycosylated CTP peptides to EPO. In another embodiment, increasing the hydrodynamic size or volume by about 128 kDa is achieved by attaching 8 non-glycosylated CTP peptides EPO. In another embodiment, increasing the hydrodynamic size or volume by about 144 kDa is achieved by attaching 9 non-glycosylated CTP peptides EPO. In another embodiment, increasing the hydrodynamic size or volume by about 160 kDa is achieved by attaching 10 non-glycosylated CTP peptides EPO.

[00059] In one embodiment, provided herein is a method of increasing the serum half-life of or the biological activity of APO-A1, the method comprising the step of increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 21 kDa as compared to an unmodified APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 42 kDa is achieved by attaching 2 non-glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 63-120 kDa is achieved by attaching 3 non-glycosylated CTP peptides to the APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 84 kDa is achieved by attaching 4 non-glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 105 kDa is achieved by attaching 5 non-

glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 126 kDa is achieved by attaching 6 non-glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 147 kDa is achieved by attaching 7 non-glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 168 kDa is achieved by attaching 8 non-glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 189 kDa is achieved by attaching 9 non-glycosylated CTP peptides to APO-A1. In another embodiment, increasing the hydrodynamic size or volume by about 210 kDa is achieved by attaching 10 non-glycosylated CTP peptides to APO-A1.

[00060] In one embodiment, provided herein is a method of increasing the serum half-life of,or the biological activity of FIX, the method comprising the step of increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 20 kDa as compared to an unmodified FIX. In another embodiment, increasing the hydrodynamic size or volume by about 40 kDa is achieved by attaching 2 non-glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 60 kDa is achieved by attaching 3 non-glycosylated CTP peptides to the FIX. In another embodiment, increasing the hydrodynamic size or volume by about 80 kDa is achieved by attaching 4 non-glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 100 kDa is achieved by attaching 5 non-glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 120 kDa is achieved by attaching 6 non-glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 140 kDa is achieved by attaching 7 non-glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 160 kDa is achieved by attaching 8 non-glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 180 kDa is achieved by attaching 9 non-glycosylated CTP peptides to FIX. In another embodiment, increasing the hydrodynamic size or volume by about 200 kDa is achieved by attaching 10 non-glycosylated CTP peptides to FIX.

[00061] In one embodiment, provided herein is a method of increasing the serum half-life of,or the biological activity of FVIIa, the method comprising the step of increasing the hydrodynamic size or volume of the polypeptide or fragment thereof by about 20 kDa as

compared to an unmodified FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 40 kDa is achieved by attaching 2 non-glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 60 kDa is achieved by attaching 3 non-glycosylated CTP peptides to the FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 80 kDa is achieved by attaching 4 non-glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 100 kDa is achieved by attaching 5 non-glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 120 kDa is achieved by attaching 6 non-glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 140 kDa is achieved by attaching 7 non-glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 160 kDa is achieved by attaching 8 non-glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 180 kDa is achieved by attaching 9 non-glycosylated CTP peptides to FVIIA. In another embodiment, increasing the hydrodynamic size or volume by about 200 kDa is achieved by attaching 10 non-glycosylated CTP peptides to FVIIA.

[00062] In one embodiment, the hydrodynamic volume increases the retention time of the protein of interest in a biological sample. In another embodiment, the hydrodynamic volume increases the area under the curve (AUC) of the protein of interest in a biological sample. In another embodiment, the biological sample is blood, target tissues (e.g., join, CNS), cerebro-spinal fluid (CSF), lymph, or sera.

[00063] In another embodiment, increasing the hydrodynamic volume increases the bioavailability of the polypeptide of interest or fragment thereof provided herein. In another embodiment, increasing the hydrodynamic volume of the polypeptide also extends the serum half-life of the polypeptide or fragment thereof.

[00064] In another embodiment, increasing the hydrodynamic volume increases the bioactivity of the polypeptide.

[00065] In another embodiment, the terms “CTP peptide,” “carboxy terminal peptide” and “CTP sequence” are used interchangeably herein. In another embodiment, the carboxy terminal peptide is a full-length CTP. In another embodiment, the carboxy terminal peptide is a truncated CTP. Each possibility represents a separate embodiment of the present invention.

[00066] In another embodiment, a signal peptide is attached to the amino terminus of the CTP, as described in US 7,553,940, which is incorporated by reference herein in its entirety.

[00067] In another embodiment, at least one CTP is attached to the polypeptide via a linker. In another embodiment, the linker is a peptide bond. In another embodiment, the fused protein forms a CTP-modified polypeptide. In one embodiment, the method of increasing a hydrodynamic volume of a polypeptides or fragments thereof comprises fusing the polypeptides or fragments thereof to at least one CTP peptide on the amino or carboxyl terminus of the polypeptides or fragments thereof. In another embodiment, the CTP is recombinantly fused to the polypeptides or fragments thereof. In another embodiment, the CTP is chemically conjugated to the polypeptides or fragments thereof.

[00068] In one embodiment, the CTP-modified polypeptide comprises a peptide that comprises fewer than 50 amino acids and at least one chorionic gonadotrophin carboxy terminal peptide, attached to an N-(amino) or a C- (carboxy) terminus of the peptide.

[00069] In one embodiment, engineered polypeptides of interest of the invention comprising at least a single CTP attached to their N-terminus and/ or C-terminus are at least equivalent to the non CTP modified polypeptides of interest, in terms of biological activity. In other embodiments, engineered polypeptides of interest of the invention comprising at least one CTP attached to their N-terminus and/ or C-terminus are at least equivalent to the non CTP modified polypeptides of interest, in terms of pharmacological measures such as pharmacokinetics and pharmacodynamics.

[00070] In one embodiment, the CTP sequence provided herein comprises: DPRFQDSSSSKAPPPSLPSPSRLPGPSDTPIL (SEQ ID NO: 1). In another embodiment, the CTP sequence comprises: SSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 2). In another embodiment, the CTP sequence comprises an amino acid sequence selected from the sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 2. In yet another embodiment, the CTP sequence is selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

[00071] In one embodiment, the carboxy terminal peptide (CTP) peptide of the present invention comprises the amino acid sequence from amino acid 112 to position 145 of human chorionic gonadotrophin. In another embodiment, the CTP sequence of the present invention comprises the amino acid sequence from amino acid 118 to position 145 of human chorionic gonadotropin, as set forth in SEQ ID NO: 2. In another embodiment, the CTP sequence also commences from any position between positions 112-118 and terminates at position 145 of

human chorionic gonadotrophin. In another embodiment, the CTP sequence peptide is 28, 29, 30, 31, 32, 33 or 34 amino acids long and commences at position 112, 113, 114, 115, 116, 117 or 118 of the CTP amino acid sequence.

[00072] In one embodiment, the truncated CTP comprises the first 10 amino acids of SEQ ID NO: 3. In another embodiment, SEQ ID NO: 3 comprises the following amino acid (AA) sequence: SSSSKAPPPSLP.

[00073] In one embodiment, the truncated CTP comprises the first 11 amino acids of SEQ ID NO: 2. In one embodiment, the truncated CTP comprises the first 12 amino acids of SEQ ID NO: 2. In one embodiment, the truncated CTP comprises the first 8 amino acids of SEQ ID NO: 2 or SEQ ID NO: 3. In one embodiment, the truncated CTP comprises the first 13 amino acids of SEQ ID NO: 2. In one embodiment, the truncated CTP comprises the first 14 amino acids of SEQ ID NO: 2. In one embodiment, the truncated CTP comprises the first 6 amino acids of SEQ ID NO: 2 or SEQ ID NO: 3. In one embodiment, the truncated CTP comprises the first 5 amino acids of SEQ ID NO: 2 or SEQ ID NO: 3.

[00074] In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 1-5 conservative amino acid substitutions as described in U.S. Pat. No. 5,712,122, which is incorporated herein by reference. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 1 conservative amino acid substitution. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 2 conservative amino acid substitutions. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 3 conservative amino acid substitutions. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 4 conservative amino acid substitutions. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 5 conservative amino acid substitutions.

[00075] In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 40% homologous to the native CTP amino acid sequence or a peptide thereof. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 50% homologous to the native CTP amino acid sequence or a peptide thereof. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 60% homologous to the native CTP amino acid sequence or a peptide

thereof. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 70% homologous to the native CTP amino acid sequence or a peptide thereof. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 80% homologous to the native CTP amino acid sequence or a peptide thereof. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 90% homologous to the native CTP amino acid sequence or a peptide thereof. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 95% homologous to the native CTP amino acid sequence or a peptide thereof. In another embodiment, the CTP peptide amino acid sequence of the present invention is at least 98% homologous to the native CTP amino acid sequence or a peptide thereof.

[00076] In another embodiment, the polynucleotide encoding the CTP peptide of the present invention is at least 70% homologous to the native human CTP DNA sequence or a peptide thereof. In another embodiment, the polynucleotide encoding the CTP peptide of the present invention is at least 80% homologous to the native human CTP DNA sequence or a peptide thereof. In another embodiment, the polynucleotide encoding the CTP peptide of the present invention is at least 90% homologous to the native CTP DNA sequence or a peptide thereof. In another embodiment, the polynucleotide encoding the CTP peptide of the present invention is at least 95% homologous to the native CTP DNA sequence or a peptide thereof. In another embodiment, the polynucleotide encoding the CTP peptide of the present invention is at least 98% homologous to the native CTP DNA sequence or a peptide thereof.

[00077] In one embodiment, at least one of the chorionic gonadotrophin CTP amino acid sequences is truncated. In another embodiment, both of the chorionic gonadotrophin CTP amino acid sequences are truncated. In another embodiment, two of the chorionic gonadotrophin CTP amino acid sequences are truncated. In another embodiment, three of the chorionic gonadotrophin CTP amino acid sequences are truncated. In another embodiment, four of the chorionic gonadotrophin CTP amino acid sequences are truncated. In another embodiment, five of the chorionic gonadotrophin CTP amino acid sequences are truncated. In another embodiment, six of the chorionic gonadotrophin CTP amino acid sequences are truncated. In another embodiment, seven of the chorionic gonadotrophin CTP amino acid sequences are truncated. In another embodiment, eight of the chorionic gonadotrophin CTP amino acid sequences are truncated. In another embodiment, two or more of the chorionic

gonadotrophin CTP amino acid sequences are truncated. In another embodiment, all of the chorionic gonadotrophin CTP amino acid sequences are truncated.

[00078] In one embodiment, the CTP peptides provided herein are attached to the polypeptides or fragments thereof provided herein via a linker. In one embodiment, one to ten CTP peptides are attached to the polypeptides or fragments thereof provided herein via a linker. In one embodiment, at least one CTP is attached to the polypeptides or fragments thereof provided herein via a linker. In another embodiment, the linker is a peptide bond.

[00079] In one embodiment, at least one of the chorionic gonadotrophin CTP amino acid sequences is glycosylated. In another embodiment, both of the chorionic gonadotrophin CTP amino acid sequences are glycosylated. In another embodiment, two of the chorionic gonadotrophin CTP amino acid sequences are glycosylated. In another embodiment, three of the chorionic gonadotrophin CTP amino acid sequences are glycosylated. In another embodiment, four of the chorionic gonadotrophin CTP amino acid sequences are glycosylated. In another embodiment, five of the chorionic gonadotrophin CTP amino acid sequences are glycosylated. In another embodiment, two or more of the chorionic gonadotrophin CTP amino acid sequences are glycosylated. In another embodiment, all of the chorionic gonadotrophin CTP amino acid sequences are glycosylated.

[00080] In one embodiment, at least one of the chorionic gonadotrophin CTP amino acid sequences is not glycosylated. In another embodiment, both of the chorionic gonadotrophin CTP amino acid sequences are not glycosylated. In another embodiment, two of the chorionic gonadotrophin CTP amino acid sequences are not glycosylated. In another embodiment, three of the chorionic gonadotrophin CTP amino acid sequences are not glycosylated. In another embodiment, four of the chorionic gonadotrophin CTP amino acid sequences are not glycosylated. In another embodiment, five of the chorionic gonadotrophin CTP amino acid sequences are not glycosylated. In another embodiment, two or more of the chorionic gonadotrophin CTP amino acid sequences are not glycosylated. In another embodiment, all of the chorionic gonadotrophin CTP amino acid sequences are not glycosylated.

[00081] In one embodiment, the glycosylated CTP sequence of the present invention comprises at least one glycosylation site. In another embodiment, the glycosylated CTP sequence of the present invention comprises two glycosylation sites. In another embodiment, the glycosylated CTP sequence of the present invention comprises three glycosylation sites. In another embodiment, the glycosylated CTP sequence of the present invention comprises

four glycosylation sites. In another embodiment, the glycosylated CTP sequence of the present invention comprises five glycosylation sites. In another embodiment, the glycosylated CTP sequence of the present invention comprises six glycosylation sites. In another embodiment, the glycosylated CTP sequence of the present invention comprises seven glycosylation sites. In another embodiment, the glycosylated CTP sequence of the present invention comprises eight glycosylation sites. In another embodiment, the CTP sequence of the present invention comprises from one to four glycosylation sites. In another embodiment, the CTP sequence of the present invention comprises from four to nine glycosylation sites. In another embodiment, the CTP sequence of the present invention comprises from six to twelve glycosylation sites.

[00082] In one embodiment, at least one of the chorionic gonadotrophin CTP amino acid sequences is fully glycosylated. In another embodiment, at least one of the chorionic gonadotrophin CTP amino acid sequences is partially glycosylated. In one embodiment, partially glycosylated indicates that at least one of the CTP glycosylation sites is glycosylated. In another embodiment, the glycosylation sites are O-glycosylation sites. In another embodiment, the glycosylation sites are N-glycosylation sites.

[00083] In one embodiment, the CTP sequence modification is advantageous in permitting the usage of lower dosages when attached to a polypeptide, drug, or agent of interest. In another embodiment, the CTP sequences modification is advantageous in permitting fewer dosages of a polypeptide, drug, or agent of interest. In another embodiment, the CTP sequences modification is advantageous in permitting a safe, long-acting effect when administering a CTP-modified polypeptide, drug, or agent of interest.

[00084] In another embodiment, modifications to polypeptides of interest and CTP peptides herein include, but are not limited to C terminus modification, polypeptide bond modification, including, but not limited to, CH2-NH, CH2-S, CH2-S=O, O=C-NH, CH2-O, CH2-CH2, S=C-NH, CH=CH or CF=CH, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further details in this respect are provided hereinunder.

[00085] In another embodiment, polypeptide bonds (-CO-NH-) within the polypeptide are substituted. In another embodiment, the polypeptide bonds are substituted by N-methylated

bonds (-N(CH3)-CO-). In another embodiment, the polypeptide bonds are substituted by ester bonds (-C(R)H-C-O-O-C(R)-N-). In another embodiment, the polypeptide bonds are substituted by ketomethylen bonds (-CO-CH2-). In another embodiment, the polypeptide bonds are substituted by α-aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH2-NH-). In another embodiment, the polypeptide bonds are substituted by hydroxyethylene bonds (-CH(OH)-CH2-). In another embodiment, the polypeptide bonds are substituted by thioamide bonds (-CS-NH-). In another embodiment, the polypeptide bonds are substituted by olefinic double bonds (-CH=CH-). In another embodiment, the polypeptide bonds are substituted by retro amide bonds (-NH-CO-). In another embodiment, the polypeptide bonds are substituted by polypeptide derivatives (-N(R)-CH2-CO-), wherein R is the "normal" side chain, naturally presented on the carbon atom. In another embodiment, these modifications occur at any of the bonds along the polypeptide chain and in one embodiment at several (2-3 bonds) at the same time.

[00086] In one embodiment, natural aromatic amino acids of the polypeptide such as Trp, Tyr and Phe, are substituted for synthetic non-natural acid such as Phenylglycine, TIC, naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr. In another embodiment, the polypeptides of the present invention include one or more modified amino acid or one or more non-amino acid monomers (e.g. fatty acid, complex carbohydrates etc).

[00087] In one embodiment, "amino acid" or "amino acid sequence" is understood to include the 20 naturally occurring amino acid; those amino acid often modified post-translationally in vivo, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acid including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. In one embodiment, "amino acid" includes both D- and L-amino acids.

[00088] In another embodiment, the engineered polypeptides or peptides of the present invention are biochemically synthesized such as by using standard solid phase techniques. In another embodiment, these biochemical methods include exclusive solid phase synthesis, partial solid phase synthesis, fragment condensation, or classical solution synthesis.

[00089] In one embodiment, recombinant protein techniques are used to generate the engineered polypeptides of interest or fragments thereof of the present invention.. In another embodiment, recombinant techniques are described by Bitter et al., (1987) Methods in

Enzymol. 153:516-544, Studier et al. (1990) Methods in Enzymol. 185:60-89, Brisson et al. (1984) Nature 310:511-514, Takamatsu et al. (1987) EMBO J. 6:307-311, Coruzzi et al. (1984) EMBO J. 3:1671-1680 and Brogli et al., (1984) Science 224:838-843, Gurley et al. (1986) Mol. Cell. Biol. 6:559-565 and Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463, which are incorporated herein by reference in their entirety.

[00090] In another embodiment, the CTP-modified polypeptide of interest or fragment thereof comprises a peptide that comprises fewer than 50 amino acids and at least one glycosylated and/or non-glycosylated chorionic gonadotrophin carboxy terminal peptide, attached to an N- or a C-terminus of the polypeptide. In one embodiment, the CTP-modified polypeptide of interest or fragment thereof provided herein comprises a peptide that comprises fewer than 40 amino acids and at least one chorionic gonadotrophin carboxy terminal peptide, attached to an N- or a C-terminus of the polypeptide. In another embodiment, the CTP-modified polypeptide of interest or fragment thereof provided herein comprises a peptide that comprises fewer than 30, 20, or 10 amino acids. In one embodiment, the polypeptide of interest or fragment thereof comprising fewer than 50 amino acids includes peptides provided herein. In another embodiment, the peptide comprising fewer than 50 amino acids is hGH, OXM, EPO, apolipoprotein A1(APO-A1), an interferon, a cytokine or a coagulation factor.

[00091] In one embodiment the polypeptide of interest or fragment thereof provided herein is an EPO. In one embodiment the polypeptide of interest or fragment thereof provided herein is an APO. In one embodiment the polypeptide of interest or fragment thereof thereof provided herein is an FVIIa. In one embodiment the polypeptide of interest or fragment thereof provided herein is an FIX. In one embodiment the polypeptide of interest or fragment thereof provided herein is an interferon. In another embodiment the polypeptide of interest or fragment thereof provided herein is an hGH. In another embodiment the polypeptide of interest or fragment thereof provided herein is a OXM. In another embodiment the polypeptide of interest or fragment thereof provided herein is a GLP-1. In another embodiment the polypeptide of interest or fragment thereof provided herein is insulin. In another embodiment the polypeptide of interest or fragment thereof provided herein is enkephalin. In another embodiment the polypeptide of interest or fragment thereof provided herein is an ACTH. In another embodiment the polypeptide of interest or fragment thereof

provided herein is a glucagon. In another embodiment the polypeptide of interest or fragment thereof provided herein is an insulin-like growth factor. In another embodiment the polypeptide of interest or fragment thereof provided herein is an epidermal growth factor. In another embodiment the polypeptide of interest or fragment thereof provided herein is an acidic or basic fibroblast growth factor. In another embodiment the polypeptide of interest or fragment thereof provided herein is a platelet-derived growth factor. In another embodiment the polypeptide of interest or fragment thereof provided herein is a granulocyte-CSF. In another embodiment the polypeptide of interest or fragment thereof provided herein is a macrophage-CSF. In another embodiment the polypeptide of interest or fragment thereof provided herein is an IL-2. In another embodiment the polypeptide of interest or fragment thereof provided herein is an IL-3. In another embodiment the polypeptide of interest or fragment thereof provided herein is a tumor necrosis factor. In another embodiment the polypeptide of interest or fragment thereof provided herein is an LHRH. In another embodiment polypeptide of interest or fragment thereof provided herein is an LHRH analog. In another embodiment the polypeptide of interest or fragment thereof provided herein is a somatostatin. In another embodiment the polypeptide of interest or fragment thereof provided herein is a growth hormone releasing factor. In another embodiment the polypeptide of interest or fragment thereof provided herein is an endorphin. In another embodiment the polypeptide of interest or fragment thereof provided herein is an alveolar surfactant protein. In another embodiment the polypeptide of interest or fragment thereof provided herein is a natriuretic factor. In another embodiment the polypeptide of interest or fragment thereof provided herein is an adhesin. In another embodiment the polypeptide of interest or fragment thereof provided herein is an angiostatin. In another embodiment the polypeptide of interest or fragment thereof provided herein is an endostatin. In another embodiment the polypeptide of interest or fragment thereof provided herein is a receptor peptide. In another embodiment the polypeptide of interest or fragment thereof provided herein is a receptor binding ligand. In another embodiment the polypeptide of interest or fragment thereof provided herein is an antibody. In another embodiment the polypeptide of interest or fragment thereof provided herein is an antibody fragment. In another embodiment the polypeptide of interest or fragment thereof provided herein is a peptide or a protein including any modified form.

[00092] In another embodiment, the polypeptide of interest or fragment thereof comprises additionally at least one CTP amino acid peptide attached on the N-terminus and/ or one CTP

amino acid peptide attached on the C-terminus. In another embodiment, the polypeptide of interest or fragment thereof is selected from the following list: insulin, Albutein/albumin, Activase altiplase/tPA, adenosine deaminase, immune globulin, glucocerebrosidase, Leukine-sargramostim/GM-CSF, G-CSF, Venoglobulin-S/IgG, Proleukin aldesleukin, DNase, factor VIII, Helixate, L-asparaginase, WinRho SDF Rh I, Retavase retaplase/tPA, Factor IX, FSH, globulin, fibrin, interleukin-11, becaplermin/PDGF, lepirudin/herudin, TNF, Thymoglobulin, factor VIIa, interferon alpha-2a, interferon alfa n-1, interferon alfa-N3, interferon beta-1b, interferon gamma-1b, Interleukin-2, HGH, or monoclonal antibodies.

[00093] In one embodiment, the polypeptide of interest or fragment thereof provided herein further comprises a signal peptide. In another embodiment, the polypeptide of interest or fragment thereof is a growth hormone. In another embodiment, the growth hormone further comprises a signal peptide. In another embodiment, following expression and secretion, the signal peptide is cleaved from the precursor engineered peptides/polypeptides resulting in the mature engineered peptides/polypeptides. In another embodiment, signal sequences include, but are not limited to the endogenous signal sequences.

[00094] In another embodiment, the polypeptides and methods of the present invention provide a growth hormone having additionally a signal peptide comprising the following amino acid sequence: MATGSRTSLLLAFGLLCLPWLQEGSA (SEQ ID NO: 4).

[00095] In another embodiment, CTP-modified or conjugated growth hormones of this invention are used in the same manner as unmodified growth hormones. In another embodiment, conjugated growth hormones of this invention have an increased circulating half-life and plasma residence time, decreased clearance, and increased clinical activity in vivo. In another embodiment, due to the improved properties of the conjugated growth hormones as described herein, these conjugates are administered less frequently than unmodified growth hormones. In another embodiment, conjugated growth hormones as described herein are administered once a week to once every two weeks. In another embodiment, conjugated growth hormones as described herein are administered once every two weeks to once every three weeks. In another embodiment, conjugated growth hormones as described herein are administered once a day to three times a week. In another embodiment, decreased frequency of administration will result in improved patient compliance leading to improved treatment outcomes, as well as improved patient quality of life. In another embodiment, compared to conventional conjugates of growth hormones linked

to poly(ethylene glycol) it has been found that growth hormone CTP conjugates having the molecular weight and linker structure of the conjugates of this invention have an improved potency, improved stability, elevated area under the curve (AUC) levels, and enhanced circulating half-life. In another embodiment, compared to conventional conjugates of growth hormones linked to poly(ethylene glycol) it has been found that growth hormones having the molecular weight and linker structure of the conjugates of this invention have an improved potency, improved stability, elevated AUC levels, enhanced circulating half-life. In another embodiment, compared to conventional conjugates of growth hormones linked to poly(ethylene glycol) it has been found that growth hormones having the optimal hydrodynamic volume of the conjugates of this invention have an improved potency, improved stability, elevated AUC levels, enhanced circulating half-life. In another embodiment, a therapeutically effective amount of a conjugated growth hormone is the amount of conjugate necessary for the in vivo measurable expected biological activity. In another embodiment, a growth hormone utilized according to the teachings of the present invention exhibits increased potency. In another embodiment, the attachment of CTP sequences to both the N- and C-termini of a growth hormone results in prolonged in-vivo activity.

[00096] In another embodiment, the growth hormone is any growth hormone known to one of skill in the art. In another embodiment, the growth hormone is a human growth hormone. In another embodiment, the nucleotide sequence and/or the amino acid sequence of a growth hormone is available in a gene bank database. In another embodiment, the growth hormone is a homologue of a growth hormone provided herein and/ or of a growth hormone provided in a gene bank database. In another embodiment, a homologue also refers to a deletion, insertion, or substitution variant, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof.

[00097] In another embodiment, the growth hormone is variant of hGH missing exons 2, 3, 4, or any combination thereof. In another embodiment, the growth hormone comprises a signal peptide. In another embodiment, the growth hormone comprises a signal cleavage site. In another embodiment, polypeptides comprising GH modified by CTPs of the present invention comprise recombinant GH.

[00098] In another embodiment, a growth hormone as described herein is a member of the superfamily of growth hormone (GH)-like cytokines. In another embodiment, a growth

hormone as described herein is human growth hormone (hGH). In another embodiment, a human growth hormone comprises the following amino acid sequence (Genbank Accession No. P01241):

MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFE
EAYIPKEQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFL
RSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNS
HNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF (SEQ ID NO: 5).

[00099] In another embodiment, a human growth hormone comprises the following amino acid sequence:

MFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQTSLCFSESI
PTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDL
EEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVE
TFLRIVQCRSVEGSCGF (SEQ ID NO: 6). In another embodiment, a human growth hormone comprises the following amino acid sequence: MFPTIPLSRLFDNAMLRAHRLHQLA (SEQ ID NO: 7). In another embodiment, an hGH comprises the following amino acid sequence:

MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFE
EAYIPKVQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFL
RSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNS
HNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF (SEQ ID NO: 8). In another embodiment, an hGH is a substitution variant in which glutamine in position 65 of hGH is substituted by a valine.

[000100]In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. AAA72260. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. AAK69708. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. CAA01435. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. CAA01329. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. CAA00380. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. AAA72555. In

another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. NP_000506.2. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. NP_072053.1. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. NP_072054.1. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. NP_072055.1. In another embodiment, a growth hormone of the invention comprises the gene bank amino acid deposited sequence under accession no. NP_072056.1.

[000101]In another embodiment, the nucleic acid molecule encoding a growth hormone as described herein encodes any amino acid sequence of a growth hormone known to one of skill in the art. In another embodiment, the nucleic acid molecule encoding a growth hormone as described herein encodes an hGH. In another embodiment, the nucleic acid molecule encoding a growth hormone comprises the gene bank nucleic acid deposited sequence under accession no. NM_000515.3. In another embodiment, the nucleic acid molecule encoding a growth hormone comprises the gene bank nucleic acid deposited sequence under accession no. NM_022559.2. In another embodiment, the nucleic acid molecule encoding a growth hormone comprises the gene bank nucleic acid deposited sequence under accession no. NM_022560.2. In another embodiment, the nucleic acid molecule encoding a growth hormone comprises the gene bank nucleic acid deposited sequence under accession no. NM_022561.2. In another embodiment, the nucleic acid molecule encoding a growth hormone comprises the gene bank nucleic acid deposited sequence under accession no. NM_022562.2.

[000102]In another embodiment, a polypeptide comprising a growth hormone of the invention comprises one CTP attached to a C-terminus of a growth hormone (hGH-CTP) and having the following amino acid sequence:

MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFE
EAYIPKEQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFL
RSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNS
HNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGFSSSSKAPPPSLPSPSRL
PGPSDTPILPQ (SEQ ID NO: 9).

[000103]In another embodiment, a polypeptide comprising a growth hormone of the invention comprises two CTPs in tandem attached to a C-terminus of a growth hormone (hGH-CTP-CTP) and having the following amino acid sequence: MATGSRTSLLLAFGLLCLPWLQEGSAFPTIPLSRLFDNAMLRAHRLHQLAFDTYQEFE EAYIPKEQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFL RSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNS HNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGFSSSSKAPPPSLPSPSRL PGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 10).

[000104]In another embodiment, a polypeptide comprising a growth hormone of the invention comprises two CTPs attached in tandem to a C-terminus of a growth hormone and one CTP attached to an N-terminus of a growth hormone (CTP-hGH-CTP-CTP) and having the following amino acid sequence: MATGSRTSLLLAFGLLCLPWLQEGSASSSSKAPPPSLPSPSRLPGPSDTPILPQFPTIPLS RLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQTSLCFSESIPTPSNREE TQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEEGIQTL MGRLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQ CRSVEGSCGFSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPI LPQ (SEQ ID NO: 11).

[000105]In another embodiment, a polypeptide comprising a growth hormone of the invention comprises two CTPs in tandem attached to a C-terminus of a growth hormone, wherein one CTP of two CTPs is truncated, and one additional CTP attached to an N-terminus of a growth hormone (tCTP-hGH-CTP-CTP) and having the following amino acid sequence: MATGSRTSLLLAFGLLCLPWLQEGSASSSSKAPPPSLPFPTIPLSRLFDNAMLRAHRLH QLAFDTYQEFEEAYIPKEQKYSFLQNPQTSLCFSESIPTPSNREETQQKSNLELLRISLL LIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIF KQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGFSSSSK APPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 12).

[000106]In another embodiment, a polypeptide comprising a growth hormone of the invention comprises one CTP attached to a C-terminus of a growth hormone and one CTP attached to an N-terminus of a growth hormone (CTP-hGH-CTP) and having the following amino acid sequence: MATGSRTSLLLAFGLLCLPWLQEGSASSSSKAPPPSLPSPSRLPGPSDTPILPQFPTIPLS

RLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQTSLCFSESIPTPSNREE
TQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEEGIQTL
MGRLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQ
CRSVEGSCGFSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 13).

[000107]In another embodiment, a polypeptide comprising a growth hormone and one CTP comprises the following amino acid sequence:
MATGSRTSLLLAFGLLCLPWLQEGSASSSSKAPPPSLPSPSRLPGPSDTPILPQFPTIPLS
RLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQTSLCFSESIPTPSNREE
TQQKSNLELLRISLLLIQSWLEPVQFLRSVFANSLVYGASDSNVYDLLKDLEEGIQTL
MGRLEDGSPRTGQIFKQTYSKFDTNSHNDDALLKNYGLLYCFRKDMDKVETFLRIVQ
CRSVEGSCGF (SEQ ID NO: 14).

[000108]In another embodiment, a polynucleotide molecule encoding a polypeptide having CTP-hGH-CTP comprises the following nucleic acid sequence:
tctagaggacatggccaccggcagcaggaccagcctgctgctggccttcggcctgctgtgcctgccatggctgcaggagggcagcg
ccagctcttcttctaaggctccacccccatctctgcccagccccagcagactgccgggccccagcgacacacccattctgccccagttc
cccaccatccccctgagcaggctgttcgacaacgccatgctgagggctcacaggctgcaccagctggcctttgacacctaccaggag
ttcgaggaagcctacatccccaaggagcagaagtacagcttcctgcagaacccccagacctccctgtgcttcagcgagagcatcccc
acccccagcaacagagaggagacccagcagaagagcaacctggagctgctgaggatctccctgctgctgatccagagctggctgg
agcccgtgcagttcctgagaagcgtgttcgccaacagcctggtgtacggcgccagcgacagcaacgtgtacgacctgctgaaggac
ctggaggagggcatccagaccctgatgggccggctggaggacggcagccccaggaccggccagatcttcaagcagacctacagc
aagttcgacaccaacagccacaacgacgacgccctgctgaagaactacgggctgctgtactgcttcagaaaggacatggacaaggtg
gagaccttcctgaggatcgtgcagtgcagaagcgtggagggcagctgcggcttcagctccagcagcaaggcccctccccgagcct
gccctccccaagcaggctgcctgggccctccgacacaccaatcctgcctcagtgatgaaggtctggatgcggccgc (SEQ ID NO: 15).

[000109]In another embodiment, a polynucleotide molecule encoding a polypeptide having CTP-hGH-CTP-CTP comprises the following nucleic acid sequence:
tctagaggacatggccaccggcagcaggaccagcctgctgctggccttcggcctgctgtgcctgccatggctgcaggagggcagcg
ccagctcttcttctaaggctccacccccatctctgcccagccccagcagactgccgggccccagcgacacacccattctgccccagttc
cccaccatccccctgagcaggctgttcgacaacgccatgctgagggctcacaggctgcaccagctggcctttgacacctaccaggag
ttcgaggaagcctacatccccaaggagcagaagtacagcttcctgcagaacccccagacctccctgtgcttcagcgagagcatcccc
acccccagcaacagagaggagacccagcagaagagcaacctggagctgctgaggatctccctgctgctgatccagagctggctgg
agcccgtgcagttcctgagaagcgtgttcgccaacagcctggtgtacggcgccagcgacagcaacgtgtacgacctgctgaaggac

ctggaggagggcatccagaccctgatgggccggctggaggacggcagccccaggaccggccagatcttcaagcagacctacagc
aagttcgacaccaacagccacaacgacgacgccctgctgaagaactacgggctgctgtactgcttcagaaaggacatggacaaggtg
gagaccttcctgaggatcgtgcagtgcagaagcgtggagggcagctgcggcttcagctccagcagcaaggcccctccccgagcct
gccctccccaagcaggctgcctgggccctccgacacaccaatcctgccacagagcagctcctctaaggcccctcctccatccctgcc
atccccctcccggctgcctggcccctctgacacccctatcctgcctcagtgatgaaggtctggatgcggccgc (SEQ ID NO:
16).

[000110]In another embodiment, a polynucleotide molecule encoding a polypeptide having CTP-hGH-CTP-CTP comprises the following nucleic acid sequence:
tctagaggacatggccaccggcagcaggaccagcctgctgctggccttcggcctgctgtgcctgccatggctgcaggagggcagcg
ccagctcttcttctaaggctccacccccgagcctgcccttccccaccatcccccctgagcaggctgttcgacaacgccatgctgagggct
cacaggctgcaccagctggcctttgacacctaccaggagttcgaggaagcctacatccccaaggagcagaagtacagcttcctgcag
aacccccagacctccctgtgcttcagcgagagcatccccacccccagcaacagagaggagacccagcagaagagcaacctggagc
tgctgaggatctccctgctgctgatccagagctggctggagcccgtgcagttcctgagaagcgtgttcgccaacagcctggtgtacgg
cgccagcgacagcaacgtgtacgacctgctgaaggacctggaggagggcatccagaccctgatgggccggctggaggacggcag
ccccaggaccggccagatcttcaagcagacctacagcaagttcgacaccaacagccacaacgacgacgccctgctgaagaactacg
ggctgctgtactgcttcagaaaggacatggacaaggtggagaccttcctgaggatcgtgcagtgcagaagcgtggagggcagctgc
ggcttcagctccagcagcaaggcccctccccgagcctgccctccccaagcaggctgcctgggccctccgacacaccaatcctgcc
acagagcagctcctctaaggcccctcctccatccctgccatcccccctcccggctgcctggccctctgacacccctatcctgcctcagt
gatgaaggtctggatgcggccgc (SEQ ID NO: 17).

[000111]In another embodiment, a growth hormone of the invention is homologous to a known sequence of a growth hormone. In another embodiment, a growth hormone of the invention is homologous to a growth hormone sequence as disclosed herein. In another embodiment, homology according to the present invention also encompasses deletions, insertions, or substitution variants, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof. In one embodiment the substitution variant is one, in which the glutamine in position 65 of hGH is substituted by a valine [Gellerfors et al., J Pharm Biomed Anal 1989, 7:173-83].

[000112]In one embodiment, the phrase "human growth hormone" (hGH) refers to a polypeptide, such as set forth in Genbank Accession No. P01241 exhibiting hGH activity (i.e., stimulation of growth).

[000113]In one embodiment, "human growth hormone" (hGH) refers to a polypeptide, such as set forth in Genbank Accession No. P01241, exhibiting hGH activity (i.e., stimulation of growth). In one embodiment, hGH of the present invention also refers to homologues. In one embodiment, hGH amino acid sequence of the present invention is at least 50% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH amino acid sequence of the present invention is at least 60% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH amino acid sequence of the present invention is at least 70% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH amino acid sequence of the present invention is at least 80% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH amino acid sequence of the present invention is at least 90% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH amino acid sequence of the present invention is at least 95% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters).

[000114]In one embodiment, the peptide of interest provided herein is oxyntomodulin. In another embodiment, oxyntomodulin (OXM) comprises the following amino acid (AA) sequence: HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA (SEQ ID NO: 18). In another embodiment, OXM consists of the amino acid sequence of SEQ ID NO: 18. In another embodiment, OXM comprises or consists of the amino acid sequence depicted in CAS No. 62340-29-8.

[000115]In one embodiment, the term oxyntomodulin further includes a homologue of a known oxyntomodulin. In one embodiment, the homologue is a functional homologue. In another embodiment, the term "functional" refers to the ability a homologue, polypeptides or

fragments thereof provided herein has to suppress appetite. The term also refers to the ability a homologue, polypeptides or fragments thereof provided herein has to extend another protein's or peptide's biological half-life. In another embodiment, the biological half-life (T½) of a protein, peptide or homologue provided herein refers to the time it takes for half of the amount of the protein, peptide or homologue to be degraded or to not be present in a biological medium in a subject. In another embodiment, the biological medium is serum, cerebospinal fluid, tissue, mucosa, and the like.

[000116]In another embodiment, OXM is human OXM or any mammal OXM. In another embodiment, OXM is also referred to as glucagon-37 or bioactive enteroglucagon. In another embodiment, OXM is a dual Polypeptide or fragments thereof. In another embodiment, OXM is a biologically active fragment of OXM. In another embodiment, biologically active OXM extends from amino acid 30 to amino acid 37 of SEQ ID NO: 18. In another embodiment, biologically active OXM extends from amino acid 19 to amino acid 37 of SEQ ID NO: 18. In another embodiment, OXM of the invention corresponds to an octapeptide from which the two C-terminal amino acids are deleted. In another embodiment, OXM of the invention corresponds to any fragment of SEQ ID NO: 18 which retains OXM activity as described herein. In another embodiment, the invention also encompasses homologues e.g., polypeptides which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 87%, at least 89%, at least 91%, at least 93%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homologous to an oxyntomodulin as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters.

[000117]In other embodiments, the term engineered oxyntomodulin refers to the amino acid sequence of a matured oxyntomodulin. In other embodiments, the term engineered oxyntomodulin refers to the amino acid sequence of the oxyntomodulin including its signal sequence or signal peptide.

[000118]In another embodiment, the polypeptides or fragments thereof provided herein comprise a signal peptide or signal sequence.

[000119]In another embodiment, "signal sequence" and "signal peptide" are used interchangeably herein. In another embodiment, "sequence" when in reference to a polynucleotide molecule can refer to a coding portion. Each possibility represents a separate embodiment of the present invention.

[000120]In one embodiment, the polypeptide or peptide of interest provided herein is erythropoietin (EPO). In another embodiment, the term "erythropoietin" refers to mammalian erythropoietin. In one embodiment, "erythropoietin" refers to human erythropoietin, such as set forth in GenBank Accession No. AAA52400.

[000121]In one embodiment, an erythropoietin or EPO sequence of the present invention also refers to homologues. In one embodiment, the erythropoietin amino acid sequence of the present invention is at least 50% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, the erythropoietin amino acid sequence of the present invention is at least 60% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, the erythropoietin amino acid sequence of the present invention is at least 70% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, the erythropoietin amino acid sequence of the present invention is at least 80% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, the erythropoietin amino acid sequence of the present invention is at least 90% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, the erythropoietin amino acid sequence of the present invention is at least 95% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters).

[000122]In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 19 having additionally at least one CTP amino acid peptide on the N-terminus and at least additional one CTP amino acid peptide on the C-terminus. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 19:

MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAE
HCSLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQ
PWEPLQLHVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRV
YSNFLRGKLKLYTGEACRTGDRSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO:
19).

[000123]In another embodiment, the EPO peptide additionally has at least one CTP amino acid peptide on the N-terminus and at least one additional CTP amino acid peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 20 having additionally at least one CTP amino acid peptide on the N-terminus and at least one CTP amino acid peptide on the C-terminus:

MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAE
HCSLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQ
PWEPLQLHVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRV
YSNFLRGKLKLYTGEACRTGDRSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSL
PSPSRLPGPSDTPILPQ (SEQ ID NO: 20).

[000124]In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 21 having additionally at least one CTP amino acid peptide on the N-terminus and at least one CTP amino acid peptide on the C-terminus:
MGVHECPAWLWLLLSLLSLPLGLPVLGSSSSKAPPPSLPSPSRLPGPSDTPILPQAPPRL
ICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQAVE
VWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKEA
ISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDRSSSSKAPPPSL
PSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 21).

[000125]In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP amino acid peptide on the N-terminus and at least one CTP amino acid peptide on the C-terminus:
MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAE
HCSLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQ
PWEPLQLHVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRV
YSNFLRGKLKLYTGEACRTGDRSSSSKAPPPSLPSPSRLPGPSDTPILPQAPPRLICDSR
VLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQAVEVWQG

LALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKEAISPPD AASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDR (SEQ ID NO: 22).

[000126]In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 23 having additionally at least one CTP amino acid peptide on the N-terminus and at least one CTP amino acid peptide on the C-terminus: MGVHECPAWLWLLLSLLSLPLGLPVLGSSSSKAPPPSLPSPSRLPGPSDTPILPQAPPRL ICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQAVE VWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKEA ISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDR (SEQ ID NO: 23).

[000127]In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 24 having additionally at least one CTP amino acid peptide on the N-terminus and at least one CTP amino acid peptide on the C-terminus: MGVHECPAWLWLLLSLLSLPLGLPVLGSSSSKAPPPSLPSPSRLPGPSDTPILPQAPPRL ICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQAVE VWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKEA ISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDRSSSSKAPPPSL PSPSRLPGPSDTPILPQ (SEQ ID NO: 24).

[000128]In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 25: MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAE HCSLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQ PWEPLQLHVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRV YSNFLRGKLKLYTGEACRTGDR (SEQ ID NO: 25).

[000129]In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 26 encoding an EPO peptide and one CTP amino acid peptide on the N-terminus and two CTP amino acid peptides on the C-terminus: tctagaggtc atcatggggg tgcacgaatg tcctgcctgg ctgtggcttc tcctgtcccttc tgtcgctc cctctgggcc tcccagtcct gggctcctct tcctcaaagg cccctccccc gagccttcca agtccatccc gactcccggg gccctcggac accccaatat taccacaagc cccaccacgc ctcatctgtg acagccgagt cctggagagg tacctcttgg aggccaagga ggccgagaat atcacgacgg gctgtgctga acactgcagc ttgaatgaga atatcactgt cccagacacc aaagttaatt tctatgcctg gaagaggatg gaggtcgggc agcaggccgt agaagtctgg cagggcctgg ccctgctgtc ggaagctgtc ctgcggggcc aggccctgtt

ggtcaactct tcccagccgt gggagcccct gcagctgcat gtggataaag ccgtcagtgg ccttcgcagc ctcaccactc tgcttcgggc tctgggagcc cagaaggaag ccatctcccc tccagatgcg gcctcagctg ctccactccg aacaatcact gctgacactt tccgcaaact cttccgagtc tactccaatt tcctccgggg aaagctgaag ctgtacacag gggaggcctg caggacaggg gacagatcct cttcctcaaa ggcccctccc ccgagccttc caagtccatc ccgactcccg gggccctccg acacaccaat cctgccacag agcagctcct ctaaggcccctcctccatcc ctgccatccc cctcccggct gcctggcccc tctgacaccc ctatcctgcc tcagtgatga aggtcttctg gatccgcggc cgc (SEQ ID NO: 26). In another embodiment, the methods of the present invention the methods of the present invention provide an amino acid sequence comprising an EPO peptide set forth in SEQ ID NO: 66 having two CTP amino acid peptides on the N-terminus: MGVHECPAWLWLLLSLLSLPLGLPVLGSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSK APPPSLPSPSRLPGPSDTPILPQAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNEN ITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQL HVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLRG KLKLYTGEACRTGDR (SEQ ID NO: 66).

[000130]In another embodiment, the methods of the present invention provide a nucleic acid sequence, set forth in SEQ ID NO: 67, encoding an EPO peptide and two CTP amino acid peptides on the N-terminus: ATGGGCGTGCACGAGTGTCCTGCTTGGCTGTGGCTGCTGCTGAGCCTGCTGTCCCT GCCTCTGGGCCTGCCTGTGCTGGGCAGCAGCAGCTCTAAGGCCCCTCCACCCAGC CTGCCCAGCCCTTCTAGACTGCCTGGCCCCAGCGACACCCCCATCCTGCCTCAGA GCAGCAGCAGCAAGGCCCCACCACCATCCCTGCCTAGCCCCAGCAGACTGCCAG GCCCTTCCGATACCCCAATCCTGCCCCAGGCCCCTCCCAGACTGATCTGCGACAG CCGGGTGCTGGAAAGATACCTGCTGGAAGCCAAAGAGGCCGAGAACATCACCAC CGGCTGCGCCGAGCACTGCAGCCTGAACGAGAATATCACCGTGCCCGACACCAA AGTGAACTTCTACGCCTGGAAGCGGATGGAAGTGGGCCAGCAGGCCGTGGAAGT GTGGCAGGGACTGGCCCTGCTGAGCGAGGCCGTGCTGAGAGGACAGGCCCTGCT GGTGAACAGCAGCCAGCCCTGGGAGCCCCTGCAGCTGCATGTGGATAAGGCCGT GTCCGGCCTGCGGAGCCTGACCACACTGCTGAGAGCCCTGGGCGCTCAGAAAGA GGCCATCTCTCCCCCTGATGCCGCCTCTGCCGCCCCTCTGAGAACCATCACCGCCG ACACCTTCCGGAAGCTGTTCCGGGTGTACAGCAACTTCCTGCGGGGCAAGCTGAA GCTGTACACCGGCGAGGCCTGCCGGACCGGCGATAGATAAGCTTGGCGCGCC (SEQ ID NO: 67).

[000131]In another embodiment, the methods of the present invention the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 68 having two CTP amino acid peptides on the N-terminus and two CTP amino acid peptides on the C-terminus: MGVHECPAWLWLLLSLLSLPLGLPVLGSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSK APPPSLPSPSRLPGPSDTPILPQAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNEN ITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQL HVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLRG KLKLYTGEACRTGDRSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLP GPSDTPILPQ (SEQ ID NO: 68).

[000132]In another embodiment, the methods of the present invention provide a nucleic acid sequence, set forth in SEQ ID NO: 69, encoding an EPO peptide and two CTP amino acid peptides on the N-terminus and two CTP amino acid peptides on the C-terminus: ATGGGCGTGCACGAGTGTCCTGCTTGGCTGTGGCTGCTGCTGAGCCTGCTGTCCCT GCCTCTGGGCCTGCCTGTGCTGGGCAGCAGCAGCTCTAAGGCCCCTCCACCCAGC CTGCCCAGCCCTTCTAGACTGCCTGGCCCCAGCGACACCCCCATCCTGCCTCAGA GCAGCAGCAGCAAGGCCCCACCACCATCCCTGCCTAGCCCCAGCAGACTGCCAG GCCCTTCCGATACCCCAATCCTGCCCCAGGCCCCTCCCAGACTGATCTGCGACAG CCGGGTGCTGGAAAGATACCTGCTGGAAGCCAAAGAGGCCGAGAACATCACCAC CGGCTGCGCCGAGCACTGCAGCCTGAACGAGAATATCACCGTGCCCGACACCAA AGTGAACTTCTACGCCTGGAAGCGGATGGAAGTGGGCCAGCAGGCCGTGGAAGT GTGGCAGGGACTGGCCCTGCTGAGCGAGGCCGTGCTGAGAGGACAGGCCCTGCT GGTGAACAGCAGCCAGCCCTGGGAGCCCCTGCAGCTGCATGTGGATAAGGCCGT GTCCGGCCTGCGGAGCCTGACCACACTGCTGAGAGCCCTGGGCGCTCAGAAAGA GGCCATCTCTCCCCCTGATGCCGCCTCTGCCGCCCCTCTGAGAACCATCACCGCCG ACACCTTCCGGAAGCTGTTCCGGGTGTACAGCAACTTCCTGCGGGGCAAGCTGAA GCTGTACACCGGCGAGGCCTGCCGGACCGGCGATAGAAGCAGCTCCAGCAAGGC TCCACCCCCCAGCCTGCCATCCCCAAGTAGACTGCCCGGGCCCTCTGACACACCT ATCCTGCCACAGTCCAGCAGCTCCAAAGCTCCCCCACCATCCCTCCCATCCCCATC CAGACTGCCTGGACCATCCGACACTCCAATTCTGCCTCAGTAAGCTTGGCGCGCC (SEQ ID NO: 69).

[000133]In one embodiment, "interferon" refers to the mammalian interferon polypeptide Type I. In one embodiment, "interferon" refers to the mammalian interferon polypeptide Type

II. In some embodiments, additional suitable interferon polypeptides as known to those of ordinary skill in the art are utilized. In some embodiments, the interferon is alpha-interferon. In some embodiments, the interferon is beta-interferon. In some embodiments, the interferon is gamma-interferon. In some embodiments, the interferon is omega-interferon. In some embodiments, the interferon is a subspecies interferon. In one embodiment, the subspecies interferon (IFN) is IFN-α2a. In one embodiment, the subspecies interferon (IFN) is IFN- α 2b. In one embodiment, the subspecies interferon (IFN) is IFN-β1a. In one embodiment, the interferon (IFN) subspecies is IFN- β1b.

[000134]In one embodiment, interferon of the present invention exhibits interferon activity, such as antiviral or antiproliferative activity. In some embodiments, GenBank accession nos. of non-limiting examples of interferons are listed in Table 1 below.

[000135]In one embodiment, an interferon of the present invention also refers to homologues. In one embodiment, an interferon amino acid sequence of the present invention is at least 50% homologous to interferon sequences listed in Table 1 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, an interferon amino acid sequence of the present invention is at least 60% homologous interferon sequences listed in Table 1 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters. In one embodiment, interferon amino acid sequence of the present invention is at least 70% homologous to interferon sequences listed in Table 1, as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters. In one embodiment, an interferon amino acid sequence of the present invention is at least 80% homologous to interferon sequences listed in Table 1, as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters. In one embodiment, interferon amino acid sequence of the present invention is at least 90% homologous to interferon sequences listed in Table 1, as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters. In one embodiment, an interferon amino acid sequence of the present invention is at least 95% homologous to interferon sequences listed in Table 1, as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters. In some embodiments, homology according to the present invention also encompasses deletions, insertions, or substitution variants, including an amino acid substitution, thereof and

biologically active polypeptide fragments thereof. In one embodiment the cysteine in position 17 of interferon β is substituted by a Serine.

[000136] Table 1 below lists examples of interferons with their respective NCBI sequence numbers

Table 1

Interferon name	*NCBI sequence number*
interferon, α1	NP_076918.1
interferon, α 10	NP_002162.1
interferon, α 13	NP_008831.2
interferon, α 14	NP_002163.1
interferon, α 16	NP_002164.1
interferon, α 17	NP_067091.1
interferon, α 2	NP_000596.2
interferon, α 21	NP_002166.1
interferon, α 4	NP_066546.1
interferon, α 5	NP_002160.1
interferon, α 6	NP_066282.1
interferon, α 7	NP_066401.2
interferon, α 8	NP_002161.2
interferon, beta precursor	NP_002167.1
interferon, ε1	NP_795372.1
interferon, γ	NP_000610.2
interferon, ε	NP_064509.1
interferon, Ω1	NP_002168.1

[000137]In another embodiment, the interferon (IFN) provided herein as the peptide or as a polypeptide is a type I interferon. In another embodiment, the interferon (IFN) is IFN-α In another embodiment, the interferon (IFN) is IFN-β. In another embodiment, the interferon (IFN) is IFN-γ. In another embodiment, an interferon (IFN) peptide as described herein comprises an amino acid sequence set forth in SEQ ID NO: 27. In another embodiment, SEQ ID NO: 27 comprises the following amino acid (AA) sequence: MTNKCLLQIALLLCFSTTALSMSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRM

NFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQI NHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILR NFYFINRLTGYLRN (SEQ ID NO: 27, Human Interferon-β1a). In another embodiment, an interferon (IFN) peptide as described herein comprises an amino acid sequence of human interferon β1a (hIFN β1a). In another embodiment, an interferon (IFN) peptide as described herein comprises an amino acid sequence set fourth in GenBank Accession No. NP_002167.1.

[000138]In another embodiment, an interferon (IFN) peptide as described herein is encoded by a nucleotide acid sequence set forth in SEQ ID NO: 28. In another embodiment, SEQ ID NO: 28 comprises the following nucleotide acid (NA) sequence: tctagaggacatgaccaacaagtgcctgctgcagatcgccctgctgctgtgcttcagcaccaccgccctgagcatgagctacaacctg ctgggcttcctgcagaggtccagcaacttccagtgccagaagctgctgtggcagctgaacggcaggctggaatactgcctgaaggac aggatgaacttcgacatcccagaggaaatcaagcagctgcagcagttccagaaggaggacgccgccctgaccatctacgagatgct gcagaacatcttcgccatcttcaggcaggacagcagcagcaccggctggaacgagaccatcgtggagaacctgctggccaacgtgt accaccagatcaaccacctgaaaaccgtgctggaagagaagctggaaaaggaggacttcaccaggggcaagctgatgagcagcct gcacctgaagaggtactacggcagaatcctgcactacctgaaggccaaggagtacagccactgcgcctggaccatcgtgagggtgg agatcctgaggaacttctacttcatcaacaggctgaccggctacctgaggaactgatgagtccgcggccgc (SEQ ID NO: 28, Human Interferon-β1a). In another embodiment, an interferon (IFN) peptide as described herein is encoded by a nucleotide acid (NA) molecule of human interferon β1a (hIFN β1a). In another embodiment, an interferon (IFN) peptide as described herein is encoded by a nucleotide acid (NA) molecule comprising a nucleotide acid sequence set fourth in GenBank Accession No. NM_002176.

[000139]In another embodiment, an interferon (IFN) peptide as described herein comprises an amino acid sequence set forth in SEQ ID NO: 29. In another embodiment, SEQ ID NO: 29 comprises the following amino acid (AA) sequence: TF*LQPFEAFALAQQVVGDTVRVVNMTNKCLLQIALLLCFSTTALSMSYNLLGFLQR SSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAI FRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYY GRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN (SEQ ID NO: 29).

[000140]In another embodiment, an interferon (IFN) peptide as described herein is encoded by a nucleotide acid sequence set forth in SEQ ID NO: 30. In another embodiment, SEQ ID

NO: 30 comprises the following nucleotide acid (NA) sequence:
acattctaactgcaacctttcgaagcctttgctctggcacaacaggtagtaggcgacactgttcgtgttgtcaacatgaccaacaagtgtct
cctccaaattgctctcctgttgtgcttctccactacagctctttccatgagctacaacttgcttggattcctacaaagaagcagcaattttcag
tgtcagaagctcctgtggcaattgaatgggaggcttgaatactgcctcaaggacaggatgaactttgacatccctgaggagattaagca
gctgcagcagttccagaaggaggacgccgcattgaccatctatgagatgctccagaacatctttgctattttcagacaagattcatctagc
actggctggaatgagactattgttgagaacctcctggctaatgtctatcatcagataaaccatctgaagacagtcctggaagaaaaactg
gagaaagaagatttcaccaggggaaaactcatgagcagtctgcacctgaaaagatattatgggaggattctgcattacctgaaggcca
aggagtacagtcactgtgcctggaccatagtcagagtggaaatcctaaggaacttttacttcattaacagacttacaggttacctccgaaa
ctga (SEQ ID NO: 30).

[000141]In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide and a CTP unit. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide and a CTP unit attached to the C-terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide and at least one CTP unit attached to the C-terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide and a CTP unit attached to the N-terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide and at least one CTP unit attached to the N-terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide, at least one CTP unit attached to the N-terminus, and/ or at least one CTP unit attached to the C-terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide, at least one CTP unit attached to the N-terminus, and two CTP units in tandem attached to the C-terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide, at least one CTP unit attached to the N-terminus, and two CTP units attached to the C-terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide, one CTP unit attached to the N-terminus, and at least two CTP units attached to the C-terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide, one CTP unit attached to the N-terminus, and at least two CTP units in tandem attached to the C-terminus.

[000142]In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide and at least three CTP units. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide and three CTP units. In another

embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide- CTP polypeptide encoded by an amino acid sequence comprising the amino acid sequence set forth in SEQ ID NO: 31. In another embodiment, SEQ ID NO: 31 comprises the following amino acid (AA) sequence:

MTNKCLLQIALLLCFSTTALSMSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRM NFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQI NHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILR NFYFINRLTGYLRNSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 31).

[000143]In another embodiment, the polypeptide as described herein comprising an interferon (IFN) peptide – and CTP is encoded by a nucleic acid molecule set forth in SEQ ID NO: 32. In another embodiment, SEQ ID NO: 32 comprises the following nucleotide acid (NA) sequence:

tctagaggacatgaccaacaagtgcctgctgcagatcgccctgctgctgtgcttcagcaccaccgccctgagcatgagctacaacctg ctgggcttcctgcagaggtccagcaacttccagtgccagaagctgctgtggcagctgaacggcaggctggaatactgcctgaaggac aggatgaacttcgacatcccagaggaaatcaagcagctgcagcagttccagaaggaggacgccgccctgaccatctacgagatgct gcagaacatcttcgccatcttcaggcaggacagcagcagcaccggctggaacgagaccatcgtggagaacctgctggccaacgtgt accaccagatcaaccacctgaaaaccgtgctggaagagaagctggaaaaggaggacttcaccaggggcaagctgatgagcagcct gcacctgaagaggtactacggcagaatcctgcactacctgaaggccaaggagtacagccactgcgcctggaccatcgtgagggtgg agatcctgaggaacttctacttcatcaacaggctgaccggctacctgaggaacagctccagcagcaaggcccctccaccttccctgcc cagtccaagccgactccctgggccctccgatacaccaattctgccacagtgatga (SEQ ID NO: 32).

[000144]In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide and two CTP units attached to its carboxy terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide – CTP (x2) encoded by an amino acid sequence comprising the amino acid sequence set forth in SEQ ID NO: 33. In another embodiment, SEQ ID NO: 33 comprises the following amino acid (AA) sequence:

MTNKCLLQIALLLCFSTTALSMSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRM NFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQI NHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILR NFYFINRLTGYLRNSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGP SDTPILPQ (SEQ ID NO: 33).

[000145]In another embodiment, the polypeptide as described herein comprising an interferon (IFN) peptide – and two CTP units attached to its carboxy terminus is encoded by a nucleic acid molecule set forth in SEQ ID NO: 34. In another embodiment, SEQ ID NO: 34 comprises the following nucleotide acid (NA) sequence:

tctagaggacatgaccaacaagtgcctgctgcagatcgccctgctgctgtgcttcagcaccaccgccctgagcatgagctacaacctg ctgggcttcctgcagaggtccagcaacttccagtgccagaagctgctgtggcagctgaacggcaggctggaatactgcctgaaggac aggatgaacttcgacatcccagaggaaatcaagcagctgcagcagttccagaaggaggacgccgccctgaccatctacgagatgct gcagaacatcttcgccatcttcaggcaggacagcagcagcaccggctggaacgagaccatcgtggagaacctgctggccaacgtgt accaccagatcaaccacctgaaaaccgtgctggaagagaagctggaaaaggaggacttcaccaggggcaagctgatgagcagcct gcacctgaagaggtactacggcagaatcctgcactacctgaaggccaaggagtacagccactgcgcctggaccatcgtgagggtgg agatcctgaggaacttctacttcatcaacaggctgaccggctacctgaggaacagctccagcagcaaggcccctccaccttccctgcc cagtccaagccgactccctgggccctccgacacaccaatcctgccacagagcagctcctctaaggcccctcctccatccctgccatcc ccctcccggctgcctggcccctctgacacccctatcctgcctcagtgatgaaggtctggatccgcggccgc (SEQ ID NO: 34).

[000146]In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide, a single CTP unit attached to the IFN's amino terminus, and two CTP units attached to the IFN's carboxy terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide, a single CTP unit attached to the IFN's amino terminus and two CTP units attached in tandem to the IFN's carboxy terminus. In another embodiment, the polypeptide as described herein comprises (from amino to carboxy termini): CTP (x1)-interferon (IFN) peptide – CTP (x2) comprising an amino acid sequence set forth in SEQ ID NO: 365. In another embodiment, SEQ ID NO: 35 comprises the following amino acid (AA) sequence:

MTNKCLLQIALLLCFSTTALSSSSSKAPPPSLPSPSRLPGPSDTPILPQMSYNLLGFLQRS SNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIF RQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYG RILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRNSSSSKAPPPSLPSPSRLPGPS DTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 35).

[000147]In another embodiment, the polypeptide as described herein comprising an interferon (IFN) peptide, a single CTP unit attached to the IFN's amino terminus and two CTP units attached to the IFN's carboxy terminus is encoded by a nucleic acid molecule set forth in SEQ ID NO: 36. In another embodiment, SEQ ID NO: 36 comprises the following nucleotide

acid (NA) sequence:

tctagaggacatgaccaacaagtgcctgctgcagatcgccctgctgctgtgcttcagcaccaccgccctgagcagcagcagctccaa
ggccccacccccccagcctgcccagccccagcagactgccaggccccagcgacacccccatcctgccccagatgagctacaacctg
ctgggcttcctgcagaggtccagcaacttccagtgccagaagctgctgtggcagctgaacggcaggctggaatactgcctgaaggac
aggatgaacttcgacatcccagaggaaatcaagcagctgcagcagttccagaaggaggacgccgccctgaccatctacgagatgct
gcagaacatcttcgccatcttcaggcaggacagcagcagcaccggctggaacgagaccatcgtggagaacctgctggccaacgtgt
accaccagatcaaccacctgaaaaccgtgctggaagagaagctggaaaaggaggacttcaccaggggcaagctgatgagcagcct
gcacctgaagaggtactacggcagaatcctgcactacctgaaggccaaggagtacagccactgcgcctggaccatcgtgagggtgg
agatcctgaggaacttctacttcatcaacaggctgaccggctacctgaggaacagctccagcagcaaggcccctccaccttccctgcc
cagtccaagccgactccctgggccctccgacacaccaatcctgccacagagcagctcctctaaggcccctcctccatccctgccatcc
ccctcccggctgcctggcccctctgacacccctatcctgcctcagtgatgaaggtctggatccgcggccgc (SEQ ID NO: 36).

[000148]In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide, a single CTP attached to the IFN's amino terminus, and a single CTP located within an IFN coding sequence. In another embodiment, the polypeptide as described herein comprises (from amino to carboxy termini): CTP (x1)-interferon (IFN) peptide (fragment 1) – CTP- interferon (IFN) peptide (fragment 2) comprising an amino acid sequence set forth in SEQ ID NO: 37. In another embodiment, SEQ ID NO: 37 comprises the following amino acid (AA) sequence:

MTNKCLLQIALLLCFSTTALSSSSSKAPPPSLPSPSRLPGPSDTPILPQMSYNLLGFLQRS
SNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIF
RQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYG
RILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRNSSSSKAPPPSLPSPSRLPGPS
DTPILPQMSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQ
KEDAALTIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKE
DFTRGKLMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN
(SEQ ID NO: 37).

[000149]In another embodiment, the polypeptide as described herein comprising an interferon (IFN) peptide, a single CTP unit attached to the IFN's amino terminus, and a single CTP unit located within the IFN coding sequence is encoded by a nucleic acid molecule set forth in SEQ ID NO: 38. In another embodiment, SEQ ID NO: 38 comprises the following nucleotide acid (NA) sequence:

tctagaggacatgaccaacaagtgcctgctgcagatcgccctgctgctgtgcttcagcaccaccgccctgagcagcagctccaa
ggccccacccccagcctgcccagccccagcaggctgccaggccccagcgacacccccatcctgccccagatgagctacaacctg
ctgggcttcctgcagaggtccagcaacttccagtgccagaaactgctgtggcagctgaacggcaggctggaatactgcctgaaggac
cggatgaacttcgacatccccgaagagatcaagcagctgcagcagttccagaaagaggacgccgccctgaccatctacgagatgctg
cagaacatcttcgccatcttcaggcaggacagcagcagcaccggctggaacgagaccatcgtggagaacctgctggccaacgtgta
ccaccagatcaaccacctgaaaaccgtgctggaagagaagctggaaaaagaggacttcaccaggggcaagctgatgagcagcctg
cacctgaagaggtactacggcagaatcctgcactacctgaaggccaaagagtacagccactgcgcctggaccatcgtgagggtgga
gatcctgcggaacttctacttcatcaacaggctgaccggctacctgaggaacagctccagcagcaaggcccctccaccctccctgccc
tccccaagcagactgcccggaccctccgacacaccaattctgccacagatgtcctacaatctgctcggatttctgcagcgctcctccaa
ctttcagtgtcagaagctcctctggcagctcaatggccgcctggaatattgtctgaaagacagaatgaattttgacatcccagaggaaatt
aaacagctccagcagtttcagaaagaagatgctgctctcacaatctatgaaatgctccagaatatctttgcaatctttcgccaggacagct
cctccaccgggtggaatgagacaattgtcgagaatctgctcgccaatgtctatcatcagatcaatcacctcaagacagtcctcgaagaa
aaactcgaaaaagaagatttcacacgcggcaaactgatgtcctccctgcatctgaagcgctactatgggcgcatcctgcattatctgaaa
gctaaagaatactcccactgtgcttggacaattgtgcgcgtcgagatcctgagaaacttttatttcattaaccgcctgacaggatacctgc
gcaactgatgaaggtctggatgcggccgc (SEQ ID NO: 38).

[000150]In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide and a single CTP unit attached to its amino terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide – CTP comprising an amino acid sequence set forth in SEQ ID NO: 39. In another embodiment, SEQ ID NO: 39 comprises the following amino acid (AA) sequence: MTNKCLLQIALLLCFSTTALSSSSSKAPPPSLPSPSRLPGPSDTPILPQMSYNLLGFLQRS SNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIF RQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYG RILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN* (SEQ ID NO: 39).

[000151]In another embodiment, the polypeptide as described herein comprising an interferon (IFN) peptide – and a single CTP attached to its amino terminus is encoded by a nucleic acid molecule set forth in SEQ ID NO: 40. In another embodiment, SEQ ID NO: 40 comprises the following nucleotide acid (NA) sequence: tctagaggacatgaccaacaagtgcctgctgcagatcgccctgctgctgtgcttcagcaccaccgccctgagcagcagctccaa ggccccacccccagcctgcccagccccagcaggctgccaggccccagcgacacccccatcctgccccagatgagctacaacctg ctgggcttcctgcagaggtccagcaacttccagtgccagaaactgctgtggcagctgaacggcaggctggaatactgcctgaaggac

cggatgaacttcgacatccccgaagagatcaagcagctgcagcagttccagaaagaggacgccgccctgaccatctacgagatgctg
cagaacatcttcgccatcttcaggcaggacagcagcagcaccggctggaacgagaccatcgtggagaacctgctggccaacgtgta
ccaccagatcaaccacctgaaaaccgtgctggaagagaagctggaaaaagaggacttcaccaggggcaagctgatgagcagcctg
cacctgaagaggtactacggcagaatcctgcactacctgaaggccaaagagtacagccactgcgcctggaccatcgtgagggtgga
gatcctgcggaacttctacttcatcaacaggctgaccggctacctgaggaactgatgagtccgcggccgc (SEQ ID NO: 40).

[000152]In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide, a single CTP unit attached to its amino terminus, and a single CTP unit attached to its carboxy terminus. In another embodiment, the polypeptide as described herein comprises an interferon (IFN) peptide – CTP comprising an amino acid sequence set forth in SEQ ID NO: 41. In another embodiment, SEQ ID NO: 41 comprises the following amino acid (AA) sequence:
MTNKCLLQIALLLCFSTTALSSSSSKAPPPSLPSPSRLPGPSDTPILPQMSYNLLGFLQRS
SNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQNIFAIF
RQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYG
RILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRNSSSSKAPPPSLPSPSRLPGPS
DTPILPQ* (SEQ ID NO: 41).

[000153]In another embodiment, the polypeptide as described herein comprising an interferon (IFN) peptide, a single CTP unit attached to its amino terminus, and a single CTP unit attached to its carboxy terminus is encoded by a nucleic acid molecule set forth in SEQ ID NO: 42. In another embodiment, SEQ ID NO: 42 comprises the following nucleotide acid (NA) sequence:
tctagaggacatgaccaacaagtgcctgctgcagatcgccctgctgctgtgcttcagcaccaccgccctgagcagcagcagctccaa
ggccccacccccagcctgcccagccccagcagactgccaggccccagcgacacccccatcctgccccagatgagctacaacctg
ctgggcttcctgcagaggtccagcaacttccagtgccagaagctgctgtggcagctgaacggcaggctggaatactgcctgaaggac
aggatgaacttcgacatcccagaggaaatcaagcagctgcagcagttccagaaggaggacgccgccctgaccatctacgagatgct
gcagaacatcttcgccatcttcaggcaggacagcagcagcaccggctggaacgagaccatcgtggagaacctgctggccaacgtgt
accaccagatcaaccacctgaaaaccgtgctggaagagaagctggaaaaggaggacttcaccaggggcaagctgatgagcagcct
gcacctgaagaggtactacggcagaatcctgcactacctgaaggccaaggagtacagccactgcgcctggaccatcgtgagggtgg
agatcctgaggaacttctacttcatcaacaggctgaccggctacctgaggaacagctccagcagcaaggcccctccaccttccctgcc
cagtccaagccgactccctgggccctccgatacaccaattctgccacagtgatgaaggtctggatgcggccgc (SEQ ID NO: 42).

[000154]In another embodiment, an interferon β peptide comprises SEQ ID NO: 43 comprising the following amino acid (AA) sequence:
MSYNLLGFLQRSSNFQSQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAAL
TIYEMLQNIFAIFRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGK
LMSSLHLKRYYGRILHYLKAKEYSHCAWTIVRVEILRNFYFINRLTGYLRN (SEQ ID NO: 43).

[000155]In one embodiment, the polypeptide or fragment thereof provide herein is a glucagon-like peptide-1. In another embodiment, glucagon-like peptide-1 is utilized according to the teachings of the present invention. In another emboiment, the attachment of CTP sequences to both the amino and carboxy termini of a "glucagon-like peptide-1" results in increased potency. In another embodiment, the attachment of CTP to both the amino and carboxy termini of a peptide results in prolonged in-vivo activity. In another embodiment, the attachment of CTP to both the amino and carboxy termini of the glucagon-like peptide-results in prolonged in-vivo activity.

[000156]In one embodiment, "glucagon-like peptide-1" (GLP-1) refers to a mammalian polypeptide. In one embodiment, "glucagon-like peptide-1" (GLP-1) refers to a human polypeptide. In another embodiment GLP-1 is cleaved from the glucagon preproprotein (Genbank ID No. NP002045) that has the ability to bind to the GLP-1 receptor and initiate a signal transduction pathway resulting in insulinotropic activity. In one embodiment, "insulinotropic activity" refers to the ability to stimulate insulin secretion in response to elevated glucose levels, thereby causing glucose uptake by cells and decreased plasma glucose levels. In some embodiments, GLP-1 polypeptides include, but are not limited to those described in U.S. Pat. No. 5,118,666; which is incorporated by reference herein.

[000157]In one embodiment, a GLP-1 of the present invention also refers to a GLP-1 homologue. In one embodiment, GLP-1 amino acid sequence of the present invention is at least 50% homologous to GLP-1 sequences set forth in Genbank ID No. NP002045 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters. In one embodiment, GLP-1 amino acid sequence of the present invention is at least 60% homologous to GLP-1 sequences set forth in Genbank ID No. NP002045 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters. In one embodiment, GLP-1 amino acid

sequence of the present invention is at least 70% homologous to GLP-1 sequences set forth in Genbank ID No. NP002045 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters. In one embodiment, GLP-1 amino acid sequence of the present invention is at least 80% homologous to GLP-1 sequences set forth in Genbank ID No. NP002045 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters. In one embodiment, GLP-1 amino acid sequence of the present invention is at least 90% homologous to GLP-1 sequences set forth in Genbank ID No. NP002045 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters. In one embodiment, GLP-1 amino acid sequence of the present invention is at least 95% homologous to GLP-1 sequences set forth in Genbank ID No. NP002045 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters.

[000158]In one embodiment, the polypeptide of interest or fragment thereof provided herein is an apolipoprotein. In another embodiment, the polypeptide or fragment thereof provided herein is an apolipoprotein A1 (APO-A1). In another embodiment, the apolipoprotein is attached to at least one CTP peptide on the N- and or the C-terminus. In another embodiment, the apolipotrotein is apolipoprotein AI, apolipoprotein AII, apolipoprotein AIV, or an analogue or variant thereof.

[000159]In one embodiment, the apolipoprotein constructs according to the present invention may broadly be looked upon as HDL analogues due to their ability to form complexes with cholesterol and other lipids and assist in the transportation of these compounds to the liver.

[000160]In another embodiment, functional equivalence to native apolipoprotein A-I, A-II or A-IV may conveniently be measured using a lipid binding assay. The ability of the CTP-modified apolipoprotein to elicit substantially the same physiological response in a mammal may conveniently be measured by measurement of the ability to perform reverse cholesterol transport in a test organism such as rabbits or rodents such as mice.

[000161]In one embodiment, the polypeptide comprising the CTP-modified apolipoprotein is capable of performing reverse cholesterol transport as well as or even better than native apolipoproteins *in-vivo*, despite the modification caused by the addition of at least one CTP. In another embodiment, in-vitro the CTP-modified apolipoprotein has lower *in-vitro*

biological activity but it is compensated by an extended half-life. In another embodiment, the plasma half-life of the CTP-modified apolipoprotein is preferably increased compared to that of the wild-type apolipoprotein. In one embodiment, the increased half-life is due to the hydrodynamic size of the apolipoprotein construct, which may reduce the rate of filtration through the kidneys.

[000162] In one embodiment, the amino acid sequence of APO-A1 is DEPPQSPWDRVKDKATVYVDVLKDSGRDYVSQFEGSAGKGLNLKLLDNWDSVTST FSKLREQLGPVTQEFWDNLEKETEGLRGEMSKDLEEVKAKVQPYLDDFQKKWQEE MELYRQKVEPLRAELQEGARQKLHELQEKLSPLGEEMRDRARAHVDALRTHLAPYS DELRQRLAARLEALKENGGARLAEYHAKATEHLSTLSEKAKPALEDLRQGLLPVLES FKVSFLSALEEYTKKLNTQ (SEQ ID NO: 44) or a homologue or variant or fragment thereof. In one embodiment, the methods of the present invention the methods of the present invention provide an amino acid sequence comprising an APO-A1 peptide having one CTP amino acid peptide on the C-terminus: MKAAVLTLAVLFLTGSQARHFWQQDEPPQSPWDRVKDLATVYVDVLKDSGRDYVS QFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQEFWDNLEKETEGLRQEMS KDLEEVKAKVQPYLDDFQKKWQEEMELYRQKVEPLRAELQEGARQKLHELQEKLS PLGEEMRDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATE HLSTLSEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTKKLNTQSSSSKAPPPSLPSPS RLPGPSDTPILPQ (SEQ ID NO: 70).

[000163] In another embodiment, the methods of the present invention provide a nucleic acid sequence, set forth in SEQ ID NO: 71, encoding an APO-A1 peptide and one CTP amino acid peptide on the C-terminus: ATGAAGGCCGCCGTGCTGACCCTGGCCGTGCTGTTTCTGACCGGCTCTCAGGCCC GGCACTTCTGGCAGCAGGACGAGCCTCCCCAGTCCCCCTGGGACAGAGTGAAGG ACCTGGCCACCGTGTACGTGGACGTGCTGAAGGACTCCGGCAGAGACTACGTGTC CCAGTTCGAGGGCTCTGCCCTGGGCAAGCAGCTGAACCTGAAGCTGCTGGACAAC TGGGACTCCGTGACCTCCACCTTCTCCAAGCTGCGCGAACAGCTGGGACCTGTGA CCCAGGAATTCTGGGACAACCTGGAAAAAGAGACAGAGGGCCTGAGACAGGAA ATGTCCAAGGACCTGGAAGAGGTCAAAGCCAAGGTGCAGCCCTACCTGGACGAC TTCCAGAAGAAATGGCAGGAAGAGATGGAACTGTACCGGCAGAAGGTGGAACCC

CTGCGGGCCGAGCTGCAGGAAGGCGCTAGACAGAAGCTGCACGAACTGCAGGAA
AAGCTGTCCCCCCTGGGCGAGGAAATGCGGGACAGAGCCAGAGCCCACGTGGAC
GCCCTGAGAACCCACCTGGCCCCCTACTCTGACGAGCTGCGGCAGAGGCTGGCCG
CCAGACTGGAAGCCCTGAAAGAGAACGGCGGAGCCCGGCTGGCCGAGTACCACG
CTAAGGCTACCGAGCACCTGTCCACCCTGTCCGAGAAGGCCAAGCCCGCCCTGGA
AGATCTGCGGCAGGGCCTGCTGCCCGTGCTGGAATCCTTCAAGGTGTCCTTCCTG
TCCGCTCTGGAAGAGTACACCAAGAAGCTGAACACCCAGTCCTCCAGCTCCAAGG
CCCCTCCACCCTCCCTGCCTAGCCCTAGTAGACTGCCTGGGCCCTCCGACACCCCC
ATCCTGCCCCAGTGATGAGGATCCGCGGCCGCGAGCTC (SEQ ID NO: 71).

[000164] In one embodiment, the methods of the present invention the methods of the present invention provide an amino acid sequence comprising an APO-A1 peptide having two CTP amino acid peptide C-terminus:
MKAAVLTLAVLFLTGSQARHFWQQDEPPQSPWDRVKDLATVYVDVLKDSGRDYVS
QFEGSALGKQLNLKLLDNWDSVTSTFSKLREQLGPVTQEFWDNLEKETEGLRQEMS
KDLEEVKAKVQPYLDDFQKKWQEEMELYRQKVEPLRAELQEGARQKLHELQEKLS
PLGEEMRDRARAHVDALRTHLAPYSDELRQRLAARLEALKENGGARLAEYHAKATE
HLSTLSEKAKPALEDLRQGLLPVLESFKVSFLSALEEYTKKLNTQSSSSKAPPPSLPSPS
RLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 72).

[000165] In another embodiment, the methods of the present invention provide a nucleic acid sequence, set forth in SEQ ID NO: 73, encoding an APO-A1 peptide and two CTP amino acid peptide on the C-terminus:
ATGAAGGCCGCCGTGCTGACCCTGGCCGTGCTGTTTCTGACCGGCTCTCAGGCCC
GGCACTTCTGGCAGCAGGACGAGCCTCCCCAGTCCCCCTGGGACAGAGTGAAGG
ACCTGGCCACCGTGTACGTGGACGTGCTGAAGGACTCCGGCAGAGACTACGTGTC
CCAGTTCGAGGGCTCTGCCCTGGGCAAGCAGCTGAACCTGAAGCTGCTGGACAAC
TGGGACTCCGTGACCTCCACCTTCTCCAAGCTGCGCGAACAGCTGGGACCTGTGA
CCCAGGAATTCTGGGACAACCTGGAAAAAGAGACAGAGGGCCTGAGACAGGAA
ATGTCCAAGGACCTGGAAGAGGTCAAAGCCAAGGTGCAGCCCTACCTGGACGAC
TTCCAGAAGAAATGGCAGGAAGAGATGGAACTGTACCGGCAGAAGGTGGAACCC
CTGCGGGCCGAGCTGCAGGAAGGCGCTAGACAGAAGCTGCACGAACTGCAGGAA
AAGCTGTCCCCCCTGGGCGAGGAAATGCGGGACAGAGCCAGAGCCCACGTGGAC

GCCCTGAGAACCCACCTGGCCCCCTACTCTGACGAGCTGCGGCAGAGGCTGGCCG
CCAGACTGGAAGCCCTGAAAGAGAACGGCGGAGCCCGGCTGGCCGAGTACCACG
CTAAGGCTACCGAGCACCTGTCCACCCTGTCCGAGAAGGCCAAGCCCGCCCTGGA
AGATCTGCGGCAGGGCCTGCTGCCCGTGCTGGAATCCTTCAAGGTGTCCTTCCTG
TCCGCTCTGGAAGAGTACACCAAGAAGCTGAACACCCAGTCCTCCAGCTCCAAGG
CCCCTCCACCCTCCCTGCCTAGCCCTAGTAGACTGCCTGGGCCCTCCGACACACC
AATCCTGCCACAGAGCAGCTCCTCTAAGGCCCCTCCTCCATCCCTGCCATCCCCCT
CCCGGCTGCCTGGCCCCTCTGACACCCCTATCCTGCCTCAGTGATGAAGGTCTGG
ATCCGCGGCCGC (SEQ ID NO: 73).

[000166]In one embodiment, a functional equivalent of an apolipoprotein or fragments thereof may be obtained by addition, substitution or deletion of at least one amino acid. When the amino acid sequence comprises a substitution of one amino acid for another, such a substitution may be a conservative amino acid substitution. Fragments of SEQ ID NO: 44 may comprise more than one such substitution, such as, for e.g., two conservative amino acid substitutions, for example three or four conservative amino acid substitutions, such as five or six conservative amino acid substitutions, for example seven or eight conservative amino acid substitutions, such as from 10 to 15 conservative amino acid substitutions, for example from 15 to 25 conservative amino acid substitution, such as from 25 to 75 conservative amino acid substitutions, for example from 75 to 125 conservative amino acid substitutions, such as from 125 to 175 conservative amino acid substitutions. Substitutions can be made within any one or more groups of predetermined amino acids.

[000167]In another embodiment, a fragment of apolipoprotein contains the lipid binding region.

[000168]"Functional equivalency" as used in the present invention is according to one preferred embodiment established by means of reference to the corresponding functionality of a predetermined fragment of the sequences provided herein.

[000169]Functional equivalents of variants of the sequences provided herein will be understood to exhibit amino acid sequences gradually differing from the preferred predetermined sequence, as the number and scope of insertions, deletions and substitutions including conservative substitutions increases. This difference is measured as a reduction in

homology between the preferred predetermined sequence and the fragment or functional equivalent.

[000170]In one embodiment, the polypeptide of interest or fragment thereof provided herein is a cytokine. In another embodiment, the cytokine is a Hematopoietin cytokine. In another embodiment, the cytokine is an Interferon cytokine. In another embodiment, the cytokine is a chemokine. In another embodiment, the cytokine is a Tumor Necrosis Factor cytokine. In another embodiment, a cytokine as used herein comprises biological activity and clinical efficacy. In another embodiment, a cytokine as used herein is a therapeutic protein.

[000171]AII fragments or functional equivalents of apolipoprotein are included within the scope of this invention, regardless of the degree of homology that they show to a preferred predetermined sequence of apolipoprotein. The reason for this is that some regions of the sequence SEQ ID NO: 44 are most likely readily mutatable, or capable of being completely deleted, without any significant effect on the binding activity of the resulting fragment. Methods of generating functionally equivalent variants of SEQ ID NO:44 are described in US Patent No. 6,897,039, incorporated herein by reference.

[000172]In one embodiment, the polypeptide of interest or fragment thereof provided herein is a coagulation factor. In another embodiment, a coagulation factor of the invention is a protein. In another embodiment, a coagulation factor of the invention is a peptide. In another embodiment, a coagulation factor of the invention is a polypeptide. In another embodiment, the coagulation factor is an enzyme. In another embodiment, the coagulation factor is a serine protease. In another embodiment, the coagulation factor is a glycoprotein. In another embodiment, the coagulation factor is a transglutaminase. In another embodiment, the coagulation factor is an inactive zymogen. In another embodiment, the coagulation factor is any coagulation factor known to one of skill in the art. In another embodiment, the coagulation factor is FVIII. In another embodiment, the coagulation factor is FV. In another embodiment, the coagulation factor is Factor XIII. In another embodiment, the coagulation factor is factor X. In another embodiment, the coagulation factor is thrombin. In another embodiment, the coagulation factor is fibrin. In another embodiment, the coagulation factor is FVIIa. In another embodiment, the coagulation factor is FVII. In another embodiment, the coagulation factor is FIX. In another embodiment, the coagulation factor is FX. In another embodiment, the coagulation factor is FXIa. In another embodiment, the coagulation factor is FXII. In another embodiment, the coagulation factor is FXa. In another embodiment, the

coagulation factor is FVa. In another embodiment, the coagulation factor is prothrombin. In another embodiment, the coagulation factor is thrombin. In another embodiment, the coagulation factor is FV. In another embodiment, the coagulation factor is FXI. In another embodiment, the coagulation factor is vWF. In another embodiment, the coagulation factor is FVIIIa. In another embodiment, the coagulation factor is B-deleted Domain FVIII (FVIIIBDD). In another embodiment, the coagulation factor is FIXa. In another embodiment, the coagulation factor is prekallikrein. In another embodiment, the coagulation factor is kallikrein. In another embodiment, the coagulation factor is FXIIa. In another embodiment, the coagulation factor is fibrinogen. In another embodiment, the coagulation factor is thrombomodulin. In another embodiment, the coagulation factor is FII.

[000173]In another embodiment, the coagulation factor is a glycoprotein. In another embodiment, the coagulation factor is a vitamin K dependent glycoprotein. In another embodiment, the coagulation factor is a vitamin K independent glycoprotein. In another embodiment, the coagulation factor is a recombinant protein. In another embodiment, the coagulation factor is a recombinant glycoprotein. In another embodiment, the coagulation factor is a recombinant glycoprotein FV. In another embodiment, the coagulation factor is a recombinant FVI. In another embodiment, the coagulation factor is a recombinant FVII. In another embodiment, the coagulation factor is a recombinant FVIII. In another embodiment, the coagulation factor is a recombinant FIX. In another embodiment, the coagulation factor is a recombinant FX. In another embodiment, the coagulation factor is a recombinant FXI. In another embodiment, the coagulation factor is a recombinant FXII. In another embodiment, the coagulation factor is a recombinant FvW. In another embodiment, the coagulation factor is a recombinant FII. In another embodiment, the coagulation factor is a recombinant FIXa. In another embodiment, the coagulation factor is a recombinant FXIa. In another embodiment, the coagulation factor is a recombinant fibrin. In another embodiment, the coagulation factor is a recombinant FVIIa. In another embodiment, the coagulation factor is a recombinant FXa. In another embodiment, the coagulation factor is a recombinant FVa. In another embodiment, the coagulation factor is a recombinant prothrombin. In another embodiment, the coagulation factor is a recombinant thrombin. In another embodiment, the coagulation factor is a recombinant FVIIIa. In another embodiment, the coagulation factor is a recombinant prekallikrein. In another embodiment, the coagulation factor is a recombinant kallikrein. In another embodiment, the coagulation factor is a recombinant FXIIa. In another embodiment,

the coagulation factor is any known recombinant coagulation factor. In another embodiment, the coagulation factor comprising a signal peptide is any known recombinant coagulation factor. In another embodiment, a coagulation factor comprises 1-10 CTP repeats attached to the C-terminus and no CTPs attached to the N-terminus. In another embodiment, the coagulation factor comprising a signal peptide is any known recombinant coagulation factor. In another embodiment, a coagulation factor comprises at least one CTP attached to the C-terminus and no CTPs attached to the N-terminus. In another embodiment, a coagulation factor comprising 1-10 CTP repeats attached to the C-terminus and no CTPs attached to the N-terminus is an engineered coagulation factor. In another embodiment, a coagulation factor comprising at least one CTP attached to the C-terminus and no CTPs attached to the N-terminus is an engineered coagulation factor. In another embodiment, a coagulation factor comprising 1-10 CTP repeats attached to the C-terminus and no CTPs attached to the N-terminus is a conjugated coagulation factor. In another embodiment, a coagulation factor comprising at least one CTP attached to the C-terminus and no CTPs attached to the N-terminus is a conjugated coagulation factor.

[000174]In another embodiment, the coagulation factor comprising a domain organization similar or identical to the domain organization of FIX, FVII, factor X, protein C and prothrombin. In another embodiment, the coagulation factor is synthesized as precursors with N-terminal propeptide. In another embodiment, the coagulation factor as used herein is in an inactive pro-enzyme form. In another embodiment, the coagulation factor is produces in hepatocytes. In another embodiment, the coagulation factor comprises a docking site for gammacarboxylase which converts glutamic acids (Glu) into gamma carboxy glutamic acids (Gla). In another embodiment, the coagulation factor is a commercially available coagulation factor.

[0033] In another embodiment, the amino acid sequence of factor VII comprises the following amino acid sequence:

MVSQALRLLCLLLGLQGCLAAVFVTQEEAHGVLHRRRRANAFLEELRPGSLE
RECKEEQCSFEEAREIFKDAERTKLFWISYSDGDQCASSPCQNGGSCKDQLQSYICFC
LPAFEGRNCETHKDDQLICVNENGGCEQYCSDHTGTKRSCRCHEGYSLLADGVSCTP
TVEYPCGKIPILEKRNASKPQGRIVGGKVCPKGECPWQVLLLVNGAQLCGGTLINTIW
VVSAAHCFDKIKNWRNLIAVLGEHDLSEHDGDEQSRRVAQVIIPSTYVPGTTNHDIAL
LRLHQPVVLTDHVVPLCLPERTFSERTLAFVRFSLVSGWGQLLDRGATALELMVLNV

PRLMTQDCLQQSRKVGDSPNITEYMFCAGYSDGSKDSCKGDSGGPHATHYRGTWYL TGIVSWGQGCATVGHFGVYTRVSQYIEWLQKLMRSEPRPGVLLRAPFP (SEQ ID NO: 45).

[000175]In another embodiment, the amino acid sequence of factor VII comprises the following amino acid sequence:

MVSQALRLLCLLLGLQGCLAAVFVTQEEAHGVLHRRRRANAFLEELRPGSLERECKE EQCSFEEAREIFKDAERTKLFWISYSDGDQCASSPCQNGGSCKDQLQSYICFCLPAFEG RNCETHKDDQLICVNENGGCEQYCSDHTGTKRSCRCHEGYSLLADGVSCTPTVEYPC GKIPILEKRNASKPQGRIVGGKVCPKGECPWQVLLLVNGAQLCGGTLINTIWVVSAA HCFDKIKNWRNLIAVLGEHDLSEHDGDEQSRRVAQVIIPSTYVPGTTNHDIALLRLHQ PVVLTDHVVPLCLPERTFSERTLAFVRFSLVSGWGQLLDRGATALELMVLNVPRLMT QDCLQQSRKVGDSPNITEYMFCAGYSDGSKDSCKGDSGGPHATHYRGTWYLTGIVS WGQGCATVGHFGVYTRVSQYIEWLQKLMRSEPRPGVLLRAPFP*GCGR. (SEQ ID NO: 46).

[000176] In another embodiment, the nucleic acid sequence encoding factor VII comprises the nucleic acid sequence:

CTCGAGGACATGGTCTCCCAGGCCCTCAGGCTCCTCTGCCTTCTGCTTGGGCTTCA GGGCTGCCTGGCTGCAGTCTTCGTAACCCAGGAGGAAGCCCACGGCGTCCTGCA CCGGCGCCGGCGCGCCAACGCGTTCCTGGAGGAGCTGCGGCCGGGCTCCCTGGA GAGGGAGTGCAAGGAGGAGCAGTGCTCCTTCGAGGAGGCCCGGGAGATCTTCAA GGACGCGGAGAGGACGAAGCTGTTCTGGATTTCTTACAGTGATGGGGACCAGTG TGCCTCAAGTCCATGCCAGAATGGGGGCTCCTGCAAGGACCAGCTCCAGTCCTAT ATCTGCTTCTGCCTCCCTGCCTTCGAGGGCCGGAACTGTGAGACGCACAAGGATG ACCAGCTGATCTGTGTGAACGAGAACGGCGGCTGTGAGCAGTACTGCAGTGACC ACACGGGCACCAAGCGCTCCTGTCGGTGCCACGAGGGGTACTCTCTGCTGGCAG ACGGGGTGTCCTGCACACCCACAGTTGAATATCCATGTGGAAAAATACCTATTCT AGAAAAAAGAAATGCCAGCAAACCCCAAGGCCGAATTGTGGGGGGCAAGGTGT GCCCCAAAGGGGAGTGTCCATGGCAGGTCCTGTTGTTGGTGAATGGAGCTCAGTT GTGTGGGGGGACCCTGATCAACACCATCTGGGTGGTCTCCGCGGCCCACTGTTTC GACAAAATCAAGAACTGGAGGAACCTGATCGCGGTGCTGGGCGAGCACGACCTC AGCGAGCACGACGGGGATGAGCAGAGCCGGCGGGTGGCGCAGGTCATCATCCCC AGCACGTACGTCCCGGGCACCACCAACCACGACATCGCGCTGCTCCGCCTGCACC

AGCCCGTGGTCCTCACTGACCATGTGGTGCCCCTCTGCCTGCCCGAACGGACGTT
CTCTGAGAGGACGCTGGCCTTCGTGCGCTTCTCATTGGTCAGCGGCTGGGGCCAG
CTGCTGGACCGTGGCGCCACGGCCCTGGAGCTCATGGTCCTCAACGTGCCCCGGC
TGATGACCCAGGACTGCCTGCAGCAGTCACGGAAGGTGGGAGACTCCCCAAATA
TCACGGAGTACATGTTCTGTGCCGGCTACTCGGATGGCAGCAAGGACTCCTGCAA
GGGGGACAGTGGAGGCCCACATGCCACCCACTACCGGGGCACGTGGTACCTGAC
GGGCATCGTCAGCTGGGGCCAGGGCTGCGCAACCGTGGGCCACTTTGGGGTGTA
CACCAGGGTCTCCCAGTACATCGAGTGGCTGCAAAAGCTCATGCGCTCAGAGCC
ACGCCCAGGAGTCCTCCTGCGAGCCCCATTTCCCTGAGGATGCGGCCGC (SEQ ID
NO: 47).

[000177]In another embodiment, the nucleic acid sequence encoding factor VII-CTP (attached to the carboxy terminus) comprises the following nucleic acid sequence:

CTCGAGGACATGGTCTCCCAGGCCCTCAGGCTCCTCTGCCTTCTGCTTGGGCTTCA
GGGCTGCCTGGCTGCAGTCTTCGTAACCCAGGAGGAAGCCCACGGCGTCCTGCA
CCGGCGCCGGCGCGCCAACGCGTTCCTGGAGGAGCTGCGGCCGGGCTCCCTGGA
GAGGGAGTGCAAGGAGGAGCAGTGCTCCTTCGAGGAGGCCCGGGAGATCTTCAA
GGACGCGGAGAGGACGAAGCTGTTCTGGATTTCTTACAGTGATGGGGACCAGTG
TGCCTCAAGTCCATGCCAGAATGGGGGCTCCTGCAAGGACCAGCTCCAGTCCTAT
ATCTGCTTCTGCCTCCCTGCCTTCGAGGGCCGGAACTGTGAGACGCACAAGGATG
ACCAGCTGATCTGTGTGAACGAGAACGGCGGCTGTGAGCAGTACTGCAGTGACC
ACACGGGCACCAAGCGCTCCTGTCGGTGCCACGAGGGGTACTCTCTGCTGGCAG
ACGGGGTGTCCTGCACACCCACAGTTGAATATCCATGTGGAAAAATACCTATTCT
AGAAAAAAGAAATGCCAGCAAACCCCAAGGCCGAATTGTGGGGGGCAAGGTGT
GCCCCAAAGGGGAGTGTCCATGGCAGGTCCTGTTGTTGGTGAATGGAGCTCAGTT
GTGTGGGGGGACCCTGATCAACACCATCTGGGTGGTCTCCGCGGCCCACTGTTTC
GACAAAATCAAGAACTGGAGGAACCTGATCGCGGTGCTGGGCGAGCACGACCTC
AGCGAGCACGACGGGGATGAGCAGAGCCGGCGGGTGGCGCAGGTCATCATCCCC
AGCACGTACGTCCCGGGCACCACCAACCACGACATCGCGCTGCTCCGCCTGCACC
AGCCCGTGGTCCTCACTGACCATGTGGTGCCCCTCTGCCTGCCCGAACGGACGTT
CTCTGAGAGGACGCTGGCCTTCGTGCGCTTCTCATTGGTCAGCGGCTGGGGCCAG
CTGCTGGACCGTGGCGCCACGGCCCTGGAGCTCATGGTCCTCAACGTGCCCCGGC
TGATGACCCAGGACTGCCTGCAGCAGTCACGGAAGGTGGGAGACTCCCCAAATA

TCACGGAGTACATGTTCTGTGCCGGCTACTCGGATGGCAGCAAGGACTCCTGCAA
GGGGGACAGTGGAGGCCCACATGCCACCCACTACCGGGGCACGTGGTACCTGAC
CGGCATCGTGAGCTGGGGCCAGGGCTGCGCCACCGTGGGCCACTTCGGCGTGTAC
ACCAGGGTGTCCCAGTACATCGAGTGGCTGCAGAAACTGATGAGAAGCGAGCCC
AGACCCGGCGTGCTGCTGAGAGCCCCCTTCCCCAGCAGCAGCTCCAAGGCCCCTC
CCCCTAGCCTGCCCAGCCCTAGCAGACTGCCTGGGCCCAGCGACACCCCCATCCT
GCCCCAGTGAGGATCCGCGGCCGC (SEQ ID NO: 48).

[000178]In another embodiment, the amino acid sequence of factor VII-CTP (attached to the carboxy terminus) comprises the following amino acid sequence:

MVSQALRLLCLLLGLQGCLAAVFVTQEEAHGVLHRRRRANAFLEELRPGSLERECKE
EQCSFEEAREIFKDAERTKLFWISYSDGDQCASSPCQNGGSCKDQLQSYICFCLPAFEG
RNCETHKDDQLICVNENGGCEQYCSDHTGTKRSCRCHEGYSLLADGVSCTPTVEYPC
GKIPILEKRNASKPQGRIVGGKVCPKGECPWQVLLLVNGAQLCGGTLINTIWVVSAA
HCFDKIKNWRNLIAVLGEHDLSEHDGDEQSRRVAQVIIPSTYVPGTTNHDIALLRLHQ
PVVLTDHVVPLCLPERTFSERTLAFVRFSLVSGWGQLLDRGATALELMVLNVPRLMT
QDCLQQSRKVGDSPNITEYMFCAGYSDGSKDSCKGDSGGPHATHYRGTWYLTGIVS
WGQGCATVGHFGVYTRVSQYIEWLQKLMRSEPRPGVLLRAPFPSSSSKAPPPSLPSPS
RLPGPSDTPILPQ* (SEQ ID NO: 49).

[000179] In another embodiment, the nucleic acid sequence encoding factor VII-CTP-CTP (attached to the carboxy terminus) comprises the following nucleic acid sequence:

CTCGAGGACATGGTCTCCCAGGCCCTCAGGCTCCTCTGCCTTCTGCTTGGGCTTCA
GGGCTGCCTGGCTGCAGTCTTCGTAACCCAGGAGGAAGCCCACGGCGTCCTGCA
CCGGCGCCGGCGCGCCAACGCGTTCCTGGAGGAGCTGCGGCCGGGCTCCCTGGA
GAGGGAGTGCAAGGAGGAGCAGTGCTCCTTCGAGGAGGCCCGGGAGATCTTCAA
GGACGCGGAGAGGACGAAGCTGTTCTGGATTTCTTACAGTGATGGGGACCAGTG
TGCCTCAAGTCCATGCCAGAATGGGGGCTCCTGCAAGGACCAGCTCCAGTCCTAT
ATCTGCTTCTGCCTCCCTGCCTTCGAGGGCCGGAACTGTGAGACGCACAAGGATG
ACCAGCTGATCTGTGTGAACGAGAACGGCGGCTGTGAGCAGTACTGCAGTGACC
ACACGGGCACCAAGCGCTCCTGTCGGTGCCACGAGGGGTACTCTCTGCTGGCAG
ACGGGGTGTCCTGCACACCCACAGTTGAATATCCATGTGGAAAAATACCTATTCT
AGAAAAAAGAAATGCCAGCAAACCCCAAGGCCGAATTGTGGGGGGCAAGGTGT
GCCCCAAAGGGGAGTGTCCATGGCAGGTCCTGTTGTTGGTGAATGGAGCTCAGTT

GTGTGGGGGGACCCTGATCAACACCATCTGGGTGGTCTCCGCGGCCCACTGTTTC
GACAAAATCAAGAACTGGAGGAACCTGATCGCGGTGCTGGGCGAGCACGACCTC
AGCGAGCACGACGGGGATGAGCAGAGCCGGCGGGTGGCGCAGGTCATCATCCCC
AGCACGTACGTCCCGGGCACCACCAACCACGACATCGCGCTGCTCCGCCTGCACC
AGCCCGTGGTCCTCACTGACCATGTGGTGCCCCTCTGCCTGCCCGAACGGACGTT
CTCTGAGAGGACGCTGGCCTTCGTGCGCTTCTCATTGGTCAGCGGCTGGGGCCAG
CTGCTGGACCGTGGCGCCACGGCCCTGGAGCTCATGGTCCTCAACGTGCCCCGGC
TGATGACCCAGGACTGCCTGCAGCAGTCACGGAAGGTGGGAGACTCCCCAAATA
TCACGGAGTACATGTTCTGTGCCGGCTACTCGGATGGCAGCAAGGACTCCTGCAA
GGGGGGACAGTGGAGGCCCACATGCCACCCACTACCGGGGCACGTGGTACCTGAC
CGGCATCGTGAGCTGGGGCCAGGGCTGCGCCACCGTGGGCCACTTCGGCGTGTA
CACCAGGGTGTCCCAGTACATCGAGTGGCTGCAGAAACTGATGAGAAGCGAGCC
CAGACCCGGCGTGCTGCTGAGAGCCCCCTTCCCCAGCAGCAGCTCCAAGGCCCCT
CCCCCTAGCCTGCCCAGCCCTAGCAGACTGCCTGGGCCCTCCGACACACCAATCC
TGCCACAGAGCAGCTCCTCTAAGGCCCCTCCTCCATCCCTGCCATCCCCCTCCCG
GCTGCCAGGCCCCTCTGACACCCCTATCCTGCCTCAGTGATGAAGGTCTGGATCC
GCGGCCGC (SEQ ID NO: 50).

[000180]In another embodiment, the amino acid sequence of factor VII-CTP-CTP (attached to the carboxy terminus) comprises the following amino acid sequence: MVSQALRLLCLLLGLQGCLAAVFVTQEEAHGVLHRRRRANAFLEELRPGSLERECKE EQCSFEEAREIFKDAERTKLFWISYSDGDQCASSPCQNGGSCKDQLQSYICFCLPAFEG RNCETHKDDQLICVNENGGCEQYCSDHTGTKRSCRCHEGYSLLADGVSCTPTVEYPC GKIPILEKRNASKPQGRIVGGKVCPKGECPWQVLLLVNGAQLCGGTLINTIWVVSAA HCFDKIKNWRNLIAVLGEHDLSEHDGDEQSRRVAQVIIPSTYVPGTTNHDIALLRLHQ PVVLTDHVVPLCLPERTFSERTLAFVRFSLVSGWGQLLDRGATALELMVLNVPRLMT QDCLQQSRKVGDSPNITEYMFCAGYSDGSKDSCKGDSGGPHATHYRGTWYLTGIVS WGQGCATVGHFGVYTRVSQYIEWLQKLMRSEPRPGVLLRAPFPSSSSKAPPPSLPSPS RLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ** (SEQ ID NO: 51).

[000181]In another embodiment, the amino acid sequence of factor VII-CTP-CTP-CTP (three attached to the carboxy terminus) comprises the following amino acid sequence: MVSQALRLLCLLLGLQGCLAAVFVTQEEAHGVLHRRRRANAFLEELRPGSLERECKE EQCSFEEAREIFKDAERTKLFWISYSDGDQCASSPCQNGGSCKDQLQSYICFCLPAFEG

RNCETHKDDQLICVNENGGCEQYCSDHTGTKRSCRCHEGYSLLADGVSCTPTVEYPC
GKIPILEKRNASKPQGRIVGGKVCPKGECPWQVLLLVNGAQLCGGTLINTIWVVSAA
HCFDKIKNWRNLIAVLGEHDLSEHDGDEQSRRVAQVIIPSTYVPGTTNHDIALLRLHQ
PVVLTDHVVPLCLPERTFSERTLAFVRFSLVSGWGQLLDRGATALELMVLNVPRLMT
QDCLQQSRKVGDSPNITEYMFCAGYSDGSKDSCKGDSGGPHATHYRGTWYLTGIVS
WGQGCATVGHFGVYTRVSQYIEWLQKLMRSEPRPGVLLRAPFPSSSSKAPPPSLPSPS
RLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSD
TPILPQ (SEQ ID NO: 52).

[000182]In another embodiment, the amino acid sequence of factor VII-CTP(X4) (four attached to the carboxy terminus) comprises the following amino acid sequence: MVSQALRLLCLLLGLQGCLAAVFVTQEEAHGVLHRRRRANAFLEELRPGSLERECKE
EQCSFEEAREIFKDAERTKLFWISYSDGDQCASSPCQNGGSCKDQLQSYICFCLPAFEG
RNCETHKDDQLICVNENGGCEQYCSDHTGTKRSCRCHEGYSLLADGVSCTPTVEYPC
GKIPILEKRNASKPQGRIVGGKVCPKGECPWQVLLLVNGAQLCGGTLINTIWVVSAA
HCFDKIKNWRNLIAVLGEHDLSEHDGDEQSRRVAQVIIPSTYVPGTTNHDIALLRLHQ
PVVLTDHVVPLCLPERTFSERTLAFVRFSLVSGWGQLLDRGATALELMVLNVPRLMT
QDCLQQSRKVGDSPNITEYMFCAGYSDGSKDSCKGDSGGPHATHYRGTWYLTGIVS
WGQGCATVGHFGVYTRVSQYIEWLQKLMRSEPRPGVLLRAPFPSSSSKAPPPSLPSPS
RLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSD
TPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 53).

[000183]In another embodiment, the amino acid sequence of factor VII-CTP(x5) (five attached to the carboxy terminus) comprises the following amino acid sequence: MVSQALRLLCLLLGLQGCLAAVFVTQEEAHGVLHRRRRANAFLEELRPGSLERECKE
EQCSFEEAREIFKDAERTKLFWISYSDGDQCASSPCQNGGSCKDQLQSYICFCLPAFEG
RNCETHKDDQLICVNENGGCEQYCSDHTGTKRSCRCHEGYSLLADGVSCTPTVEYPC
GKIPILEKRNASKPQGRIVGGKVCPKGECPWQVLLLVNGAQLCGGTLINTIWVVSAA
HCFDKIKNWRNLIAVLGEHDLSEHDGDEQSRRVAQVIIPSTYVPGTTNHDIALLRLHQ
PVVLTDHVVPLCLPERTFSERTLAFVRFSLVSGWGQLLDRGATALELMVLNVPRLMT
QDCLQQSRKVGDSPNITEYMFCAGYSDGSKDSCKGDSGGPHATHYRGTWYLTGIVS
WGQGCATVGHFGVYTRVSQYIEWLQKLMRSEPRPGVLLRAPFPSSSSKAPPPSLPSPS
RLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSD

TPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 54).

[000184]In another embodiment, the nucleic acid sequence encoding factor IX comprises the following nucleic acid sequence:

GCGATCGCCATGCAGCGCGTGAACATGATCATGGCAGAATCACCAGGCCTCATC
ACCATTGCCTTTTAGGATATCTACTCAGTGCTGAATGTACAGTTTTTCTTGATCAT
GAAAACGCCAACAAAATTCTGAATCGGCCAAAGAGGTATAATTCAGGTAAATTG
GAAGAGTTTGTTCAAGGGAACCTTGAGAGAGAATGTATGGAAGAAAAGTGTAGT
TTTGAAGAAGCACGAGAAGTTTTTGAAAACACTGAAAGAACAACTGAATTTTGG
AAGCAGTATGTTGATGGAGATCAGTGTGAGTCCAATCCATGTTTAAATGGCGGCA
GTTGCAAGGATGACATTAATTCCTATGAATGTTGGTGTCCCTTTGGATTTGAAGG
AAAGAACTGTGAATTAGATGTAACATGTAACATTAAGAATGGCAGATGCGAGCA
GTTTTGTAAAAATAGTGCTGATAACAAGGTGGTTTGCTCCTGTACTGAGGGATAT
CGACTTGCAGAAAACCAGAAGTCCTGTGAACCAGCAGTGCCATTTCCATGTGGA
AGAGTTTCTGTTTCACAAACTTCTAAGCTCACCCGTGCTGAGACTGTTTTTCCTGA
TGTGGACTATGTAAATTCTACTGAAGCTGAAACCATTTTGGATAACATCACTCAA
AGCACCCAATCATTTAATGACTTCACTCGAGTTGTTGGTGGAGAAGATGCCAAAC
CAGGTCAATTCCCTTGGCAGGTTGTTTTGAATGGTAAAGTTGATGCATTCTGTGG
AGGCTCTATCGTTAATGAAAAATGGATTGTAACTGCTGCCCACTGTGTTGAAACT
GGTGTTAAAATTACAGTTGTCGCAGGTGAACATAATATTGAGGAGACAGAACAT
ACAGAGCAAAAGCGAAATGTGATTCGAATTATTCCTCACCACAACTACAATGCA
GCTATTAATAAGTACAACCATGACATTGCCCTTCTGGAACTGGACGAACCCTTAG
TGCTAAACAGCTACGTTACACCTATTTGCATTGCTGACAAGGAATACACGAACAT
CTTCCTCAAATTTGGATCTGGCTATGTAAGTGGCTGGGGAAGAGTCTTCCACAAA
GGGAGATCAGCTTTAGTTCTCCAGTACCTTAGAGTTCCACTTGTTGACCGAGCCA
CATGTCTTCGATCTACAAAGTTCACCATCTATAACAACATGTTCTGTGCTGGCTTC
CATGAAGGAGGTAGAGATTCATGTCAAGGAGATAGTGGGGGACCCCATGTTACT
GAAGTGGAAGGGACCAGTTTCTTAACTGGAATTATTAGCTGGGGTGAAGAGTGT
GCAATGAAAGGCAAATATGGAATATATACCAAGGTATCCCGGTATGTCAACTGG
ATTAAGGAAAAAACAAAGCTCACTTGAACGCGGCCGC (SEQ ID NO: 55).

[000185]In another embodiment, the amino acid sequence of factor IX comprises the following amino acid sequence:

MQRVNMIMAESPGLITICLLGYLLSAECTVFLDHENANKILNRPKRYNSGKLEEFVQG
NLERECMEEKCSFEEAREVFENTERTTEFWKQYVDGDQCESNPCLNGGSCKDDINSY
ECWCPFGFEGKNCELDVTCNIKNGRCEQFCKNSADNKVVCSCTEGYRLAENQKSCE
PAVPFPCGRVSVSQTSKLTRAETVFPDVDYVNSTEAETILDNITQSTQSFNDFTRVVG
GEDAKPGQFPWQVVLNGKVDAFCGGSIVNEKWIVTAAHCVETGVKITVVAGEHNIE
ETEHTEQKRNVIRIIPHHNYNAAINKYNHDIALLELDEPLVLNSYVTPICIADKEYTNIF
LKFGSGYVSGWGRVFHKGRSALVLQYLRVPLVDRATCLRSTKFTIYNNMFCAGFHE
GGRDSCQGDSGGPHVTEVEGTSFLTGIISWGEECAMKGKYGIYTKVSRYVNWIKEKT
KLT* (SEQ ID NO: 56).

[000186]In another embodiment, the nucleic acid sequence encoding factor IX-CTP (attached to the carboxy terminus) comprises the following nucleic acid sequence:
GCGATCGCCATGCAGCGCGTGAACATGATCATGGCAGAATCACCAGGCCTCATC
ACCATCTGCCTTTTAGGATATCTACTCAGTGCTGAATGTACAGTTTTTCTTGATCA
TGAAAACGCCAACAAAATTCTGAATCGGCCAAAGAGGTATAATTCAGGTAAATT
GGAAGAGTTTGTTCAAGGGAACCTTGAGAGAGAATGTATGGAAGAAAAGTGTAG
TTTTGAAGAAGCACGAGAAGTTTTTGAAAACACTGAAAGAACAACTGAATTTTG
GAAGCAGTATGTTGATGGAGATCAGTGTGAGTCCAATCCATGTTTAAATGGCGGC
AGTTGCAAGGATGACATTAATTCCTATGAATGTTGGTGTCCCTTTGGATTTGAAG
GAAAGAACTGTGAATTAGATGTAACATGTAACATTAAGAATGGCAGATGCGAGC
AGTTTTGTAAAAATAGTGCTGATAACAAGGTGGTTTGCTCCTGTACTGAGGGATA
TCGACTTGCAGAAAACCAGAAGTCCTGTGAACCAGCAGTGCCATTTCCATGTGGA
AGAGTTTCTGTTTCACAAACTTCTAAGCTCACCCGTGCTGAGACTGTTTTTCCTGA
TGTGGACTATGTAAATTCTACTGAAGCTGAAACCATTTTGGATAACATCACTCAA
AGCACCCAATCATTTAATGACTTCACTCGAGTTGTTGGTGGAGAAGATGCCAAAC
CAGGTCAATTCCCTTGGCAGGTTGTTTTGAATGGTAAAGTTGATGCATTCTGTGG
AGGCTCTATCGTTAATGAAAAATGGATTGTAACTGCTGCCCACTGTGTTGAAACT
GGTGTTAAAATTACAGTTGTCGCAGGTGAACATAATATTGAGGAGACAGAACAT
ACAGAGCAAAAGCGAAATGTGATTCGAATTATTCCTCACCACAACTACAATGCA
GCTATTAATAAGTACAACCATGACATTGCCCTTCTGGAACTGGACGAACCCTTAG
TGCTAAACAGCTACGTTACACCTATTTGCATTGCTGACAAGGAATACACGAACAT
CTTCCTCAAATTTGGATCTGGCTATGTAAGTGGCTGGGGAAGAGTCTTCCACAAA
GGGAGATCAGCTTTAGTTCTTCAGTACCTTAGAGTTCCACTTGTTGACCGAGCCA

CATGTCTTCGATCTACAAAGTTCACCATCTATAACAACATGTTCTGTGCTGGCTTC
CATGAAGGAGGTAGAGATTCATGTCAAGGAGATAGTGGGGGACCCCATGTTACT
GAAGTGGAAGGGACCAGTTTCTTAACTGGAATTATTAGCTGGGGTGAAGAGTGT
GCAATGAAAGGCAAATATGGAATATATACCAAGGTATCCCGGTATGTCAACTGG
ATTAAGGAAAAAACAAAGCTCACTAGCTCCAGCAGCAAGGCCCCTCCCCCGAGC
CTGCCCTCCCCAAGCAGGCTGCCTGGGCCCTCCGACACACCAATCCTGCCACAGT
GATGAAGGTCTGGATCCGCGGCCGC (SEQ ID NO: 57).

[000187] In another embodiment, the amino acid sequence of factor IX-CTP (attached to the carboxy terminus) comprises the following amino acid sequence:

MQRVNMIMAESPGLITICLLGYLLSAECTVFLDHENANKILNRPKRYNSGKLEEFVQG
NLERECMEEKCSFEEAREVFENTERTTEFWKQYVDGDQCESNPCLNGGSCKDDINSY
ECWCPFGFEGKNCELDVTCNIKNGRCEQFCKNSADNKVVCSCTEGYRLAENQKSCE
PAVPFPCGRVSVSQTSKLTRAETVFPDVDYVNSTEAETILDNITQSTQSFNDFTRVVG
GEDAKPGQFPWQVVLNGKVDAFCGGSIVNEKWIVTAAHCVETGVKITVVAGEHNIE
ETEHTEQKRNVIRIIPHHNYNAAINKYNHDIALLELDEPLVLNSYVTPICIADKEYTNIF
LKFGSGYVSGWGRVFHKGRSALVLQYLRVPLVDRATCLRSTKFTIYNNMFCAGFHE
GGRDSCQGDSGGPHVTEVEGTSFLTGIISWGEECAMKGKYGIYTKVSRYVNWIKEKT
KLTSSSSKAPPPSLPSPSRLPGPSDTPILPQ** (SEQ ID NO: 58).

[000188]In another embodiment, the nucleic acid sequence encoding factor IX-CTP-CTP (attached to the carboxy terminus) comprises the following nucleic acid sequence:

GCGATCGCCATGCAGCGCGTGAACATGATCATGGCAGAATCACCAGGCCTCATC
ACCATCTGCCTTTTAGGATATCTACTCAGTGCTGAATGTACAGTTTTTCTTGATCA
TGAAAACGCCAACAAAATTCTGAATCGGCCAAAGAGGTATAATTCAGGTAAATT
GGAAGAGTTTGTTCAAGGGAACCTTGAGAGAGAATGTATGGAAGAAAAGTGTAG
TTTTGAAGAAGCACGAGAAGTTTTTGAAAACACTGAAAGAACAACTGAATTTTG
GAAGCAGTATGTTGATGGAGATCAGTGTGAGTCCAATCCATGTTTAAATGGCGGC
AGTTGCAAGGATGACATTAATTCCTATGAATGTTGGTGTCCCTTTGGATTTGAAG
GAAAGAACTGTGAATTAGATGTAACATGTAACATTAAGAATGGCAGATGCGAGC
AGTTTTGTAAAAATAGTGCTGATAACAAGGTGGTTTGCTCCTGTACTGAGGGATA
TCGACTTGCAGAAAACCAGAAGTCCTGTGAACCAGCAGTGCCATTTCCATGTGGA
AGAGTTTCTGTTTCACAAACTTCTAAGCTCACCCGTGCTGAGACTGTTTTTCCTGA
TGTGGACTATGTAAATTCTACTGAAGCTGAAACCATTTTGGATAACATCACTCAA

AGCACCCAATCATTTAATGACTTCACTCGAGTTGTTGGTGGAGAAGATGCCAAAC
CAGGTCAATTCCCTTGGCAGGTTGTTTTGAATGGTAAAGTTGATGCATTCTGTGG
AGGCTCTATCGTTAATGAAAAATGGATTGTAACTGCTGCCCACTGTGTTGAAACT
GGTGTTAAAATTACAGTTGTCGCAGGTGAACATAATATTGAGGAGACAGAACAT
ACAGAGCAAAAGCGAAATGTGATTCGAATTATTCCTCACCACAACTACAATGCA
GCTATTAATAAGTACAACCATGACATTGCCCTTCTGGAACTGGACGAACCCTTAG
TGCTAAACAGCTACGTTACACCTATTTGCATTGCTACAAGGAATACACGAACATC
TTCCTCAAATTTGGATCTGGCTATGTAAGTGGCTGGGGAAGAGTCTTCCACAAAG
GGAGATCAGCTTTAGTTCTTCAGTACCTTAGAGTTCCACTTGTTGACCGAGCCAC
ATGTCTTCGATCTACAAAGTTCACCATCTATAACAACATGTTCTGTGCTGGCTTCC
ATGAAGGAGGTAGAGATTCATGTCAAGGAGATAGTGGGGGACCCCATGTTACTG
AAGTGGAAGGGACCAGTTTCTTAACTGGAATTATTAGCTGGGGTGAAGAGTGTG
CAATGAAAGGCAAATATGGAATATATACCAAGGTATCCCGGTATGTCAACTGGA
TTAAGGAAAAAACAAAGCTCACTAGCTCCAGCAGCAAGGCCCCTCCCCCGAGCC
TGCCCTCCCCAAGCAGGCTGCCTGGGCCCTCCGACACACCAATCCTGCCACAGAG
CAGCTCCTCTAAGGCCCCTCCTCCATCCCTGCCATCCCCCTCCCGGCTGCCTGGCC
CCTCTGACACCCCTATCCTGCCTCAGTGATGAAGGTCTGGATCCGCGGCCGC (SEQ
ID NO: 59).

[000189] In another embodiment, the amino acid sequence of factor IX-CTP-CTP (attached to the carboxy terminus) comprises the following amino acid sequence:
MQRVNMIMAESPGLITICLLGYLLSAECTVFLDHENANKILNRPKRYNSGKLEEFVQG
NLERECMEEKCSFEEAREVFENTERTTEFWKQYVDGDQCESNPCLNGGSCKDDINSY
ECWCPFGFEGKNCELDVTCNIKNGRCEQFCKNSADNKVVCSCTEGYRLAENQKSCE
PAVPFPCGRVSVSQTSKLTRAETVFPDVDYVNSTEAETILDNITQSTQSFNDFTRVVG
GEDAKPGQFPWQVVLNGKVDAFCGGSIVNEKWIVTAAHCVETGVKITVVAGEHNIE
ETEHTEQKRNVIRIIPHHNYNAAINKYNHDIALLELDEPLVLNSYVTPICIADKEYTNIF
LKFGSGYVSGWGRVFHKGRSALVLQYLRVPLVDRATCLRSTKFTIYNNMFCAGFHE
GGRDSCQGDSGGPHVTEVEGTSFLTGIISWGEECAMKGKYGIYTKVSRYVNWIKEKT
KLTSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ**
(SEQ ID NO: 60).

[000190]In another embodiment, the amino acid sequence of factor IX-CTP(x3) (three attached to the carboxy terminus) comprises the following amino acid sequence:

MQRVNMIMAESPGLITICLLGYLLSAECTVFLDHENANKILNRPKRYNSGKLEEFVQG
NLERECMEEKCSFEEAREVFENTERTTEFWKQYVDGDQCESNPCLNGGSCKDDINSY
ECWCPFGFEGKNCELDVTCNIKNGRCEQFCKNSADNKVVCSCTEGYRLAENQKSCE
PAVPFPCGRVSVSQTSKLTRAETVFPDVDYVNSTEAETILDNITQSTQSFNDFTRVVG
GEDAKPGQFPWQVVLNGKVDAFCGGSIVNEKWIVTAAHCVETGVKITVVAGEHNIE
ETEHTEQKRNVIRIIPHHNYNAAINKYNHDIALLELDEPLVLNSYVTPICIADKEYTNIF
LKFGSGYVSGWGRVFHKGRSALVLQYLRVPLVDRATCLRSTKFTIYNNMFCAGFHE
GGRDSCQGDSGGPHVTEVEGTSFLTGIISWGEECAMKGKYGIYTKVSRYVNWIKEKT
KLTSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSS
KAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 61).

[000191]In another embodiment, the amino acid sequence of factor IX-CTP(x4) (four attached to the carboxy terminus) comprises the following amino acid sequence:

MQRVNMIMAESPGLITICLLGYLLSAECTVFLDHENANKILNRPKRYNSGKLEEFVQG
NLERECMEEKCSFEEAREVFENTERTTEFWKQYVDGDQCESNPCLNGGSCKDDINSY
ECWCPFGFEGKNCELDVTCNIKNGRCEQFCKNSADNKVVCSCTEGYRLAENQKSCE
PAVPFPCGRVSVSQTSKLTRAETVFPDVDYVNSTEAETILDNITQSTQSFNDFTRVVG
GEDAKPGQFPWQVVLNGKVDAFCGGSIVNEKWIVTAAHCVETGVKITVVAGEHNIE
ETEHTEQKRNVIRIIPHHNYNAAINKYNHDIALLELDEPLVLNSYVTPICIADKEYTNIF
LKFGSGYVSGWGRVFHKGRSALVLQYLRVPLVDRATCLRSTKFTIYNNMFCAGFHE
GGRDSCQGDSGGPHVTEVEGTSFLTGIISWGEECAMKGKYGIYTKVSRYVNWIKEKT
KLTSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSS
KAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQ (SEQ ID NO: 62).

[000192]In another embodiment, the amino acid sequence of factor IX-CTP(x5) (five attached to the carboxy terminus) comprises the following amino acid sequence:

MQRVNMIMAESPGLITICLLGYLLSAECTVFLDHENANKILNRPKRYNSGKLEEFVQG
NLERECMEEKCSFEEAREVFENTERTTEFWKQYVDGDQCESNPCLNGGSCKDDINSY
ECWCPFGFEGKNCELDVTCNIKNGRCEQFCKNSADNKVVCSCTEGYRLAENQKSCE
PAVPFPCGRVSVSQTSKLTRAETVFPDVDYVNSTEAETILDNITQSTQSFNDFTRVVG
GEDAKPGQFPWQVVLNGKVDAFCGGSIVNEKWIVTAAHCVETGVKITVVAGEHNIE
ETEHTEQKRNVIRIIPHHNYNAAINKYNHDIALLELDEPLVLNSYVTPICIADKEYTNIF
LKFGSGYVSGWGRVFHKGRSALVLQYLRVPLVDRATCLRSTKFTIYNNMFCAGFHE

GGRDSCQGDSGGPHVTEVEGTSFLTGIISWGEECAMKGKYGIYTKVSRYVNWIKEKT
KLTSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSS
KAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSLPSPSRLPGPSDTPILPQSSSSKAPPPSL
PSPSRLPGPSDTPILPQ (SEQ ID NO: 63).

[000193]In another embodiment, furin is added to a cell expressing the coagulation factor-CTP of the invention. In another embodiment, furin increases the production efficiency of a coagulation factor-CTP of the invention in a cell. In another embodiment, furin is co-transfected with the vector comprising the coding sequence of the coagulation factor-CTP of the invention. In another embodiment, furin is encoded by a separate vector. In another embodiment, furin and a coagulation factor-CTP are encoded by one vector. In another embodiment, the coding sequence of furin is inserted into pCI-DHFR. In another embodiment, the coding sequence of furin is engineered in pCI-dhfr/smaI+NotI, Furin/AsisI F.I.+NotI.

[000194]In another embodiment, the nucleic acid sequence encoding furin comprises the following nucleic acid sequence:

tctagagtcgacccCGCCATGGAGCTGAGGCCCTGGTTGCTATGGGTGGTAGCAGCAACA
GGAACCTTGGTCCTGCTAGCAGCTGATGCTCAGGGCCAGAAGGTCTTCACCAACA
CGTGGGCTGTGCGCATCCCTGGAGGCCCAGCGGTGGCCAACAGTGTGGCACGGA
AGCATGGGTTCCTCAACCTGGGCCAGATCTTCGGGGACTATTACCACTTCTGGCA
TCGAGGAGTGACGAAGCGGTCCCTGTCGCCTCACCGCCCGCGGCACAGCCGGCT
GCAGAGGGAGCCTCAAGTACAGTGGCTGGAACAGCAGGTGGCAAAGCGACGGA
CTAAACGGGACGTGTACCAGGAGCCCACAGACCCCAAGTTTCCTCAGCAGTGGT
ACCTGTCTGGTGTCACTCAGCGGGACCTGAATGTGAAGGCGGCCTGGGCGCAGG
GCTACACAGGGCACGGCATTGTGGTCTCCATTCTGGACGATGGCATCGAGAAGA
ACCACCCGGACTTGGCAGGCAATTATGATCCTGGGGCCAGTTTTGATGTCAATGA
CCAGGACCCTGACCCCCAGCCTCGGTACACACAGATGAATGACAACAGGCACGG
CACACGGTGTGCGGGGGAAGTGGCTGCGGTGGCCAACAACGGTGTCTGTGGTGT
AGGTGTGGCCTACAACGCCCGCATTGGAGGGGTGCGCATGCTGGATGGCGAGGT
GACAGATGCAGTGGAGGCACGCTCGCTGGGCCTGAACCCCAACCACATCCACAT
CTACAGTGCCAGCTGGGGCCCCGAGGATGACGGCAAGACAGTGGATGGGCCAGC
CCGCCTCGCCGAGGAGGCCTTCTTCCGTGGGGTTAGCCAGGGCCGAGGGGGGCT
GGGCTCCATCTTTGTCTGGGCCTCGGGGAACGGGGGCCGGGAACATGACAGCTG

CAACTGCGACGGCTACACCAACAGTATCTACACGCTGTCCATCAGCAGCGCCAC
GCAGTTTGGCAACGTGCCGTGGTACAGCGAGGCCTGCTCGTCCACACTGGCCACG
ACCTACAGCAGTGGCAACCAGAATGAGAAGCAGATCGTGACGACTGACTTGCGG
CAGAAGTGCACGGAGTCTCACACGGGCACCTCAGCCTCTGCCCCCTTAGCAGCCG
GCATCATTGCTCTCACCCTGGAGGCCAATAAGAACCTCACATGGCGGGACATGC
AACACCTGGTGGTACAGACCTCGAAGCCAGCCCACCTCAATGCCAACGACTGGG
CCACCAATGGTGTGGGCCGGAAAGTGAGCCACTCATATGGCTACGGGCTTTTGG
ACGCAGGCGCCATGGTGGCCCTGGCCCAGAATTGGACCACAGTGGCCCCCCAGC
GGAAGTGCATCATCGACATCCTCACCGAGCCCAAAGACATCGGGAAACGGCTCG
AGGTGCGGAAGACCGTGACCGCGTGCCTGGGCGAGCCCAACCACATCACTCGGC
TGGAGCACGCTCAGGCGCGGCTCACCCTGTCCTATAATCGCCGTGGCGACCTGGC
CATCCACCTGGTCAGCCCCATGGGCACCCGCTCCACCCTGCTGGCAGCCAGGCCA
CATGACTACTCCGCAGATGGGTTTAATGACTGGGCCTTCATGACAACTCATTCCT
GGGATGAGGATCCCTCTGGCGAGTGGGTCCTAGAGATTGAAAACACCAGCGAAG
CCAACAACTATGGGACGCTGACCAAGTTCACCCTCGTACTCTATGGCACCGCCCC
TGAGGGGCTGCCCGTACCTCCAGAAAGCAGTGGCTGCAAGACCCTCACGTCCAG
TCAGGCCTGTGTGGTGTGCGAGGAAGGCTTCTCCCTGCACCAGAAGAGCTGTGTC
CAGCACTGCCCTCCAGGCTTCGCCCCCCAAGTCCTCGATACGCACTATAGCACCG
AGAATGACGTGGAGACCATCCGGGCCAGCGTCTGCGCCCCCTGCCACGCCTCAT
GTGCCACATGCCAGGGGCCGGCCCTGACAGACTGCCTCAGCTGCCCCAGCCACG
CCTCCTTGGACCCTGTGGAGCAGACTTGCTCCCGGCAAAGCCAGAGCAGCCGAG
AGTCCCCGCCACAGCAGCAGCCACCTCGGCTGCCCCCGGAGGTGGAGGCGGGGC
AACGGCTGCGGGCAGGGCTGCTGCCCTCACACCTGCCTGAGGTGGTGGCCGGCC
TCAGCTGCGCCTTCATCGTGCTGGTCTTCGTCACTGTCTTCCTGGTCCTGCAGCTG
CGCTCTGGCTTTAGTTTTCGGGGGGTGAAGGTGTACACCATGGACCGTGGCCTCA
TCTCCTACAAGGGGCTGCCCCCTGAAGCCTGGCAGGAGGAGTGCCCGTCTGACTC
AGAAGAGGACGAGGGCCGGGGCGAGAGGACCGCCTTTATCAAAGACCAGAGCG
CCCTCTGAACGCGGCCGC (SEQ ID NO: 64).

[000195]In another embodiment, the amino acid sequence of furin comprises the following amino acid sequence:

MELRPWLLWVVAATGTLVLLAADAQGQKVFTNTWAVRIPGGPAVANSVARKHGFL
NLGQIFGDYYHFWHRGVTKRSLSPHRPRHSRLQREPQVQWLEQQVAKRRTKRDVYQ

EPTDPKFPQQWYLSGVTQRDLNVKAAWAQGYTGHGIVVSILDDGIEKNHPDLAGNY
DPGASFDVNDQDPDPQPRYTQMNDNRHGTRCAGEVAAVANNGVCGVGVAYNARI
GGVRMLDGEVTDAVEARSLGLNPNHIHIYSASWGPEDDGKTVDGPARLAEEAFFRG
VSQGRGGLGSIFVWASGNGGREHDSCNCDGYTNSIYTLSISSATQFGNVPWYSEACSS
TLATTYSSGNQNEKQIVTTDLRQKCTESHTGTSASAPLAAGIIALTLEANKNLTWRD
MQHLVVQTSKPAHLNANDWATNGVGRKVSHSYGYGLLDAGAMVALAQNWTTVA
PQRKCIIDILTEPKDIGKRLEVRKTVTACLGEPNHITRLEHAQARLTLSYNRRGDLAIH
LVSPMGTRSTLLAARPHDYSADGFNDWAFMTTHSWDEDPSGEWVLEIENTSEANNY
GTLTKFTLVLYGTAPEGLPVPPESSGCKTLTSSQACVVCEEGFSLHQKSCVQHCPPGF
APQVLDTHYSTENDVETIRASVCAPCHASCATCQGPALTDCLSCPSHASLDPVEQTCS
RQSQSSRESPPQQQPPRLPPEVEAGQRLRAGLLPSHLPEVVAGLSCAFIVLVFVTVFLV
LQLRSGFSFRGVKVYTMDRGLISYKGLPPEAWQEECPSDSEEDEGRGERTAFIKDQSA
L* (SEQ ID NO: 65).

[000196]In some embodiments, the term coagulation factor further includes homologues of known coagulation factors which have a coagulating activity. In some embodiments, homology according to the present invention also encompasses deletions, insertions, or substitution variants, including an amino acid substitution, thereof and biologically active polypeptide fragments thereof.

[000197]In another embodiment, the invention includes homologues of a coagulation factor having a coagulation activity. In another embodiment, the invention includes homologues of a coagulation factor as described herein having a coagulation activity. In another embodiment, homologues e.g., polypeptides which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 87%, at least 89%, at least 91%, at least 93%, at least 95% or more say 99% homologous to a coagulation factor as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters.

[000198]In another embodiment, the invention includes homologues of furin. In another embodiment, homologues e.g., polypeptides which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 87%, at least 89%, at least 91%, at least 93%, at least 95% or more say 99% homologous to a furin as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters.

[000199]In one embodiment, the homologues of polypeptides or fragments thereof provided herein also refer to deletion, insertion, or substitution variants, including amino acid substitution, thereof and biologically active polypeptide fragments thereof.

[000200]In another embodiment, three chorionic gonadotrophin carboxy terminal peptides are attached to the C-terminus of the peptide or polypeptide provided herein. In another embodiment, four chorionic gonadotrophin carboxy terminal peptides are attached to the C-terminus of the polypeptide or fragment thereof provided herein. In another embodiment, five chorionic gonadotrophin carboxy terminal peptides are attached to the C-terminus of the polypeptide or fragment thereof provided herein. In another embodiment, 1-10 CTP are attached to the amino or C-terminus of polypeptide or fragment thereof provided herein. In another embodiment, 1-10 CTP are attached to the N-terminus of polypeptide or fragment thereof provided herein. In another embodiment, 1-10 CTP are attached to the C-terminus of polypeptide or fragment thereof provided herein.

[000201]It is to be understood that the compositions and methods of the present invention comprising the elements or steps as described herein may, in another embodiment, consist of those elements or steps, or in another embodiment, consist essentially of those elements or steps. In another embodiment, the term "comprise" refers to the inclusion of the indicated active agent, such as the CTP-modified polypeptide or fragment thereof, as well as inclusion of other active agents, and pharmaceutically or physiologically acceptable carriers, excipients, emollients, stabilizers, etc., as are known in the pharmaceutical industry. In another embodiment, the term "consisting essentially of" refers to a composition, whose only active ingredient is the indicated active ingredient, however, other compounds may be included which are for stabilizing, preserving, etc. the formulation, but are not involved directly in the therapeutic effect of the indicated active ingredient. In another embodiment, the term "consisting essentially of" may refer to components which facilitate the release of the active ingredient. In another embodiment, the term "consisting" refers to a composition, which contains the active ingredient and a pharmaceutically acceptable carrier or excipient.

[000202]In one embodiment, the methods provided herein comprise a polypeptide or a fragment thereof attached to at least one gonadotrophin carboxy terminal peptides (CTPs) attached at the N- or C-terminus of the polypeptide or fragments thereof.

[000203]In another embodiment, the methods provided herein comprise a polypeptide or a fragment thereof attached to 1 to 3 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/ or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or a fragment thereof attached to 1 to 5 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/ or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or a fragment thereof attached to 1 to 10 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/ or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or a fragment thereof attached to 2 to 3 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/ or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or a fragment thereof attached to 2 to 5 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/ or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or a fragment thereof attached to 2 to 10 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/ or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or a fragment thereof attached to 3 to 5 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/ or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or a fragment thereof attached to 3 to 8 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/ or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or a fragment thereof attached to 3 to 10 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/ or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or fragments thereof attached to 6 to 10 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or fragments thereof attached to 1 gonadotrophin carboxy terminal peptides (CTPs) attached at the N- and/or C-terminus of the polypeptide or fragments thereof. In another embodiment, the methods provided herein comprise a polypeptide or fragments thereof attached to 2, 3, 4, 5, 6, 7, 8, 9, or 10 gonadotrophin carboxy terminal peptides (CTPs)

attached at the N- and/or C-terminus of the polypeptide or fragments thereof. In another embodiment, the CTP peptides are tandemly attached on either side (N-terminus or C-terminus) of the polypeptide or fragments thereof.

[000204]In one embodiment, provided herein is an expression vector comprising the polynucleotide provided herein. In another embodiment, provided herein is a cell comprising the expression vector. In another embodiment, provided herein is a composition comprising the expression vector.

[000205]In another embodiment, the invention provides a composition comprising the cell as described herein. In another embodiment, the cell is a eukaryotic cell. In another embodiment, the cell is a prokaryotic cell.

[000206]In one embodiment, a CTP sequence at the C-terminal end ofa polypeptide or fragments thereof provides enhanced protection against degradation of the polypeptide or fragments thereof. In another embodiment, a CTP sequence at the C-terminal end of the polypeptide or fragments thereof provides enhanced protection against clearance. In another embodiment, a CTP sequence at the C-terminal end of the polypeptide or fragments thereof provides prolonged clearance time. In another embodiment, a CTP sequence at the C-terminal end of the polypeptide or fragments thereof enhances its Cmax. In another embodiment, a CTP sequence at the C-terminal end of the polypeptide or fragments thereof enhances its Tmax. In another embodiment, a CTP sequence at the C-terminal end of the polypeptide or fragments thereof prolongs its T½. In another embodiment, aCTP sequence at the C-terminal end of the polypeptide or fragments thereof prolongs its AUC.

[000207]In another embodiment, a conjugated polypeptide or fragment thereof of the invention is used in the same manner as an unmodified polypeptide or fragment thereof. In another embodiment, a polypeptide or fragment thereof of the invention is used in the same manner as an unmodified polypeptide or fragment thereof. In another embodiment, a polypeptide or fragment thereof of this invention has an increased circulating half-life and plasma residence time, decreased clearance, and increased clinical activity in vivo. In another embodiment, due to the improved properties of the polypeptide or fragment thereof provided herein, this conjugate is administered less frequently than the polypeptide or fragment thereof.

[000208]In another embodiment, decreased frequency of administration results in improved treatment strategy, which in one embodiment, leads to improved patient compliance leading

to improved treatment outcomes, as well as improved patient quality of life. In another embodiment, compared to conventional conjugates, the conjugates provided herein having the hydrodynamic volume further provided herein have an improved *in vivo* potency, improved stability, elevated AUC levels, and enhanced circulating half-life.

[000209]In another embodiment, the present invention further provides a pharmaceutical composition comprising a CTP-modified polypeptide comprising of one glycosylated gonadotropin carboxy terminal peptides (CTPs) attached to the N-terminus and two glycosylated CTP attached to the C-terminus of the polypeptide or fragments thereof.

[000210]In another embodiment, it is to be understood that combinations of glycosylated and non-glycosylated CTPs can be used in modifying the polypeptides of interest or fragments thereof, further provided herein. Such combinations can include, for example, at least one non-glycosylated CTP attached to the N-terminus of the polypeptide or fragments thereof, and at least one glycosylated CTP attached to the C-terminus of the same, and vice versa. Moreover, and in another embodiment, combinations of at least one glycosylated CTP and at least one non-glycosylated CTP attached to the same terminus (N- or C-terminus) are also envisioned. These combinations may also include truncations of the glycosylated and/or non-glycosylated CTP. Such combinations can be determined by a skilled artisan guided by the invention provided herein so as to arrive at an optimal hydrodynamic volume or hydrodynamic size for the polypeptides of interest or fragments thereof. As a result the polypeptides of interest or fragments thereof having an optimal hydrodynamic volume can possess the optimally desired characteristics, i.e., improved potency, improved stability, elevated AUC levels, enhanced bioavailability and enhanced circulating half-life.

[000211]In one embodiment, provided herein is a pharmaceutical composition comprising a therapeutically effective amount of the CTP-modified polypeptide of interest or fragment thereof provided herein. In another embodiment, a therapeutically effective amount of CTP-modified polypeptides or fragments thereof is determined according to factors such as the specific condition being treated, the condition of the patient being treated, as well as the other ingredients in the composition. In another embodiment, the therapeutic efficacy of the CTP-modified polypeptide or fragment thereof is optimally adjusted by adding or removing glycosylated and/ or non-glycosylated CTP peptides in order to arrive at the optimal hydrodynamic volume. It is to be understood that in doing so, a skilled artisan can arrive at a

CTP-modified polypeptide or fragment thereof that possesses the optimal therapeutic efficacy.

[000212]In one embodiment, the CTP-modified polypeptide or fragment thereof provided herein has therapeutic uses. In another embodiment, the CTP-modified polypeptide or fragment thereof provided herein has prophylactic uses.

[000213]In one embodiment, the terms "reducing, reduction, lowering, etc." when used in relation to the methods provided herein refer to 100% reduction from a previously measured or determined level or from a normal level. In another embodiment, the reduction is by 89-99% from a previously determined level. In another embodiment, the reduction is by 79-88% from a previously determined level. In another embodiment, the reduction is by 69-78% from a previously determined level. In another embodiment, the reduction is by 59-68% from a previously determined level. In another embodiment, the reduction is by 49-58% from a previously determined level. In another embodiment, the reduction is by 39-48% from a previously determined level. In another embodiment, the reduction is by 29-38% from a previously determined level. In another embodiment, the reduction is by 19-28% from a previously determined level. In another embodiment, the reduction is by 9-18% from a previously determined level. In another embodiment, the reduction is by 5-8% from a previously determined level. In another embodiment, the reduction is by 1-4% from a previously determined level.

[000214]In one embodiment, tissue-specific promoters suitable for use with the present invention include sequences which are functional in one or more specific cell populations. Examples include, but are not limited to, promoters such as albumin that is liver-specific [Pinkert et al., (1987) Genes Dev. 1:268-277], lymphoid-specific promoters [Calame et al., (1988) Adv. Immunol. 43:235-275]; in particular promoters of T-cell receptors [Winoto et al., (1989) EMBO J. 8:729-733] and immunoglobulins; [Banerji et al. (1983) Cell 33729-740], neuron-specific promoters such as the neurofilament promoter [Byrne et al. (1989) Proc. Natl. Acad. Sci. USA 86:5473-5477], pancreas-specific promoters [Edlunch et al. (1985) Science 230:912-916] or mammary gland-specific promoters such as the milk whey promoter (U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Inducible promoters suitable for use with the present invention include, for example, the tetracycline-inducible promoter (Srour, M.A., et al., 2003. Thromb. Haemost. 90: 398-405).

[000215]In one embodiment, the phrase “a polynucleotide molecule” refers to a single or double stranded nucleic acid sequence which is isolated and provided in the form of an RNA sequence, a complementary polynucleotide sequence (cDNA), a genomic polynucleotide sequence and/or a composite polynucleotide sequences (e.g., a combination of the above).

[000216]In one embodiment, a "complementary polynucleotide sequence" refers to a sequence, which results from reverse transcription of messenger RNA using a reverse transcriptase or any other RNA-dependent DNA polymerase. In one embodiment, the sequence can be subsequently amplified in vivo or in vitro using a DNA polymerase.

[000217]In one embodiment, a "genomic polynucleotide sequence" refers to a sequence derived (isolated) from a chromosome and thus it represents a contiguous portion of a chromosome.

[000218]In one embodiment, a "composite polynucleotide sequence" refers to a sequence, which is at least partially complementary and at least partially genomic. In one embodiment, a composite sequence can include some exonal sequences required to encode the polypeptide of the present invention, as well as some intronic sequences interposing therebetween. In one embodiment, the intronic sequences can be of any source, including of other genes, and typically includes conserved splicing signal sequences. In one embodiment, intronic sequences include cis-acting expression regulatory elements.

[000219]In one embodiment, polynucleotides of the present invention are prepared using PCR techniques, or any other method or procedure known to one skilled in the art. In another embodiment, the procedure involves the ligation of two different DNA sequences (See, for example, “Current Protocols in Molecular Biology”, eds. Ausubel et al., John Wiley & Sons, 1992).

[000220]In one embodiment, polynucleotides of the present invention which encode the engineered polypeptides of interest or fragments thereof provided herein are inserted into expression vectors (i.e., a nucleic acid construct) to enable expression of the recombinant peptide/polypeptide. In one embodiment, the expression vector of the present invention includes additional sequences which render this vector suitable for replication and integration in prokaryotes. In one embodiment, the expression vector of the present invention includes additional sequences which render this vector suitable for replication and integration in eukaryotes. In another embodiment, the expression vector of the present invention includes a shuttle vector which renders this vector suitable for replication and integration in both

prokaryotes and eukaryotes. In another embodiment, cloning vectors comprise transcription and translation initiation sequences (e.g., promoters, enhances) and transcription and translation terminators (e.g., polyadenylation signals).

[000221]In one embodiment, a variety of prokaryotic or eukaryotic cells can be used as host-expression systems to express the CTP-modified polypeptides or fragments thereof provided herein. In another embodiment, these include, but are not limited to, microorganisms, such as bacteria transformed with a recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vector containing the polypeptide coding sequence; yeast transformed with recombinant yeast expression vectors containing the polypeptide coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors, such as Ti plasmid, containing the polypeptide coding sequence.

[000222]In one embodiment, non-bacterial expression systems are used (e.g., mammalian expression systems such as CHO cells) to express the polypeptide or fragment thereof provided herein. In another embodiment, the expression vector used to express polynucleotides of the present invention in mammalian cells is pCI-dhfrr vector. Construction of the pCI-dhfrr vector is described, according to one embodiment, in Example's Materials and Methods, below.

[000223]In one embodiment, in bacterial systems of the present invention, a number of expression vectors can be advantageously selected depending upon the use intended for the polypeptide expressed. In one embodiment, large quantities of polypeptide are desired. In one embodiment, vectors that direct the expression of high levels of the protein product, possibly as a fusion with a hydrophobic signal sequence, which directs the expressed product into the periplasm of the bacteria or the culture medium where the protein product is readily purified are desired. In one embodiment, certain fusion proteins are engineered with a specific cleavage site to aid in recovery of the polypeptide. In one embodiment, vectors adaptable to such manipulation include, but are not limited to, the pET series of E. coli expression vectors [Studier et al., Methods in Enzymol. 185:60-89 (1990)].

[000224]In one embodiment, yeast expression systems are used. In one embodiment, a number of vectors containing constitutive or inducible promoters can be used in yeast as disclosed in U.S. Pat. Application. No: 5,932,447, which is incorporated by reference herein

in its entirety. In another embodiment, vectors which promote integration of foreign DNA sequences into the yeast chromosome are used.

[000225]In one embodiment, the expression vector of the present invention can further include additional polynucleotide sequences that allow, for example, the translation of several proteins from a single mRNA such as an internal ribosome entry site (IRES) and sequences for genomic integration of the promoter-chimeric polypeptide.

[000226]In one embodiment, mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Strategene, pTRES which is available from Clontech, and their derivatives.

[000227]In one embodiment, expression vectors containing regulatory elements from eukaryotic viruses such as retroviruses are used in the present invention. SV40 vectors include pSVT7 and pMT2. In another embodiment, vectors derived from bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include pHEBO, and p2O5. Other exemplary vectors include pMSG, pAV009/A+, pMTO10/A+, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

[000228]In one embodiment, recombinant viral vectors are useful for *in vivo* expression of the peptides/polypeptides of the present invention since they offer advantages such as lateral infection and targeting specificity. In one embodiment, lateral infection is inherent in the life cycle of, for example, a retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. In one embodiment, the result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. In one embodiment, viral vectors are produced that are unable to spread laterally. In one embodiment, this characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

[000229]In one embodiment, various methods can be used to introduce the expression vector of the present invention into cells. Such methods are generally described in Sambrook et al.,

Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., Gene Targeting, CRC Press, Ann Arbor Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and Gilboa et at. [Biotechniques 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992, incorporated herein by reference, for positive-negative selection methods.

[000230]In one embodiment, introduction of nucleic acid by viral infection offers several advantages over other methods such as lipofection and electroporation, since higher transfection efficiency can be obtained due to the infectious nature of viruses.

[000231]It will be appreciated that the engineered polypeptides or fragments thereof of provided herein can also be expressed from a nucleic acid construct administered to the individual employing any suitable mode of administration (e.g., subcutaneous administration, oral administration, intra-nasal administration, intra-venal administration, or in vivo gene therapy). In one embodiment, the nucleic acid construct is introduced into a suitable cell via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination, etc.) and an expression system as needed and then the modified cells are expanded in culture and returned to the individual (i.e., ex vivo gene therapy).

[000232]In one embodiment, plant expression vectors are used. In one embodiment, the expression of a polypeptide coding sequence is driven by a number of promoters. In another embodiment, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV [Brisson et al., Nature 310:511-514 (1984)], or the coat protein promoter to TMV [Takamatsu et al., EMBO J. 6:307-311 (1987)] are used. In another embodiment, plant promoters are used such as, for example, the small subunit of RUBISCO [Coruzzi et al., EMBO J. 3:1671-1680 (1984); and Brogli et al., Science 224:838-843 (1984)] or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B [Gurley et al., Mol. Cell. Biol. 6:559-565 (1986)]. In one embodiment, constructs are introduced into plant cells using Ti plasmid, Ri plasmid, plant viral vectors, direct DNA transformation, microinjection, electroporation and other techniques well known to the skilled artisan. See, for example, Weissbach & Weissbach [Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463 (1988)]. Other

expression systems such as insects and mammalian host cell systems, which are well known in the art, can also be used by the present invention.

[000233]It will be appreciated that other than containing the necessary elements for the transcription and translation of the inserted coding sequence (encoding the polypeptide), the expression construct of the present invention can also include sequences engineered to optimize stability, production, purification, yield or activity of the expressed polypeptide.

[000234]In one embodiment, transformed cells are cultured under effective conditions, which allow for the expression of high amounts of recombinant engineered oxyntomodulin peptides. In another embodiment, effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. In one embodiment, an effective medium refers to any medium in which a cell is cultured to produce the recombinant polypeptide of the present invention. In another embodiment, a medium typically includes an aqueous solution having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes and petri plates. In another embodiment, culturing is carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. In another embodiment, the determination of culturing conditions are within the expertise of one of ordinary skill in the art.

[000235]In one embodiment, depending on the vector and host system used for production, the resultant polypeptide or fragment thereof or, in another embodiment, the resultant CTP-modified polypeptide or fragment thereof provided herein are expressed within a recombinant cell for glycosylation of the CTP to take place, are secreted into the fermentation medium, or are retained on the outer surface of a mammalian cell.

[000236]In one embodiment, following a predetermined time in culture, recovery of the recombinant polypeptide or fragment thereof is effected.

[000237]In one embodiment, the phrase "recovering the recombinant engineered polypeptide or fragment thereof" refers to collecting the whole fermentation medium containing the polypeptide or fragment thereof and need not imply additional steps of separation or purification. In another embodiment, additional steps of separation or purification well known in the art are carried out in order to recover the recombinant engineered polypeptide or fragment thereof.

[000238]In one embodiment, engineered polypeptides or fragments thereof or variants thereof provided herein are purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization.

[000239]To facilitate recovery, the expressed coding sequence can be engineered to encode the polypeptide or fragment thereof provided herein and fused cleavable moiety. Further, a fusion protein can be designed so that the polypeptide can be readily isolated by affinity chromatography; e.g., by immobilization on a column specific for the cleavable moiety. A cleavage site is engineered between the engineered polypeptides or fragments thereof and the cleavable moiety and the polypeptide can be released from the chromatographic column by treatment with an appropriate enzyme or agent that specifically cleaves the fusion protein at this site [e.g., see Booth et al., Immunol. Lett. 19:65-70 (1988); and Gardella et al., J. Biol. Chem. 265:15854-15859 (1990)].

[000240]In one embodiment, the engineered peptide or polypeptide provided herein is retrieved in "substantially pure" form.

[000241]In one embodiment, the phrase "substantially pure" refers to a purity that allows for the effective use of the protein in the applications described herein.

[000242]The engineered polypeptide or fragment thereof provided herein can also be synthesized using in vitro expression systems. In another embodiment, *in vitro* synthesis methods are well known in the art and the components of the system are commercially available.

[000243]In one embodiment, the recombinant engineered polypeptides or fragments thereof are synthesized and purified; their therapeutic efficacy can be assayed either *in vivo* or *in vitro*. The binding activities of the recombinant engineered polypeptides or fragments thereof of the present invention can be ascertained using various assays as known to one of skill in the art.

[000244] In another embodiment, the polypeptides or fragments thereof of the present invention can be provided to the individual per se. In one embodiment, the engineered polypeptides or fragments thereof provided herein can be provided to the individual as part of a pharmaceutical composition where it is mixed with a pharmaceutically acceptable carrier.

[000245]In another embodiment, a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

[000246]In another embodiment, "active ingredient" refers to the polypeptide sequence of interest, which is accountable for the biological effect.

[000247]In another embodiment, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which are interchangeably used herein refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases. In one embodiment, one of the ingredients included in the pharmaceutically acceptable carrier can be for example polyethylene glycol (PEG), a biocompatible polymer with a wide range of solubility in both organic and aqueous media (Mutter et al. (1979)).

[000248]In another embodiment, the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. In one embodiment, excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

[000249]Techniques for formulation and administration of drugs are found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

[000250]Various embodiments of dosage ranges are contemplated by this invention. The dosage of the CTP-modified polypeptide or fragments thereof provided herein, in one embodiment, is in the range of 0.005-100 mg/day. In another embodiment, the dosage is in the range of 0.005-5 mg/day. In another embodiment, the dosage is in the range of 0.01-50 mg/day. In another embodiment, the dosage is in the range of 0.1-20 mg/day. In another embodiment, the dosage is in the range of 0.1-10 mg/day. In another embodiment, the dosage is in the range of 0.01-5 mg/day. In another embodiment, the dosage is in the range of 0.001-0.01 mg/day. In another embodiment, the dosage is in the range of 0.001-0.1 mg/day. In another embodiment, the dosage is in the range of 0.1-5 mg/day. In another embodiment, the dosage is in the range of 0.5-50 mg/day. In another embodiment, the dosage is in the range of 0.2-15 mg/day. In another embodiment, the dosage is in the range of 0.8-65 mg/day. In

another embodiment, the dosage is in the range of 1-50 mg/day. In another embodiment, the dosage is in the range of 5-10 mg/day. In another embodiment, the dosage is in the range of 8-15 mg/day. In another embodiment, the dosage is in a range of 10-20 mg/day. In another embodiment, the dosage is in the range of 20-40 mg/day. In another embodiment, the dosage is in a range of 60-120 mg/day. In another embodiment, the dosage is in the range of 12-40 mg/day. In another embodiment, the dosage is in the range of 40-60 mg/day. In another embodiment, the dosage is in a range of 50-100mg/day. In another embodiment, the dosage is in a range of 1-60 mg/day. In another embodiment, the dosage is in the range of 15-25 mg/day. In another embodiment, the dosage is in the range of 5-10 mg/day. In another embodiment, the dosage is in the range of 55-65 mg/day.

[000251]In another embodiment, the dosage is in a range of 50-500 mg/day. In another embodiment, the dosage is in a range of 50-150 mg/day. In another embodiment, the dosage is in a range of 100-200 mg/day. In another embodiment, the dosage is in a range of 150-250 mg/day. In another embodiment, the dosage is in a range of 200-300 mg/day. In another embodiment, the dosage is in a range of 250-400 mg/day. In another embodiment, the dosage is in a range of 300-500 mg/day. In another embodiment, the dosage is in a range of 350-500 mg/day.

[000252]In one embodiment, the dosage is 20 mg/day. In one embodiment, the dosage is 30 mg/day. In one embodiment, the dosage is 40 mg/day. In one embodiment, the dosage is 50 mg/day. In one embodiment, the dosage is 0.01 mg/day. In another embodiment, the dosage is 0.1 mg/day. In another embodiment, the dosage is 1 mg/day. In another embodiment, the dosage is 0.530 mg/day. In another embodiment, the dosage is 0.05 mg/day. In another embodiment, the dosage is 50 mg/day. In another embodiment, the dosage is 10 mg/day. In another embodiment, the dosage is 20-70 mg/day. In another embodiment, the dosage is 5 mg/day.

[000253]In one embodiment, the dosage of the CTP-modified polypeptides or fragments thereof is 1-5 mg/day. In one embodiment, the dosage of the CTP-modified polypeptides or fragments thereof is 1-3 mg/day. In another embodiment, the dosage of the CTP-modified polypeptides or fragments thereof is 2 mg/day.

[000254]In another embodiment, the dosage is 1-90 mg/day. In another embodiment, the dosage is 1-90 mg/2 days. In another embodiment, the dosage is 1-90 mg/3 days. In another embodiment, the dosage is 1-90 mg/4 days. In another embodiment, the dosage is 1-90 mg/5

days. In another embodiment, the dosage is 1-90 mg/6 days. In another embodiment, the dosage is 1-90 mg/week. In another embodiment, the dosage is 1-90 mg/9 days. In another embodiment, the dosage is 1-90 mg/11 days. In another embodiment, the dosage is 1-90 mg/14 days.

[000255]In another embodiment, the CTP-modified polypeptide or fragment thereof dosage is 10-50 mg/day. In another embodiment, the dosage is 10-50 mg/2 days. In another embodiment, the dosage is 10-50 mg/3 days. In another embodiment, the dosage is 10-50 mg/4 days. In another embodiment, the dosage is 10-50 micrograms mg/5 days. In another embodiment, the dosage is 10-50 mg/6 days. In another embodiment, the dosage is 10-50 mg/week. In another embodiment, the dosage is 10-50 mg/9 days. In another embodiment, the dosage is 10-50 mg/11 days. In another embodiment, the dosage is 10-50 mg/14 days.

[000256]In another embodiment, the doses can be given as mg/kg or units/kg. In another embodiment, dosage of the CTP-modified polypeptide or fragments thereof provided herein, in one embodiment, is in the range of 0.005-100 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.005-5 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.01-50 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.1-20 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.1-10 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.01-5 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.001-0.01 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.001-0.1 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.1-5 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.5-50 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.2-15 mg/kg or units/kg. In another embodiment, the dosage is in the range of 0.8-65 mg/kg or units/kg. In another embodiment, the dosage is in the range of 1-50 mg/kg or units/kg. In another embodiment, the dosage is in the range of 5-10 mg/kg or units/kg. In another embodiment, the dosage is in the range of 8-15 mg/kg or units/kg. In another embodiment, the dosage is in a range of 10-20 mg/kg or units/kg. In another embodiment, the dosage is in the range of 20-40 mg/kg or units/kg. In another embodiment, the dosage is in a range of 60-120 mg/kg or units/kg. In another embodiment, the dosage is in the range of 12-40 mg/kg or units/kg. In another embodiment, the dosage is in the range of 40-60 mg/kg or units/kg. In another embodiment, the dosage is in a range of 50-100mg/kg or units/kg. In another embodiment, the dosage is in a range of 1-60 mg/kg or units/kg. In another

embodiment, the dosage is in the range of 15-25 mg/kg or units/kg. In another embodiment, the dosage is in the range of 5-10 mg/kg or units/kg. In another embodiment, the dosage is in the range of 55-65 mg/kg or units/kg.

[000257]In another embodiment, a polypeptide comprising polypeptides or fragments thereof provided herein and at least one CTP unit is formulated in an intranasal dosage form. In another embodiment, a polypeptide comprising polypeptides or fragments thereof provided herein and at least one CTP unit is formulated in an injectable dosage form. In another embodiment, a polypeptide comprising polypeptides or fragments thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 0.0001 mg to 0.6 mg. In another embodiment, a polypeptide comprising polypeptides or fragments thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 0.001 mg to 0.005 mg. In another embodiment, a polypeptide comprising polypeptides or fragments thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 0.005 mg to 0.01 mg. In another embodiment, a polypeptide comprising polypeptides or fragments thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 0.01 mg to 0.3 mg. In another embodiment, a polypeptide comprising polypeptides or fragments thereof provided herein and at least one CTP unit is administered to a subject in a dose in a dose ranging from 0.2 mg to 0.6 mg. In another embodiment, the polypeptides or fragments thereof provided herein is free of CTPs on its amino terminus.

[000258]In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 0.2 mg to 2 mg. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 2 mg to 6 mg. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 4 mg to 10 mg. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 5 mg and 15 mg.

[000259]In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 0.2 mg to 2 mg. In another embodiment, a polypeptide comprising a polypeptide or fragment

thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 2 mg to 6 mg. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 4 mg to 10 mg. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject in a dose ranging from 5 mg and 15 mg.

[000260]In another embodiment, the dosage of CTP-modified polypeptides or fragments thereof provided herein is such that it contains 65% of the amount of the agonist than that administered using the non-CTP-modified polypeptides or fragments thereof. In another embodiment, the dosage of CTP-modified polypeptides or fragments thereof provided herein is such that it contains 55% of the amount of the agonist than that administered using the non-CTP-modified polypeptides or fragments thereof. In another embodiment, the dosage of CTP-modified polypeptides or fragments thereof provided herein is such that it contains 45% of the amount of the agonist than that administered using the non-CTP-modified polypeptides or fragments thereof. In another embodiment, the dosage of CTP-modified polypeptides or fragments thereof provided herein is such that it contains 10% of the amount of the agonist than that administered using the non-CTP-modified polypeptides or fragments thereof. In another embodiment, the dosage of CTP-modified polypeptides or fragments thereof provided herein is such that it contains 25% of the amount of the agonist than that administered using the non-CTP-modified polypeptides or fragments thereof. In another embodiment, the dosage of CTP-modified polypeptides or fragments thereof provided herein is such that it contains 35% of the amount of the agonist than that administered using the non-CTP-modified polypeptides or fragments thereof. In another embodiment, the dosage of CTP-modified polypeptides or fragments thereof provided herein is such that it contains 75% of the amount of the agonist than that administered using the non-CTP-modified polypeptides or fragments thereof. In another embodiment, the dosage of CTP-modified polypeptides or fragments thereof provided herein is such that it contains 100% of the amount of the agonist than that administered using the non-CTP-modified agonist. However, even if the dosage contains the same amount of agonist as non-CTP-modified polypeptides or fragments thereof, it is still advantageous to subjects in that it will be administered less frequently because of its increased half-life compared to normal agonist.

[000261]In another embodiment, a therapeutically effective amount of a conjugated polypeptide or fragments thereof provided herein is between 50-500 IU per kg body weight administered once a day. In another embodiment, a therapeutically effective amount of a conjugated polypeptide or fragment thereof is 150-250 IU per kg body weight, administered once a day. In another embodiment, a pharmaceutical composition comprising a conjugated polypeptide or fragment thereof is formulated at a strength effective for administration by various means to a human patient.

[000262]In one embodiment, the CTP-modified polypeptide comprising polypeptides or fragments thereof provided herein is administered in an amount effective to bring circulating polypeptides or fragments thereof's activity to 20-30 IU/dL in a subject. In another embodiment, the CTP-modified polypeptide comprising polypeptides or fragments thereof provided herein is administered in an amount effective to bring circulating polypeptides or fragments thereof's activity to 25-50 IU/dL in a subject. In another embodiment, the CTP-modified polypeptide comprising polypeptides or fragments thereof provided herein is administered in an amount effective to bring circulating polypeptides or fragments thereof's activity to 50-100 IU/dL in a subject. In another embodiment, the CTP- modified polypeptide comprising polypeptides or fragments thereof provided herein is administered in an amount effective to bring circulating polypeptides' or fragments thereof's activity to 100-200 IU/dL in a subject. In another embodiment, the CTP-modified polypeptide comprising polypeptides or fragments thereof provided herein is administered in an amount effective to bring circulating polypeptides or fragments thereof's activity to 10-50 IU/dL in a subject. In another embodiment, the CTP-modified polypeptide comprising polypeptides or fragments thereof provided herein is administered in an amount effective to bring circulating polypeptides or fragments thereof's activity to 20-100 IU/dL in a subject.

[000263]In one embodiment, the CTP-modified polypeptides or fragments thereof provided herein are administered to a subject on a weekly basis. In another embodiment, the CTP-modified polypeptides or fragments thereof provided herein are administered to a subject twice a week. In another embodiment, the CTP-modified polypeptides or fragments thereof provided herein are administered to a subject on a fortnightly (once every two weeks) basis. In another embodiment, the CTP-modified polypeptides or fragments thereof provided herein are administered to a subject twice a month. In another embodiment, the CTP-modified polypeptides or fragments thereof provided herein are administered to a subject once a month.

In another embodiment, the CTP-modified polypeptides or fragments thereof provided herein are administered to a subject on a daily basis. In another embodiment, the CTP-modified polypeptides or fragments thereof provided herein are administered to a subject every two days. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject once every three days.

[000264]In another embodiment, a polypeptide comprising a polypeptide or fragment thereof and at least one CTP unit is administered to a subject once every four days. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject once every five days. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject once every six days. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject once every 7-14 days. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject once every 10-20 days. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject once every 5-15 days. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is administered to a subject once every 15-30 days.

[000265]In another embodiment, the methods of the invention include increasing the compliance of patients afflicted with chronic illnesses that are in need of a polypeptide or fragments thereof provided herein therapy. In another embodiment, by increasing the hydrodynamic size of a CTP-modified polypeptide or fragment thereof by a factorprovided herein. In another embodiment, the methods of the invention enable reduction in the dosing frequency of the polypeptide or fragment thereof.

[000266]In another embodiment, the term compliance comprises adherence. In another embodiment, the methods of the invention include increasing the compliance of patients in need of a therapy by increasing the hydrodynamic size of a polypeptide or fragment thereof by a factor or increment provided herein which results in reducing the frequency of administration of the polypeptide or fragment thereof. In another embodiment, reduction in the frequency of administration of the polypeptide or fragment thereof provided herein is achieved due to the CTP modifications and a subsequent increase hydrodynamic size by a

factor or increment provided herein, which renders the polypeptide or fragments thereof more stable. In another embodiment, reduction in the frequency of administration of the polypeptide or fragment thereof provided herein is achieved due to the CTP modifications and subsequent increase in hydrodynamic size by a factor or increment provided herein, which increase the half-life (T1/2) of the polypeptide or fragments thereof. In another embodiment, reduction in the frequency of administration of the polypeptide or fragments thereof provided herein is achieved by increasing the hydrodynamic size of a polypeptide or fragment thereof by a factor or incrementprovided herein which results in increasing the clearance time or reducing the clearance rate of the polypeptide or fragment thereof provided herein.

[000267]In another embodiment, reduction in the frequency of administration of a polypeptide or fragment thereof provided herein is achieved by increasing the hydrodynamic size of a polypeptide or fragment thereof by a factor or increment provided herein which results in increasing the AUC measure of the polypeptide or fragments thereof.

[000268]Oral administration, in one embodiment, comprises a unit dosage form comprising tablets, capsules, lozenges, chewable tablets, suspensions, emulsions and the like. Such unit dosage forms comprise a safe and effective amount of the desired polypeptides or fragments thereof provided herein, each of which is in one embodiment, from about 0.7 or 3.5 mg to about 280 mg/70 kg, or in another embodiment, about 0.5 or 10 mg to about 210 mg/70 kg. The pharmaceutically-acceptable carriers suitable for the preparation of unit dosage forms for peroral administration are well-known in the art. In another embodiment, tablets typically comprise conventional pharmaceutically-compatible adjuvants as inert diluents, such as calcium carbonate, sodium carbonate, mannitol, lactose and cellulose; binders such as starch, gelatin and sucrose; disintegrants such as starch, alginic acid and croscarmelose; lubricants such as magnesium stearate, stearic acid and talc. In one embodiment, glidants such as silicon dioxide can be used to improve flow characteristics of the powder-mixture. In one embodiment, coloring agents, such as the FD&C dyes, can be added for appearance. Sweeteners and flavoring agents, such as aspartame, saccharin, menthol, peppermint, and fruit flavors, are useful adjuvants for chewable tablets. Capsules typically comprise one or more solid diluents disclosed above. In another embodiment, the selection of carrier components depends on secondary considerations like taste, cost, and shelf stability, which are not critical for the purposes of this invention, and can be readily made by a person skilled in the art.

[000269]In one embodiment, the oral dosage form comprises predefined release profile. In one embodiment, the oral dosage form of the present invention comprises an extended release tablets, capsules, lozenges or chewable tablets. In one embodiment, the oral dosage form of the present invention comprises a slow release tablets, capsules, lozenges or chewable tablets. In one embodiment, the oral dosage form of the present invention comprises an immediate release tablets, capsules, lozenges or chewable tablets. In one embodiment, the oral dosage form is formulated according to the desired release profile of the pharmaceutical active ingredient as known to one skilled in the art.

[000270]Peroral compositions, in another embodiment, comprise liquid solutions, emulsions, suspensions, and the like. In another embodiment, pharmaceutically-acceptable carriers suitable for preparation of such compositions are well known in the art. In another embodiment, liquid oral compositions comprise from about 0.001% to about 0.933% of the desired compound or compounds, or in another embodiment, from about 0.01% to about 10%.

[000271]In one embodiment, compositions for use in the methods of this invention comprise solutions or emulsions, which in another embodiment are aqueous solutions or emulsions comprising a safe and effective amount of the compounds of the present invention and optionally, other compounds, intended for topical intranasal administration. In another embodiment, the compositions comprise from about 0.001% to about 10.0% w/v of a subject compound, more preferably from about 00.1% to about 2.0, which is used for systemic delivery of the compounds by the intranasal route.

[000272]In another embodiment, a polypeptide comprising a polypeptide or fragment thereof and at least one CTP unit is injected into the muscle (intramuscular injection). In another embodiment, a polypeptide comprising a polypeptide or fragment thereof and at least one CTP unit is injected below the skin (subcutaneous injection). In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is injected into the muscle. In another embodiment, a polypeptide comprising a polypeptide or fragment thereof provided herein and at least one CTP unit is injected into the skin. In another embodiment, a polypeptide or fragment thereof provided herein as described herein is administered via systemic administration. In another embodiment, a polypeptide or fragment thereof provided herein as described herein is administered by intravenous injection. In another embodiment, administration can be parenteral, pulmonary, oral, topical, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, transnasal,

intraocular, ophthalmic, epidural, buccal, rectal, transmucosal, intestinal or parenteral delivery, including intramedullary injections as well as intrathecal or direct intraventricular administration.

[000273]In another embodiment, the preparation is administered in a local rather than systemic manner, for example, via injection of the preparation directly into a specific region of a patient's body.

[000274]In one embodiment, the route of administration may be enteral. In another embodiment, the route may be conjunctival, transdermal, intradermal, intra-arterial, vaginal, rectal, intratumoral, parcanceral, transmucosal, intramuscular, intravascular, intraventricular, intracranial, intra-nasal, sublingual, or a combination thereof.

[000275]In another embodiment, the pharmaceutical compositions are administered by intravenous, intra-arterial, or intramuscular injection of a liquid preparation. In another embodiment, liquid formulations include solutions, suspensions, dispersions, emulsions, oils and the like. In one embodiment, the pharmaceutical compositions are administered intravenously, and are thus formulated in a form suitable for intravenous administration. In another embodiment, the pharmaceutical compositions are administered intra-arterially, and are thus formulated in a form suitable for intra-arterial administration. In another embodiment, the pharmaceutical compositions are administered intramuscularly, and are thus formulated in a form suitable for intramuscular administration.

[000276]Further, and in another embodiment, the pharmaceutical compositions are administered topically to body surfaces, and are thus formulated in a form suitable for topical administration. Suitable topical formulations include gels, ointments, creams, lotions, drops and the like. For topical administration, the compounds of the present invention are combined with an additional appropriate therapeutic agent or agents, prepared and applied as solutions, suspensions, or emulsions in a physiologically acceptable diluent with or without a pharmaceutical carrier.

[000277]In one embodiment, pharmaceutical compositions of the present invention are manufactured by processes well known in the art, for e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[000278]In one embodiment, pharmaceutical compositions for use in accordance with the present invention is formulated in a conventional manner using one or more physiologically

acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. In one embodiment, formulation is dependent upon the route of administration chosen.

[000279]In one embodiment, injectables of the invention are formulated in aqueous solutions. In one embodiment, injectables of the invention are formulated in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. In another embodiment, for transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[000280]In one embodiment, the preparations described herein are formulated for parenteral administration, for e.g., by bolus injection or continuous infusion. In another embodiment, formulations for injection are presented in unit dosage form, for e.g., in ampoules or in multidose containers with optionally, an added preservative. In another embodiment, compositions are suspensions, solutions or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[000281]The compositions also comprise, in another embodiment, preservatives, such as benzalkonium chloride and thimerosal and the like; chelating agents, such as edetate sodium and others; buffers such as phosphate, citrate and acetate; tonicity agents such as sodium chloride, potassium chloride, glycerin, mannitol and others; antioxidants such as ascorbic acid, acetylcystine, sodium metabisulfote and others; aromatic agents; viscosity adjustors, such as polymers, including cellulose and derivatives thereof; and polyvinyl alcohol and acid and bases to adjust the pH of these aqueous compositions as needed. The compositions also comprise, in another embodiment, local anesthetics or other actives. The compositions can be used as sprays, mists, drops, and the like.

[000282]In one embodiment, pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients can be prepared as appropriate oil or water based injection suspensions. Suitable lipophilic solvents or vehicles include, in another embodiment, fatty oils such as sesame oil, or synthetic fatty acid esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions contain, in another embodiment, substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. In another embodiment, the suspension also contains suitable stabilizers or agents

which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

[000283]In another embodiment, the active compound can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez- Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; J. E. Diederichs and al., Pharm./nd. 56 (1994) 267- 275).

[000284]In another embodiment, the pharmaceutical composition delivered in a controlled release system is formulated for intravenous infusion, implantable osmotic pump, transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump is used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity to the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, for e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984). Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990).

[000285]In another embodiment, the active ingredient is in powder form for constitution with a suitable vehicle, for e.g., sterile, pyrogen-free water based solution, before use. Compositions are formulated, in another embodiment, for atomization and inhalation administration. In another embodiment, compositions are contained in a container with attached atomizing means.

[000286]In one embodiment, the preparation of the present invention is formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

[000287]In one embodiment, pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. In another embodiment, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

[000288]In one embodiment, determination of a therapeutically effective amount is well within the capability of those skilled in the art.

[000289]Some examples of substances which can serve as pharmaceutically-acceptable carriers or components thereof are sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and methyl cellulose; powdered tragacanth; malt; gelatin; talc; solid lubricants, such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols such as propylene glycol, glycerine, sorbitol, mannitol, and polyethylene glycol; alginic acid; emulsifiers, such as the Tween™ brand emulsifiers; wetting agents, such sodium lauryl sulfate; coloring agents; flavoring agents; tableting agents, stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; and phosphate buffer solutions. The choice of a pharmaceutically-acceptable carrier to be used in conjunction with the compound is basically determined by the way the compound is to be administered. If the subject compound is to be injected, in one embodiment, the pharmaceutically-acceptable carrier is sterile, physiological saline, with a blood-compatible suspending agent, the pH of which has been adjusted to about 7.4.

[000290]In addition, the compositions further comprise binders (e.g., acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g., cornstarch, potato starch, alginic acid, silicon dioxide, croscarmelose sodium, crospovidone, guar gum, sodium starch glycolate), buffers (e.g., Tris-HCl., acetate, phosphate) of various pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g. sodium lauryl sulfate), permeation enhancers, solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g. hydroxypropyl cellulose, hyroxypropylmethyl cellulose), viscosity increasing agents (e.g. carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum), sweeteners (e.g. aspartame, citric acid), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), lubricants (e.g. stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g. colloidal silicon dioxide), plasticizers (e.g. diethyl phthalate, triethyl citrate), emulsifiers (e.g. carbomer, hydroxypropyl cellulose, sodium lauryl sulfate), polymer coatings (e.g., poloxamers or poloxamines), coating and film forming agents (e.g. ethyl cellulose, acrylates, polymethacrylates) and/or adjuvants.

[000291]Typical components of carriers for syrups, elixirs, emulsions and suspensions include ethanol, glycerol, propylene glycol, polyethylene glycol, liquid sucrose, sorbitol and water. For a suspension, typical suspending agents include methyl cellulose, sodium carboxymethyl cellulose, cellulose (e.g. Avicel™, RC-591), tragacanth and sodium alginate; typical wetting agents include lecithin and polyethylene oxide sorbitan (e.g. polysorbate 80). Typical preservatives include methyl paraben and sodium benzoate. In another embodiment, peroral liquid compositions also contain one or more components such as sweeteners, flavoring agents and colorants disclosed above.

[000292]The compositions also include incorporation of the active material into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts.) Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of *in vivo* clearance.

[000293]Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors.

[000294]In one embodiment, compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline. In another embodiment, the modified compounds exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds. In one embodiment, modifications also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. In another embodiment, the desired in vivo biological activity is achieved by the administration of such polymer-compound abducts less frequently or in lower doses than with the unmodified compound.

[000295]Preparation of effective amount or dose can be estimated initially from in vitro assays. In one embodiment, a dose can be formulated in animal models and such information can be used to more accurately determine useful doses in humans.

[000296]In one embodiment, toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. In one embodiment, the data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. In one embodiment, the dosages vary depending upon the dosage form employed and the route of administration utilized. In one embodiment, the exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. [See e.g., Fingl, et al., (1975) "The Pharmacological Basis of Therapeutics", Ch. 1 p.1].

[000297]In one embodiment, depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

[000298]In one embodiment, the amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

[000299]In one embodiment, compositions including the preparation of the present invention formulated in a compatible pharmaceutical carrier are also prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

[000300]In another embodiment, a polypeptide or fragment thereof or a variant (CTP-modified) form thereof, as described herein, is lyophilized (i.e., freeze-dried) preparation in combination with complex organic excipients and stabilizers such as nonionic surface active agents (i.e., surfactants), various sugars, organic polyols and/or human serum albumin. In another embodiment, a pharmaceutical composition comprises a lyophilized polypeptide or fragment thereof provided herein, prepared in sterile water for injection. In another embodiment, a pharmaceutical composition comprises a lyophilized polypeptide or fragments thereof provided herein, is prepared in sterile PBS for injection. In another embodiment, a pharmaceutical composition comprises a lyophilized polypeptide or fragment thereof provided herein, prepared in sterile 0.9% NaCl for injection.

[000301]In another embodiment, the pharmaceutical composition comprises a CTP-modified polypeptide or fragment thereof as described herein and complex carriers such as human serum albumin, polyols, sugars, and anionic surface active stabilizing agents. In another

embodiment, the pharmaceutical composition comprises a CTP-polypeptide or fragment thereof and lactobionic acid and an acetate/glycine buffer. In another embodiment, the pharmaceutical composition comprises a CTP-modified polypeptide or fragment thereof as described herein and amino acids, such as arginine or glutamate that increase the solubility of interferon compositions in water. In another embodiment, the pharmaceutical composition comprises a lyophilized CTP-modified polypeptide or fragments thereof as described herein and glycine or human serum albumin (HSA), a buffer (e g. acetate) and an isotonic agent (e.g. NaCl). In another embodiment, the pharmaceutical composition comprises a lyophilized CTP-modified polypeptide or fragments thereof, as described herein and phosphate buffer, glycine and HSA.

[000302]In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof, as described herein is stabilized when placed in buffered solutions having a pH between about 4 and 7.2. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof is in a buffered solution having a pH between about 4 and 8.5. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof is in a buffered solution having a pH between about 6 and 7. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof is in a buffered solution having a pH of about 6.5. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof as described herein is stabilized with an amino acid as a stabilizing agent and in some cases a salt (if the amino acid does not contain a charged side chain).

[000303]In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof as described herein is a liquid composition comprising a stabilizing agent at between about 0.3% and 5% by weight which is an amino acid.

[000304]In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof as described herein provides dosing accuracy and product safety. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof as described herein provides a biologically active, stable liquid formulation for use in injectable applications. In another embodiment, the

pharmaceutical composition comprises a non-lyophilized CTP-modified polypeptide or fragments thereof as described herein.

[000305]In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragment thereof provided herein provides a liquid formulation permitting storage for a long period of time in a liquid state facilitating storage and shipping prior to administration.

[000306]In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof, provided herein comprises solid lipids as matrix material. In another embodiment, the injectable pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof, as described herein comprises solid lipids as matrix material. In another embodiment, the production of lipid microparticles by spray congealing was described by Speiser (Speiser and al., Pharm. Res. 8 (1991) 47-54) followed by lipid nanopellets for peroral administration (Speiser EP 0167825 (1990)). In another embodiment, lipids, which are used, are well tolerated by the body (e. g. glycerides composed of fatty acids which are present in the emulsions for parenteral nutrition).

[000307]In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragment thereof, as described herein comprises polymeric microparticles. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragment thereof, as described herein comprises nanoparticles. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragment thereof, as described herein comprises liposomes. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragment thereof, as described herein comprises lipid emulsion. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragment thereof, as described herein comprises microspheres. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragment thereof, as described herein comprises lipid nanoparticles. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragment thereof, as described herein comprises lipid nanoparticles comprising amphiphilic lipids. In another embodiment, the pharmaceutical composition comprising a CTP-modified polypeptide or fragments thereof, as described herein comprises lipid nanoparticles comprising a drug, a lipid matrix and a surfactant. In another embodiment, the lipid matrix has a monoglyceride content which is at least 50% w/w.

[000308]In one embodiment, compositions of the present invention are presented in a pack or dispenser device, such as an FDA approved kit, which contain one or more unit dosage forms containing the active ingredient. In one embodiment, the pack, for example, comprise metal or plastic foil, such as a blister pack. In one embodiment, the pack or dispenser device is accompanied by instructions for administration. In one embodiment, the pack or dispenser is accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, in one embodiment, is labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert.

[000309]In one embodiment, it will be appreciated that the polypeptides or fragments thereof provided herein can be provided to the individual with additional active agents to achieve an improved therapeutic effect as compared to treatment with each agent by itself. In another embodiment, measures (e.g., dosing and selection of the complementary agent) are taken to avoid adverse side effects which are associated with combination therapies.

[000310]In one embodiment, the term "about," means in quantitative terms plus or minus 5%, or in another embodiment plus or minus 10%, or in another embodiment plus or minus 15%, or in another embodiment plus or minus 20%.

[000311]The term "subject" refers in one embodiment to a mammal including a human in need of therapy for, or susceptible to, a condition or its sequelae. The subject may include dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice and humans. The term "subject" does not exclude an individual that is normal in all respects.

[000312]Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Materials and Methods:

Production of different CTP-modified proteins

[000313] The DNA coding region of human growth hormone (hGH), erythropoietin (EPO), APO-A1, Factor IX and Factor VII were ligated to the DNA sequence of CTP peptide. The CTP peptide was fused to the N-terminus and/ or C-terminus in single copy or in tandem, as detailed in Table 2. The engineered plasmids were transfected and expressed in CHO cell line that enable proper structuring of the O-glycans, which play a critical role in increasing the hydrodynamic volume of the proteins (see Table 5). The different proteins were purified according to custom processes that were developed uniquely for each protein, as detailed below:

[000314]*CTP-EPO-CTP-CTP:* The clarified harvest was loaded on a Blue Sepharose column. The eluted product was diluted and loaded on a Q Sepharose column. The eluted fraction from the Q Sepharose column was processed by ultrafiltration using an Amicon centrifugal device (30 kDa cut-off) and was dialyzed. The concentrated and dialyzed fraction was loaded on a Phenyl Sepharose column. The eluted fraction from Phenyl Sepharose was processed by ultrafiltration with an Amicon centrifugal device (30 kDa cut-off) and dialyzed against PBS pH 7.

[000315]*CTP-CTP-EPO and CTP-CTP-EPO-CTP-CTP:* Clarified harvest was loaded onto a DEAE Sepharose column and eluted. The eluted fraction was conditioned with Ammonium Sulfate and was loaded on a Phenyl Sepharose HS column. Elution Phenyl was concentrated and dialyzed. The next two columns are in a flow-through mode: Hydroxyapatite type I 40 µ and SP Sepharose. The final product was concentrated, dialyzed and stored at -20°C.

[000316]*APO-CTP* and *APO-CTP-CTP:* These two versions of APO proteins were purified with an affinity column (Capture Select Apo, Bac). The clarified harvest was diluted 1:1 with PBS and eluted from the column. The elution was concentrated and dialyzed against PBS and stored at -80°C.

[000317]*CTP-hGH-CTP-CTP:* The clarified harvest is filtered using UFDF1. Virus inactivation is accomplished.. The first chromatography is an anion exchange chromatography, DEAE Sepharose FF. The resin of the second chromatography is Phenyl Sepharose. The eluate pool of the second chromatography is diafiltrated and concentrated in UFDF-2. The UFDF-2 step is followed by two more chromatographies, Ceramic Hydroxyapatite Type I 40 µM and SP Sepharose FF, in a flow-through mode. Nanofiltration was performed. The product solution is concentrated to 41 ± 1 mg/mL and dialyzed.

[000318]*FIX-CTP-CTP-CTP:* Tris-HCl, pH 9 was added to clarified harvest. The first chromatography column was carried out using an anion exchange, Q column. The next column was Heparin Hyper D. The eluted fraction was adjusted to final concentration of 10mM Sodium Phosphate with a final pH of 6.8. The last chromatography step was performed on CHT resin. The eluted fraction was concentrated and dialyzed againt TBS pH 7.5.

[000319]*FIX-CTP-CTP-CTP-CTP:* The clarified harvest is concentrated and dialyzed. The only chromatography step is an affinity chromatography, immobilized Jacalin. The eluted product was concentrated and dialyzed against TBS pH 7.5.

[000320]*FIX-CTP-CTP-CTP-CTP-CTP:* The clarified harvest is concentrated and dialyzed. The only chromatography step is an affinity chromatography, immobilized Jacalin The eluted product was concentrated and dialyzed against TBS pH 7.5.

[000321] *APOA1-CTP-CTP:* The clarified harvest was concentrated and dialyzed. The first chromatography was carried out using an anion exchange chromatography, DEAE Sepharose FF column. The second chromatography step was performed on Immobilized Jacalin resinThe eluate was diafiltrated and concentrated in UFDF-2 against TBS pH 7.4.

[000322]*APOA1-CTP:* The first chromatography was carried out using an affinity chromatography, Capture-Select APO-AI column.. The second chromatography step was performed on Immobilized Jacalin resinThe eluate was diafiltrated and concentrated in UFDF-2 against TBS pH 7.4.

[000323]*APOA1:* The diluted harvest was loaded on affinity chromatography, Capture-Select APO-AI. The eluate was diafiltrated and concentrated in UFDF-2 against TBS pH 7.4.

[000324]*FVIIa-CTP-CTP-CTP:* The clarified harvest was concentrated and dialyzed. Virus inactivation was accomplished The first chromatography was carried out usig an affinity column, VII Select. The eluted fraction was diluted before loading on the next column-Ceramic Hydroxyapatite (CHT). The CHT eluate was loaded on Phenyl Sepharose column. The eluate was diafiltrated and was activated on an anion exchange chromatography Column. The column is than washed and the product was eluted. Nanofiltration was performed.

[000325]*FVIIa-CTP-CTP-CTP-CTP-CTP:* The clarified harvest was concentrated and dialyzed. The first chromatography was carried out using an affinity column, VII Select. The

eluted fraction was loaded on the next column- Ceramic Hydroxyapatite (CHT). The column was washed and the product was eluted. The CHT eluate was loaded on Phenyl Sepharose column. The eluate is diafiltrated and concentrated. Factor VII was activated on an anion exchange chromatography. The column was then washed and the product was eluted.

[000326]Table 2: Schematic description of CTP-modified protein

Plasmid schematic description
CTP-hGH-CTP-CTP (MOD-4023)
CTP-CTP-EPO
CTP-EPO-CTP-CTP
CTP-CTP-EPO-CTP-CTP
APO -CTP
APO -CTP- CTP
FIX-CTP-CTP-CTP
FIX-CTP-CTP-CTP-CTP
FIX-CTP-CTP-CTP-CTP-CTP
FVIIa-CTP-CTP-CTP
FVIIa-CTP-CTP-CTP-CTP-CTP

Deglycosylation of CTP-modified proteins

[000327]Deglycosylation of CTP-modified proteins was performed using Glyko Sialidase A (cat. no. PZ PZGK80040, Prozyme), O-glycanase (cat. no. PZ PZGK80090, Prozyme) and N-glycanase (cat. no. PZGKE-5006A, Prozyme). Proteins were digested for 2h (at 37°C) with sialidasa A, followed by digestion with O-glycanase and if needed with N-glycanase for overnight.

Molecular weight determination by MALDI-TOF

[000328] Molecular weights (Mw) of the CTP-modified proteins were measured by MALDI-TOF technology using the REFLEX-IV model (Bruker Daltonics, Bremen, Germany). Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) is a technique in which a co-precipitate of an UV-light absorbing matrix and a biomolecule like proteins or peptide is irradiated by a laser pulse. The ionized biomolecules are accelerated in an electric field and enter the flight tube. During the flight in this tube, different molecules are separated according to their mass to charge ratio and reach the

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detector at different times. In this way each molecule yields a distinct signal that can be converted to molecular weight. The method is used for characterization of different proteins and peptide with molecular masses between 400 and 350,000 Da. It is a very sensitive method, which allows the detection of low (10-15 to 10-18 mole) quantities of sample with an accuracy of 0.1 - 0.01 %. The measurements were performed at the Analytical Research Services Unit (Ben-Gurion University, Beer-Sheva, Israel).

Analysis of hydrodynamic size by HPLC using SEC column

[000329]The hydrodynamic size of the proteins were measured by HPLC (Dionex UltiMate 3000) using TSKgel G2000SW SEC column (cat. no. 08540, TosoHaas)for hGH, Epo and Apo native and related CTP- modified proteins or a TSKgel G3000WXL SEC column (cat. no. 08541, TosoHaas) for Factor IX and Factor VII native and related CTP-modified proteins. The H Mw calibration kit (Cat. No. 151-1901, BioRad) was used for measuring proteins size The results were fitted to a logarithmic fit (y=a*ln X +b) and the hydrodynamic sizes of the different proteins were calculated.

RESULTS

Example 1: Production of different CTP-modified proteins.

[000330] Eleven different CTP-modified proteins were transfected and expressed in a CHO cell line. The various harvests were purified according to the methods described above. The purified proteins are shown in Figures 1 & 2.

Example 2: Analysis of molecular weight by MALDI-TOF method

[000331] The molecular weight of different glycosylated and non-glycosylated CTP-modified proteins was determined using MALDI-TOF technology and was compared to their corresponding native proteins' (intact proteins which are not fused to CTP, namely, Biotropin for hGH, EPREX® for Epo, ApoAI, Mononine® for Factor IX and Novoseven® for FVIIa) molecular weight Table 3). The measured Mw for all native and non-glycosylated proteins was in good agreement with the theoretical Mw, which is based on the proteins' amino acid sequences. The increments in the molecular weights per one copy of non-glycosylated and

glycosylated CTP were calculated and are depicted in Figures 3A and 3B, respectively. The contribution of one copy of CTP to the molecular weight was calculated as follows: First, the increment in the molecular weight was calculated by subtracting the measured, or the theoretical -in the case of native hGH-, molecular weight of the native proteins from the measured molecular weight of their corresponding CTP-modified proteins. Then, the calculated increment was divided by the number of CTP copies for each protein. For example, MOD-4023 (hGH that was fused to one copy of CTP at the N-terminus and to 2 copies of CTP in tandem at the C-terminus) has molecular weight of 38,128, while the native hGH has a theoretical molecular weight of 22,000. The difference between those two proteins is 16.13 kDa which means that the contribution of each glycosylated CTP is 5.4 kDa (16.13 divided by 3 copies of CTP). The average contribution of one copy of non-glycosylated CTP in all measured proteins is 2.76 kDa±0.103 (Figure3A, Table 3). This result is aligned with the theoretical Mw of a single CTP, which is 2.78 kDa. The glycosylated CTP contributes an average of 4.76kDa±0.422 to the Mw (Figure3B, Table 3), without significant differences between the various measured proteins.

[000332]Table 3: MALDI-TOF results of non-glycosylated and glycosylated CTP-modified proteins and their corresponding native proteins. ND – Not determined.

Proteins Description	Theoretical Mw (based on protein backbone excluding glycan contribution)	Non-glycosylated proteins. MALDI-TOF results (Da)	Glycosylated proteins. MALDI-TOF results (Da)	Increment of molecular size per one copy of non-glycosylated CTP	Increment of molecular size per one copy of glycosylated CTP
Biotropin(rhGH)	22000	ND	ND	ND	ND
CTP-hGH-CTP-CTP (MOD-4023)	30469.4	30525	38128	2.8	5.4
EPREX®(rEPO)	18396	18246	29160	0.0	0.0
CTP-CTP-EPO	23956	23690	37074	2.7	4.0
CTP-EPO-CTP-CTP	26736	27300	43547.8	3.0	4.8
APO AI	28078	28021.5	28024.5	0.0	0.0
APO -CTP	30858	30686.5	32505	2.7	4.5

APO CTP-CTP	33638	33569	36710	2.8	4.3
Mononine® (rFIX)	48695.6	47172	53270	0.0	0.0
FIX-CTP-CTP-CTP	57036	55626.5	68876	2.8	5.2
FIX-CTP-CTP-CTP-CTP	59816.2	58346.5	73552.5	2.8	5.1
FIX-CTP-CTP-CTP-CTP-CTP	62596.2	61051.5	77797	2.8	4.9
Novoseven® (rFVIIa)	47222.6	45899	50310.4	0.0	
FVIIa-CTP-CTP-CTP	58343.1	53755.5	64302	2.6	4.7
FVIIa-CTP-CTP-CTP-CTP-CTP	61123.2	59266	74431	2.7	4.8
			Ave.	**2.76**	**4.76**
			SD	**0.103**	**0.422**
			%CV	**3.72**	**8.87**

Example 3: Analysis of hydrodynamic size by HPLC method

[000333]Hydrodynamic volume is the major parameter influencing the retention time (RT) of proteins when passed through size exclusion column. Therefore, protein sizes were calculated by SEC column using HMw Gel filtration calibration kit (cat. no. 151-1901, BioRad). The retention time of the standards were measured in both SEC TSK 2000 and TSK 3000 columns and the % relative error (%RE) for each column was calculated in order to determine the precision of the analytical methods. The %RE of the obsereved Mw of the calibration proteins were calculated and compared to the known and expected Mw of the calibration proteins. The results of the calculated Mw for the calibration curve and the %RE are presented in Table 4a for TSK 2000 SEC column and in Table 4b for TSK 3000 SEC column. The results show that the %RE was below or equal to 20% (≤20%), indicating high precision for a broad range of a protein's determined molecular weight.

[000334]Table 4a: H Mw calibration curve results and calculated %RE using TSK 2000. The expected molecular weight of the calibration curve proteins were provided by the commercial kit (H Mw calibration kit BioRad Cat. No. 151-1901) that was used.

Standard proteins	Expected M.W.	R.T.	Observed M.W.	%RE
Gamma-globulin	158,000	15.535	155279.16	-1.72
Ovalbumin	44,000	18.535	52924.39	20.28

Myoglobulin	17,000	22.315	13635.14	-19.79
Vitamin B12	1,350	28.61	1424.88	5.55

[000335]Table 4b: H Mw calibration curve results and calculated %RE using TSK 3000 column. The expected Mw of the calibration curve proteins were provided by the commercial kit that was used (H Mw calibration kit BioRad Cat. No. 151-1901).

Standard proteins	Expected M.W.	R.T.	Observed M.W.	%RE
Tyroglobulin	670,000	11.925	753,500	12.46
Gamma-globulin	158,000	16.250	126,808	-19.74
Ovalbumin	44,000	18.702	46,172	4.94
Myoglobulin	17,000	21.012	17,824	4.85

[000336]In order to determine the contribution of glycosylated CTP to the hydrodynamic volume of CTP-modified proteins, various CTP-modified proteins were analyzed by SEC column and their hydrodynamic sizes were calculated. The corresponding recombinant proteins: Biotropin (rhGH), EPREX® (rEPO), ApoAI, Mononine® (rFIX) and Novoseven® (rFVIIa) were analyzed in parallel to their CTP-modified corresponding proteins in order to calculate the contribution of glycosylated CTP to the protein (Table 5, Figure 4). Figure 4A presents the total increment of hydrodynamic size of CTP-modified proteins to the native proteins as was measured by SEC column.

[000337]Table 5: SEC-HPLC results and calculated increament of one copy of CTP of CTP-modified proteins and their corresponding native proteins.

Glycosylated proteins	SEC-HPLC Mw (Da)	Increase in kDa per glycosylated CTP
Biotropin (rhGH)	21116	NA
CTP-hGH-CTP-CTP (MOD-4023)	107750	28.9
EPREX® (rEPO)	79014	NA
CTP-CTP-EPO	146616	33.8
CTP-EPO-CTP-CTP	168032	29.7
CTP-CTP-EPO-CTP-CTP	199970	30.2
APO	62086	NA

APO -CTP	100233	38.1
APO CTP-CTP	141094	39.5
Mononine® (rFIX)	117553	NA
FIX-CTP-CTP-CTP	261982	48.1
FIX-CTP-CTP-CTP-CTP	329362	53.0
FIX-CTP-CTP-CTP-CTP-CTP	381095	52.7
Novoseven® (rFVIIa)	76706	NA
FVIIa -CTP-CTP-CTP	206645	43.3
FVIIa -CTP-CTP-CTP-CTP-CTP	325602	49.8

[000338]The increment in the molecular weight of one copy of glycosylated CTP was calculated by subtracting the measured hydrodynamic size of the native proteins from the measured hydrodynamic size of their corresponding CTP-modified proteins. Then, the calculated increment was divided by the number of CTP copies for each protein. The calculated contributions of one copy of glycosylated CTPs to the molecular weight of various proteins are presented in Figure 4B. The various proteins exhibit increments that range between 29 kDa to 53 kDa per one copy of glycosyted CTP.

[000339]Interestingly and unexpectedly, the contribution of one copy of glycosylated CTP of the FIX and FVIIa was markedly higher with a contribution of 43-53 kDa (per one copy of CTP) compared to other measured proteins (Table 5). The increment in the hydrodynamic size per one copy of glycosylated CTP is much higher than the calculated contribution of 4.76 kDa per one copy of glycosylated CTP to the molecular weight, as measured by MALDI-TOF. The differences in the calculated Mw between the methods results from the fact that while MALDI-TOF is measuring the actual Mw of the protein, the SEC-HPLC measurement is affected by the protein's hydrodynamic volume, suggesting that glycosylated CTP is substantially increasing the hydrodynamic volume of proteins to which it is attached. The magnitude in the hydrodynamic volume is about 6-11 fold higher compared to the calculated contribution per CTP measured by MALDI-TOF. Of note, the contribution of CTP to the hydrodynamic size of the protein being modified was lower for hGH and CTP-modified EPO variants (around 30kDa) but slightly higher for FIX and FVII-CTP modified variants, and was surprisingly not affected by the number of CTPs being added to the particular protein.

[000340]In addition, the contribution of non-glycosylated CTP to the protein's Mw was determined by SEC-HPLC (Table 6, Figures 5A & 5B). The de-glycosylation was performed by incubating the proteins with Sialidase A (remove sialic acid) for 2h at 37°C followed by adding O-glycanase (to remove O-glycans). In the case of EPREX® (rEPO), Mononine® (rFIX), NovoSeven® (rFVII) and their corresponding CTP-modified proteins, which contain N-glycans, N-glycanase was added for an over-night digestion to remove N-glycans. The contribution of non-glycosylated CTP to the hydrodynamic size or volume of various proteins was calculated and compared to their corresponding native proteins, i.e., the contribution of non-glycosylated CTP-modified polypeptides to the hydrodynamic volume were calculated by comparing the hydrodynamic volume of the non-glycosylated CTP-modified polypeptides to that of the non-glycosylated corresponding native protein. For example, for EPO, the N- and O-glycans were removed from Eprex®, and the increase in hydrodynamic volume of the CTP-modified Epo variants were calculated and compared to their molecular weight).

[000341]Figure 5A depicts the increment in the hydrodynamic size of the intact proteins, while Figure 5B depicts the contribution of one copy of non-glycosylated CTP-modified proteins. Remarkably, non-glycosylated CTP increases the hydrodynamic size of the CTP-modified proteins when compared to the corresponding native proteins. The calculated contribution of one copy of non-glycosylated CTP was different between the various proteins, ranging between 8 kDa to 21 kDa per one copy of non glycosylated CTP (Table 6). Considering that the theoretical molecular weight of CTP, which consists of 28 amino acids, is 2.78 kDa and the measured molecular weight (by MALDI-TOF) was also about 2.76 kDa, these results suggest that the contribution of non-glycosylated CTP to the molecular weight is higher than expected. Further, and as similarly observed for glycosylated CTP, the hydrodynamic volume was also observed to be much higher than the expected for non-glycolysated CTP. Overall, attaching CTP to a protein results in an increase in hydrodynamic volume that is attributable to both the CTP backbone and the CTP glycans.

[000342]It was also observed that number of CTPs added to a particular protein did not affect the contribution to the hydrodynamic size of the same. The most significant increment of non-glycosylated CTP was observed for Apo, FIX and FVII that have copies of CTP in the C-terminus of the protein. This finding that adding CTP at the C-terminus leads to a higher contribution to hydrodynamic volume was unexpected. Interestingly and unexpectedly the

contribution of one copy of non-glycosylated CTP of Apo, FIX and FVIIa CTP- modified proteins was very similar and measured ~20 kDa (Table 6) but the contribution of glycosylated CTP of the coagulation factors was significantly higher compared to Apo glycosylated CTP (Table 5).

[000343]Table 6: SEC-HPLC results and calculated increament of one copy of CTP of non-glycosylated CTP-modified proteins and their corresponding native proteins.

Non-glycosylated proteins	SEC-HPLC Mw (Da)	Increase in kDa per non-glycosylated CTP
Biotropin(rhGH)	21116	NA
CTP-hGH-CTP-CTP (MOD-4023)	45480	8.1
EPREX® (rEPO)	18083	NA
CTP-CTP-EPO	49472	15.7
CTP-EPO-CTP-CTP	65991	16.0
CTP-CTP-EPO-CTP-CTP	85228	16.8
APO	61267	NA
APO -CTP	82846	21.6
APO CTP-CTP	104007	21.4
Mononine® (rFIX)	79539	NA
FIX-CTP-CTP-CTP	138132	19.5
FIX-CTP-CTP-CTP-CTP	160115	20.1
FIX-CTP-CTP-CTP-CTP-CTP	186677	21.4
Novoseven® (rFVIIa)	52570	NA
FVIIa-CTP-CTP-CTP	107321	18.3
FVIIa-CTP-CTP-CTP-CTP-CTP	158706	21.2

[000344]This study showed that one copy of glycosylated CTP contributes at least 28 (kDa) to the hydrodynamic volume while the increment of Mw is 4.76 (kDa) 0.422, as determined using SEC-HPLC and MALDI-TOF, respectively. This unexpected magnitude in the hydrodynamic volume of CTP-modified proteins is likely the reason for the observed extended serum half-life and enhancement of biological activity of CTP-modified proteins. The non-glycosylated CTP contributes at least 8 (kDa) to the hydrodynamic volume, while the molecular weight increment was 2.76 (kDa) 0.103. Interestingly, the Mw of non-

glycosylated and glycosylated CTP peptide as was measured by MALDI-TOF was similar between all of the proteins. Further, the hydrodynamic volume of glycosylated and non-glycosylated CTP peptide-modified proteins as measured by SEC-HPLC were different. These findings suggest that although the Mw of the CTP peptide is similar when fused to different proteins in different position, it causes an unexpected increase of the hydrodynamic volume of different proteins to which it is attached, as evidence by the SEC column measurements.

[000345]Having described preferred embodiments of the invention with reference to the accompanying drawings, it is to be understood that the invention is not limited to the precise embodiments, and that various changes and modifications may be effected therein by those skilled in the art without departing from the scope or spirit of the invention as defined in the appended claims.

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.