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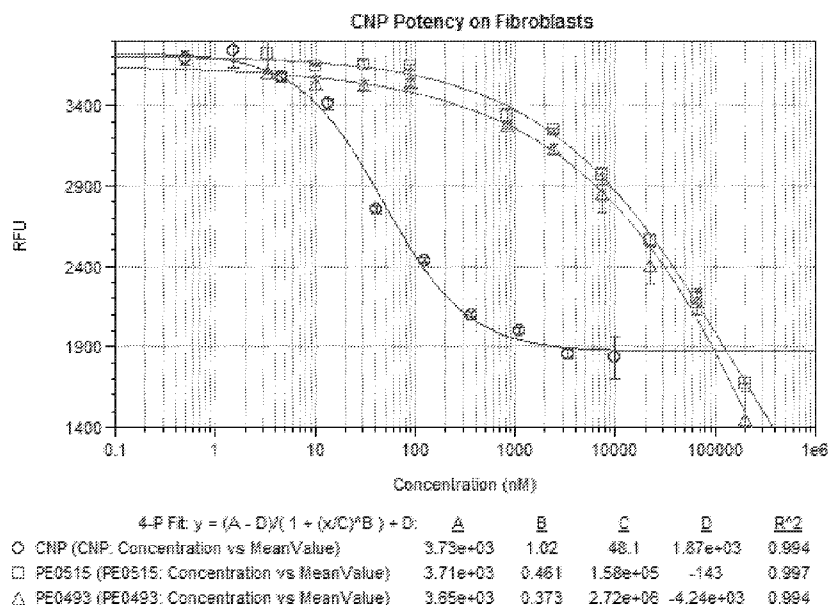
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(54) Title: ELP FUSION PROTEINS FOR CONTROLLED AND SUSTAINED RELEASE

FIG. 1A Potency of CNP constructs



(57) Abstract: The present disclosure provides pharmaceutical formulations for sustained release, and methods for delivering a treatment regimen with a combination of sustained release and long half-life formulations. The disclosure provides improved pharmacokinetics for peptide and small molecule drugs.

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ELP FUSION PROTEINS FOR CONTROLLED AND SUSTAINED RELEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This Application claims the benefit of U.S. Provisional Application No. 62/442,057 filed January 4, 2017 and U.S. Provisional Application No. 62/332,803 filed May 6, 2016, the contents of each of which are hereby incorporated by reference in their entireties.

FIELD OF INVENTION

[0002] The present disclosure relates to pharmaceutical formulations for sustained release, and methods for delivering a treatment regimen with the sustained release formulations.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0003] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: a computer readable format copy of the sequence listing (filename: PHAS_033_02WO_SeqList_ST25.txt, date recorded: May 1, 2017, file size 287 kilobytes).

BACKGROUND

[0004] The effectiveness of peptide and small molecule drugs is often limited by the half-life of such drugs in the circulation, as well as difficulties in obtaining substantially constant plasma levels. For example, the incretin GLP-1 must be administered at relatively high doses to counter its short half-life in the circulation, and these high doses are associated with nausea, among other things (Murphy and Bloom, Nonpeptidic glucagon-like peptide 1 receptor agonists: A magic bullet for diabetes? *PNAS* 104 (3):689-690 (2007)). Further, the peptide agent vasoactive intestinal peptide (VIP) exhibits a half-life, in some estimates, of less than one minute, making this agent impractical for pharmaceutical use (Domschke *et al.*, Vasoactive intestinal peptide in man: pharmacokinetics, metabolic and circulatory effects, *Gut* 19:1049-1053 (1978); Henning and Sawmiller, Vasoactive intestinal peptide: cardiovascular effects, *Cardiovascular Research* 49:27-37 (2001)). A short plasma half-life for peptide drugs is often due to fast renal clearance as well as to enzymatic degradation during systemic circulation.

SUMMARY OF THE INVENTION

[0005] The present disclosure provides pharmaceutical formulations for sustained release, and methods for delivering a treatment regimen with the sustained release formulations. The disclosure thereby provides improved pharmacokinetics for peptide and small molecule drugs.

[0006] In some aspects, the disclosure provides a sustained release pharmaceutical formulation. The formulation includes a therapeutic agent for systemic administration, where the therapeutic agent includes an active agent and an amino acid sequence capable of forming a reversible matrix at the body temperature of a subject. The reversible matrix is formed from hydrogen bonds (*e.g.*, intra- and/or intermolecular hydrogen bonds) as well as from hydrophobic contributions. The formulation further includes one or more pharmaceutically acceptable excipients and/or diluents. The matrix provides for a slow absorption to the circulation from an injection site. The sustained release, or slow absorption from the injection site, is due to a slow reversal of the matrix as the concentration dissipates at the injection site. Once product moves into the circulation, the formulation confers long half-life and improved stability. Thus, a unique combination of slow absorption and long half-life is achieved leading to a desirable PK profile with a low peak to trough (C_{max} to C_{min}) and delayed or late T_{max} .

[0007] In certain embodiments, the amino acid sequence capable of forming a reversible matrix at the body temperature of a subject is an Elastin-Like-Peptide (ELP) sequence. The ELP sequence includes or consists of structural peptide units or sequences that are related to, or mimics of, repeat sequence present in the elastin protein. The ELP amino acid sequence may exhibit a visible and reversible inverse phase transition in the selected formulation. That is, the amino acid sequence may be structurally disordered and highly soluble in the formulation below a transition temperature (T_t), but exhibit a sharp (2-3°C range) disorder-to-order phase transition when the temperature of the formulation is raised above the T_t . In some embodiments, the present disclosure provides therapeutic agents having transition temperatures between about 26°C and about 37°C. In addition to temperature, length of the ELP polymer, amino acid composition of the ELP, ionic strength, pH, pressure, selected solvents, presence of organic solutes, and protein concentration may also affect the transition properties, and these may be tailored for the desired absorption profile. In some embodiments the protein concentration and salt concentration affect the transition

properties (e.g. transition temperature). Exemplary sequences or structures for the ELP amino acid sequence forming the matrix are disclosed herein.

[0008] In certain embodiments, the active agent for systemic administration is a protein or peptide, which may have a short circulatory half-life, such as from about 30 seconds to about 1 hour, to about 2 hours, or to about 5 hours. In some embodiments, the protein or peptide has a circulatory half-life of from 30 seconds to about 10 hours. The therapeutic agent may be a recombinant fusion protein between the protein active agent and the amino acid sequence capable of forming the matrix. Exemplary peptide active agents include apelin, arginase, C-type natriuretic peptide (CNP), a GLP-1 receptor antagonist, a GLP-2 receptor agonist, hepcidin, IGF-1, urodilatin, thymosin β 4, TNF-related apoptosis-inducing ligand (TRAIL), Parathyroid hormone fragments (e.g. residues 1-34), full length parathyroid hormone, Adrenocorticotrophic hormone, Coversin, Kisspeptin, kisspeptin fragments (e.g. amino acid residues 1-10 or amino acid residues 1-54), Annexin A1-derived peptides (e.g. amino acid residues 2-26), FGF21, or derivatives, analogs, mimetics, combinations, or fragments thereof. Small molecule drugs for delivery in accordance with the disclosure are disclosed herein. By providing a slow absorption from the injection site, renal clearance and degradation can be controlled, thereby achieving the desired PK profile.

[0009] In other aspects, the disclosure provides methods for delivering a sustained release regimen of an active agent. The methods include administering the formulation described herein to a subject in need, wherein the formulation is administered from about 1 to about 8 times per month. In some embodiments, the formulation is administered about weekly, and may be administered subcutaneously or intramuscularly (for example). In some embodiments, the site of administration is not a pathological site, that is, the therapeutic agent is not administered directly to the intended site of action.

DESCRIPTION OF THE DRAWINGS

[0010] **Figure 1 A-B** shows the potency of CNP constructs.

[0011] **Figure 2A-C** shows the effect on growth of subcutaneous injection of five week old male FVB/nJ mice ($n = 12/\text{group}$) with PE0552 or saline (control) three times per week. Effect on growth was determined by measuring nose to tail length (A), nose to anus (B) and length of tail (C). The graphs show mean measurements and standard error for each group.

[0012] **Figure 3A-C** shows the effect on growth of subcutaneous injection of three week old male FVB/nJ mice ($n = 11/\text{group}$) with PE9206, PE9216, PE9306, PE9326 or saline (control)

daily for 3 weeks. Effect on growth was determined by measuring nose to tail length (A), nose to anus length (B), and tail length (C). The graphs show the mean change in growth versus baseline over the 3 week period.

[0100] **Figure 4** shows the effect on small intestine weight of subcutaneous injection of PE0503 or GLP-2 (A2G) over an eleven-day dosing period in male Sprague Dawley rats (200 – 220 g, n = 12/group). Dosing was as follows: Vehicle group dosed with saline daily; PE0503 Q1D dosed with PE0503 at 1.3 mg/kg once per day; PE0503 Q2D dosed with PE0503 at 5.1 mg/kg every other day; PE0503 Q4D dosed with PE0503 at 20.5 mg/kg once every four days; GLP-2(A2G) TID dosed twice per day with 0.1 mg/kg GLP2(A2G).

DETAILED DESCRIPTION

[0013] The present disclosure provides pharmaceutical formulations for sustained release, and methods for delivering a treatment regimen with the sustained release formulations. In certain embodiments, the pharmaceutical compositions disclosed herein have enhanced efficacy, bioavailability, circulatory half-life, persistence, degradation resistance, etc. The disclosure thereby provides improved pharmacokinetics for active agents, such as peptides and small molecule drugs, including a relatively flat PK profile with a low ratio of peak to trough, and/or a long T_{max}. The PK profile can be maintained with a relatively infrequent administration schedule, such as from one to eight injections per month in some embodiments.

[0014] In some aspects, the disclosure provides sustained release pharmaceutical formulations. The formulation includes therapeutic agents for systemic administration, where the therapeutic agent includes an active agent and an amino acid sequence capable of forming a matrix or coacervate at the body temperature of a subject. The reversible matrix is formed from hydrogen bonds (*e.g.*, intra- and/or intermolecular hydrogen bonds) as well as from hydrophobic contributions. The formulation further includes one or more pharmaceutically acceptable excipients and/or diluents. The matrix provides for a slow absorption to the circulation from an injection site. Without being bound by theory, this slow absorption is due to the slow reversal of the matrix or coacervate at the periphery of the injection site depot. The slow absorption profile provides for a flat PK profile, as well as convenient and comfortable administration regimen. For example, in various embodiments, the plasma concentration of the active agent over the course of

days (*e.g.*, from 2 to about 60 days, or from about 4 to about 30 days) does not change by more than a factor of 20, or by more than a factor of about 10, or by more than a factor of about 5, or by more than a factor of about 3. Generally, this flat PK profile is seen over a plurality of (substantially evenly spaced) administrations, such as at least about 2, at least about 5, or at least about 10 administrations of the formulation. In some embodiments, the slow absorption is manifest by a T_{max} (time to maximum plasma concentration) of greater than about 5 hours, greater than about 10 hours, greater than about 20 hours, greater than about 30 hours, or greater than about 50 hours.

AMINO ACID SEQUENCES FORMING A REVERSIBLE MATRIX

[0015] The sustained release, or slow absorption from the injection site, is controlled by the amino acid sequence capable of forming a hydrogen-bonded matrix or coacervate at the body temperature of the subject.

[0016] In some embodiments, the amino acid sequence contains structural units that form hydrogen-bonds through protein backbone groups and/or side chain groups, and which may contribute hydrophobic interactions to matrix formation. In some embodiments, the amino acid side chains do not contain hydrogen bond donor groups, with hydrogen bonds being formed substantially through the protein backbone. Exemplary amino acids include proline, alanine, valine, glycine, and isoleucine, and similar amino acids. In some embodiments, the structural units are substantially repeating structural units, so as to create a substantially repeating structural motif, and substantially repeating hydrogen-bonding capability. In these and other embodiments, the amino acid sequence contains at least about 10%, at least about 20%, at least about 40%, or at least about 50% proline, which may be positioned in a substantially repeating pattern. In this context, a substantially repeating pattern means that at least about 50% or at least about 75% of the proline residues of the amino acid sequence are part of a definable structural unit. In still other embodiments, the amino acid sequence contains amino acids with hydrogen-bond donor side chains, such as serine, threonine, and/or tyrosine. In some embodiments, the repeating sequence may contain from one to about four proline residues, with remaining residues independently selected from non-polar residues, such as glycine, alanine, leucine, isoleucine, and valine. Non-polar or hydrophobic residues may contribute hydrophobic interactions to the formation of the matrix.

[0017] In other embodiments, the amino acid sequence capable of forming the matrix at body temperature may include a random coil or non-globular extended structure. For example, the amino acid sequence capable of forming the matrix at body temperature may comprise an amino acid sequence disclosed in U.S. Patent Publication No. 2008/0286808, WIPO Patent Publication No. 2008/155134, and U.S. Patent Publication No. 2011/0123487, each of which is hereby incorporated by reference.

[0018] In some embodiments the amino acid sequence includes an unstructured recombinant polymer of at least 40 amino acids. The unstructured polymer may include more than about 100, about 150, about 200 or more contiguous amino acids. In some embodiments, the amino acid sequence forms a random coil domain. In particular, a polypeptide or amino acid polymer having or forming “random coil conformation” substantially lacks a defined secondary and tertiary structure. In some embodiments, the unstructured polymer is defined as a polymer having at least 40 amino acids where the total number of glycine (G), aspartate (D), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) residues constitutes more than about 80% of the total amino acids in the polymer. In some embodiments, at least 50% of the amino acids are devoid of secondary structure as determined by the Chou-Fasman algorithm.

[0019] The amino acid sequences may form a “gel-like” state upon injection at a temperature higher than the storage temperature. Exemplary sequences have repeating peptide units, and/or may be relatively unstructured at the lower temperature, and achieve a hydrogen-bonded, structured, state at the higher temperature.

Elastin-Like Peptides (ELPs)

[0020] In some embodiments, the amino acid sequence capable of forming a matrix at body temperature is a peptide having repeating units of from four to ten amino acids. The repeating unit may form one, two, or three hydrogen bonds in the formation of the matrix. In certain embodiments, the amino acid sequence capable of forming a matrix at body temperature is an amino acid sequence of silk, elastin, collagen, keratin, or mimic thereof, or an amino acid sequence disclosed in U.S. Patent 6,355,776, which is hereby incorporated by reference.

[0021] In certain embodiments, the amino acid sequence is an Elastin-Like-Peptide (ELP) sequence. The ELP sequence includes or consists of structural peptide units or sequences that are related to, or mimics of, the elastin protein. The ELP sequence is constructed from structural units of from three to about twenty amino acids, or in some embodiments, from about four to about ten

amino acids, such as about four, about five or about six amino acids. The length of the individual structural units may vary or may be uniform. Exemplary structural units are defined by SEQ ID NOS: 1-13 (below), which may be employed as repeating structural units, including tandem-repeating units, or may be employed in some combination. Thus, the ELP may comprise or consist essentially of structural unit(s) selected from SEQ ID NOS: 1-13, as defined below.

[0022] In some embodiments, including embodiments in which the structural units are ELP units, the amino acid sequence includes or consists essentially of from about 1 to about 500 structural units, or in certain embodiments about 9 to about 200 structural units, or in certain embodiments about 10 to 200 structural units, or in certain embodiments about 50 to about 200 structural units, or in certain embodiments from about 80 to about 200 structural units, or from about 80 to about 150 structural units. In some embodiments, the structural units are ELP units defined by one or more of SEQ ID NOS: 1-13. In some embodiments, the ELP includes a combination of units defined by SEQ ID NOS: 1-13. Thus, the structural units collectively may have a length of from about 50 to about 2000 amino acid residues, or from about 100 to about 800 amino acid residues, or from about 200 to about 700 amino acid residues, or from about 400 to about 600 amino acid residues. In exemplary embodiments, the amino acid sequence of the ELP structural unit includes or consists essentially of about 3 structural units, of about 7 structural units, of about 9 structural units, of about 10 structural units, of about 15 structural units, of about 20 structural units, of about 40 structural units, of about 80 structural units, of about 90 structural units, of about 100 structural units, of about 120 structural units, of about 140 structural units, about 144 structural units, of about 160 structural units, of about 180 structural units, of about 200 structural units, or of about 500 structural units. In exemplary embodiments, the structural units collectively have a length of about 45 amino acid residues, of about 90 amino acid residues, of about 100 amino acid residues, of about 200 amino acid residues, of about 300 amino acid residues, of about 400 amino acid residues, of about 500 amino acid residues, of about 600 amino acid residues, of about 700 amino acid residues, of about 720 amino acid residues, of about 800 amino acid residues, or of about 1000 amino acid residues.

[0023] The amino acid sequence may exhibit a visible and reversible inverse phase transition with the selected formulation. That is, the amino acid sequence may be structurally disordered and highly soluble in the formulation below a transition temperature (T_t), but exhibit a sharp (2-3°C range) disorder-to-order phase transition, or coacervation, when the temperature of

the formulation is raised above the Tt. In addition to temperature, length of the amino acid polymer, amino acid composition, ionic strength, pH, pressure, selected solvents, presence of organic solutes, and protein concentration may also affect the transition properties, and these may be tailored in the formulation for the desired absorption profile. Absorption profile can be easily tested by determining plasma concentration or activity of the active agent over time.

[0024] In certain embodiments, the ELP component(s) may be formed of structural units, including but not limited to:

- (a) the tetrapeptide Val-Pro-Gly-Gly, or VPGG (SEQ ID NO: 1);
- (b) the tetrapeptide Ile-Pro-Gly-Gly, or IPGG (SEQ ID NO: 2);
- (c) the pentapeptide Val-Pro-Gly-X-Gly (SEQ ID NO: 3), or VPGXG, where X is any natural or non-natural amino acid residue except proline, and where X optionally varies among polymeric or oligomeric repeats;
- (d) the pentapeptide Ala-Val-Gly-Val-Pro, or AVGVVP (SEQ ID NO: 4);
- (e) the pentapeptide Ile-Pro-Gly-X-Gly, or IPGXG (SEQ ID NO: 5), where X is any natural or non-natural amino acid residue, and where X optionally varies among polymeric or oligomeric repeats;
- (e) the pentapeptide Ile-Pro-Gly-Val-Gly, or IPGVG (SEQ ID NO: 6);
- (f) the pentapeptide Leu-Pro-Gly-X-Gly, or LPGXG (SEQ ID NO: 7), where X is any natural or non-natural amino acid residue, and where X optionally varies among polymeric or oligomeric repeats;
- (g) the pentapeptide Leu-Pro-Gly-Val-Gly, or LPGVG (SEQ ID NO: 8);
- (h) the hexapeptide Val-Ala-Pro-Gly-Val-Gly, or VAPGVG (SEQ ID NO: 9);
- (i) the octapeptide Gly-Val-Gly-Val-Pro-Gly-Val-Gly, or GVGVPGVG (SEQ ID NO: 10);

(j) the nonapeptide Val-Pro-Gly-Phe-Gly-Val-Gly-Ala-Gly, or VPGFGVGAG (SEQ ID NO: 11);

(k) the nonapeptides Val-Pro-Gly-Val-Gly-Val-Pro-Gly-Gly, or VPGVGVPGG (SEQ ID NO: 12); and

(l) the pentapeptide Xaa-Pro-Gly-Val-Gly, or XPGVG (SEQ ID NO: 13) where X is any natural or non-natural amino acid residue, and where X optionally varies among polymeric or oligomeric repeats.

[0025] Such structural units defined by SEQ ID NOS: 1-13 may form structural repeating units, or may be used in combination to form an ELP. In some embodiments, the ELP component is formed entirely (or almost entirely) of one or a combination of (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) structural units selected from SEQ ID NOS: 1-13. In other embodiments, at least about 75%, or at least about 80%, or at least about 90% of the ELP component is formed from one or a combination of structural units selected from SEQ ID NOS: 1-13, and which may be present as repeating units.

[0026] In certain embodiments, the ELP contains repeat units, including tandem repeating units, of Val-Pro-Gly-X-Gly (SEQ ID NO: 3), where X is as defined above, and where the percentage of Val-Pro-Gly-X-Gly (SEQ ID NO: 3) units taken with respect to the entire ELP component (which may comprise structural units other than VPGXG (SEQ ID NO: 3)) is greater than about 50%, or greater than about 75%, or greater than about 85%, or greater than about 95% of the ELP. The ELP may contain motifs of 5 to 15 structural units (*e.g.* about 9 or about 10 structural units) of SEQ ID NO: 3, with the guest residue X varying among at least 2 or at least 3 of the units in the motif. The guest residues may be independently selected, such as from non-polar or hydrophobic residues, such as the amino acids V, I, L, A, G, and W (and may be selected so as to retain a desired inverse phase transition property). In certain embodiments, the guest residues are selected from V, G, and A.

[0027] In certain embodiments, the ELP contains repeat units, including tandem repeating units, of Xaa-Pro-Gly-Val-Gly (SEQ ID NO: 13), where X is as defined above, and where the percentage of Xaa-Pro-Gly-Val-Gly (SEQ ID NO: 13) units taken with respect to the entire ELP component (which may comprise structural units other than XPGVG (SEQ ID NO: 13)) is greater than about 50%, or greater than about 75%, or greater than about 85%, or greater than about 95%

of the ELP. The ELP may contain motifs of 5 to 15 structural units (*e.g.* about 9 or about 10 structural units) of SEQ ID NO: 13, with the guest residue X varying among at least 2 or at least 3 of the units in the motif. The guest residues may be independently selected, such as from non-polar or hydrophobic residues, such as the amino acids V, I, L, A, G, and W (and may be selected so as to retain a desired inverse phase transition property). In certain embodiments, the guest residues are selected from V and A.

[0028] In certain embodiments, the ELP contains repeating units, including tandem repeating units of any of SEQ ID NOs: 1-13 either alone or in combination. In some embodiments, the ELP contains repeats of two or more of any of SEQ ID NOs: 1-13 in combination. In certain embodiments, the ELP contains repeats of SEQ ID NO: 3 and SEQ ID NO: 13. In some embodiments, the ELP contains repeats of SEQ ID NO: 3 and SEQ ID NO: 13, wherein the guest residues are independently selected, such as from non-polar or hydrophobic residues, such as the amino acids V, I, L, A, G, and W (and may be selected so as to retain a desired inverse phase transition property). In certain embodiments, the guest residues are selected from V, G, and A.

[0029] In some embodiments, the ELP includes 9-mers including nine copies of one or more ELP structural units disclosed herein. In some embodiments, the ELP includes 9-mers including nine copies of a pentapeptide disclosed herein. In some embodiments, the ELP includes 9-mers including SEQ ID NOs: 3 and 13 in any combination. In some embodiments, the ELP includes a sequence alternating between SEQ ID NOs: 3 and 13. ELPs of varying numbers of 9-mers can be combined to produce ELPs with, for instance, 18, 27, 36, 45, 54, 63, 72, 81, 90, 99, 108, 117, 126, 135, 144, 153, 162, 171, or 180 copies of the 9-mer.

[0030] In certain embodiments, the ELP includes 9-mers including SEQ ID NO: 3, wherein the guest residue is selected from V, G, and A. In certain embodiments, the ELP includes 9-mers including SEQ ID NO: 3, wherein V, G, and A are in the ratio of 7:2:0 (α). In certain embodiments, the ELP includes 9-mers including SEQ ID NO:3, wherein V, G, and A are in the ratio of 7:0:2 (β v1). In certain embodiments, the ELP includes 9-mers including SEQ ID NO:3, wherein V, G, and A are in the ratio of 6:0:3 (β v2). In certain embodiments, the ELP includes 9-mers including SEQ ID NO:3, wherein V, G, and A are in the ratio of 5:2:2 (γ). In certain embodiments, the ELP includes 9-mers including SEQ ID NO: 13, wherein the guest residue is selected from V, G, and A. In certain embodiments, the ELP includes 9-mers including SEQ ID

NO:13, wherein V, G, and A are in the ratio of 5:0:4 (delta). Exemplary 9-mers are disclosed in **Table 1**. **Table 2** demonstrates the transition temperatures of several exemplary 9-mers.

[0031] **Table 1 Guest residue ratios in exemplary 9-mers.** The ELP polymers have hydrophobicities between the 10-mer ELP 1 series (least hydrophobic) and the 10-mer ELP 4 series (most hydrophobic).

ELP series	Pentamer motif	Guest residue ratio
1 series	VPGXG	5 Val : 3 Gly : 2 Ala
alpha	VPGXG	7 Val : 2 Gly : 0 Ala
beta v1	VPGXG	7 Val : 0 Gly : 2 Ala
beta v2	VPGXG	6 Val : 0 Gly : 3 Ala
gamma	VPGXG	5 Val : 2 Gly : 2 Ala
delta	XPGVG	5 Val : 0 Gly : 4 Ala
	VPGXG	6 Val : 3 Gly : 0 Ala
	VPGXG	6 Val : 2 Gly : 1 Ala
	VPGXG	6 Val : 1 Gly : 2 Ala
	VPGXG	6 Val : 0 Gly : 3 Ala
	VPGXG	7 Val : 1 Gly : 1 Ala
	VPGXG	8 Val : 0 Gly : 1 Ala
	VPGXG	8 Val : 1 Gly : 0 Ala
4 series	VPGXG	10 Val : 0 Gly : 0 Ala

[0032] **Table 2** Comparison of measured transition temperatures of exemplary 9-mers to ELP1 series. The inflection of turbidity measured using a Cary spectrophotometer is the result of the ELP biopolymer phase transitioning.

ELP series (10mg/ml)	Transition temp
1 series (pPB1023)	37°C
alpha (pPE0253)	29°C
beta v1 (pPE0254)	28°C

beta v2 (pPE0311)	31°C
gamma (pPE0255)	29°C
delta (pPE0256)	35°C
4 series (pPE0002)	26°C

[0033] In some embodiments, the ELP includes combinations of the 9-mers listed in **Table 1**. In some embodiments, the ELP includes combinations of the alpha, beta v1, beta v2, and/or delta 9-mers. For example, the gamma ELP is constructed by alternating between an alpha 9-mer and a beta v1 9-mer for 16 copies until a 144mer is constructed. In certain embodiments, the ELP includes combinations of alpha and beta v1 9-mers. In certain embodiments, the ELP includes combinations of alpha and beta v2 9-mers. In certain embodiments, the ELP includes combinations of alpha and delta 9-mers. In certain embodiments, the ELP includes combinations of beta v1 and beta v2 9-mers. In certain embodiments, the ELP includes combinations of beta v1 and delta 9-mers. In certain embodiments, the ELP includes combinations of beta v2 and delta 9-mers. In certain embodiments, the ELP includes combinations of alpha, beta v1, and beta v2 9-mers. In certain embodiments, the ELP includes combinations of alpha, beta v1, and delta 9-mers. In certain embodiments, the ELP includes combinations of alpha, beta v2, and delta 9-mers. For example, in particular arrangements, the ELPbeta v2 may include the following guest residues in structural units iterated in the following sequence: A-V-A-V-V-A-V-A-V. The iterated sequence may be repeated sequentially in the ELP about 10 times, about 12 times, about 15 times, about 16 times, about 20 times, about 25 times, about 30 times, or about 35 times or more. In some aspects, the ELP contains about 10 to about 20 iterated sequences. In other aspects, the ELP contains about 15 to 20 iterated sequences. In some aspects, the ELP contains about 16 iterated sequences.

[0034] In some embodiments, the ELP includes 10-mers including ten copies of one or more ELP structural units disclosed herein. In some embodiments, the ELP includes 10-mers including ten copies of a pentapeptide disclosed herein. In some embodiments, the ELP includes 10-mers including SEQ ID NOs: 3 and 13 in any combination. In some embodiments, the ELP includes a sequence alternating between SEQ ID NOs: 3 and 13. ELPs of varying numbers of 10-mers can be combined to produce ELPs with, for instance, 20, 30, 40, 60, 90, 100, 120, 150, 160, or 200 copies of the 10-mer. Exemplary 10-mers are disclosed in **Table 3**.

[0035] **Table 3 Guest residue ratios in exemplary 10-mers.** The ELP polymers have hydrophobicities between the ELP 1 series (least hydrophobic) and the ELP 4 series (most hydrophobic).

ELP series	Pentamer motif	Guest residue ratio
1 series	VP <u>G</u> XG	5 Val : 3 Gly : 2 Ala
	VP <u>G</u> XG	5 Val : 4 Gly : 1 Ala
	VP <u>G</u> XG	5 Val : 5 Gly : 0 Ala
	VP <u>G</u> XG	5 Val : 2 Gly : 3 Ala
	VP <u>G</u> XG	5 Val : 1 Gly : 4 Ala
	VP <u>G</u> XG	5 Val : 0 Gly : 5 Ala
	VP <u>G</u> XG	6 Val : 4 Gly : 0 Ala
	VP <u>G</u> XG	6 Val : 3 Gly : 1 Ala
	VP <u>G</u> XG	6 Val : 2 Gly : 2 Ala
	VP <u>G</u> XG	6 Val : 1 Gly : 3 Ala
	VP <u>G</u> XG	6 Val : 0 Gly : 4 Ala
	VP <u>G</u> XG	7 Val : 3 Gly : 0 Ala
	VP <u>G</u> XG	7 Val : 2 Gly : 1 Ala
	VP <u>G</u> XG	7 Val : 1 Gly : 2 Ala
	VP <u>G</u> XG	7 Val : 0 Gly : 3 Ala
	VP <u>G</u> XG	8 Val : 2 Gly : 0 Ala
	VP <u>G</u> XG	8 Val : 0 Gly : 2 Ala
	VP <u>G</u> XG	8 Val : 1 Gly : 1 Ala
	VP <u>G</u> XG	9 Val : 1 Gly : 1 Ala
	VP <u>G</u> XG	9 Val : 0 Gly : 1 Ala
4 series	VP <u>G</u> XG	10 Val : 0 Gly : 0 Ala

[0036] In some embodiments, the ELP may form a β -turn structure. Exemplary peptide sequences suitable for creating a β -turn structure are described in International Patent Application PCT/US96/05186, which is hereby incorporated by reference in its entirety. For example, the fourth residue (X) in the sequence VPGXG (SEQ ID NO: 3), can be varied without eliminating the formation of a β -turn.

[0037] The structure of exemplary ELPs may be described using the notation $ELP_k [X_i Y_j - n]$, where k designates a particular ELP repeat unit, the bracketed capital letters are single letter amino acid codes, and their corresponding subscripts designate the relative ratio of each guest residue X in the structural units (where applicable), and n describes the total length of the ELP in number of the structural repeats. For example, ELP1 $[V_5 A_2 G_3 - 10]$ designates an ELP component containing 10 repeating units of the pentapeptide VPGXG (SEQ ID NO: 3), where X is valine, alanine, and glycine at a relative ratio of about 5:2:3; ELP1 $[K_1 V_2 F_1 - 4]$ designates an ELP component containing 4 repeating units of the pentapeptide VPGXG (SEQ ID NO: 3), where X is lysine, valine, and phenylalanine at a relative ratio of about 1:2:1; ELP1 $[K_1 V_7 F_1 - 9]$ designates a polypeptide containing 9 repeating units of the pentapeptide VPGXG (SEQ ID NO: 3), where X is lysine, valine, and phenylalanine at a relative ratio of about 1:7:1; ELP1 $[V - 5]$ designates a polypeptide containing 5 repeating units of the pentapeptide VPGXG (SEQ ID NO: 3), where X is valine; ELP1 $[V - 20]$ designates a polypeptide containing 20 repeating units of the pentapeptide VPGXG (SEQ ID NO: 3), where X is valine; ELP2 $[5]$ designates a polypeptide containing 5 repeating units of the pentapeptide AVGVP (SEQ ID NO: 4); ELP3 $[V - 5]$ designates a polypeptide containing 5 repeating units of the pentapeptide IPGXG (SEQ ID NO: 5), where X is valine; ELP4 $[V - 5]$ designates a polypeptide containing 5 repeating units of the pentapeptide LPGXG (SEQ ID NO: 7), where X is valine.

[0038] With respect to ELP, the T_t is a function of the hydrophobicity of the guest residue. Thus, by varying the identity of the guest residue(s) and their mole fraction(s), ELPs can be synthesized that exhibit an inverse phase transition over a broad range of temperatures. Thus, the T_t at a given ELP length may be decreased by incorporating a larger fraction of hydrophobic guest residues in the ELP sequence. Examples of suitable hydrophobic guest residues include valine, leucine, isoleucine, phenylalanine, tryptophan and methionine. Tyrosine, which is moderately hydrophobic, may also be used. Conversely, the T_t may be increased by incorporating residues, such as those selected from: glutamic acid, cysteine, lysine, aspartate, alanine, asparagine, serine, threonine, glycine, arginine, and glutamine.

[0039] For polypeptides having a molecular weight $> 100,000$, the hydrophobicity scale disclosed in PCT/US96/05186 (which is hereby incorporated by reference in its entirety) provides one means for predicting the approximate T_t of a specific ELP sequence. For polypeptides having a molecular weight $< 100,000$, the T_t may be predicted or determined by the following quadratic

function: $T_t = M_0 + M_1X + M_2X^2$ where X is the MW of the fusion protein, and $M_0 = 116.21$; $M_1 = -1.7499$; $M_2 = 0.010349$.

[0040] The ELP in some embodiments is selected or designed to provide a T_t ranging from about 10 to about 37°C, such as from about 20 to about 37°C, or from about 25°C to about 37°C. In some embodiments, the transition temperature at physiological conditions (*e.g.*, 0.9% saline) is from about 34°C to 36°C, to take into account a slightly lower peripheral body temperature.

[0041] In certain embodiments, the ELP includes $[VPGXG]_m$, where m is any number from 1 to 200. In certain embodiments, the ELP includes $[VPGXG]_m$, where m is any number from 1 to 200, and each X is selected from V, G, and A. In certain embodiments, the ELP includes $[VPGXG]_m$, where m is any number from 1 to 200, each X is selected from V, G, and A, and wherein the ratio of V:G:A may be about 5:3:2. In certain embodiments, the ELP includes $[VPGXG]_{60}$, where each X is selected from V, G, and A, and wherein the ratio of V:G:A may be about 5:3:2. In certain embodiments, the ELP includes $[VPGXG]_{90}$, where each X is selected from V, G, and A, and wherein the ratio of V:G:A may be about 5:3:2. For example, the amino acid sequence capable of forming the hydrogen-bonded matrix at body temperature includes $[VPGXG]_{120}$, where each X is selected from V, G, and A, and wherein the ratio of V:G:A may be about 5:3:2. As shown herein, 120 structural units of this ELP can provide a transition temperature at about 37°C with about 5 to 15 mg/ml (*e.g.*, about 10 mg/ml) of protein. At concentrations of about 50 to about 100 mg/mL the phase transition temperature is about 35.5 degrees centigrade (just below body temperature), which allows for peripheral body temperature to be just less than 37°C. In some embodiments, the ELP may include $[VPGXG]_{144}$, where each X is selected from V, G, and A, and wherein the ratio of V:G:A may be about 5:3:2. In some embodiments, the ELP includes $[VPGXG]_{180}$, where each X is selected from V, G, and A, and wherein the ratio of V:G:A may be about 5:3:2.

[0042] In certain embodiments, the ELP includes $[VPGXG]_m$, where m is any number from 1 to 200, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:2:0. In certain embodiments, the ELP includes $[VPGXG]_{60}$, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:2:0. In certain embodiments, the ELP includes $[VPGXG]_{90}$, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:2:0. In certain embodiments, the ELP includes $[VPGXG]_{108}$, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:2:0. In certain embodiments, the ELP includes

[VPGXG]₁₂₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:2:0. In certain embodiments, the ELP includes [VPGXG]₁₄₄, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:2:0. In certain embodiments, the ELP includes [VPGXG]₁₈₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:2:0.

[0043] In certain embodiments, the ELP includes [VPGXG]_m, where m is any number from 1 to 200, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:0:2. In certain embodiments, the ELP includes [VPGXG]₆₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:0:2. In certain embodiments, the ELP includes [VPGXG]₉₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:0:2. In certain embodiments, the ELP includes [VPGXG]₁₀₈, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:0:2. In certain embodiments, the ELP includes [VPGXG]₁₂₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:0:2. In certain embodiments, the ELP includes [VPGXG]₁₄₄, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:0:2. In certain embodiments, the ELP includes [VPGXG]₁₈₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 7:0:2.

[0044] In certain embodiments, the ELP includes [VPGXG]_m, where m is any number from 1 to 200, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 6:0:3. In certain embodiments, the ELP includes [VPGXG]₆₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 6:0:3. In certain embodiments, the ELP includes [VPGXG]₉₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 6:0:3. In certain embodiments, the ELP includes [VPGXG]₁₀₈, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 6:0:3. In certain embodiments, the ELP includes [VPGXG]₁₂₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 6:0:3. In certain embodiments, the ELP includes [VPGXG]₁₄₄, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 6:0:3. In certain embodiments, the ELP includes [VPGXG]₁₈₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 6:0:3.

[0045] In certain embodiments, the ELP includes [VPGXG]_m, where m is any number from 1 to 200, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about

5:2:2. In certain embodiments, the ELP includes [VPGXG]₆₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:2:2. In certain embodiments, the ELP includes [VPGXG]₉₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:2:2. In certain embodiments, the ELP includes [VPGXG]₁₀₈, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:2:2. In certain embodiments, the ELP includes [VPGXG]₁₂₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:2:2. In certain embodiments, the ELP includes [VPGXG]₁₄₄, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:2:2. In certain embodiments, the ELP includes [VPGXG]₁₈₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:2:2.

[0046] In certain embodiments, the ELP includes [VPGXG]_m, where m is any number from 1 to 200, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 10:0:0. In certain embodiments, the ELP includes [VPGXG]₆₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 10:0:0. In certain embodiments, the ELP includes [VPGXG]₉₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 10:0:0. In certain embodiments, the ELP includes [VPGXG]₁₀₈, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 10:0:0. In certain embodiments, the ELP includes [VPGXG]₁₂₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 10:0:0. In certain embodiments, the ELP includes [VPGXG]₁₄₄, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 10:0:0. In certain embodiments, the ELP includes [VPGXG]₁₈₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 10:0:0.

[0047] In certain embodiments, the ELP includes [VPGXG]_m, where m is any number from 1 to 100, where each X is selected from V and L, and wherein the ratio of V:L is about 3:7 or about 4:6 or about 1:1 or about 6:4 or about 3:7. In certain embodiments, the ELP includes [VPGXG]₆₀, where each X is selected from V and L, and wherein the ratio of V:L is about 3:7 or about 4:6 or about 1:1 or about 6:4 or about 3:7. In certain embodiments, the ELP includes [VPGXG]₅₀, where each X is selected from V and L, and wherein the ratio of V:L is about 3:7 or about 4:6 or about 1:1 or about 6:4 or about 3:7. In certain embodiments, the ELP includes [VPGXG]₄₀, where each X is selected from V and L, and wherein the ratio of V:L is about 3:7 or about 4:6 or about 1:1 or about 6:4 or about 3:7. In certain embodiments, the ELP includes [VPGXG]₃₀, where each X is

selected from V and L, and wherein the ratio of V:L is about 3:7 or about 4:6 or about 1:1 or about 6:4 or about 3:7. In certain embodiments, the ELP includes [VPGXG]₂₀, where each X is selected from V and L, and wherein the ratio of V:L is about 3:7 or about 4:6 or about 1:1 or about 6:4 or about 3:7.

[0048] In certain embodiments, the ELP includes [XPGVG]_m, where m is any number from 1 to 200. In certain embodiments, the ELP includes [XPGVG]_m, where m is any number from 1 to 200, and each X is selected from V, G, and A. In certain embodiments, the ELP includes [XPGVG]_m, where m is any number from 1 to 200, each X is selected from V, G, and A and wherein the ratio of V:G:A is about 5:0:4. In certain embodiments, the ELP includes [XPGVG]₆₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:0:4. In certain embodiments, the ELP includes [XPGVG]₉₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:0:4. In certain embodiments, the ELP includes [XPGVG]₁₂₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:0:4. In certain embodiments, the ELP includes [XPGVG]₁₄₄, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:0:4. In certain embodiments, the ELP includes [XPGVG]₁₈₀, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is about 5:0:4.

[0049] In certain embodiments, the ELP includes [VPGVG]_m where m is any number from 1 to 200. In some embodiments, the ELP includes [VPGVG]₆₀, [VPGVG]₉₀, or [VPGVG]₁₂₀. As shown herein, 120 structural units of this ELP can provide a transition temperature at about 37°C with about 0.005 to about 0.05 mg/ml (*e.g.*, about 0.01 mg/ml) of protein. Alternatively, the ELP includes [VPGXG]₁₄₄ or [XPGVG]₁₄₄. As shown herein (**Table 2**), 144 structural units of either of these ELPs can provide a transition temperature at 28°C to 35°C inclusive.

[0050] In various embodiments, the intended subject is human, and the body temperature is about 37°C, and thus the therapeutic agent is designed to provide a sustained release at or near this temperature (*e.g.* between about 28°C to about 37°C). A slow release into the circulation with reversal of hydrogen bonding and/or hydrophobic interactions is driven by a drop in concentration as the product diffuses at the injection site, even though body temperature remains constant. In other embodiments, the subject is a non-human mammal, and the therapeutic agent is designed to exhibit a sustained release at the body temperature of the mammal, which may be from about 30 to about 40°C in some embodiments, such as for certain domesticated pets (*e.g.*, dog or cat) or

livestock (e.g., cow, horse, sheep, or pig). Generally, the Tt is higher than the storage conditions of the formulation (which may be from 2 to about 25°C, or from 15 to 22°C), such that the therapeutic agent remains in solution for injection.

[0051] In some embodiments, the ELP can provide a transition temperature at a range of 27°C to 36°C inclusive. In some embodiments, the ELP can provide a transition temperature at a range of 28 °C to 35°C inclusive. In some embodiments, the ELP can provide a transition temperature at a range of 29 °C to 34°C inclusive. In some embodiments, the ELP can provide a transition temperature at a range of 27°C to 33°C inclusive. In some embodiments, the ELP can provide a transition temperature at a range of 30°C to 33°C inclusive. In some embodiments, the ELP can provide a transition temperature at a range of 31°C to 31°C inclusive. In some embodiments, the ELP can provide a transition temperature of 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, 35°C, or 36°C. In some embodiments, the ELP can provide a transition temperature at a range of 28 °C to 35 °C inclusive at a protein concentration of 10 mg/mL in 110 mM NaCl.

[0052] Elastin-like-peptide (ELP) protein polymers and recombinant fusion proteins can be prepared as described in U.S. Patent Publication No. 2010/0022455, which is hereby incorporated by reference. In some embodiments, the ELP protein polymers are constructed through recursive ligation to rapidly clone DNA encoding highly repetitive polypeptides of any sequence and specified length over a large range of molecular weights. In a single cycle, two halves of a parent plasmid, each containing a copy of an oligomer, are ligated together, thereby dimerizing the oligomer and reconstituting a functional plasmid. This process is carried out recursively to assemble an oligomeric gene with the desired number of repeats. For example, one ELP structural subunit (e.g. a pentapeptide or a 9-mer of pentapeptides) is inserted into a vector. The vector is digested, and another ELP structural unit (e.g. a pentapeptide or a 9-mer of pentapeptides) is inserted. Each subsequent round of digestion and ligation doubles the number of ELP structural units contained in the resulting vector until the ELP polymer is the desired length. By varying the number of pentapeptides in the initial structural unit, ELPs of varying length can easily be constructed. Alternative means of construction (i.e. other than recursive ligation) can be used to produce alternative lengths of ELP.

[0053] In some embodiments, the vector contains one or more additional amino acids or ELP structural unit repeats. For example, pPE0248 adds an additional pentamer repeat to the N terminus of the 144mer with valine in the guest position and an additional pentamer to the C

terminus with a tryptophan in the guest residue position. The tryptophan may be used as a means to increase the extinction coefficient of the molecule, allowing for better measurement of absorbance, for instance at 280nm, which can be useful for determination of protein concentration, or for monitoring protein content during purification. The pentamers added to either end can also be designed so as the encoding DNA contains restriction enzyme recognition sites for cloning of fusion partners on to either end of the ELP coding sequence.

[0054] In some embodiments, the therapeutic agent includes an active agent and one or more ELPs. In some embodiments, the therapeutic agent includes an active agent with one or more ELPs at either the N- or C-terminus. In some embodiments, the therapeutic agent includes an active agent with one or more ELPs at both the N- or C-termini. In some embodiments, the ELPs are approximately the same size. In some embodiments, the ELPs differ in size. In some embodiments, an ELP at one terminus is larger than an ELP at the other terminus. In some embodiments, an ELP at the N-terminus is larger than an ELP at the C-terminus. In some embodiments, an ELP at the C-terminus is larger than an ELP at the N-terminus.

ACTIVE AGENTS

Protein Active Agents

[0055] In various embodiments, the active agent is a protein or peptide, which by itself may have a short circulatory half-life, such as from about 30 seconds to about 1 hour. The therapeutic agent may be a recombinant fusion protein between the protein active agent and the amino acid sequence capable of forming the hydrogen-bonded matrix at the body temperature of the subject (e.g. an ELP). Any appropriate protein active agent may be used in the therapeutic agents of the present disclosure. In various embodiments, the protein active agent is Activin receptor 2A extracellular domain, Adrenocorticotrophic hormone (ACTH), alpha-2 macroglobulin, alpha-MSH/Afamelanotide, Amylin (pramlintide), Angiotensin (1-7), Annexin A1, Apelin, Arginase, Asparaginase, Bradykinin B2 receptor antagonist, Compstatin, Coversin, CTLA-4, C-type natriuretic peptide, cenderitide (CD-NP), Elafin, Exendin-4, Fibroblast growth factor (FGF)-18/sprifermin, FGF-19, FGF-21, Galanin, Granulocyte colony stimulating factor (G-CSF), Ghrelin, GLP-1/GIP dual agonist, GLP-1/glucagon dual agonist, GLP-1/GLP-2 dual agonist, Glucagon, Glucagon-like peptide (GLP)-1 receptor antagonist, GLP-2, Granulocyte macrophage colony stimulating factor (GM-CSF), Hecpudin, Human growth hormone (hGH),

hGH antagonist, Icatibant, Insulin-like growth factor (IGF)-1, Interleukin 1 receptor antagonist (IL-1Ra), Infestin-4, Kisspeptin, L4F peptide, Lacritin, Parathyroid hormone (PTH), Parathyroid hormone-related protein (PTHrP), PYY, Relamorelin, Relaxin, Somatostatin, Thioredoxin, Thymosin β 4, TNF-related apoptosis-inducing ligand (TRAIL), Urate oxidase, Urodilatin, Uroguanylin, Parathyroid hormone fragments (e.g. residues 1-34), full length parathyroid hormone, Adrenocorticotrophic hormone, Coversin, Kisspeptin, kisspeptin fragments (e.g. amino acid residues 1-10 or amino acid residues 1-54), Annexin A1-derived peptides (e.g. amino acid residues 2-26), analogs, derivatives, mimetics, fragments, combinations, or functional variants thereof. In some embodiments, the present disclosure provides therapeutic agents including a protein active agent and an amino acid sequence providing sustained release. In some embodiments, the amino acid sequence providing sustained release is an Elastin-like peptide (ELP).

[0056] The half-life of protein active agents can be extended by a variety of means, including increasing the size and thus the hydrodynamic volume of the protein active agent, adding modified or unnatural amino acids, conjugation of moieties (e.g. pegylation), the addition of synthetic sequences (e.g. XTEN® sequences, PASylation®), carboxy-terminal extension from hCG (CTP), addition of albumin-binding sequences (e.g. AlbuDAb®), conjugation of albumin-binding compounds, e.g. fatty acids, post-translational modifications such as N-glycosylation and fusion to other peptides, or fusion with a mammalian heterologous protein, such as albumin, transferrin, or antibody Fc sequences. Such sequences are described in US Patent No. 7,238,667 (particularly with respect to albumin conjugates), US Patent No. 7,176,278 (particularly with respect to transferrin conjugates), and US Patent No. 5,766,883.

[0057] In some embodiments, the disclosure provides mimetics, analogs, derivatives, variants, or mutants of one or more active protein agents disclosed herein. In some embodiments, the mimetic, analog, derivative, variant, or mutant contains one or more amino acid substitutions compared to the amino acid sequence of the native therapeutic peptide agent. In some embodiments, one to 20 amino acids are substituted. In some embodiments, the mimetic, analog, derivative, variant, or mutant contains about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 amino acid substitutions compared to the amino acid sequence of the native therapeutic peptide agent. In some embodiments, the mimetic, analog, derivative, variant, or mutant contains one or more amino acid deletions compared to the amino acid sequence

of the native therapeutic peptide agent. In some embodiments, one to 20 amino acids are deleted compared to the amino acid sequence of the native protein agent. In some embodiments, the mimetic, analog, derivative, variant, or mutant has about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 amino acid deletions compared to the amino acid sequence of the native protein agent. In some embodiments, one to ten amino acids are deleted at either terminus compared to the amino acid sequence of the native protein agent. In some embodiments, one to ten amino acids are deleted from both termini compared to the amino acid sequence of the native protein agent. In some embodiments, the amino acid sequence of the mimetic, analog, derivative, variant, or mutant is at least about 70% identical to the amino acid sequence of the native protein agent. In some embodiments, the amino acid sequence of the mimetic, analog, derivative, variant, or mutant is about 70%, about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the amino acid sequence of the native protein agent. Percentage identity can be calculated using the alignment program EMBOSS Needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1.

[0058] In some embodiments, the disclosure provides for co-formulation of any two or more active agents disclosed herein. In some embodiments, the co-formulation includes two or more peptide active agents and small molecule active agents. In some embodiments, the co-formulation includes two or more small molecule active agents. In some embodiments, the co-formulation includes two or more peptide active agents. In some embodiments, one or more of the active agents in the co-formulation is not conjugated to an ELP. In some embodiments, all of the active agents in the co-formulation are conjugated to an ELP.

Apelin

[0059] In certain embodiments, the protein active agent is apelin, analogs, mimetics, derivatives, fragments, or functional variants thereof. Human apelin is an endogenous ligand for the Apelin Receptor (APJ Class A G-protein coupled receptor). Apelin is expressed in several cell types including human cardiomyocytes, endothelial cells, and vascular smooth muscle cells. The *apelin* gene has been identified in various species, including human, dog, bovine, rat, mouse, rhesus monkey, and zebra fish and codes for an apelin preproprotein of 77 amino acids. Spanning 1726 base pairs of genomic DNA with 3 exons, the apelin locus is highly conserved between

species. In apelin preproprotein processing, a signal peptide corresponding to residues 1-22 of the apelin preproprotein is cleaved off, resulting in a 55 amino acid apelin proprotein, which is processed into several active molecular forms including apelin-12, apelin-13, apelin-16, apelin-17 and apelin-36. (Kawamata *et al.*, 2001, *Biochim Biophys Acta* 1538:162-71). Such active molecular forms are collectively referred to as apelin and are named according to their length and/or modification state. The full length mature peptide, Apelin-36, is a 36 amino acid long peptide derived from the 55 amino acid long apelin proprotein (Tatemoto *et al.*, 1998, *Biochem. Biophys. Res. Comm.* 251:471-476, 1998) and corresponds to residues 42-77 of the preproprotein. Apelin-17 and apelin-13 are derived from the carboxy (C)-terminal end of apelin. Apelin-17 corresponds to residues 61-77 of the apelin proprotein. Apelin-13 corresponds to residues 65-77 of the apelin proprotein. The apelin proteins may include amino acid derivatives or modifications. For example, the most active isoform is the pyroglutamated form of apelin-13 (pyr-apelin 13).

[0060] Apelin is an endogenous vasodilator and inotrope which may lower blood pressure by antagonizing the renin-angiotensin system, possibly via formation of receptor heterotrimers.

[0061] In certain embodiments of the disclosure, the active agent includes an amino acid sequence providing sustained release fused or conjugated to apelin, mimetics, analogs, derivatives, fragments, or functional variants thereof. In some embodiments, the amino acid sequence providing sustained release is ELP. In certain embodiments, the apelin is a mammalian apelin. In some embodiments, the apelin is human apelin (*e.g.*, SEQ ID NO: 39), which is the full length mature human apelin peptide, apelin-36 (SEQ ID NO: 39). In other embodiments, the apelin is a truncation of apelin-36 including, but not limited to, apelin-16, apelin-17 (SEQ ID NO: 41), apelin-13 (SEQ ID NO: 43), and apelin-12 (SEQ ID NO: 44). In some embodiments, the apelin includes one or more modified amino acids. In some embodiments, the apelin includes one or more amino acid derivatives. In some embodiments, the apelin is pyr-apelin 13 (SEQ ID NO: 42).

[0101] In some embodiments, the apelin is a functional analog of mammalian apelin, including functional fragments truncated at the N-terminus and/or the C-terminus of apelin by from about 1 to about 30 amino acids, including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, up to about 20 amino acids, up to about 25 amino acids, or up to about 30 amino acids. In other embodiments, functional variants contain from about 1 to about 30 amino acid insertions, deletions, and/or substitutions with respect to a native or truncated sequence (*e.g.*, SEQ ID NOs: 39, 41, 42, 43, or 44). For

example, functional variants may have up to about 3 amino acid, up to about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, up to about 20 amino acid, up to about 25 amino acid, or up to about 30 amino acid insertions, deletions, and/or substitutions with respect to a native or truncated sequence (*e.g.*, SEQ ID NOs: 39, 41, 42, 43, or 44). Protein activity may be confirmed or assayed using any available assay. In other embodiments, the apelin has at least about 75% identity, about 80% identity, about 90% identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99% identity with a native or truncated sequence (*e.g.*, SEQ ID NOs: 39, 41, 42, 43, or 44). Percentage identity can be calculated using the alignment program EMBOSS needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1. In some embodiments, the apelin may contain additional chemical modifications known in the art. In some embodiments, the functional analog of apelin is Elabella/Toddler or an analog or derivative thereof (Yang *et al.*, 2015, *Trends in Pharmacol. Sci.* 36:560-567)

[0102] In certain embodiments, the therapeutic agent includes an ELP fused to the N-terminus or the C-terminus of apelin, mimetics, analogs, derivatives, fragments, or functional variants thereof. In some aspects, the therapeutic agent includes an ELP fused to the N-terminus of human apelin-36 (SEQ ID NO: 40). In some embodiments, the apelin is in a fusion protein with more than one ELP sequence. In some embodiments, the apelin has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-terminus of the apelin includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the apelin includes fewer than about 90 repeating structural units. In other aspects, the ELP at the C-terminus and/or the N-terminus of the apelin includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at the N- and C-termini are approximately the same size. In other embodiments, the two or more ELPs at the N- and C-termini differ in size. In some embodiments, the ELP at the N-terminus of the apelin is larger than the ELP at the C-terminus of the apelin. In other embodiments, the ELP at the C-terminus of the apelin is larger than the ELP at the N-terminus of the apelin.

[0103] In other aspects, the present disclosure provides methods for treating or preventing diseases including, but not limited to, heart failure, pulmonary arterial hypertension (PAH), ischemic heart disease, diabetes, cancer, obesity, cardiovascular defects, or pathological conditions

involving angiogenesis (*e.g.*, cancer) by administering an ELP and an apelin. In some embodiments, the therapeutic agents disclosed herein may be administered to provide effects including, but not limited to, vasodilatory effects (*e.g.*, mediated by endothelial Nitric Oxide Synthase (eNOS)), positive inotropic effects (*e.g.*, due to increased intracellular calcium and increased myofilament calcium sensitivity), protection against ischemia-reperfusion injury (*e.g.* via Nitric Oxide (NO)-dependent and NO-independent pathways), and APJ receptor-mediated cardiac hypertrophy (*e.g.*, β -arrestin mediated, rather than G protein mediated). The methods include administering a therapeutic agent including an ELP and an apelin (as described above) to a patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (*e.g.*, dog, cat, cow, or horse). Preferably, the patient is human.

[0104] In some embodiments, treatment with an apelin therapeutic agent according to the present disclosure may also be combined with one or more pharmacologically active substances, *e.g.* selected from agents for the treatment and/or prevention of complications and disorders resulting from or associated with various diseases, including without limitation heart failure, PAH, ischemic heart disease, diabetes, cancer, obesity, cardiovascular defects, or pathological conditions involving angiogenesis (*e.g.*, cancer). In additional embodiments, treatment with an apelin therapeutic agent according to the present disclosure may be combined with one or more pharmacologically active substances, *e.g.* selected from agents providing effects including, but not limited to, vasodilatory effects (*e.g.*, mediated by endothelial Nitric Oxide Synthase (eNOS)), positive inotropic effects (*e.g.* due to increased intracellular calcium and increased myofilament calcium sensitivity), protection against ischemia-reperfusion injury (*e.g.*, via Nitric Oxide (NO)-dependent and NO-independent pathways), and APJ receptor-mediated cardiac hypertrophy (*e.g.*, β -arrestin mediated, rather than G protein mediated).

Arginase

[0105] In some embodiments, the protein active agent is Arginase (EC 3.5.3.1; L-arginine amidinohydrolase), isozymes, fragments, or functional variants thereof. Arginase is a 35 kDa key enzyme of the urea cycle that catalyzes the conversion of L-arginine to ornithine and urea, which is the final cytosolic reaction of urea formation in the mammalian liver. Arginase serves three important functions: production of urea, production of ornithine, and regulation of substrate arginine levels for nitric oxide synthase (Jenkinson *et al.*, 1996, *Comp. Biochem. Physiol.* 114B:107-132; Kanyo *et al.*, 1996, *Nature* 383:554-557; Christianson, 1997, *Prog. Biophys.*

Molec. Biol. 67:217-252). Urea production provides a mechanism to excrete nitrogen in the form of a highly soluble, non-toxic compound, thus avoiding the potentially dangerous consequences of high ammonia levels. L-ornithine is a precursor for the biosynthesis of polyamines, spermine, and spermidine, which have important roles in cell proliferation and differentiation. Finally, arginase modulates production of nitric oxide by regulating the levels of arginine present within tissues.

[0106] In general, arginase is expressed in liver, kidney and testis of urea-producing animals (e.g. mammals, elasmobranchs, amphibians, and turtles). In most mammals, including humans, the family of arginase proteins includes the isozymes arginase I, which is mainly expressed in the liver cells, and arginase II, which is mainly expressed in the kidney and erythrocytes.

[0107] In some embodiments, a deficiency in human liver arginase may result in argininemia, an inherited autosomal recessive disorder accompanied by hyperammonemia, a condition where excess arginine leads to seizures, impaired cognitive abilities, and neuronal impairment. Clinically significant hyperargininemia results from mutations in the *arginase I* gene (Cederbaum S. D. *et al.*, 1979, *Pediat. Res.* 13:827-833; Cederbaum S. *et al.*, 1977, *J. Pediatr.* 90:569-573; Michel V. V. *et al.*, 1978, *Clin. Genet.* 13:61-67). Due to an arginase deficiency, arginine cannot be degraded into urea and participate in the ornithine metabolism cycle, resulting in blood arginine levels about 7 to about 10 times higher than normal, increased arginine levels in the cerebrospinal fluid, increased urine production, and increased urea excretion of creatinine. Arginase I deficient patients may present with spasticity, growth retardation, progressive mental impairment and episodic hyperammonemia (Cederbaum S.D. *et al.*, 1979, *Pediat Res* 13:827-833; Cederbaum S.D., *et al.*, 1977, *J. Pediatr* 90:569-573; Thomas K.R. and Capecchi M.R., 1987, *Cell* 51:503-512).

[0108] In certain embodiments of the disclosure, the arginase therapeutic agent includes an amino acid sequence providing sustained release fused or conjugated to an arginase, mimetics, analogs, derivatives, fragments, or functional variants thereof. In some embodiments, the amino acid agent providing sustained release is an ELP.

[0109] In certain embodiments, the arginase is a mammalian arginase. In some embodiments, the arginase is arginase I. In other embodiments, the arginase is arginase II. In some embodiments, the arginase is human arginase (SEQ ID NO: 45).

[0110] In some embodiments, the arginase is a functional analog of mammalian arginase, including functional fragments truncated at the N-terminus and/or the C-terminus of arginase by from about 1 to about 50 amino acids, including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, up to about 20 amino acids, up to about 25 amino acids, up to about 30 amino acids, up to about 35 amino acids, up to about 40 amino acids, up to about 45 amino acids, or up to about 50 amino acids. In other embodiments, functional variants contain from about 1 to about 50 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 45). For example, functional variants may have up to about 3 amino acid, about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, up to about 20 amino acid, up to about 25 amino acid, up to about 30 amino acid, up to about 35 amino acid, up to about 40 amino acid, up to about 45 amino acid, or up to about 50 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 45). Protein activity may be confirmed or assayed using any available assay. In other embodiments, the arginase has at least about 75% identity, about 80% identity, about 90% identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99% identity with a native sequence (*e.g.*, SEQ ID NO: 45). Percentage identity can be calculated using the alignment program EMBOSS needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1. In some embodiments, the arginase may contain additional chemical modifications known in the art. In some embodiments, the arginase may utilize manganese as a cofactor, instead of cobalt.

[0111] In certain embodiments, the therapeutic agent includes an ELP fused to the N-terminus or the C-terminus of an arginase, mimetics, analogs, derivatives, fragments, or functional variants thereof. In some embodiments, the therapeutic agent includes an ELP fused to the C-terminus of human arginase (SEQ ID NO: 46). In some embodiments, the arginase is in a fusion protein with more than one ELP sequence. In some embodiments, the arginase has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-terminus of the arginase includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the arginase includes fewer than about 90 repeating structural units. In other aspects, the ELP at the C-terminus and/or the N-

terminus of the arginase includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at the N- and C- termini are approximately the same size. In other embodiments, the two or more ELPs at the N- and C- termini differ in size. In some embodiments, the ELP at the N-terminus of the arginase is larger than the ELP at the C-terminus of the arginase. In other embodiments, the ELP at the C-terminus of the arginase is larger than the ELP at the N-terminus of the arginase.

[0112] In other aspects, the present disclosure provides methods for treating or preventing diseases associated with, or caused by, a defect in an arginase gene or arginase gene expression, such as, for example, urea cycle diseases; hypertension; hypotension; hyperammonemia; episodic hyperammonemia; defects in biosynthesis of proline, glutamate, nitric oxide and ornithine; hyperargininemia and its related spasticity; growth retardation; progressive mental impairment; prostate disease, prostate cancer, prostatitis or benign prostatic hyperplasia or hypertrophy, prostate damage; kidney disease, and kidney damage; cancers including, without limitation, non-Hodgkin's lymphoma, hepatocarcinomas, melanomas or renal cell carcinomas; autoimmune disease; fibrotic diseases; erectile dysfunction; pulmonary hypertension; atherosclerosis; renal disease; asthma; T-cell dysfunction; ischemia reperfusion injury; neurodegenerative diseases; wound healing; arginine-dependent hyperplasia; tumors; hepatocarcinomas; melanomas; renal cell carcinomas; HCC; or melanoma. The methods include administering a therapeutic agent including an ELP and an arginase (as described above) to a patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (e.g., dog, cat, cow, or horse). Preferably, the patient is human.

[0113] In some embodiments, treatment with an arginase therapeutic agent according to the present disclosure may also be combined with one or more pharmacologically active substances, e.g. selected from agents for the treatment and/or prevention of complications and disorders resulting from or associated with various diseases, including without limitation hypertension; hypotension; hyperammonemia; episodic hyperammonemia; defects in biosynthesis of proline, glutamate, nitric oxide and ornithine; hyperargininemia and its related spasticity; growth retardation; progressive mental impairment; prostate disease, prostate cancer; prostatitis or benign prostatic hyperplasia or hypertrophy; prostate damage; kidney disease; kidney damage; cancers including, without limitation, non-Hodgkin's lymphoma, hepatocarcinomas, melanomas or renal cell carcinomas; autoimmune disease; fibrotic diseases; erectile dysfunction; pulmonary

hypertension; atherosclerosis; renal disease; asthma; T-cell dysfunction; ischemia reperfusion injury; neurodegenerative diseases; wound healing; arginine-dependent hyperplasia; tumors; hepatocarcinomas; melanomas; renal cell carcinomas; HCC; or melanoma.

C-type Natriuretic Peptide (CNP)

[0114] In some embodiments, the protein active agent is C-type natriuretic peptide (CNP), mimetics, analogs, derivatives, fragments, or functional variants thereof. Natriuretic peptides play a role in cardiovascular homeostasis, diuresis, natriuresis, and vasodilation. The natriuretic peptide family consists of three structurally related peptides: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). These small, single chain peptides (ANP, BNP, and CNP) have a 17-amino acid loop structure (Levin et al., *N. Engl. J. Med.*, 339: 863-870 (1998)) and play important roles in multiple biological processes. ANP and BNP are produced primarily within the muscle cells of the heart, and have important roles in cardiovascular homeostasis (*Science*, 252: 120-123 (1991)). CNP is expressed more widely, including in the central nervous system, reproductive tract, bone and endothelium of blood vessels (*Hypertension*, 49: 419-426 (2007)), and acts, for example, as a regulator of bone growth, a vasodilator and inotrope, and have cardiovascular effects.

[0115] In humans, CNP is initially produced from the natriuretic peptide precursor C (NPPC) gene as a single chain 126-amino acid pre-pro polypeptide (Sudoh, T. *et al.*, *Biochem. Biophys. Res. Commun.*, 1990, 168: 863-870). Removal of the signal peptide yields pro-CNP, and further cleavage by the endoprotease furin generates an active 53-amino acid peptide (CNP-53), which is secreted and cleaved again to produce the mature 22-amino acid peptide (CNP-22) (Wu, *J. Biol. Chem.*, 2003, 278: 25847-852). CNP-53 and CNP-22 differ in their distribution, with CNP-53 predominating in tissues, while CNP-22 is mainly found in plasma and cerebrospinal fluid (J. Alfonzo, *Recept. Signal. Transduct. Res.*, 2006, 26: 269-297). The predominant bioactive form of CNP is CNP-22.

[0116] Natriuretic peptides act mainly through two receptors: natriuretic peptide receptor-A (NPR-A) and natriuretic peptide receptor B (NPR-B). These receptors have cytoplasmic guanylyl cyclase domains, which are activated upon ANP, BNP, or CNP binding and lead to accumulation of intracellular cGMP. Both CNP-53 and CNP-22 bind similarly to NPR-B and they can both induce cGMP production in a dose-dependent and similar fashion (Yeung, V.T., *Peptides*, 1996, 17: 101-106). A third receptor, NPR-C, binds each of the natriuretic peptides with high

affinity and functions primarily to capture the peptides from the extracellular compartment and deposit the peptides into lysosomes, where they are degraded (Maak, T. *et al.*, *Science*, 1987, 238: 675-678).

[0117] CNP binds to and activates NPR-B resulting in increased intracellular cyclic guanosine monophosphate (cGMP) levels. Downstream signaling mediated by cGMP generation influences a diverse array of biological processes including endochondral ossification (the process that governs longitudinal long-bone growth).

[0118] In certain embodiments of the disclosure, the therapeutic agent includes an amino acid sequence providing sustained release fused or conjugated to CNP, fragments, or functional variants thereof. In some embodiments, the amino acid sequence providing sustained release is ELP.

[0119] In certain embodiments, the CNP is a mammalian CNP. In some embodiments, the CNP is human CNP-53 (SEQ ID NO: 51). In other embodiments, the CNP is CNP-22 (SEQ ID NO: 47). In other embodiments, the CNP is CNP-37 (SEQ ID NO: 49). In yet other embodiments, the CNP is CNP-39 (SEQ ID NO: 52).

[0120] In some embodiments, the CNP is a fragment or functional variant of mammalian CNP, including functional fragments truncated at the N-terminus and/or the C-terminus of CNP by from about 1 to about 30 amino acids, including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, up to about 20 amino acids, up to about 25 amino acids, or up to about 30 amino acids. In other embodiments, functional variants contain from about 1 to about 30 amino acid insertions, deletions, and/or substitutions with respect to a native or truncated sequence (*e.g.*, SEQ ID NOs: 47, 49, 51, or 52). For example, functional variants may have up to about 3 amino acid, up to about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, up to about 20 amino acid, up to about 25 amino acid, or up to about 30 amino insertions, deletions, and/or substitutions with respect to a native or truncated sequence (*e.g.*, SEQ ID NOs: 47, 49, 51, or 52). Protein activity may be confirmed or assayed using any available assay. In other embodiments, the CNP has at least about 75% identity, about 80% identity, about 90% identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99% identity with a native or truncated sequence (*e.g.*, SEQ ID NOs: 47, 49, 51, or 52). Percentage identity can be calculated using the alignment program EMBOSS needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The

following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1. In some embodiments, the CNP may contain additional chemical modifications known in the art.

[0121] In some aspects, the therapeutic agent includes an ELP fused to the N-terminus or the C-terminus of CNP, fragments, or functional variants thereof. In some embodiments, the therapeutic agent includes an ELP fused to the N-terminus of CNP-22 (SEQ ID NO: 48). In another aspect, the therapeutic agent includes an ELP fused to the N-terminus of CNP-37 (SEQ ID NO: 50). In some embodiments, the therapeutic agent includes an ELP fused to the C-terminus of CNP (e.g. SEQ ID NO: 57 or 98). In some embodiments, the therapeutic agent includes an ELP fused to a pro-CNP (SEQ ID NO: 56). In some embodiments, the CNP is in a fusion protein with more than one ELP sequence. In some embodiments, the CNP has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-terminus of the CNP includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the CNP includes fewer than about 90 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the CNP includes fewer than about 40 repeating structural units. In other aspects, the ELP at the C-terminus and/or the N-terminus of the CNP includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at the N- and C- termini are approximately the same size. In other embodiments, the two or more ELPs at the N- and C- termini differ in size. In some embodiments, the ELP at the N-terminus of the CNP is larger than the ELP at the C-terminus of the CNP. In other embodiments, the ELP at the C-terminus of the CNP is larger than the ELP at the N-terminus of the CNP.

[0122] In some embodiments, the ELP is attached to the C-terminus and/or the N-terminus of the CNP via a linker (SEQ ID NOs: 83 and 55). In some embodiments, the linker comprises a tripeptide GGS sequence (SEQ ID NO: 87). In some aspects, the linker comprises a single copy of the tripeptide GGS (SEQ ID NO: 87). In another aspect, the linker comprises multiple repeats of the tripeptide GGS. In some embodiments, the linker comprises two repeats of the GGS sequence (SEQ ID NO: 102). In some embodiments, the linker comprises three repeats of the GGS sequence (SEQ ID NO: 103). In some embodiments, the linker comprises a pentapeptide GGGGS sequence (SEQ ID NO: 93). In some aspects, the linker comprises a single copy of the pentapeptide GGGGS (SEQ ID NO: 93). In another aspect, the linker comprises multiple repeats

of the pentapeptide GGGGS. In some embodiments, the linker comprises two repeats of the GGGGS sequence (SEQ ID NO: 104). In some embodiments, the linker comprises three repeats of the GGGGS sequence (SEQ ID NO: 105). In some embodiments, the linker comprises a pentapeptide PAPAP sequence (SEQ ID NO: 94). In some aspects, the linker comprises a single copy of the pentapeptide PAPAP. In another aspect, the linker comprises multiple repeats of the pentapeptide PAPAP. In some embodiments, the linker comprises two repeats of the PAPAP sequence (SEQ ID NO: 106). In some embodiments, the linker comprises three repeats of the PAPAP sequence (SEQ ID NO: 107). In some embodiments, the linker comprises a pentapeptide EAAAK sequence (SEQ ID NO: 95). In some aspects, the linker comprises a single copy of the pentapeptide EAAAK. In another aspect, the linker comprises multiple repeats of the pentapeptide EAAAK. In some embodiments, the linker comprises two repeats of the EAAAK sequence (SEQ ID NO: 108). In some embodiments, the linker comprises three repeats of the EAAAK sequence (SEQ ID NO: 109).

[0123] In some embodiments, the ELP is attached to the C-terminus and/or the N-terminus of the CNP via a peptide sequence comprising a protease recognition site. In some embodiments, the protease recognition site is cleaved *in vivo*, thereby releasing the CNP. In some embodiments, the protease recognition site is cleaved at the injection site, thereby releasing the CNP. In some embodiments, the protease recognition site is cleaved in the circulation, thereby releasing the CNP. In some embodiments, the protease recognition site is cleaved in the joint in proximity to the growth plate, thereby releasing the CNP where it is required and minimizing side effects. Protease recognition sites include those for Factor Xa (e.g. IEGR↓, IDGR↓, GR↓), thrombin (e.g. LVPR↓GS, LVPR↓GF), cathepsin (e.g. cathepsin K, RKPR↓G, RKLR↓G), matrix metalloprotease (MMP) (e.g. PLGL↓WAG “consensus” recognition sequence for MMP1, MMP2, MMP3, MMP7, MMP8, and MMP9 (Eckhard *et al.* (2016) *Matrix Biology* 49, 37-60). Analysis of the preferred cleavage site for human cathepsin K by Chou *et al.* (2006. *J. Biol. Chem.* **281**, 12824-12832) suggests alternative cleavage sites that can be used, e.g. P3 could be K, G, H or M, P2 could be P, I or L, P1 could be Q, R or K.

[0124] In other aspects, the present disclosure provides methods for treating or preventing diseases including, but not limited to, diseases associated with regulation of bone growth (e.g., skeletal dysplasias including dwarfism and Achondroplasia); juvenile idiopathic arthritis; osteoarthritis; cardiovascular diseases; diseases affecting cardiovascular homeostasis, diuresis,

natriuresis, or vasodilation; diseases of the central nervous system, diseases of the reproductive tract; or diseases affecting the endothelium of blood vessels. The methods include administering a therapeutic agent including an ELP and a CNP (as described above) to a patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (e.g., dog, cat, cow, or horse). Preferably, the patient is human.

[0125] In some embodiments, treatment with a CNP therapeutic agent according to the present disclosure may also be combined with one or more pharmacologically active substances, e.g. selected from agents for the treatment and/or prevention of complications and disorders resulting from or associated with various diseases including, without limitation, diseases affecting regulation of bone growth (e.g., skeletal dysplasias including dwarfism and Achondroplasia); juvenile idiopathic arthritis; osteoarthritis; cardiovascular diseases; diseases affecting cardiovascular homeostasis, diuresis, natriuresis, or vasodilation; diseases of the central nervous system, diseases of the reproductive tract; or diseases affecting the endothelium of blood vessels.

Glucagon-Like Peptide (GLP)-1 Receptor Antagonists

[0126] In certain embodiments of the disclosure, the therapeutic agent includes an ELP component fused or conjugated to a GLP-1 receptor antagonist, such as exendin-4 lacking amino acids 1-8, derivatives, analogs, mimetics, fragments, or functional variants thereof.

[0127] Human GLP-1 is a 37 amino acid residue peptide originating from preproglucagon which is synthesized in the L-cells in the distal ileum, in the pancreas, and in the brain. Processing of preproglucagon to give GLP-1 (7-36) amide, GLP-1 (7-37) and GLP-2 occurs mainly in the L-cells.

[0128] After processing in the intestinal L-cells, GLP-1 is released into the circulation, most notably in response to a meal. The plasma concentration of GLP-1 rises from a fasting level of approximately 15 pmol/L to a peak postprandial level of 40 pmol/L.

[0129] In some aspects, the protein active agents of the present invention are GLP-1 receptor antagonists. In some embodiments, the GLP-1 receptor antagonists are N-terminal and/or C-terminal fragments of GLP-1. In certain embodiments, the GLP-1 receptor antagonists include the amino acid sequence: DVSSYLEGQAAKEFIWLKGR (SEQ ID NOS: 54, 64, and 65), or fragments thereof. In some aspects, the GLP-1 receptor antagonist is GLP-1 (9-31) (SEQ ID NO:

54). In other aspects, the GLP-1 receptor antagonist is GLP-1 (9-29) (SEQ ID NO: 64). In yet other aspects, the GLP-1 receptor antagonist is GLP-1-exendin-4 (SEQ ID NO: 65).

[0130] In other embodiments, the GLP-1 receptor antagonists are N-terminal and/or C-terminal fragments of exendin-4. In certain embodiments, the GLP-1 receptor antagonists comprise the following amino acid sequence: DLSKQMEEEEAVRLFIEWLKNGGP (SEQ ID NOs: 27, 28, 60, 61, 62, and 63), or a fragment thereof. In certain embodiments, the exendin-4 fragments include, but are not limited to, exendin-4 (9-39) (SEQ ID NO: 27), exendin-4 (9-31) (SEQ ID NO: 28), exendin-4 (9-30) (SEQ ID NO: 60), M-exendin-4 (9-39) (SEQ ID NO: 62), M-exendin-4 (9-31) (SEQ ID NO: 61), or M-exendin-4 (9-30) (SEQ ID NO: 63).

[0131] In other embodiments, the GLP-1 receptor antagonists include the amino acid sequence: DVSSYLEGQAAKEFIWLKGR (SEQ ID NOs: 66 and 67), or a fragment thereof. In some aspects, the GLP-1 receptor antagonist is Jant-4 (9-30) (SEQ ID NO: 66). In another aspect, the GLP-1 receptor antagonist is Jant-4 (9-39) (SEQ ID NO: 67).

[0132] In certain embodiments, the GLP-1 receptor antagonist is a mammalian GLP-1 receptor antagonist. In some embodiments, the GLP-1 receptor antagonist is a human GLP-1 receptor antagonist (*e.g.*, SEQ ID NOs: 27, 28, 54, 56, 60, 61, 62, 63, 64, 65, 66, or 67). In some embodiments, the GLP-1 receptor antagonist is a functional analog of mammalian GLP-1 receptor antagonist, including functional fragments truncated at the N-terminus and/or C-terminus of a GLP-1 receptor antagonist by from about 1 to about 30 amino acids, including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, up to about 20 amino acids, up to about 25 amino acids, or up to about 30 amino acids. In other embodiments, functional variants contain from about 1 to about 30 amino acid insertions, deletions, and/or substitutions with respect to a native or truncated sequence (*e.g.*, SEQ ID NOs: 27, 28, 54, 56, 60, 61, 62, 63, 64, 65, 66, or 67). For example, functional variants may have up to about 3 amino acid, up to about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, up to about 20 amino acid, up to about 25 amino acid, or up to about 30 amino acid insertions, deletions, and/or substitutions with respect to a native or truncated sequence (*e.g.*, SEQ ID NOs: 27, 28, 54, 56, 60, 61, 62, 63, 64, 65, 66, or 67). Protein activity may be confirmed or assayed using any available assay. In other embodiments, the GLP-1 receptor antagonist has an amino acid sequence with at least about 75% identity, about 80% identity, about 90% identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99%

identity with a native or truncated sequence (*e.g.*, SEQ ID NOs: 27, 28, 54, 56, 60, 61, 62, 63, 64, 65, 66, or 67). Percentage identity can be calculated using the alignment program EMBOSS needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1. In some embodiments, the GLP-1 receptor antagonist may contain additional chemical modifications known in the art.

[0133] In certain embodiments, the therapeutic agent includes an ELP fused to C-terminus of GLP-1 receptor antagonist, analogs, mimetics, derivatives, fragments, or functional variants thereof. In some embodiments, the therapeutic agent includes an ELP fused to the N-terminus of a human GLP-1 receptor antagonist (*e.g.*, SEQ ID NOs: 58 and 59). In some embodiments, the GLP-1 receptor antagonist is in a fusion protein with more than one ELP sequence. In some embodiments, the GLP-1 receptor antagonist has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-terminus of the GLP-1 receptor antagonist includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the GLP-1 receptor antagonist includes fewer than about 90 repeating structural units. In other aspects, the ELP at the C-terminus and/or the N-terminus of the GLP-1 receptor antagonist includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at the N- and C-termini are approximately the same size. In other embodiments, the two or more ELPs at the N- and C-termini differ in size. In some embodiments, the ELP at the N-terminus of the GLP-1 receptor antagonist is larger than the ELP at the C-terminus of the GLP-1 receptor antagonist. In other embodiments, the ELP at the C-terminus of the GLP-1 receptor antagonist is larger than the ELP at the N-terminus of the GLP-1 receptor antagonist.

[0134] In other embodiments the GLP-1 receptor antagonist therapeutic agent is used to treat diseases including, but not limited to, diabetes (type 1 or 2); metabolic disease; obesity; diseases resulting from excessive insulin secretion including, but not limited to, hypoglycemia associated with hyperinsulinemia; postgastric surgery acquired hyperinsulinemic hypoglycemia; or hyperinsulinism, such as congenital hyperinsulinism or acquired hyperinsulinism following gastric surgery, for instance gastric surgery to treat obesity. The methods include administering a therapeutic agent comprising an ELP and a GLP-1 receptor antagonist (as described above) to a

patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (e.g., dog, cat, cow, or horse). Preferably, the patient is human.

[0135] In some embodiments, the treatment with a GLP-1 receptor antagonist therapeutic agent according to the present disclosure may also be combined with one or more pharmacologically active substances, e.g. selected from agents for the treatment and/or prevention of various complications and disorders, resulting from or associated with diseases including, without limitation, diabetes (type 1 or 2); metabolic disease; obesity; diseases resulting from excessive insulin secretion including, but not limited to, hypoglycemia associated with hyperinsulinemia; postgastric surgery acquired hyperinsulinemic hypoglycemia; or hyperinsulinism, such as congenital hyperinsulinism or acquired hyperinsulinism following gastric surgery, for instance gastric surgery to treat obesity.

Glucagon-Like Peptide (GLP)-2 Receptor Agonists

[0136] In certain embodiments of the disclosure, the therapeutic agent includes an ELP fused or conjugated to a GLP-2 receptor agonist, derivative, analog, mimetic, fragment, or functional variant thereof. In some embodiments, the GLP-2 receptor agonist is GLP-2.

[0137] GLP-2 is a 33-amino-acid peptide released from the posttranslational processing of proglucagon in the enteroendocrine L cells of the intestine and in specific regions of the brainstem. It is co-secreted together with GLP-1, oxyntomodulin and glicentin, in response to nutrient ingestion. GLP-2 shows remarkable homology in terms of amino acid sequence to glucagon and GLP-1. Different mammalian forms of GLP-2 are also highly conserved. For example, the human GLP-2 (hGLP-2) and degu (a south American rodent) GLP-2 differ from rat GLP-2 (rGLP-2) by one and three amino acids respectively. GLP-2 binds to a single G protein-coupled receptor belonging to the class II glucagon secretin family. The GLP-2 receptor is localized in the small intestine, colon and stomach, which are sites that are known to be responsive to GLP-2 (Yusta *et al.*, 2000, *Gastroenterology*, 119: 744-55).

[0138] In certain embodiments of the disclosure, the active agent includes an amino acid sequence providing sustained release fused or conjugated to a GLP-2 receptor agonist, derivatives, analogs, mimetics, fragments, or functional variants thereof. In certain embodiments of the disclosure, the active agent includes an amino acid sequence providing sustained release fused or conjugated to a GLP-2 receptor agonist and hGH. In some embodiments, the GLP-2 receptor

agonist and the hGH are attached at different termini of the amino acid sequence providing sustained release. In some embodiments, the GLP-2 receptor agonist is attached to the amino terminus of the amino acid sequence providing sustained release and the hGH is attached at the carboxy terminus. In some embodiments, the hGH is attached to the amino terminus of the amino acid sequence providing sustained release and the GLP-2 receptor agonist is attached at the carboxy terminus. In some embodiments, the amino acid sequence providing sustained release is ELP.

[0139] In certain embodiments, the GLP-2 receptor agonist is a mammalian GLP-2 receptor agonist, such as a human GLP-2 receptor agonist. In some embodiments, the GLP-2 receptor agonist is human GLP-2 peptide (SEQ ID NO: 68). In other embodiments, the GLP-2 receptor agonist is a GLP-2 peptide analog wherein the Alanine (A) in position 2 of the N-terminus of GLP-2 peptide has been replaced by Glycine (G) (SEQ ID NO: 70). In certain embodiments, the GLP-2 receptor agonist includes a sequence HXDGSFSDEMNTILDNLAARDFINWLIQTKITD (SEQ ID NO: 74), wherein X is Alanine (A), Glycine (G), Leucine (L), Isoleucine (I), or Valine (V). In some embodiments, X is Alanine (A) or Glycine (G).

[0140] In some embodiments, the GLP-2 receptor agonist is a functional variant of a mammalian GLP-2 peptide, including functional fragments truncated at the C-terminus of human GLP-2 peptide by from about 1 to about 25 amino acids, including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, up to about 20 amino acids, or up to about 25 amino acids. In other embodiments, functional variants may contain from about 1 to about 25 amino acid insertions, deletions, and/or substitutions with respect to a native or consensus sequence (*e.g.*, SEQ ID NOs: 68 or 74). For example, functional variants may have up to about 3 amino acid, up to about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, up to about 20 amino acid, or up to about 25 amino acid insertions, deletions, and/or substitutions with respect to a native or consensus sequence (*e.g.*, SEQ ID NOs: 68 or 74). Protein activity may be confirmed or assayed using any available assay. In other embodiments, the GLP-2 receptor agonist has at least about 75% identity, about 80% identity, about 90% identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99% identity with a native or consensus sequence (*e.g.*, SEQ ID NOs: 68 or 74). Percentage identity can be calculated using the alignment program EMBOSS needle, available at

http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1. In some embodiments, the GLP-2 receptor agonist may contain additional chemical modifications known in the art.

[0141] In certain embodiments, the therapeutic agent includes an ELP fused to the N-terminus or the C-terminus of a GLP-2 receptor agonist, fragments, or functional variants thereof. In some embodiments, the therapeutic agent includes an ELP fused to the C-terminus of a GLP-2 peptide (SEQ ID NOs: 69, 71, and 73).

[0142] In some embodiments, the GLP-2 receptor agonist is in a fusion protein with more than one ELP sequence. In some embodiments, the GLP-2 receptor agonist has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-terminus of the GLP-2 receptor agonist includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the GLP-2 receptor agonist includes fewer than about 90 repeating structural units. In other aspects, the ELP at the C-terminus and/or the N-terminus of the GLP-2 receptor agonist includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at the N- and C-termini are approximately the same size. In some embodiments, the two or more ELPs at the N- and C-termini differ in size. In some embodiments, the ELP at the N-terminus of the GLP-2 receptor agonist is larger than the ELP at the C-terminus of the GLP-2 receptor agonist. In other embodiments, the ELP at the C-terminus of the GLP-2 receptor agonist is larger than the ELP at the N-terminus of the GLP-2 receptor agonist. In some embodiments, the ELP is attached to the C-terminus and/or the N-terminus of the GLP-2 receptor agonist via a linker (SEQ ID NOs: 71 and 73). In some embodiments, the linker comprises a tripeptide GGS sequence (SEQ ID NO: 87). In some aspects, the linker comprises a single copy of the tripeptide GGS. In another aspect, the linker comprises multiple repeats of the tripeptide GGS. In some embodiments, the linker comprises two repeats of the GGS sequence (SEQ ID NO: 102). In some embodiments, the linker comprises three repeats of the GGS sequence (SEQ ID NO: 103). In some embodiments, the linker comprises a pentapeptide GGGGS sequence (SEQ ID NO: 93). In some aspects, the linker comprises a single copy of the pentapeptide GGGGS (SEQ ID NO: 93). In another aspect, the linker comprises multiple repeats of the pentapeptide GGGGS. In some embodiments, the linker comprises two repeats of the GGGGS sequence (SEQ ID NO: 104). In some embodiments, the

linker comprises three repeats of the GGGGS sequence (SEQ ID NO: 105). In some embodiments, the linker comprises a pentapeptide PAPAP sequence (SEQ ID NO: 94). In some aspects, the linker comprises a single copy of the pentapeptide PAPAP. In another aspect, the linker comprises multiple repeats of the pentapeptide PAPAP. In some embodiments, the linker comprises two repeats of the PAPAP sequence (SEQ ID NO: 106). In some embodiments, the linker comprises three repeats of the PAPAP sequence (SEQ ID NO: 107). In some embodiments, the linker comprises a pentapeptide EAAAK sequence (SEQ ID NO: 95). In some aspects, the linker comprises a single copy of the pentapeptide EAAAK. In another aspect, the linker comprises multiple repeats of the pentapeptide EAAAK. In some embodiments, the linker comprises two repeats of the EAAAK sequence (SEQ ID NO: 108). In some embodiments, the linker comprises three repeats of the EAAAK sequence (SEQ ID NO: 109).

[0143] In other aspects, the present disclosure provides methods for treating or preventing intestinal diseases including, but not limited to, intestinal diseases such as Inflammatory Bowel Disease (IBD), such as Ulcerative Colitis (UC) and Crohn's Disease (CD), or Short Bowel Syndrome (SBS); chemotherapy-induced mucositis; chemotherapy-induced diarrhea; or ischemia-reperfusion injury. The methods include administering a therapeutic agent including an ELP and a GLP-2 receptor agonist (as described above) to a patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (e.g., dog, cat, cow, or horse). Preferably, the patient is human. In some embodiments, treatment with a GLP-2 receptor agonist according to the present disclosure may also be combined with one or more pharmacologically active substances, e.g. selected from agents for the treatment and/or prevention of various complications and disorders, resulting from or associated with various diseases including without limitation intestinal diseases including, but not limited to, intestinal diseases such as IBD (such as UC or CD) or SBS; chemotherapy-induced mucositis; chemotherapy-induced diarrhea; or ischemia-reperfusion injury.

Hepcidin

[0144] In certain embodiments, the active agent is Hepcidin (aka LEAP-1 (liver-expressed antimicrobial peptide)), derivatives, analogs, mimetics, fragments, or functional variants thereof. Hepcidin is a 8 kDa peptide hormone produced by hepatocytes in response to inflammation or to rising levels of iron in the blood.

[0145] A hepcidin cDNA encoding an 83 amino acid pre-propeptide in mice and an 84 amino acid pre-propeptide in rat and human was identified in a search for liver specific genes that were regulated by iron (Pigeon *et al.*, 2001, *J. Biol. Chem.* 276:7811 (2001)). The 24 residue N-terminal signal peptide is first cleaved to produce pro-hepcidin, which is then further processed to produce mature hepcidin which is found in both blood and urine. In human urine, the predominant form of hepcidin contains 25 amino acids, although shorter 22 and 20 amino acid peptides are also present.

[0146] Human hepcidin is a 25-amino acid peptide (Hep25). (See Krause *et al.*, 2000, *FEBS Lett* 480:147-150, and Park *et al.*, 2001, *J. Biol. Chem.* 276:7806-7810). The structure of the bioactive 25-amino acid form of hepcidin is a simple hairpin with 8 cysteines that form 4 disulfide bonds (Jordan *et al.*, 2009, *J. Biol. Chem.* 284:24155-67).

[0147] Hepcidin binds to its receptor, the iron export channel ferroportin, and causes its internalization and degradation, thereby reducing ferroportin mediated release of iron into the blood. Internalization further reduces iron transport across gut mucosa and uptake by liver and macrophages. Hepcidin expression is increased in acute and chronic inflammation which decreases iron availability for erythropoiesis. In certain embodiments, the hepcidin therapeutic agent includes an amino acid sequence providing sustained release fused or conjugated to hepcidin, fragments, or functional variants thereof. In some embodiments, the amino acid sequence providing sustained release is ELP.

[0148] In certain embodiments, the hepcidin is a mammalian hepcidin. In some embodiments, the mammalian hepcidin is a human hepcidin (SEQ ID NO: 75). In some embodiments, the mammalian hepcidin includes the N-terminal fragment of Hepcidin, DTHFPICIF (SEQ ID NO: 88) which is critical to activity. In certain embodiments, the present invention provides peptide fragments that mimic the hepcidin activity of Hep25, the bioactive human 25-amino acid form. Such peptides are referred to as “mini-hepcidins.”

[0149] In some embodiments, the hepcidin is a functional variant of human hepcidin, including functional fragments truncated at the C-terminus of hepcidin by from about 1 to about 20 amino acids, including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, or up to about 20 amino acids. In other embodiments, functional variants contain from about 1 to about 20 amino acid insertions, deletions, and/or substitutions at the C-terminus with respect to the native sequence (*e.g.*, SEQ ID

NO: 75). For example, functional variants may have up to about 3 amino acid, up to about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, or up to about 20 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 75).

[0150] Protein activity may be confirmed or assayed using any available assay. As used herein, in some embodiments, a compound having “hepcidin activity” indicates the compound has the ability to lower plasma iron concentrations in subjects (*e.g.* mice or humans), when administered thereto (*e.g.* parenterally injected or orally administered), in a dose-dependent and time-dependent manner. (*See, e.g.*, as demonstrated in Rivera *et al.*, 2005, *Blood*, 106: 2196-2199). In some embodiments, the peptides of the present invention have *in vitro* activity as assayed by the ability to cause the internalization and degradation of ferroportin in a ferroportin-expressing cell line as taught in Nemeth *et al.*, 2006, *Blood*, 107: 328-333. *In vitro* activity may be measured for example by the dose-dependent loss of fluorescence of cells engineered to display ferroportin fused to green fluorescent protein as taught in Nemeth *et al.*, 2006, *Blood*, 107: 328-33.

[0151] In some embodiments, the hepcidin has at least about 75% identity, about 80% identity, about 90% identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99% identity with a native sequence (*e.g.*, SEQ ID NO: 75). Percentage identity can be calculated using the alignment program EMBOSS needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1. In some embodiments, the hepcidin may contain additional chemical modifications known in the art.

[0152] In certain embodiments, the therapeutic agent includes an ELP fused to the N-terminus or the C-terminus of hepcidin, fragments, or functional variants thereof. In some embodiments, the therapeutic agent includes an ELP fused to the N-terminus of human hepcidin (SEQ ID NO: 76). In other embodiments, the therapeutic agent includes an ELP fused to the C-terminus of human hepcidin (SEQ ID NO: 77).

[0153] In some embodiments, the hepcidin is in a fusion protein with more than one ELP sequence. In some embodiments, the hepcidin has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-terminus of the hepcidin includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the hepcidin includes fewer than about 90 repeating structural units. In

other aspects, the ELP at the C-terminus and/or the N-terminus of the hepcidin includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at the N- and C- termini are approximately the same size. In other embodiments, the two or more ELPs at the N- and C- termini differ in size. In some embodiments, the ELP at the N-terminus of the hepcidin is larger than the ELP at the C-terminus of the hepcidin peptide. In some embodiments, the ELP at the C-terminus of the hepcidin is larger than the ELP at the N-terminus of the hepcidin.

[0154] In other aspects, the present disclosure provides methods for treating or preventing intestinal diseases including, but not limited to, iron overload diseases such as hereditary hemochromatosis (HH) or iron-loading anemias; type 2 hemochromatosis; cardiac diseases such as myocardial siderosis or heart failure; diseases causing endocrine damage; liver diseases such as hepatic cirrhosis or hepatocellular carcinoma; diabetes; beta-thalassemia; acute severe infections; or polycythemia vera. The methods include administering a therapeutic agent including an ELP and a hepcidin (as described above) to a patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (e.g., dog, cat, cow, or horse). Preferably, the patient is human.

[0155] In some embodiments, treatment with a hepcidin therapeutic agent according to the present disclosure may also be combined with one or more pharmacologically active substances, e.g. selected from agents for the treatment and/or prevention of various complications and disorders, resulting from or associated with various diseases, including without limitation iron overload diseases such as hereditary hemochromatosis (HH) or iron-loading anemias; type 2 hemochromatosis; cardiac diseases such as myocardial siderosis or heart failure; diseases causing endocrine damage; liver diseases such as hepatic cirrhosis or hepatocellular carcinoma; diabetes; beta-thalassemia; acute severe infections; or polycythemia vera.

Insulin-like Growth Factor-1 (IGF-1)

[0156] In some embodiments, the protein active agent is IGF-1, derivatives, analogs, mimetics, fragments, or functional variants thereof. IGF-I is a single chain peptide present in plasma and other body fluids as well as many cells/tissues which comprises 70 amino acids, including 3 disulphide bonds, and stimulates proliferation of a wide range of cell types, and mediates some of the effects of growth hormone.

[0157] IGF-1 has both systemic and local effects and is mostly associated with different specific binding proteins, four of which are termed IGFBP1, IGFBP2, IGFBP3 and IGFBP4.

These binding proteins modulate the biological functions and availability of IGF-1 in both a positive and negative manner.

[0158] IGF-1 acts mainly by interactions with the IGF-type 1 receptor exposed on the outer surface of plasma membranes in many different cell types. IGF-1R is expressed on many different cellular types and therefore several organism's tissues depend on the action of IGF-1: liver, kidneys, lungs, muscles, bone tissue and cartilage, as well as nerve tissue.

[0159] In certain embodiments, the IGF-1 therapeutic agent of the present disclosure includes an amino acid sequence providing sustained release fused or conjugated to IGF-1, fragments, or functional variants thereof. In some embodiments, the amino acid sequence providing sustained release is ELP.

[0160] In certain embodiments, the IGF-1 is a mammalian IGF-1. In some embodiments, the mammalian IGF-1 is a human IGF-1 (SEQ ID NO: 78). In some embodiments, the IGF-1 is a fragment or functional variant of human IGF-1, including functional fragments truncated at the N-terminus and/or C-terminus of IGF-1 by from about 1 to about 50 amino acids, including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, up to about 20 amino acids, up to about 25 amino acids, up to about 30 amino acids, up to about 35 amino acids, up to about 40 amino acids, up to about 45 amino acids, or up to about 50 amino acids. In other embodiments, fragments or functional variants contain from about 1 to about 50 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 78). For example, fragments or functional variants may have up to about 3 amino acid, up to about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, up to about 20 amino acid, up to about 25 amino acid, up to about 30 amino acid, up to about 35 amino acid, up to about 40 amino acid, up to about 45 amino acid, or up to about 50 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 78). Protein activity may be confirmed or assayed using any available assay. In other embodiments, the IGF-1 derivative, analog, mimetic, fragment, or functional variant thereof has at least about 75% identity, about 80% identity, about 90% identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99% identity with a native sequence (*e.g.*, SEQ ID NO: 78). Percentage identity can be calculated using the alignment program EMBOSS needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62;

Gap Open = 10; Gap Extension = 0.1. In some embodiments, the IGF-1 derivative, analog, mimetic, fragment, or functional variant thereof may contain additional chemical modifications known in the art.

[0161] In certain embodiments, the therapeutic agent includes an ELP fused to the N-terminus or the C-terminus of IGF-1, derivatives, analogs, mimetics, fragments, or functional variants thereof. In certain embodiments, the therapeutic agent includes an ELP fused to the C-terminus of IGF-1 (SEQ ID NOs: 79 and 80).

[0162] In some embodiments, the IGF-1 derivative, analog, mimetic, fragment, or functional variant thereof is in a fusion protein with more than one ELP sequence. In some embodiments, the IGF-1 has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-terminus of the IGF-1 includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the IGF-1 includes fewer than about 90 repeating structural units. In other aspects, the ELP at the C-terminus and/or the N-terminus of the IGF-1 includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at the N- and C-termini are approximately the same size. In other embodiments, the two or more ELPs at the N- and C-termini differ in size. In some embodiments, the ELP at the N-terminus of the IGF-1 is larger than the ELP at the C-terminus of the IGF-1. In other embodiments, the ELP at the C-terminus of the IGF-1 is larger than the ELP at the N-terminus of the IGF-1.

[0163] In other aspects, the present disclosure provides methods for treating or preventing disease including, but not limited to, primary IGF-1 deficiency; neuropathy; neurological diseases; cancer; kidney disease; liver disease; diseases of the lungs; diabetes; growth defects; treatment of children with GH gene deletion who have developed neutralizing antibodies to recombinant GH; Laron's syndrome, or Laron-type dwarfism; heart disease; or stroke. The methods include administering a therapeutic agent comprising an ELP and an IGF-1 (as described above) to a patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (e.g., dog, cat, cow, or horse). Preferably, the patient is human.

[0164] In some embodiments, treatment with an IGF-1 compound according to the present disclosure may also be combined chemotherapy and irradiation, as well as with one or more pharmacologically active substances, e.g. selected from agents for the treatment and/or prevention of complications and disorders resulting from or associated with various diseases, including,

without limitation, primary IGF-1 deficiency; neuropathy; neuronal diseases; cancer; kidney disease; liver disease; diseases of the lungs; diabetes; growth defects; treatment of children with GH gene deletion who have developed neutralizing antibodies to recombinant GH; Laron's syndrome, or Laron-type dwarfism; heart disease; or stroke.

Urodilatin

[0165] In some embodiments, the protein active agent is urodilatin, derivatives, analogs, mimetics, fragments, or functional variants thereof. Urodilatin is a 32 amino acid peptide hormone which is a natriuretic peptide receptor A (NPR-A) agonist. Urodilatin is the renal isoform of atrial natriuretic peptide (ANP). It is formed by a differential processing of the ANP prohormone in the kidney, as opposed to all other tissues, where instead of 126 amino acid prohormone being cleaved between amino acid 98 and 99 to form ANP and kaliuretic peptide, the prohormone is cleaved between amino acid 95 and 96. The cleavage of the ANP prohormone in the kidney results in 4 amino acids from the C-terminal end of kaliuretic peptide (*i.e.*, threonine-alanine-proline-arginine) being attached to the N-terminus of ANP. Therefore, urodilatin is a 32-amino acid-containing peptide with the same structure as the 28-amino acid-containing ANP, except for the addition of four amino acids (TAPR) at the N-terminal extension.

[0166] Urodilatin is produced in physiological quantities in the kidney, differentially processed and secreted into the urine and forms the basis for a paracrine system regulating water and sodium reabsorption at the level of the collecting duct (Forssmann, W.-G. *et al.*, 1998, *Histochemistry and Cell Biology* 110(4): 335-357). Urodilatin causes diuresis through increasing renal blood flow. It is secreted in response to increased mean arterial pressure and increased blood volume from the cells of the distal tubule and collecting duct. It is important in oliguric patients (such as those with acute renal failure and chronic renal failure) as it lowers serum creatinine and increases urine output.

[0167] Urodilatin is more potent than ANP and has a half-life twice as long as ANP, which may be due to the N-terminus of urodilatin being resistant to deactivation by neutral endopeptidase. In certain embodiments, the urodilatin therapeutic agent of the present disclosure includes an amino acid sequence providing sustained release fused or conjugated to urodilatin, fragments, or functional variants thereof. In some embodiments, the amino acid sequence providing sustained release is ELP.

[0168] In certain embodiments, the urodilatin is a mammalian urodilatin. In some embodiments, the mammalian urodilatin is human urodilatin (SEQ ID NO: 84). In some embodiments, the urodilatin is a derivative, analog, mimetic, fragment, or functional variant of mammalian urodilatin, including functional fragments truncated at the C-terminus of urodilatin by from about 1 to about 25 amino acids, including, for example, by including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, up to about 20 amino acids, or up to about 25 amino acids. In other embodiments, the urodilatin, derivatives, analogs, mimetics, fragments, or functional variants contain from about 1 to about 25 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 84). For example, the urodilatin, derivatives, analogs, mimetics, fragments, or functional variants may have up to about 3 amino acid, up to about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, up to about 20 amino acid, or up to about 25 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 84). Protein activity may be confirmed or assayed using any available assay. In other embodiments, the urodilatin, derivatives, analogs, mimetics, fragments, or functional variants have at least about 75% identity, about 80% identity, about 90% identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99% identity with a native sequence (*e.g.*, SEQ ID NO: 84). Percentage identity can be calculated using the alignment program EMBOSS needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1. In some embodiments, urodilatin, derivatives, analogs, mimetics, fragments, or functional variants may contain additional chemical modifications known in the art.

[0169] In certain embodiments, the therapeutic agent includes an ELP fused to the N-terminus or the C-terminus of a urodilatin, derivatives, analogs, mimetics, fragments, or functional variants thereof. In some embodiments, the therapeutic agent includes an ELP fused to the C-terminus of human urodilatin (SEQ ID NO: 85). In other embodiments, the therapeutic agent includes an ELP fused to the N-terminus of human urodilatin (SEQ ID NO: 86).

[0170] In some embodiments, the urodilatin, derivative, analog, mimetic, fragment, or functional variant is in a fusion protein with more than one ELP sequence. In some embodiments, the urodilatin, derivative, analog, mimetic, fragment, or functional variant has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-

terminus of the urodilatin, derivative, analog, mimetic, fragment, or functional variant includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the urodilatin, derivative, analog, mimetic, fragment, or functional variant includes fewer than about 90 repeating structural units. In other aspects, the ELP at the C-terminus and/or the N-terminus of the urodilatin, derivative, analog, mimetic, fragment, or functional variant includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at the N- and C- termini are approximately the same size. In other embodiments, the two or more ELPs at the N- and C- termini differ in size. In some embodiments, the ELP at the N-terminus of the urodilatin is larger than the ELP at the C-terminus of the urodilatin, derivative, analog, mimetic, fragment, or functional variant. In other embodiments, the ELP at the C-terminus of the urodilatin is larger than the ELP at the N-terminus of the urodilatin, derivative, analog, mimetic, fragment, or functional variant.

[0171] In other aspects, the present disclosure provides methods for treating or preventing diseases including, but not limited to, heart diseases such as acute heart failure (AHF); or kidney disease such as acute renal failure or chronic renal failure. The methods include administering a therapeutic agent including an ELP and a urodilatin (as described above) to a patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (e.g., dog, cat, cow, or horse). Preferably, the patient is human.

[0172] In some embodiments, treatment with a urodilatin according to the present disclosure may also be combined with one or more pharmacologically active substances, including, but not limited to, diuretics and agents for the treatment and/or prevention of complications and disorders resulting from or associated with various diseases including without limitation heart diseases such as AHF; or kidney disease such as acute renal failure or chronic renal failure.

Thymosin β 4

[0173] In some embodiments, the protein active agent is Thymosin β 4, derivatives, analogs, mimetics, fragments, or functional variants thereof. Thymosins refer to a family of biochemically and functionally distinct proteins that were originally identified from the thymus, and are now known to be present in many other tissues, and which have a variety of different physiological functions. More than 20 isoforms of β -thymosin have been identified in different species, and among humans three β -thymosins have been identified including thymosin β 4,

thymosin β 10, and thymosin β 15, all of which share significant amino acid sequence homology. Despite this homology, each of these thymosin beta proteins is a distinct gene product with different functions.

[0174] Thymosin β 4, the most abundant of the β -thymosins, is a highly conserved, water-soluble acidic polypeptide. Thymosin β 4 was initially identified as a protein that is up-regulated during endothelial cell migration and differentiation *in vitro*. The mammalian gene encoding thymosin β -4 localizes to the X-chromosome. Human thymosin β -4, X-linked, has 44 amino acids, and escapes X-inactivation by being processed into a 43 amino acid peptide, with a molecular weight of 4.9 kDa, by removal of the first methionyl residue (Girardi, M., *et al.*, 2003, *Immunology* 109: 1-7). Thymosin β -4 is localized to both the cytoplasm and the nucleus of cells. Thymosin β -4 is present in many tissues, and has multiple biological functions. It potently regulates actin polymerization, stimulates tissue remodeling, cell differentiation, and cell and tissue healing after injury, and is also involved in the expression of a number of inflammatory chemokines and cytokines.

[0175] In certain embodiments, the Thymosin β 4 therapeutic agent of the present disclosure includes an amino acid sequence providing sustained release fused or conjugated to Thymosin β 4, fragments, or functional variants thereof. In some embodiments, the amino acid sequence providing sustained release is ELP.

[0176] In certain embodiments, the Thymosin β 4, derivative, analog, mimetic, fragment, or functional variant is a mammalian Thymosin β 4. In some embodiments, the mammalian Thymosin β 4 is human Thymosin β 4 (SEQ ID NO: 89). In some embodiments, the Thymosin β 4 is a derivative, analog, mimetic, fragment, or functional variant of mammalian Thymosin β 4, including functional fragments truncated at the N-terminus and/or C-terminus of Thymosin β 4 by from about 1 to about 35 amino acids, including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, up to about 20 amino acids, up to about 25 amino acids, up to about 30 amino acids, or up to about 35 amino acids. In other embodiments, Thymosin β 4, derivatives, analogs, mimetics, fragments, or functional variants contain from about 1 to about 35 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 89). For example, Thymosin β 4, derivatives, analogs, mimetics, fragments, or functional variants may have up to about 3 amino acid, up to about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, up to about 20 amino

acid, up to about 25 amino acid, up to about 30 amino acid, or up to about 35 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 89). Protein activity may be confirmed or assayed using any available assay. In other embodiments, the Thymosin β 4, derivatives, analogs, mimetics, fragments, or functional variants have at least about 75% identity, about 80% identity, about 90% identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99% identity with a native sequence (*e.g.*, SEQ ID NO: 89). Percentage identity can be calculated using the alignment program EMBOSS needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1. In some embodiments, the Thymosin β 4, derivatives, analogs, mimetics, fragments, or functional variants may contain additional chemical modifications known in the art.

[0177] In certain embodiments, the therapeutic agent includes an ELP fused to the N-terminus or the C-terminus of Thymosin β 4, derivatives, analogs, mimetics, fragments, or functional variants thereof. In some embodiments, the therapeutic agent includes an ELP fused to the C-terminus of Thymosin β 4 (SEQ ID NO: 90).

[0178] In some embodiments, the Thymosin β 4, derivative, analog, mimetic, fragment, or functional variant is in a fusion protein with more than one ELP sequence. In some embodiments, the Thymosin β 4, derivative, analog, mimetic, fragment, or functional variant has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-terminus of the Thymosin β 4, derivative, analog, mimetic, fragment, or functional variant includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the Thymosin β 4, derivative, analog, mimetic, fragment, or functional variant includes fewer than about 90 repeating structural units. In other aspects, the ELP at the C-terminus and/or the N-terminus of the Thymosin β 4, derivative, analog, mimetic, fragment, or functional variant includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at the N- and C-termini are approximately the same size. In other embodiments, the two or more ELPs at the N- and C-termini differ in size. In some embodiments, the ELP at the N-terminus of the Thymosin β 4, derivative, analog, mimetic, fragment, or functional variant is larger than the ELP at the C-terminus of Thymosin β 4. In other embodiments, the ELP at the C-terminus of the Thymosin β 4, derivative, analog, mimetic, fragment, or functional variant is larger than the ELP at the N-terminus.

[0179] In other aspects, the present disclosure provides methods for treating or preventing disease including, but not limited to, heart failure; pulmonary hypertension; ischemic heart disease; dry eye; or liver fibrosis. The methods include administering a therapeutic agent including an ELP and a Thymosin β 4 (as described above) to a patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (*e.g.*, dog, cat, cow, or horse). Preferably, the patient is human.

[0180] In some embodiments, treatment with a Thymosin β 4 according to the present disclosure may also be combined with one or more pharmacologically active substances, *e.g.* selected from agents for the treatment and/or prevention of complications and disorders resulting from or associated with various diseases including, without limitation, heart failure; pulmonary hypertension; ischemic heart disease; dry eye; or liver fibrosis.

TRAIL

[0181] In certain embodiments, the protein active agent is TRAIL (TNF-related apoptosis-inducing ligand), derivatives, analogs, mimetics, fragments, or functional variants thereof.

[0182] The extrinsic cell death pathway is triggered by ligand-receptor interactions that lead to intracellular signaling events, which ultimately result in the death of the target cell (apoptosis). One such ligand is a member of the tumor necrosis factor (TNF) superfamily, TRAIL or Apo2L (Wiley, S. R., *et al.*, 1995, *Immunity* 3:673-682; Pitti, R. M., *et al.*, 1996, *J. Biol. Chem.* 271: 12687-12690). TRAIL is a type II membrane protein of 281 amino acids. Its extracellular region comprises amino acid residues 114-281 and, upon cleavage by proteases, forms soluble sTRAIL molecule of 20 kDa size, which is also biologically active. Endogenous TRAIL exists as a homotrimer which is a critical requirement for its biological function. Activated T lymphocytes and NK cells express high levels of TRAIL.

[0183] TRAIL protein acts, for example, by binding to and activating pro-apoptotic TRAIL surface receptors 1 and 2 (TRAIL-R1/R2 or DR4 and DR5). Both TRAIL and sTRAIL are capable of triggering apoptosis via interaction with TRAIL receptors present on target cells.

[0184] In certain embodiments, the TRAIL therapeutic agent of the present disclosure includes an amino acid sequence providing sustained release fused or conjugated to TRAIL, fragments, or functional variants thereof. In some embodiments, the amino acid sequence providing sustained release is ELP.

[0185] In certain embodiments, the TRAIL is a mammalian TRAIL. In some embodiments, the mammalian TRAIL is human TRAIL (SEQ ID NO: 91). In some embodiments, the TRAIL may be a derivative, analog, mimetic, fragment, or functional variant thereof of mammalian TRAIL, including functional fragments truncated at the N-terminus and/or C-terminus of TRAIL by from about 1 to about 160 amino acids, including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, up to about 20 amino acids, up to about 25 amino acids, up to about 30 amino acids, up to about 35 amino acids, up to about 40 amino acids, up to about 45 amino acids, up to about 50 amino acids, up to about 55 amino acids, up to about 60 amino acids, up to about 65 amino acids, up to about 70 amino acids, up to about 75 amino acids, up to about 80 amino acids, up to about 85 amino acids, up to about 90 amino acids, up to about 95 amino acids, up to about 100 amino acids, up to about 105 amino acids, up to about 110 amino acids, up to about 115 amino acids, up to about 120 amino acids, up to about 125 amino acids, up to about 130 amino acids, up to about 135 amino acids, up to about 140 amino acids, up to about 145 amino acids, up to about 150 amino acids, up to about 155 amino acids, or up to about 160 amino acids. TRAIL, derivatives, analogs, mimetics, fragments, or functional variants may contain from about 1 to about 160 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 91). For example, TRAIL, derivatives, analogs, mimetics, fragments, or functional variants may have up to about 3 amino acid, up to about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, up to about 20 amino acid, up to about 25 amino acid, up to about 30 amino acid, up to about 35 amino acid, up to about 40 amino acid, up to about 45 amino acid, up to about 50 amino acid, up to about 55 amino acid, up to about 60 amino acid, up to about 65 amino acid, up to about 70 amino acid, up to about 75 amino acid, up to about 80 amino acid, up to about 85 amino acid, up to about 90 amino acid, up to about 95 amino acid, up to about 100 amino acid, up to about 105 amino acid, up to about 110 amino acid, up to about 115 amino acid, up to about 120 amino acid, up to about 125 amino acid, up to about 130 amino acid, up to about 135 amino acid, up to about 140 amino acid, up to about 145 amino acid, up to about 150 amino acid, up to about 155 amino acid, or up to about 160 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 91). Protein activity may be confirmed or assayed using any available assay. In other embodiments, the TRAIL, derivative, analog, mimetic, fragment, or functional variant has at least about 75% identity, about 80% identity, about 90%

identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99% identity with a native sequence (*e.g.*, SEQ ID NO: 91). Percentage identity can be calculated using the alignment program EMBOSS needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1. In some embodiments, the TRAIL, derivative, analog, mimetic, fragment, or functional variant may contain additional chemical modifications known in the art.

[0186] In certain embodiments, the therapeutic agent includes an ELP fused to the N-terminus or the C-terminus of the TRAIL, derivative, analog, mimetic, fragment, or functional variant thereof. In some embodiments, the therapeutic agent includes an ELP fused to the N-terminus of TRAIL (SEQ ID NO: 92).

[0187] In some embodiments, the TRAIL, derivative, analog, mimetic, fragment, or functional variant is in a fusion protein with more than one ELP sequence. In some embodiments, the TRAIL, derivative, analog, mimetic, fragment, or functional variant has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-terminus of the TRAIL, derivative, analog, mimetic, fragment, or functional variant includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the TRAIL, derivative, analog, mimetic, fragment, or functional variant includes fewer than about 90 repeating structural units. In other aspects, the ELP at the C-terminus and/or the N-terminus of the TRAIL, derivative, analog, mimetic, fragment, or functional variant includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at the N- and C-termini are approximately the same size. In other embodiments, the two or more ELPs at the N- and C-termini differ in size. In some embodiments, the ELP at the N-terminus of the TRAIL, derivative, analog, mimetic, fragment, or functional variant is larger than the ELP at the C-terminus. In other embodiments, the ELP at the C-terminus of the TRAIL is larger than the ELP at the N-terminus.

[0188] In other aspects, the present disclosure provides methods for treating or preventing diseases including, but not limited to, tumorigenesis and various types of cancer; non-alcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH); cirrhosis; post-transplant liver fibrosis or cirrhosis in post-orthotopic liver transplant (POLT) recipients as a result of recurrent hepatitis C virus (HCV) infection; pancreatic fibrosis. The methods include administering a

therapeutic agent including an ELP and a TRAIL (as described above) to a patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (e.g., dog, cat, cow, or horse). Preferably, the patient is human.

[0189] In some embodiments, treatment with a TRAIL according to the present disclosure may also be combined with chemotherapy or radiation therapy. In other embodiments, the treatment with a TRAIL according to the present disclosure may also be combined with one or more pharmacologically active substances, e.g. selected from agents for the treatment and/or prevention of complications and disorders associated with various diseases, including without limitation tumorigenesis and various types of cancer; non-alcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH); cirrhosis; post-transplant liver fibrosis or cirrhosis in post-orthotopic liver transplant (POLT) recipients as a result of recurrent hepatitis C virus (HCV) infection; pancreatic fibrosis.

Fibroblast growth factor 21 (FGF21)

[0190] Fibroblast growth factor 21 (FGF21) is a member of the FGF family which produces beneficial effects on lipid levels, body weight and glucose metabolism in animals. Overexpression of FGF21 in transgenic mice has been shown to result in reduced glucose and triglyceride levels, and resistance to diet-induced obesity. (Kharitonov *et al.* (2005), *J. Clin. Invest.* 115; 1627-1635). The administration of exogenous FGF21 to rodents and primates results in normalization of blood glucose levels, reduced triglyceride and cholesterol levels, improved glucose tolerance and improved insulin sensitivity (Kharitonov *et al.* (2007), *Endocrinol.* 48:774-781). FGF21 administration in experimental animal models has been shown to reduce body weight and body fat by increasing energy expenditure, physical activity, and metabolic rate. (Long and Kharitonov (2011) *Biochim. Biophys. Acta* 1812:791-795).

[0191] FGF21 signaling is mediated through its interaction with a receptor complex that includes β Klotho (KLB) and one of three different FGF receptors (FGFR1c, FGFR2c or FGFR3c) (Ogawa *et al.* (2007), *Proc. Natl. Acad. Sci. USA* 104:7432-7437; Suzuki *et al.* (2008), *Mol. Endocrinol.* 22:1006-1014). It is believed that the main functional receptor for FGF21 signaling *in vivo* is the KLB/FGFR1c complex (this complex is referred to herein as "FGF21R").

[0192] Pharmacological activation of FGF21 signaling has been proposed for the treatment of various diseases and disorders in humans including type 2 diabetes, obesity, dyslipidemia, non-

alcoholic fatty liver disease (nonalcoholic steatohepatitis, NASH), and other metabolic conditions (Gimeno and Moller (2014) *Trends Endocrinol. Metab.* 25, 303-311). An FGF analog, LY2405319 (Kharitonov *et al.* (2013) *PLOS One*, 8, e58575) was evaluated in a clinical trial in patients with type 2 diabetes and obesity (Gaich *et al.* (2013) *Cell Metabolism* 18, 333-340). Hecht *et al.* (2012, *PLOS One*, 7(11): e49345) developed a long-acting FGF21 analog fused to the Fc domain of human IgG1.

[0193] In certain embodiments, the FGF21 therapeutic agent of the present disclosure includes an amino acid sequence providing sustained release fused or conjugated to FGF21, fragments, or functional variants thereof. In some embodiments, the amino acid sequence providing sustained release is ELP.

[0194] In certain embodiments, the FGF21 is a mammalian FGF21. In some embodiments, the mammalian FGF21 is human FGF21 (SEQ ID NO: 110). In some embodiments, the FGF21 may be a derivative, analog, mimetic, fragment, or functional variant thereof of mammalian FGF21, including functional fragments truncated at the N-terminus and/or C-terminus of FGF21 by from about 1 to about 160 amino acids, including, for example, by up to about 3 amino acids, up to about 5 amino acids, up to about 10 amino acids, up to about 15 amino acids, up to about 20 amino acids, up to about 25 amino acids, up to about 30 amino acids, up to about 35 amino acids, up to about 40 amino acids, up to about 45 amino acids, up to about 50 amino acids, up to about 55 amino acids, up to about 60 amino acids, up to about 65 amino acids, up to about 70 amino acids, up to about 75 amino acids, up to about 80 amino acids, up to about 85 amino acids, up to about 90 amino acids, up to about 95 amino acids, up to about 100 amino acids, up to about 105 amino acids, up to about 110 amino acids, up to about 115 amino acids, up to about 120 amino acids, up to about 125 amino acids, up to about 130 amino acids, up to about 135 amino acids, up to about 140 amino acids, up to about 145 amino acids, up to about 150 amino acids, up to about 155 amino acids, or up to about 160 amino acids. FGF21, derivatives, analogs, mimetics, fragments, or functional variants may contain from about 1 to about 160 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 110). For example, FGF21, derivatives, analogs, mimetics, fragments, or functional variants may have up to about 3 amino acid, up to about 5 amino acid, up to about 10 amino acid, up to about 15 amino acid, up to about 20 amino acid, up to about 25 amino acid, up to about 30 amino acid, up to about 35 amino acid, up to about 40 amino acid, up to about 45 amino acid, up to about 50 amino acid,

up to about 55 amino acid, up to about 60 amino acid, up to about 65 amino acid, up to about 70 amino acid, up to about 75 amino acid, up to about 80 amino acid, up to about 85 amino acid, up to about 90 amino acid, up to about 95 amino acid, up to about 100 amino acid, up to about 105 amino acid, up to about 110 amino acid, up to about 115 amino acid, up to about 120 amino acid, up to about 125 amino acid, up to about 130 amino acid, up to about 135 amino acid, up to about 140 amino acid, up to about 145 amino acid, up to about 150 amino acid, up to about 155 amino acid, or up to about 160 amino acid insertions, deletions, and/or substitutions with respect to a native sequence (*e.g.*, SEQ ID NO: 110). Protein activity may be confirmed or assayed using any available assay. In other embodiments, the FGF21, derivative, analog, mimetic, fragment, or functional variant has at least about 75% identity, about 80% identity, about 90% identity, about 95% identity, about 96% identity, about 97% identity, about 98% identity, or about 99% identity with a native sequence (*e.g.*, SEQ ID NO: 110). Percentage identity can be calculated using the alignment program EMBOS needle, available at http://www.ebi.ac.uk/Tools/psa/emboss_needle/. The following default parameters may be used for Pairwise alignment: Protein Weight Matrix = BLOSUM62; Gap Open = 10; Gap Extension = 0.1. In some embodiments, the TRAIL, derivative, analog, mimetic, fragment, or functional variant may contain additional chemical modifications known in the art.

[0195] In certain embodiments, the therapeutic agent includes an ELP fused to the N-terminus or the C-terminus of the FGF21, derivative, analog, mimetic, fragment, or functional variant thereof. In some embodiments, the therapeutic agent includes an ELP fused to the N-terminus of FGF21 (SEQ ID NO: 110).

[0196] In some embodiments, the FGF21, derivative, analog, mimetic, fragment, or functional variant is in a fusion protein with more than one ELP sequence. In some embodiments, the FGF21, derivative, analog, mimetic, fragment, or functional variant has one or more ELPs at both the N- and C-termini. In some embodiments, the ELP at the C-terminus and/or the N-terminus of the FGF21, derivative, analog, mimetic, fragment, or functional variant includes about 90 to about 180 repeating structural units. In other embodiments, the ELP at the C-terminus and/or the N-terminus of the FGF21, derivative, analog, mimetic, fragment, or functional variant includes fewer than about 90 repeating structural units. In other aspects, the ELP at the C-terminus and/or the N-terminus of the FGF21, derivative, analog, mimetic, fragment, or functional variant includes greater than about 180 repeating structural units. In some embodiments, the two or more ELPs at

the N- and C- termini are approximately the same size. In other embodiments, the two or more ELPs at the N- and C- termini differ in size. In some embodiments, the ELP at the N-terminus of the FGF21, derivative, analog, mimetic, fragment, or functional variant is larger than the ELP at the C-terminus. In other embodiments, the ELP at the C-terminus of the FGF21 is larger than the ELP at the N-terminus.

[0197] In some embodiments, the therapeutic agent includes an ELP fused to the N-terminus of the FGF21, derivative, analog, mimetic, fragment, or functional variant thereof and a GLP-1 agonist is fused to the N-terminus of the ELP (SEQ ID NOs: 133 and 134). Such a therapeutic agent combines the benefits of both FGF21 and GLP-1 activity for the treatment of diabetes, obesity and related disorders.

[0198] In other aspects, the present disclosure provides methods for treating or preventing diseases including, but not limited to, tumorigenesis and various types of cancer; non-alcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH); cirrhosis; post-transplant liver fibrosis or cirrhosis in post-orthotopic liver transplant (POLT) recipients as a result of recurrent hepatitis C virus (HCV) infection; pancreatic fibrosis. The methods include administering a therapeutic agent including an ELP and an FGF21 (as described above) to a patient in need of such treatment. Generally, the patient may be a human or non-human animal patient (e.g., dog, cat, cow, or horse). Preferably, the patient is human.

[0199] In some embodiments, treatment with a FGF21 according to the present disclosure may also be combined with one or more pharmacologically active substances, e.g. selected from agents for the treatment and/or prevention of complications and disorders associated with various diseases, including without limitation tumorigenesis and various types of cancer; non-alcoholic fatty liver disease (NAFLD); nonalcoholic steatohepatitis (NASH); cirrhosis; post-transplant liver fibrosis or cirrhosis in post-orthotopic liver transplant (POLT) recipients as a result of recurrent hepatitis C virus (HCV) infection; pancreatic fibrosis.

FORMULATIONS

[0200] The present disclosure provides sustained release formulations including a therapeutic agent disclosed herein and one or more pharmaceutically acceptable excipients and/or diluents. For example, such excipients include salts, and other excipients that may act to stabilize hydrogen bonding. Any appropriate excipient known in the art may be used. Exemplary excipients

include, but are not limited to, amino acids such as histidine, glycine, or arginine; glycerol; sugars, such as sucrose; surface active agents such as polysorbate 20 and polysorbate 80; citric acid; sodium citrate; antioxidants; salts including alkaline earth metal salts such as sodium, potassium, and calcium; counter ions such as chloride and phosphate; sugar alcohols (e.g. mannitol); preservatives; sugar alcohols (e.g. mannitol, sorbitol); and buffering agents. Exemplary salts include sodium chloride, potassium chloride, magnesium chloride, calcium chloride, sodium phosphate dibasic, sodium phosphate monobasic, sodium phosphate, and potassium phosphate.

[0201] The therapeutic agent is formulated at a pH, ionic strength, and generally with excipients sufficient to enable the formation of the matrix at body temperature (*e.g.*, 37°C, or at from 34 to 36°C in some embodiments). The therapeutic agent is generally prepared such that it does not form the matrix at storage conditions. The formulation can be stored frozen, refrigerated or at room temperature. Storage conditions are generally less than the transition temperature of the formulation, such as less than about 32°C, or less than about 30°C, or less than about 27°C, or less than about 25°C, or less than about 20°C, or less than about 15°C. For example, the formulation may be isotonic with blood or have an ionic strength that mimics physiological conditions. For example, the formulation may have an ionic strength of at least that of 25 mM Sodium Chloride, or at least that of 30 mM Sodium chloride, or at least that of 40 mM Sodium Chloride, or at least that of 50 mM Sodium Chloride, or at least that of 75 mM Sodium Chloride, or at least that of 100 mM Sodium Chloride, or at least that of 150 mM Sodium Chloride. In certain embodiments, the formulation has an ionic strength equivalent to that of 0.9% saline (154 mM sodium chloride).

[0202] In some embodiments, the formulation is stable at storage conditions. Storage conditions may be any conditions used to stably store a formulation. In some embodiments, the formulation is refrigerated. In some embodiments, the formulation is frozen. In some embodiments, the storage conditions include temperatures of less than about 30°C. In some embodiments, the storage conditions include temperatures of about 2°C to about 8°C. In some embodiments, the storage conditions include temperatures below 0°C. In some embodiments, the storage conditions include temperatures of about -15°C to about -80°C.

[0203] Stability can be measured using any appropriate means in the art. Generally, a stable formulation is one that shows less than a 5% increase in degradation products or impurities. In some embodiments, the formulation is stable for at least about 1 month, at least about 2 months,

at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, or at least about one year or more at the storage conditions. In some embodiments, the formulation is stable for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about one year, or at least about two years or more at 2-8 °C. In some embodiments, the formulation is stable for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about one year, or at least about two years or more at 25 °C. In some embodiments, the formulation is stable for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, at least about 6 months, at least about one year, or at least about two years or more at -15°C to about -80°C.

[0204] In some embodiments, the formulation includes two or more of calcium chloride, magnesium chloride, potassium chloride, potassium phosphate monobasic, sodium chloride, sodium phosphate dibasic, sodium phosphate monobasic, histidine, arginine, glycine, glycerol, antimicrobial preservative (e.g. metacresol), tonicity-adjusting agent (e.g. mannitol), glacial acetic acid, sodium acetate trihydrate; sucrose, sodium phosphate monobasic monohydrate, sodium phosphate dibasic heptahydrate, zinc, m-cresol, phenol, sorbitol, polysorbate 80, and polysorbate 20.

[0205] In some embodiments, the formulation includes histidine or another amino acid at a range of about 10 mM to about 100 mM histidine. In some embodiments, the formulation includes histidine or another amino acid at a range of about 10 mM to about 30 mM histidine. In some embodiments, the formulation includes histidine or another amino acid at a range of about 15 mM to about 25 mM histidine. In some embodiments, the formulation includes NaCl at a range of about 10 mM to about 165 mM NaCl. In some embodiments, the formulation includes between about 50 mM and about 165 mM NaCl. In some embodiments, the formulation includes between about 54 mM and about 162 mM NaCl. In some embodiments, the formulation includes between about 110 mM and about 162 mM NaCl. In some embodiments, the formulation includes sodium phosphate at a range of about 1 mM to about 20 mM. In some embodiments, the formulation includes sodium phosphate at a range of about 5 mM to about 15 mM. In some embodiments, the formulation includes sodium phosphate monobasic at a range of about 2mM to about 10mM. In some embodiments, the formulation includes sodium phosphate monobasic at a range of about 4 mM to about 8 mM. In some embodiments, the formulation includes sodium phosphate dibasic at

a range of about 1mM to about 10 mM. In some embodiments, the formulation includes sodium phosphate dibasic at a range of about 2 mM to about 7 mM. In some embodiments, the formulation includes sodium phosphate dibasic at a range of about 2 mM to about 5 mM. In some embodiments, the formulation includes polysorbate 20 at a range of about 0.01% to about 0.2%. In some embodiments, the formulation includes polysorbate 80 at a range of about 0.01% to about 0.2%. In some embodiments, the formulation includes sodium phosphate, sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, and polysorbate 20. In some embodiments, the formulation includes about 10mM sodium phosphate (about 7mM sodium phosphate monobasic and about 3mM sodium phosphate dibasic), about 110mM sodium chloride, and about 0.1% polysorbate 20.

[0206] In some embodiments, the formulation is formulated at physiological pH. In some embodiments, the formulation is formulated at a pH in the range of about 5.5 to about 7.5. In some embodiments, the formulation is formulated at a pH in the range of about 6.0 to about 7.0. In some embodiments, the formulation is formulated at a pH in the range of about 6.5 to about 7.0. In some embodiments, formulations with a lower pH demonstrate improved formulation stability compared to formulations at a higher pH. In some embodiments, formulations with a pH of about 6.5 demonstrate improved stability compared to formulations with a pH of about 7.0. In some embodiments, formulations with a pH of about 6.0 demonstrate improved stability compared to formulations with a pH of about 6.5. In some embodiments, formulations with a lower pH maintain a higher percentage of monomers compared to formulations at a higher pH. In some embodiments, formulations with a pH of about 6.5 maintain a higher percentage of monomers compared to formulations with a pH of about 7.0. In some embodiments, formulations with a pH of about 6.0 maintain a higher percentage of monomers compared to formulations with a pH of about 6.5.

[0207] The protein concentration of the therapeutic agent in the formulation is tailored to drive the formation of the matrix at the temperature of administration. For example, higher protein concentrations help drive the formation of the matrix, and the protein concentration needed for this purpose varies depending on the ELP series used. For example, in embodiments using an ELP1-120, or amino acid sequences with comparable transition temperatures, the protein is present in the range of about 1 mg/mL to about 200 mg/mL, or is present in the range of about 30 mg/mL to about 150 mg/mL. In embodiments using an ELP4-120, or amino acid sequences with comparable

transition temperatures, the protein is present in the range of about 0.005 mg/mL to about 10 mg/mL, or is present in the range of about 0.01 mg/mL to about 5 mg/mL.

[0208] In some embodiments, the therapeutic agent may be present in the range of about 0.5 mg/mL to about 200 mg/mL, or is present in the range of about 30 mg/mL to about 150 mg/mL. In some embodiments, the therapeutic agent is present in the range of about 50 mg/mL to about 125 mg/mL, or the range of about 75 mg/mL to about 110 mg/mL. In some embodiments, the therapeutic agent is present at a concentration of about 100 mg/mL.

[0209] In some aspects, the disclosure provides a method for delivering a sustained release regimen of an active agent disclosed herein. The method includes administering the pharmaceutical composition described herein to a subject in need, wherein the pharmaceutical composition is administered from about 1 to about 8 times per month. In some embodiments, the pharmaceutical composition is administered about 1 time, about 2 times, about 3 times, and/or about 4 times per month. In some embodiments, the pharmaceutical composition is administered weekly. In some embodiments, the pharmaceutical composition is administered daily. In some embodiments, the pharmaceutical composition is administered from one to three times weekly. In some embodiments, the pharmaceutical composition is administered once every two weeks. In some embodiments, the pharmaceutical composition is administered from one to two times a month. In particular embodiments, the pharmaceutical composition is administered about 1 time per month. In some embodiments, the pharmaceutical composition is administered about once every 2 months, about once every 3 months, about once every 4 months, about once every 5 months, and/or about once every 6 months. The pharmaceutical composition can be packaged in the form of pre-filled pens or syringes for administration once per week, twice per week, or from one to eight times per month, or alternatively filled in conventional vials and the like.

[0210] In some embodiments, the formulation is administered about monthly, and may be administered subcutaneously or intramuscularly. In some embodiments, the formulation is administered about weekly, and may be administered subcutaneously or intramuscularly. In some embodiments, the site of administration is not a pathological site, for example, is not the intended site of action.

[0211] In some embodiments, the pharmaceutical compositions disclosed herein are administered chronically. In some embodiments, the pharmaceutical compositions disclosed herein are administered for about 6 months, for about 7 months, for about 8 months, for about 9

months, for about 10 months, for about 11 months, for about 1 year, for about 2 years, for about 3 years, for about 4 years, for about 5 years, for about 10 years or more. The pharmaceutical compositions may be administered at any required dose and/or frequency disclosed herein.

[0212] In some embodiments, the pharmaceutical compositions disclosed herein are administered until disease or disorder symptoms improve. In some embodiments, the pharmaceutical compositions disclosed herein are administered until disease or disorder symptoms are ameliorated, delayed, and/or cured.

[0213] In some embodiments, the pharmaceutical compositions disclosed herein are administered before the patient begins to exhibit one or more disease or disorder symptoms. In some embodiments, the pharmaceutical compositions disclosed herein are administered at the onset of disease or disorder symptoms.

[0214] The therapeutic agent is formulated generally for “systemic delivery,” meaning that the agent is not delivered locally to a pathological site or a site of action. Instead, the agent is absorbed into the bloodstream from the injection site, where the agent acts systemically or is transported to a site of action via the circulation. The therapeutic agent may be administered by any known route, such as for example, orally, intravenously, intramuscularly, nasally, subcutaneously, intra-vaginally, and intra-rectally. In some embodiments, the formulation is generally for subcutaneous administration. In some embodiments, the pharmacokinetic (PK) parameters are prolonged when the agent is administered subcutaneously. In some embodiments, the half-life of the fusion protein is prolonged. In some embodiments, the PK parameters when the agent is administered subcutaneously are prolonged compared with the agent administered by other means (e.g. intravenously). In some embodiments, the depot of the agent is prolonged when the agent is administered subcutaneously compared with the agent administered by other means (e.g. intravenously). By providing a slow absorption from the injection site, renal clearance and degradation can be controlled, thereby achieving the desired PK profile.

[0215] Advantageously, the compositions provide for prolonged pharmacokinetic exposure due to sustained release of the active agent. In particular aspects, the maximal exposure level may be achieved at about 10 hours, about 24 hours, about 48 hours or about 72 hours after administration; typically the maximum exposure level is achieved between about 10 hours and about 48 hours after administration. After the maximal exposure level is achieved the compositions may achieve a sustained rate of release whereby a substantial percentage of the

maximal level is obtained for a period of time. For example, the sustained rate may about 50%, about 60%, about 70%, about 80%, about 90% or about 100% of the maximal exposure level. Exemplary periods of time for maintaining the sustained rate are about 3 days, about 4 days, about 5 days, about 6 days, about 1 week, about 2 weeks, about 4 weeks, about 6 weeks, or about 8 weeks, after the maximal exposure level is achieved. Subsequently, the sustained rate may lower to a reduced exposure rate. Such reduced exposure rates may be about 5%, about 10%, about 20%, about 30%, about 40%, about 50% or about 60% of the maximal exposure level. For example, in one embodiment (PE0256) a maximal exposure level of 1000 ng/mL is obtained within about 1-2 days. After this period, a sustained rate of about 70-100% of the maximal exposure level is maintained until about days 10-12 whereupon a reduced exposure rate from about 60% decreasing down to about 10% is obtained for the remainder of the study.

[0216] In various embodiments, the plasma concentration of the active agent does not change by more than a factor of about 20, or a factor of about 10, or a factor of about 5, or a factor of about 3 over the course of a plurality of administrations, such as at least 2, at least about 5, or at least about 10 administrations of the formulation. In some embodiments, the plasma concentration of the active agent does not change by more than a factor of about 20, or a factor of about 10, or a factor of about 5, or a factor of about 3 between each administration. In some embodiments, there is some accumulation until steady state is reached (e.g. after about 3 to about 4 administrations). The administrations are substantially evenly spaced, such as, for example, about daily, or about once per week, or from one to about five times per month, or about once every two months, or about once every three months. In other embodiments, the dose may be steadily increased over several administrations, so steady state is reached after 5 or more administrations.

[0217] The pharmaceutical compositions disclosed herein may be administered in smaller doses and/or less frequently than unfused or unconjugated counterparts. While one of skill in the art can determine the desirable dose in each case, a suitable dose of the therapeutic agent for achievement of therapeutic benefit, may, for example, be in a range of about 1 microgram (μg) to about 100 milligrams (mg) per kilogram body weight of the recipient per dose, preferably in a range of about 10 μg to about 50 mg per kilogram body weight per dose and most preferably in a range of about 10 μg to about 50 mg per kilogram body weight per dose. In some embodiments, the pharmaceutical composition is administered at a low dose. In some embodiments, the

pharmaceutical composition is administered at a dose between 1 mg per kilogram per body weight per dose to about 9 mg per kilogram per body weight per dose. In some embodiments, the pharmaceutical composition is administered at about 1 mg per kilogram body weight per dose, about 3 mg per kilogram body weight per dose, and/or about 9 mg per kilogram body weight per dose. The desired dose may be presented as one dose or two or more sub-doses administered at appropriate intervals throughout the day. These sub-doses can be administered in unit dosage forms, for example, containing from about 10 μ g to about 1000 mg, preferably from about 50 μ g to about 500 mg, and most preferably from about 50 μ g to about 250 mg of active ingredient per unit dosage form. Alternatively, if the condition of the recipient so requires, the doses may be administered as a continuous infusion.

[0218] In certain embodiments, the subject is a human, but in other embodiments may be a non-human mammal, such as a domesticated pet (*e.g.*, dog or cat), or livestock or farm animal (*e.g.*, horse, cow, sheep, or pig).

[0219] It should be understood that singular forms such as “a,” “an,” and “the” are used throughout this application for convenience, however, except where context or an explicit statement indicates otherwise, the singular forms are intended to include the plural. All numerical ranges should be understood to include each and every numerical point within the numerical range, and should be interpreted as reciting each and every numerical point individually. The endpoints of all ranges directed to the same component or property are inclusive, and intended to be independently combinable.

[0220] The term “about” when used in connection with a referenced numeric indication means the referenced numeric indication plus or minus up to 10% of that referenced numeric indication. For example, the language “about 50” covers the range of 45 to 55.

[0221] As used herein, the word “include,” and its variants, is intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that may also be useful in the materials, compositions, devices, and methods of this technology. Similarly, the terms “can” and “may” and their variants are intended to be non-limiting, such that recitation that an embodiment can or may comprise certain elements or features does not exclude other embodiments of the present technology that do not contain those elements or features. Although the open-ended term “comprising,” as a synonym of terms such as including, containing, or having, is used herein to describe and claim the disclosure, the present technology, or embodiments

thereof, may alternatively be described using more limiting terms such as “consisting of” or “consisting essentially of” the recited ingredients.

[0222] As used herein, "half-life" (which generally refers to in vivo half-life or circulatory half-life) is the period of time that is required for a 50% diminution of bioactivity of the active agent to occur. In some embodiments, this term includes both prolonged exposure and a long half-life (e.g. both a slow uptake from the injection site and retardation of clearance compared to the unconjugated peptide).

[0223] Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present disclosure, the preferred methods and materials are described herein.

[0224] Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present disclosure, the preferred methods and materials are described herein.

[0225] This disclosure is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1 – Preparation of CNP ELP constructs

[0226] DNA encoding a 37 amino acid version of the CNP sequence was synthesized, digested with restriction enzymes *Bgl*II/*Eco*RI, and then sub-cloned into plasmid pPE0003 to provide plasmid pPE0493, placing the CNP sequence on the C-terminus of the ELP1-120 sequence.

[0227] DNA encoding a 37 amino acid version of the CNP sequence was synthesized, digested with restriction enzymes *Xba*I and *Bsr*GI and then sub-cloned into plasmid pPE0003 cut with *Xba*I and *Acc*65I to provide plasmid pPE0531, placing the CNP sequence on the N-terminus of the ELP1-120 sequence.

[0228] DNA encoding a 37 amino acid version of the CNP sequence was synthesized to include a glycine and serine repeat linker (Gly-Gly-Ser-Gly-Gly-Ser). This was digested with restriction enzymes *XbaI* and *BsrGI* and then sub-cloned into plasmid pPE0003 cut with *XbaI* and *Acc65I* to provide plasmid pPE0552, placing the CNP sequence on the N-terminus of the ELP1-120 sequence with linker positioned between the two.

[0229] DNA encoding a 22 amino acid version of the CNP sequence was synthesized, digested with restriction enzymes *BglII* and *EcoRI*, and then sub-cloned into plasmid pPE0003 to provide plasmid pPE0514, placing the CNP sequence on the C-terminus of the ELP1-120 sequence.

[0230] DNA encoding a 22 amino acid version of the CNP sequence was synthesized, digested with restriction enzymes *XbaI* and *BsrGI* and then sub-cloned into plasmid pPE0003 cut with *XbaI* and *Acc65I* to provide plasmid pPE0550, placing the CNP sequence on the N-terminus of the ELP1-120 sequence.

[0231] DNA encoding a 22 amino acid version of the CNP sequence was synthesized which includes a glycine and serine repeat linker (Gly-Gly-Ser-Gly-Gly-Ser). This was digested with restriction enzymes *XbaI* and *BsrGI* and then sub-cloned into plasmid pPE0003 cut with *XbaI* and *Acc65I* to provide plasmid pPE0565, placing the CNP sequence on the N-terminus of the ELP1-120 sequence with linker positioned between the two

Example 2 – Potency determination of CNP constructs

[0232] Potency of purified CNP constructs described in **Example 1** was demonstrated utilizing B-type natriuretic receptor (NPRB) expressing primary uterine fibroblast cells and a cGMP Fluorescent Assay Kit (CatchPoint, Molecular Devices, Sunnyvale, CA). When the receptor becomes activated, it causes generation of cGMP within the cells. These cells are lysed and the amount of cGMP is detected via a competitive immunoassay for cGMP. The cGMP in each sample competes with a horse radish peroxidase (HRP)-labeled cGMP conjugate for binding sites on the anti-cGMP antibodies. In the absence of cGMP, most of the conjugate is bound to the antibody. Increasing concentration of cGMP competitively decreases the amount of bound conjugate, decreasing measured HRP activity.

[0233] The day prior to the assay, primary uterine fibroblast cells were plated on a 96-well tissue culture plate and incubated overnight at 37°C, 5% CO₂. The following day, serial dilutions of CNP constructs were prepared in Dulbecco's phosphate-buffered saline (DPBS). The cells were

rinsed with Krebs Ringer Bicarbonate Buffer (KRB) and then incubated at room temperature with 2mM 3-isobutyl-1-methylxanthine (IBMX) in KRB for 10 min to prevent the degradation of cGMP. The serial dilutions of samples were then added to the plate in duplicate and the plate was incubated at 37°C, 5% CO₂ for 40 min. Cell lysis buffer was then added to each well to lyse the cells and release the cGMP. These samples were then transferred to the cGMP assay plate. Both anti-cGMP antibody and HRP-cGMP were added to the assay plate and incubated at room temperature for 2 hr. The plate was then washed with cGMP wash buffer. Following the wash, stoplight red substrate was added to each well and the plate was incubated for 1 hr at room temperature. The plate was then read on a fluorescence plate reader with 530 nm excitation, 590 nm emission, and 570 nm cutoff.

[0234] The constructs in which the CNP was positioned at the N-terminus of the fusion protein were more potent than those in which the CNP was positioned at the C-terminus. The most potent construct was PE0552, which was approximately 30-fold less potent than the CNP peptide. See **Figure 1A-B**.

[0235] The PE0552 construct was injected subcutaneously three times per week in normal mice (strain FVB/nj) for up to five weeks. The mice were 3-5 weeks of age at the start of the experiment. The effect on linear growth of the mice was determined by measuring tail length, nose to anal length and nose to tail length after each week of dosing in comparison to a control group injected with normal saline. **Figure 2A-C** shows that both dose levels of PE0552 resulted in a faster rate of linear growth.

Example 3 – Preparation of protease-cleavable CNP ELP constructs

[0236] Five different genes were synthesized each containing a 37 amino acid version of the CNP sequence and one of five different protease cleavage sites (**Table 4**).

Protease	Cleavage site	Construct	SEQ ID NOs:
Factor Xa	Ile-Glu-Gly-Arg/	PE9206	121 & 122
Thrombin	Leu-Val-Pro-Arg / Gly-Ser	PE9216	123 & 124
Cathepsin K site 1	Arg-Lys-Pro-Arg / Gly	PE9306	125 & 126
Cathepsin K site 2	Arg-Lys-Leu-Arg / Gly	PE9316	127 & 128
Matrix metalloprotease consensus	Pro-Leu-Gly / Leu-Trp-Ala-Gly	PE9326	129 & 130

[0237] Each gene sequence was digested with restriction enzymes *XbaI/BsrGI*, and then sub-cloned into plasmid pPE0003 digested with *XbaI/Acc65i* to provide plasmids pPE9206, pPE9216, pPE9306, pPE9316, and pPE9326. These fusions place the CNP sequence on the N-terminus of the ELP1-120 sequence with the different protease-sensitive site between the two to enable cleavage to release the CNP sequence *in vivo*.

[0238] Potency of purified CNP constructs described in **Example 3** was demonstrated utilizing B-type natriuretic receptor (NPRB) expressing primary uterine fibroblast cell as described in **Example 2**. Pretreatment of the protease-sensitive CNP ELP constructs increased potency compared to untreated construct due to liberation of the CNP moiety.

[0239] The above constructs were injected daily in normal mice (strain FVB/nj) for three weeks. The mice were 3 weeks of age at the start of the experiment. The effect on linear growth of the mice was determined by measuring tail length, nose to anal length and nose to tail length after each week of dosing in comparison to a control group injected with normal saline. **Figure 3A-C** shows that only PE9306 resulted in a faster rate of linear growth. There was no significant effect on body weight.

[0240] Additional exemplary constructs were made comprising CNP53 at the N-terminus linked to ELP1-120 via a cathepsin K cleavage site linker (PE9446; SEQ ID NOs: 111 and 112), CNP22 at the N-terminus linked to ELP1-120 via a cathepsin K cleavage site linker (PE9456; SEQ ID NOs: 113 and 114), CNP37 at the C-terminus linked to ELP1-120 via a cathepsin K cleavage site linker (PE9486; SEQ ID NOs: 115 and 116), CNP53 at the C-terminus linked to ELP1-120 via a cathepsin K cleavage site linker (PE9496; SEQ ID NOs: 117 and 118), and CNP22 at the C-terminus linked to ELP1-120 via a cathepsin K cleavage site linker (PE9506; SEQ ID NOs: 119 and 120).

Example 4 -- Preparation of GLP-2 ELP construct

[0241] DNA encoding the GLP-2 sequence was synthesized to incorporate the A2G mutation for increased DPP-IV resistance. This was digested with restriction enzymes *XbaI* and *BsrGI* and then sub-cloned into plasmid pPE0003 cut with *XbaI* and *Acc65I* to provide plasmid pPE0503. The fusion places the GLP-2 sequence on the N-terminus of the ELP1-120 sequence.

[0242] Purified PE0503 protein was analysed in a cell-based cAMP potency assay and shown to have an EC₅₀ of 1.75 nM, compared to 0.24 nM for GLP-2 peptide.

Example 5 -- Evaluation of GLP-2 ELP construct in vivo

[0243] Male Sprague Dawley rats (200 – 220 g, n = 12/group) were injected subcutaneously with PE0503 or GLP-2 (A2G) peptide over an eleven-day dosing period as shown in **Table 5**.

Table 5

Compound	Dosing frequency	Dosing days	Dose (mg/kg/dose)	Dose (nmol/kg/dose)
Saline	Daily	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	NA	NA
PE0503	Daily	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	1.3	25
PE0503	Every other day	1, 3, 5, 7, 9, 11	5.1	100
PE0503	Every fourth day	1, 5, 9	20.5	400
GLP-1 (A2G)	Twice a day	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	0.1	25

[0244] The animals were sacrificed on day 12 and a mid-line incision was made, the small intestines were removed, stretched to their maximum length and measured. The fecal material was

then flushed from the lumen and the small intestine weight was recorded. **Figure 4** shows that treatment with PE0503 resulted in a highly significant increase in small intestine weight compared to placebo, even when dosed every fourth day (Q4D). Histological analysis of sections of the small intestine indicated a clear increase in villus height compared to vehicle (saline), as expected for a GLP-2 receptor agonist. Since the half-life is significantly longer in humans, the data verify that PE0503 is suitable for dosing once per week or less in humans.

Example 6 — Preparation of FGF21 ELP constructs

[0245] The FGF21 gene sequence was synthesized, digested with restriction enzymes *BglI/EcoRI*, and then sub-cloned into plasmid pPE0003 to provide plasmid pPE9183 (SEQ ID NOs: 131 and 132), placing the FGF21 sequence on the C-terminus of the ELP1-120 sequence.

[0246] The FGF21 gene sequence was synthesized, digested with restriction enzymes *BglI/EcoRI*, and then sub-cloned into plasmid pPB1023 to provide plasmid pPE9193 (SEQ ID NOs: 133 and 134), placing the FGF21 sequence on the C-terminus of the ELP1-120 sequence. This creates a dual agonist with GLP-1 on one end of the ELP polymer and FGF21 on the other.

INCORPORATION BY REFERENCE

[0247] All patents and publications referenced herein are hereby incorporated by reference in their entireties, including the publications disclosed below.

[0248] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure.

[0249] This application incorporates by reference the following publications and applications in their entireties for all purposes: US 2001/0034050 A1; US 2009/0220455; US 8,334,257; US 2013/0310538; US 2013/0172274; US 2011/0236384; US 6,582,926; US 7,429,458; US 7,364,859; US 8,178,495; US 2013/0079277; US 2013/0085099; US 2013/0143802; US 2014/0024600; US 2011/0178017; US 7,709,227; US 2011/0123487; US 8,729,018; US 2014/0171370; US 2013/0150291; WO/2014/113434; US 2014/0213516,

PCT/US2015/061955; and US Provisional Application Nos. 62/113,943, 62/145,770, and 62/150,679.

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CLAIMS

What is claimed is:

1. A sustained release pharmaceutical formulation comprising:
a therapeutic agent for systemic administration, the therapeutic agent comprising a protein active agent and at least 60 elastin-like peptide (ELP) structural units selected from any one of SEQ ID NOs: 1-13,
wherein the protein active agent is selected from the group consisting of apelin, arginase, C-type natriuretic peptide (CNP), a Glucagon-like peptide (GLP)-1 receptor antagonist, a Glucagon-like peptide (GLP)-2 receptor agonist, hepcidin, insulin-like growth factor-1 (IGF-1), urodilatin, thymosin β 4, TNF-related apoptosis-inducing ligand (TRAIL), FGF21, or fragments, or derivatives thereof.
2. The pharmaceutical formulation of claim 1, wherein the formulation provides slow absorption from an injection site upon administration.
3. The pharmaceutical formulation of claim 2, wherein the formulation provides a flat PK profile upon administration, as compared to the PK profile for the active agent in the absence of the amino acid sequence forming a reversible matrix.
4. The pharmaceutical formulation of claim 3, wherein the PK profile has a low peak to trough (C_{max} to C_{min}) and delayed or late T_{max} .
5. The pharmaceutical formulation of any one of claims 1 to 4, wherein a reversible matrix formed at body temperature reverses as protein concentration decreases.
6. The pharmaceutical formulation of claim 1, wherein the ELP comprises SEQ ID NO: 3 wherein X is selected from Val, Gly, and Ala.
7. The pharmaceutical formulation of claim 6, where each X is selected from V, G, and A, and wherein the ratio of V:G:A is selected from the group consisting of

- a) 5:3:2;
 - b) 7:2:0;
 - c) 7:0:2;
 - d) 6:0:3;
 - e) 5:2:2; and
 - f) 10:0:0.
8. The pharmaceutical formulation of claim 1, wherein the ELP comprises SEQ ID NO: 13 wherein X is selected from Val, Gly, and Ala.
9. The pharmaceutical formulation of claim 8, where each X is selected from V, G, and A, and wherein the ratio of V:G:A may be about 5:0:4.
10. The pharmaceutical formulation of claim 6, wherein the ELP comprises 40 to 180 repeating units of VPGXG (SEQ ID NO: 3), where each X is selected from V, G, and A, and wherein the ratio of V:G:A is selected from the group consisting of:
- a) 5:3:2;
 - b) 7:2:0;
 - c) 7:0:2;
 - d) 6:0:3; and
 - e) 5:2:2.
11. The pharmaceutical formulation of claim 10, wherein X is selected from Val, Gly, and Ala at a ratio of about 6:0:3.
12. The pharmaceutical formulation of claim 8, wherein the ELP comprises 40 to 180 repeating units of XPGVG (SEQ ID NO: 13), where each X is selected from V, G, and A, and wherein the ratio of V:G:A may be about 5:0:4.
13. The pharmaceutical formulation of any one of claims 1 to 12, wherein the subject is human.

14. The pharmaceutical formulation of any one of claims 1 to 12, wherein the subject is a non-human mammal.
15. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is a recombinant fusion protein between the protein active agent and ELP.
16. The pharmaceutical formulation of claim 1, wherein the protein active agent has a circulatory half-life in the range of from about 30 seconds to about 10 hours, or about 30 seconds to about 1 hour.
17. The pharmaceutical formulation of claim 16, wherein the formulation is a co-formulation comprising at least two of apelin, arginase, CNP, a GLP-1 receptor antagonist, a GLP-2 receptor agonist, hepcidin, IGF-1, urodilatin, thymosin β 4, and TRAIL, FGF21, or fragments, or derivatives thereof.
18. The pharmaceutical formulation of any one of claims 1 to 14 and 16 to 17, wherein the therapeutic agent is a chemical conjugate between the protein active agent and ELP.
19. The pharmaceutical formulation of any one of claims 1 to 18, wherein the therapeutic agent is present in the range of about 0.5 mg/mL to about 200 mg/mL.
20. The pharmaceutical formulation of claim 19, wherein the therapeutic agent is present in the range of about 30 mg/mL to about 150 mg/mL.
21. The pharmaceutical formulation of claim 20, wherein the therapeutic agent is present in the range of about 50 mg/mL to about 125 mg/mL, or about 75 mg/mL to about 110 mg/mL.
22. The pharmaceutical formulation of claim 21, wherein the therapeutic agent is present in the amount of about 100 mg/mL.

23. The pharmaceutical composition of any one of claims 1 to 22, wherein the therapeutic agent does not form a phase-transitioned matrix at storage conditions.
24. The pharmaceutical composition of claim 23, wherein the storage conditions are less than about 40°C, or less than about 37°C, less than about 30°C, less than about 27°C, less than about 25°C, less than about 0°C, less than about -15°C, or less than about -60°C.
25. The pharmaceutical formulation of claim 24, wherein the formulation is stable for more than 1 month at the storage conditions.
26. The pharmaceutical formulation of claim 25, wherein the formulation is stable for more than about 1 month at a temperature selected from the group consisting of:
- a. about 25°C;
 - b. about 2°C;
 - c. about 8°C;
 - d. about -15°C; and
 - e. about -80°C.
27. The pharmaceutical formulation of any of claims 1-26, wherein the formulation comprises two or more of calcium chloride, magnesium chloride, potassium chloride, potassium phosphate monobasic, sodium chloride, polysorbate 20, polysorbate 80, sodium phosphate, sodium phosphate monobasic, histidine, and sodium phosphate dibasic.
28. The pharmaceutical formulation of claim 27, wherein the formulation comprises sodium phosphate, sodium chloride and polysorbate 20.
29. The pharmaceutical formulation of claim 27, wherein the formulation comprises sodium chloride and histidine.
30. The pharmaceutical formulation of claim 28, wherein the formulation comprises 10 mM sodium phosphate, 110 mM sodium chloride, and 0.1% polysorbate 20.

31. The pharmaceutical formulation of any one of claims 1 to 30, wherein the formulation is packaged in the form of pre-dosed pens or syringes for administration about once per week, about twice per week, or from one to eight times per month.
32. A sustained release pharmaceutical formulation comprising:
a therapeutic agent, the therapeutic agent comprising a protein active agent and an ELP amino acid sequence comprising 60 to 180 repeating units of VPGXG (SEQ ID NO: 3) or XPGVG (SEQ ID NO: 13), where each X is selected from V, G, and A, and wherein the ELP exhibits a transition temperature between 26°C and 37°C,
wherein the protein active agent is apelin, arginase, C-type natriuretic peptide (CNP), a Glucagon-like peptide (GLP)-1 receptor antagonist, a Glucagon-like peptide (GLP)-2 receptor agonist, hepcidin, Insulin-like growth factor-1 (IGF-1), urodilatin, Thymosin β 4, TNF-related apoptosis-inducing ligand (TRAIL), FGF21, or fragments, or derivatives thereof.
33. The pharmaceutical formulation of claim 32, wherein the formulation provides slow absorption from an injection site upon administration.
34. The pharmaceutical formulation of claim 33, wherein the formulation provides a flat PK profile upon administration, as compared to the PK profile for the active agent in the absence of said amino acid sequence.
35. The pharmaceutical formulation of claim 33, wherein the PK profile has a low peak to trough ($C_{\max}$ to $C_{\min}$) and delayed or late $T_{\max}$.
36. The pharmaceutical formulation of any one of claims 32 to 35, wherein a reversible matrix formed at body temperature reverses as protein concentration decreases.
37. The pharmaceutical formulation of any one of claims 32 to 36, wherein V, G, and A are at a ratio selected from the group consisting of
a) 5:3:2;

- b) 7:2:0;
- c) 7:0:2;
- d) 6:0:3;
- e) 5:2:2; and
- f) 5:0:4.

38. The pharmaceutical formulation of claim 32, wherein the ELP comprises an ELP unit comprising at least 60 repeating units of VPGXG (SEQ ID NO: 3), where each X is V.
39. The pharmaceutical formulation of claim 32, wherein the therapeutic agent is a recombinant fusion protein between the protein active agent and said amino acid sequence.
40. The pharmaceutical formulation of claim 32, wherein the protein active agent has a circulatory half-life in the range of from about 30 seconds to about 10 hours, or about 30 second to about 1 hour.
41. The pharmaceutical formulation of claim 32, wherein the protein active agent is selected from the group consisting of apelin, arginase, CNP, a GLP-1 receptor antagonist, a GLP-2 receptor agonist, hepcidin, IGF-1, urodilatin, thymosin β 4, TRAIL, FGF21, or fragments, or derivatives thereof.
42. The pharmaceutical formulation of claim 32, wherein the formulation is a co-formulation comprising at least two of apelin, arginase, CNP, a GLP-1 receptor antagonist, a GLP-2 receptor agonist, hepcidin, IGF-1, urodilatin, thymosin β 4, TRAIL, FGF21, or fragments or derivatives thereof.
43. The pharmaceutical formulation of any one of claims 32 to 38 and 40 to 42, wherein the therapeutic agent is a chemical conjugate between the protein active agent and said ELP amino acid sequence.

44. The pharmaceutical formulation of any one of claims 32 to 43, wherein the therapeutic agent is present in the range of about 0.5 mg/mL to about 200 mg/mL.
45. The pharmaceutical formulation of claim 44, wherein the therapeutic agent is present in the range of about 30 mg/mL to about 150 mg/mL.
46. The pharmaceutical formulation of claim 45, wherein the therapeutic agent is present in the range of about 50 mg/mL to about 125 mg/mL, or the range of about 75 mg/mL to about 110 mg/mL.
47. The pharmaceutical formulation of claim 46, wherein the therapeutic agent is present in the range of about 100 mg/mL.
48. The pharmaceutical composition of any one of claims 32 to 47, wherein the therapeutic agent does not form a matrix at storage conditions.
49. The pharmaceutical composition of claim 48, wherein the storage conditions are less than about 40°C, or less than about 37°C, or less than about 30°C, less than about 27°C, or less than about 25°C, less than about 0°C, less than about -15°C, or less than about -60°C.
50. The pharmaceutical formulation of any one of claims 32-49, wherein the formulation comprises two or more of calcium chloride, magnesium chloride, potassium chloride, potassium phosphate monobasic, sodium chloride, sodium chloride, polysorbate 20, polysorbate 80, sodium phosphate, sodium phosphate monobasic, histidine and sodium phosphate dibasic.
51. The pharmaceutical formulation of claim 50, wherein the formulation comprises sodium phosphate, sodium chloride and polysorbate 20.
52. The pharmaceutical formulation of claim 50, wherein the formulation comprises sodium chloride and histidine.

53. The pharmaceutical formulation of claim 51, wherein the formulation comprises 10 mM sodium phosphate, 110 mM sodium chloride, and 0.1% polysorbate 20.
54. The pharmaceutical formulation of any one of claims 32 to 53, wherein the formulation is packaged in the form of pre-dosed pens or syringes for administration once per week, twice per week, or from one to eight times per month.
55. A method for delivering a sustained release regimen of a therapeutic agent, comprising, administering the formulation of any one of claims 1 to 54 to a subject in need.
56. The method of claim 55, wherein the therapeutic agent is administered about 1 to about 8 times per month.
57. The method of any one of claims 55 to 56, wherein the formulation is administered about weekly.
58. The method of any one of claims 55 to 57, wherein the formulation is administered subcutaneously or intramuscularly.
59. The method of any one of claims 55 to 58, wherein the site of administration is not a pathological site.
60. The pharmaceutical formulation of claim 1, wherein the protein active agent is human apelin and the ELP comprises at least 60 repeating units of VPGXG (SEQ ID NO:3).
61. The pharmaceutical formulation of claim 60, wherein the ELP is fused to the C terminus of human apelin.
62. The pharmaceutical formulation of claim 60, wherein the ELP is fused to the N terminus of human apelin.

63. The pharmaceutical formulation of claim 60, wherein the human apelin is SEQ ID NO: 39.
64. The pharmaceutical formulation of claim 60, wherein the human apelin is SEQ ID NO: 41.
65. The pharmaceutical formulation of claim 60, wherein the human apelin is SEQ ID NO: 42.
66. The pharmaceutical formulation of claim 60, wherein the human apelin is SEQ ID NO: 43.
67. The pharmaceutical formulation of claim 60, wherein the human apelin is SEQ ID NO: 44.
68. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 40.
69. The pharmaceutical formulation of claim 1, wherein the protein active agent is human arginase and the ELP comprises at least 60 repeating units of VPGXG (SEQ ID NO:3).
70. The pharmaceutical formulation of claim 69, wherein the ELP is fused to the C terminus of human arginase.
71. The pharmaceutical formulation of claim 69, wherein the ELP is fused to the N terminus of human arginase.
72. The pharmaceutical formulation of claim 69, wherein the human arginase is SEQ ID NO: 45.
73. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 46.
74. The pharmaceutical formulation of claim 1, wherein the protein active agent is human CNP and the ELP comprises at least 60 repeating units of VPGXG (SEQ ID NO:3).
75. The pharmaceutical formulation of claim 74, wherein the ELP is fused to the C terminus of human CNP.
76. The pharmaceutical formulation of claim 74, wherein the ELP is fused to the N terminus of human CNP.
77. The pharmaceutical formulation of claim 74, wherein the human CNP is SEQ ID NO: 47.

78. The pharmaceutical formulation of claim 74, wherein the human CNP is SEQ ID NO: 49.
79. The pharmaceutical formulation of claim 74, wherein the human CNP is SEQ ID NO: 51.
80. The pharmaceutical formulation of claim 74, wherein the human CNP is SEQ ID NO: 52.
81. The pharmaceutical formulation of any one of claims 74-80, wherein the CNP and the ELP are separated by a linker containing a protease cleavage site.
82. The pharmaceutical formulation of claim 81, wherein cleavage at said protease cleavage site enables the release of the CNP *in vivo*.
83. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 48.
84. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 83.
85. The pharmaceutical formulation of claim 1, wherein the protein active agent is a human GLP-1 receptor antagonist and the ELP is SEQ ID NO: 14.
86. The pharmaceutical formulation of claim 85, wherein the ELP is fused to the C terminus of human GLP-1 receptor antagonist.
87. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 54.
88. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 64.
89. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 65.
90. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 27.

91. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 28.
92. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 60.
93. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 61.
94. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 62.
95. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 63.
96. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 66.
97. The pharmaceutical formulation of claim 85, wherein the human GLP-1 receptor antagonist is SEQ ID NO: 67.
98. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 57.
99. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 58.
100. The pharmaceutical formulation of any one of claims 1 to 30, wherein the therapeutic agent is SEQ ID NO: 59.
101. The pharmaceutical formulation of claim 1, wherein the protein active agent is human GLP-2 receptor agonist and the ELP comprises at least 60 repeating units of VPGXG (SEQ ID NO:3).

102. The pharmaceutical formulation of claim 101, wherein the ELP is fused to the C terminus of human GLP-2 receptor agonist.
103. The pharmaceutical formulation of claim 101, wherein the ELP is fused to the N terminus of human GLP-2 receptor agonist.
104. The pharmaceutical formulation of claim 101, wherein the human GLP-2 receptor agonist is SEQ ID NO: 68.
105. The pharmaceutical formulation of claim 101, wherein the human GLP-2 receptor agonist is SEQ ID NO: 70.
106. The pharmaceutical formulation of claim 101, wherein the human GLP-2 receptor agonist is SEQ ID NO: 74, wherein X is A, G, L, I, or V.
107. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 69.
108. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 71.
109. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 73.
110. The pharmaceutical formulation of claim 1, wherein the protein active agent is human hepcidin and the ELP comprises at least 60 repeating units of VPGXG (SEQ ID NO:3).
111. The pharmaceutical formulation of claim 110, wherein the ELP is fused to the C terminus of human hepcidin.
112. The pharmaceutical formulation of claim 110, wherein the ELP is fused to the N terminus of human hepcidin.

113. The pharmaceutical formulation of claim 110, wherein the human hepcidin is SEQ ID NO: 75.
114. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 76.
115. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 77.
116. The pharmaceutical formulation of claim 1, wherein the protein active agent is IGF-1 and the ELP comprises at least 60 repeating units of VPGXG (SEQ ID NO:3).
117. The pharmaceutical formulation of claim 1, wherein the protein active agent is human IGF-1 and the ELP comprises at least 60 repeating units of XPGVG (SEQ ID NO: 13).
118. The pharmaceutical formulation of claim 116 or claim 117, wherein the ELP is fused to the C terminus of human IGF-1.
119. The pharmaceutical formulation of claim 116 or claim 117, wherein the ELP is fused to the N terminus of human IGF-1.
120. The pharmaceutical formulation of claim 116 or claim 117, wherein the human IGF-1 is SEQ ID NO: 78.
121. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 79.
122. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 80.
123. The pharmaceutical formulation of claim 1, wherein the protein active agent is human urodilatin and the ELP comprises at least 60 repeating units of VPGXG (SEQ ID NO: 3).

124. The pharmaceutical formulation of claim 124, wherein the ELP is fused to the C terminus of human urodilatin.
125. The pharmaceutical formulation of claim 124, wherein the ELP is fused to the N terminus of human urodilatin.
126. The pharmaceutical formulation of claim 124, wherein the human urodilatin is SEQ ID NO: 84.
127. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 85.
128. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 86.
129. The pharmaceutical formulation of claim 1, wherein the protein active agent is human Thymosin β 4 and the ELP comprises at least 60 repeating units of VPGXG (SEQ ID NO:3).
130. The pharmaceutical formulation of claim 129, wherein the ELP is fused to the C terminus of human Thymosin β 4.
131. The pharmaceutical formulation of claim 129, wherein the ELP is fused to the N terminus of human Thymosin β 4.
132. The pharmaceutical formulation of claim 129, wherein the human Thymosin β 4 is SEQ ID NO: 89.
133. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 90.
134. The pharmaceutical formulation of claim 1, wherein the protein active agent is human TRAIL and the ELP comprises at least 60 repeating units of VPGXG (SEQ ID NO:3).

135. The pharmaceutical formulation of claim 134, wherein the ELP is fused to the C terminus of human TRAIL.
136. The pharmaceutical formulation of claim 134, wherein the ELP is fused to the N terminus of human TRAIL.
137. The pharmaceutical formulation of claim 134, wherein the human TRAIL is SEQ ID NO: 89.
138. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 92.
139. The pharmaceutical formulation of claim 1, wherein the therapeutic agent is SEQ ID NO: 98.
140. A therapeutic protein comprising:
a protein active agent and at least 60 elastin-like peptide (ELP) structural units selected from any one of SEQ ID NOs: 1-13,
wherein the protein active agent is selected from the group consisting of apelin, arginase, C-type natriuretic peptide (CNP), a Glucagon-like peptide (GLP)-1 receptor antagonist, a Glucagon-like peptide (GLP)-2 receptor agonist, hepcidin, insulin-like growth factor-1 (IGF-1), urodilatin, thymosin β 4, TNF-related apoptosis-inducing ligand (TRAIL), FGF21, or fragments, or derivatives thereof.
141. A therapeutic protein comprising:
a therapeutic agent, the therapeutic agent comprising a protein active agent and an ELP amino acid sequence comprising 60 to 180 repeating units of VPGXG (SEQ ID NO: 3) or XPGVG (SEQ ID NO: 13), where each X is selected from V, G, and A, and wherein the ELP exhibits a transition temperature between 26°C and 37°C,
wherein the protein active agent is apelin, arginase, C-type natriuretic peptide (CNP), a Glucagon-like peptide (GLP)-1 receptor antagonist, a Glucagon-like peptide (GLP)-2 receptor agonist, hepcidin, Insulin-like growth factor-1 (IGF-1), urodilatin, Thymosin β 4, TNF-related apoptosis-inducing ligand (TRAIL), FGF21, or fragments, or derivatives thereof.

FIGURES

FIG. 1A Potency of CNP constructs

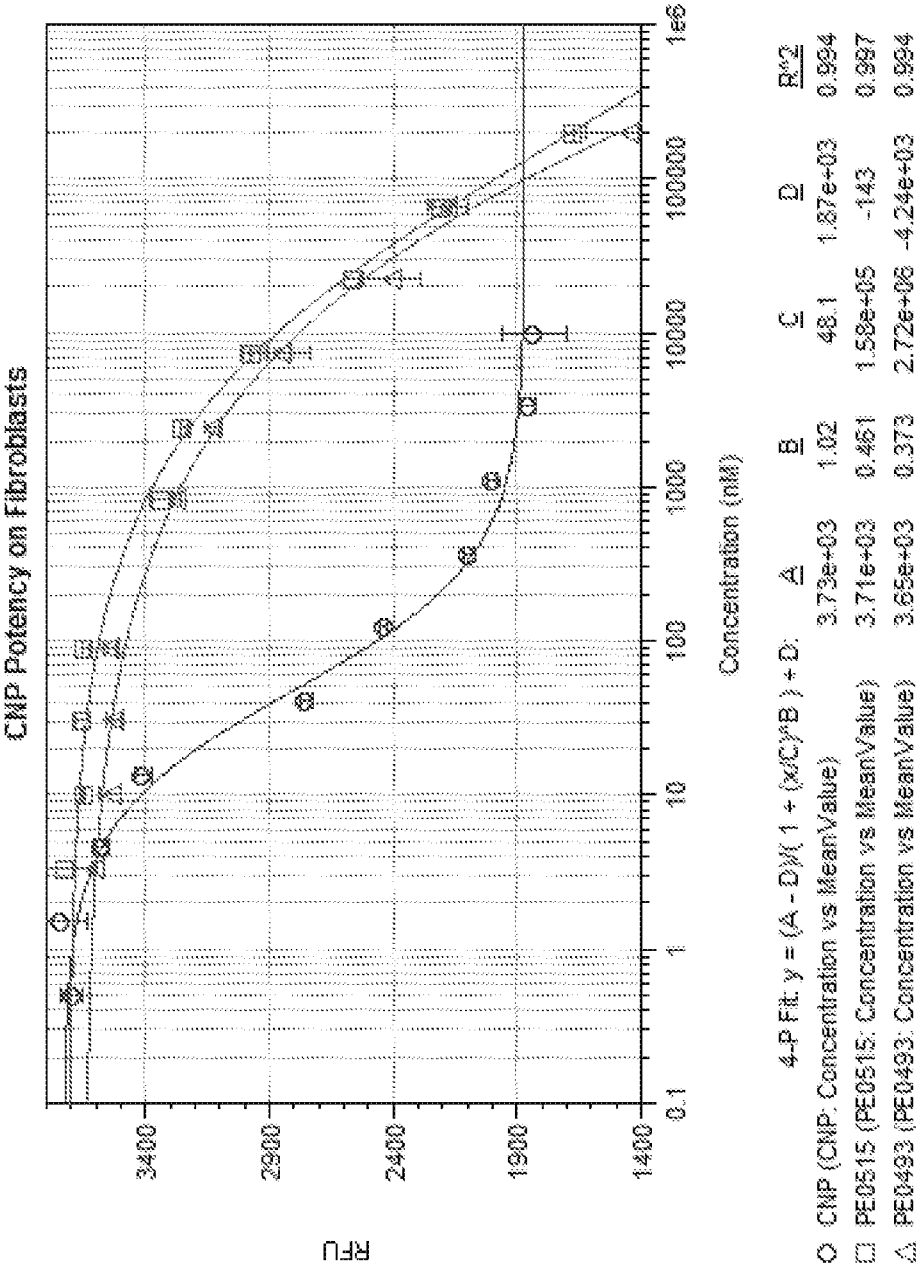
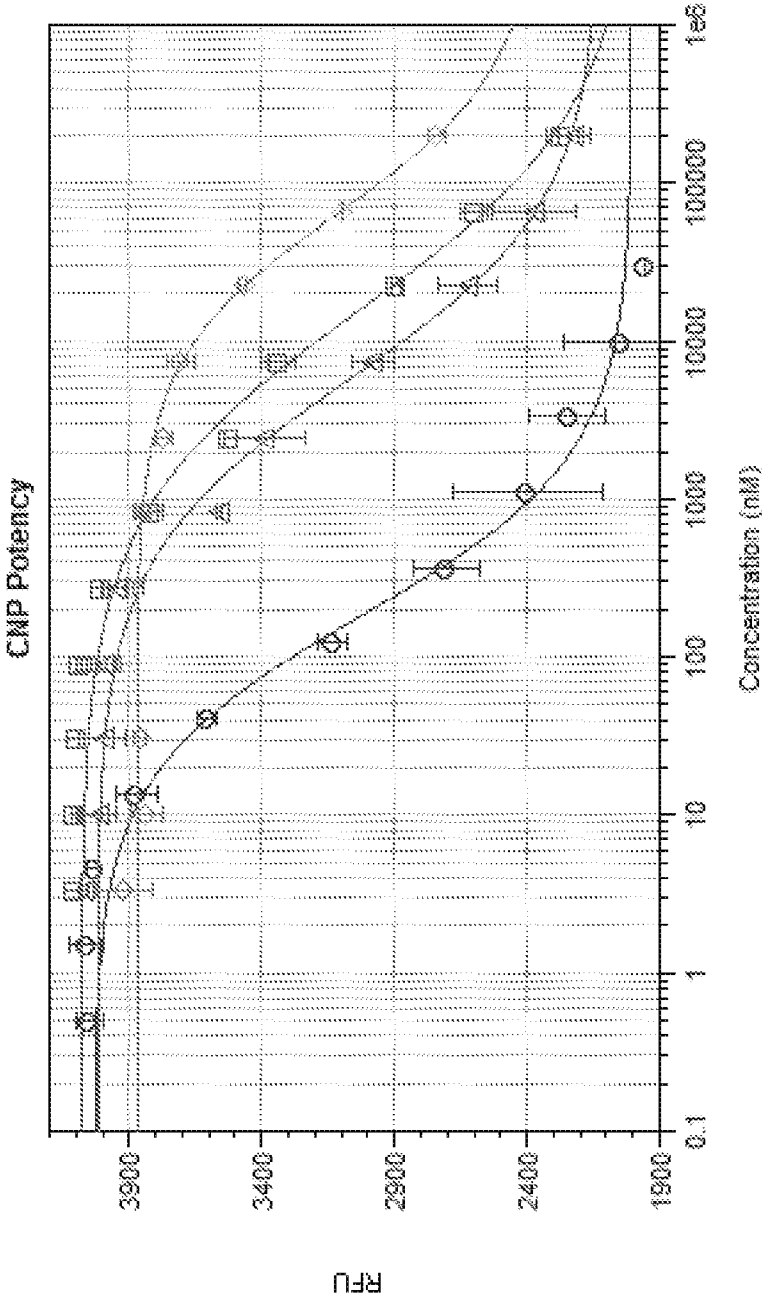


FIG. 1B



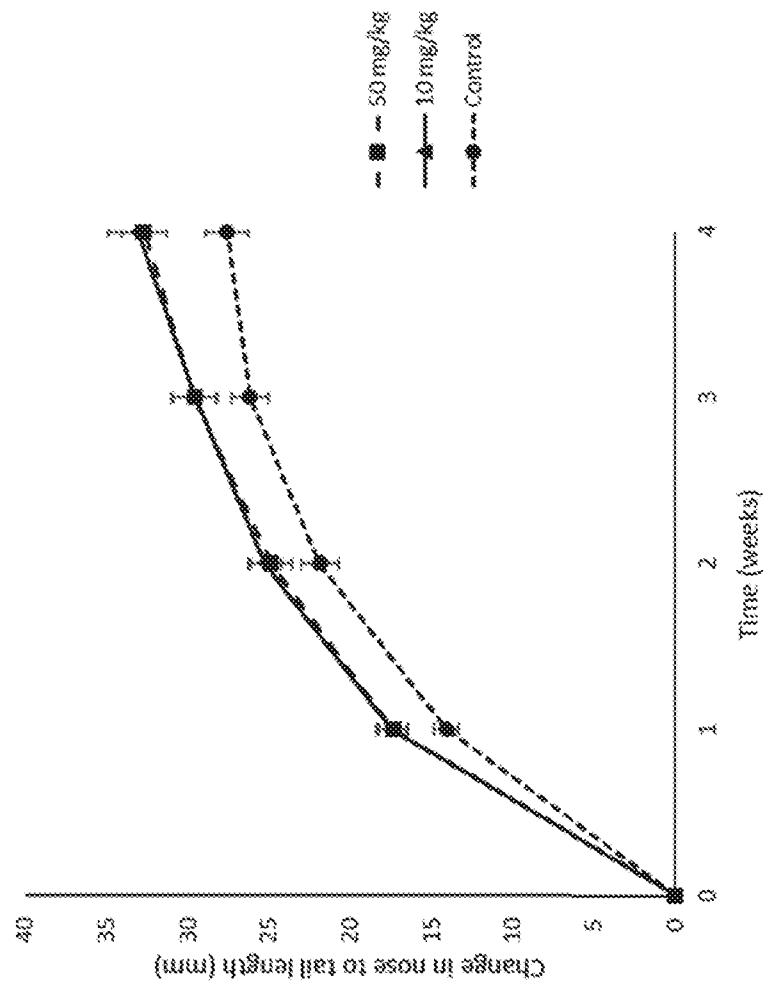


FIG. 2A

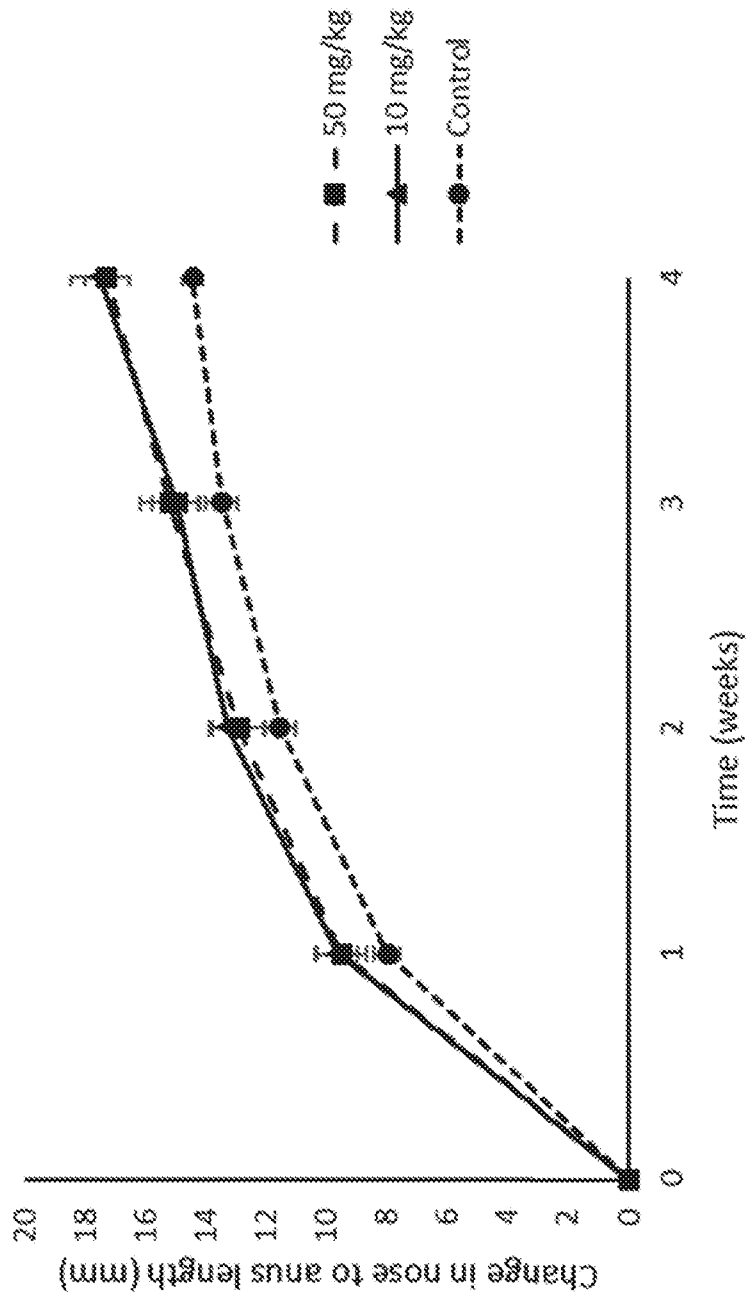


FIG. 2B

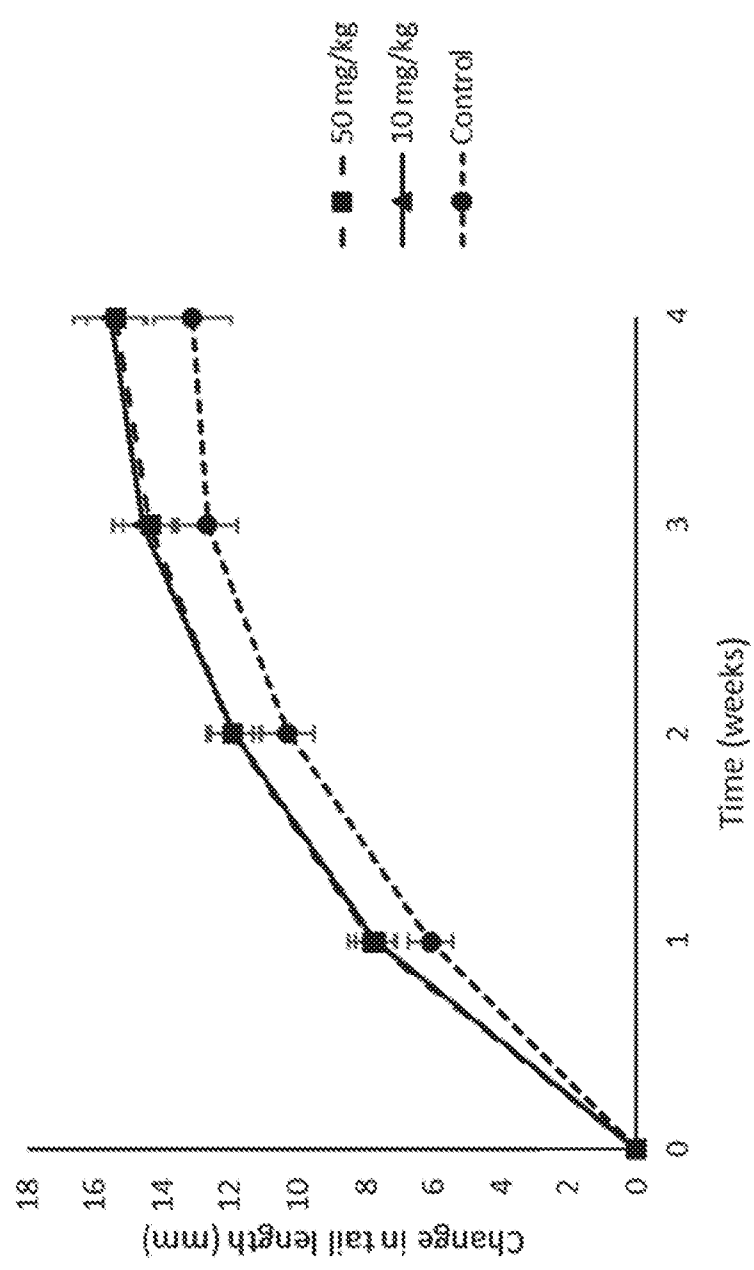


FIG. 2C

Fig 3A

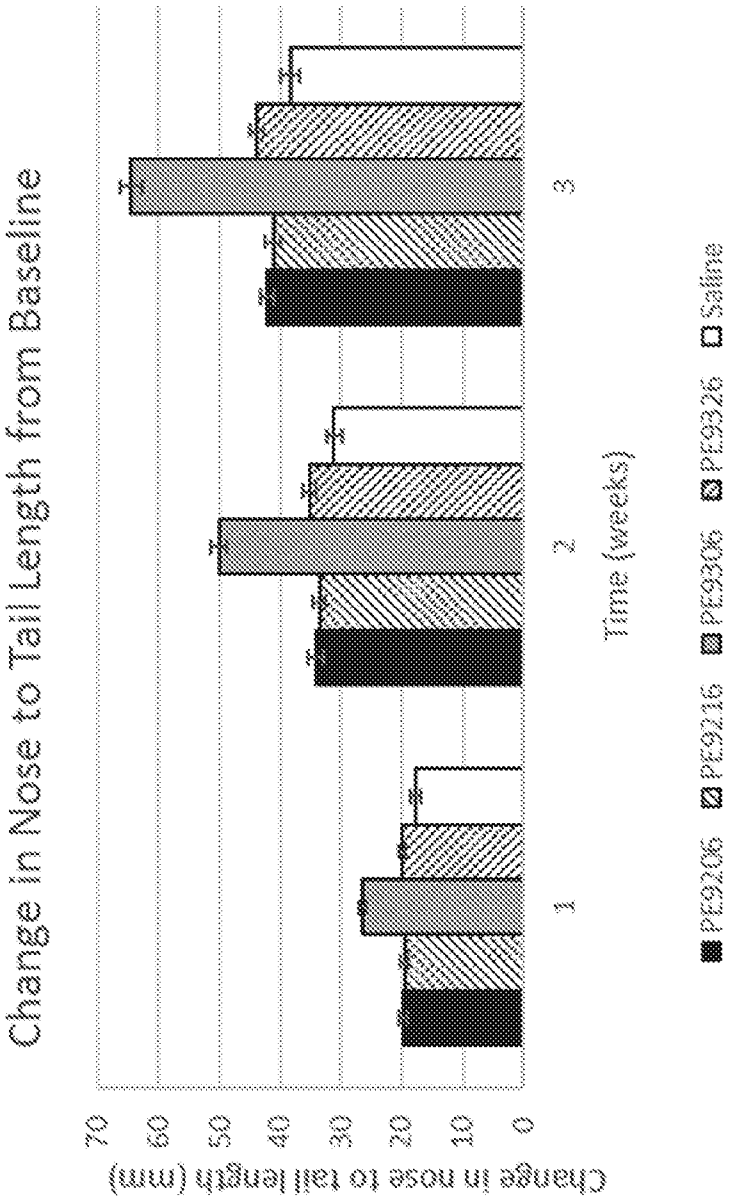


Fig 3B

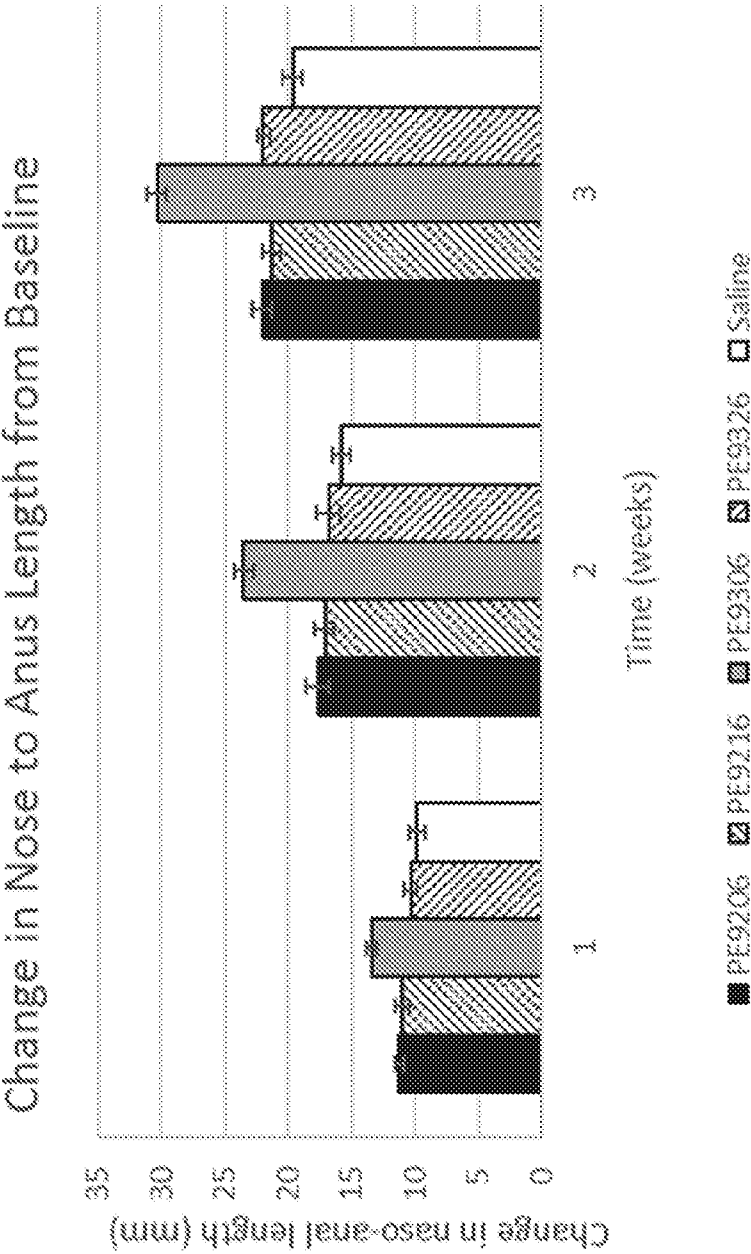


Fig 3C

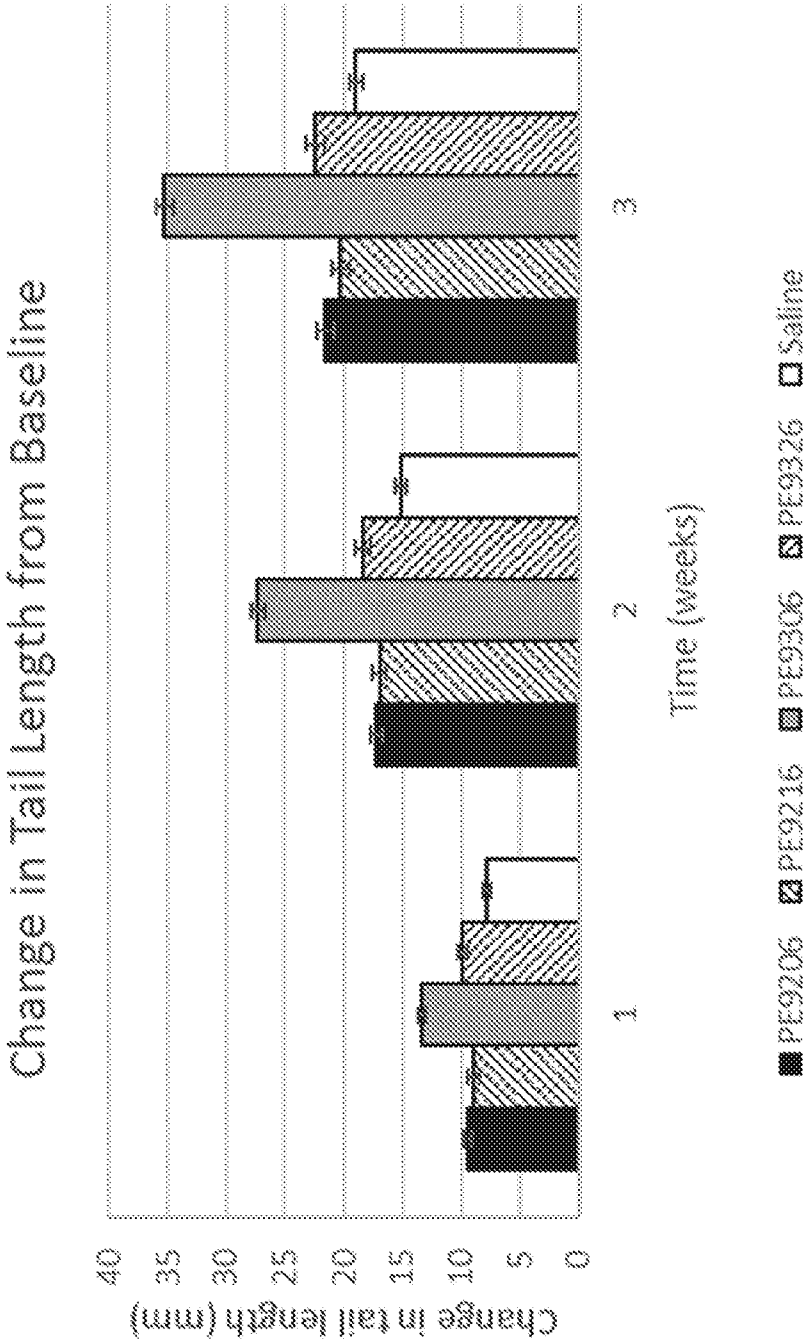
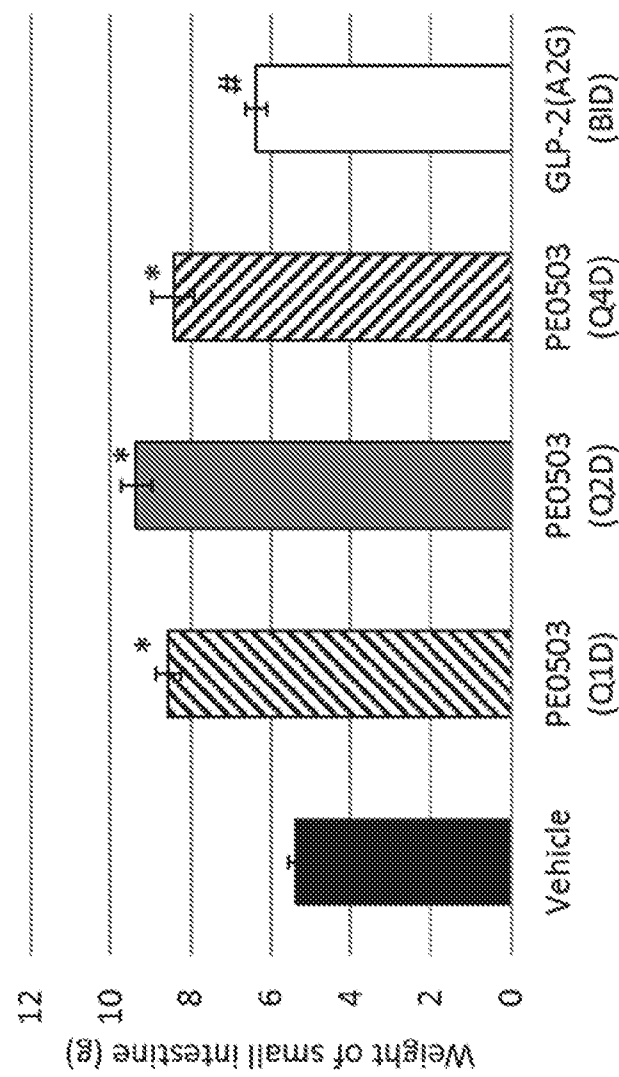


Figure 4. Effect of PE0503 on small intestine weight in normal rats



* = significantly different from vehicle, $p < 0.0001$ by t-test and significantly different from GLP-2(A2G), $p < 0.005$
= significantly different from vehicle, $p < 0.005$ by t-test

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/030403

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - A61K 9/22; A61K 38/00; A61K 47/42 (2017.01)
 CPC - A61K 38/00; A61K 38/26; A61K 47/48292 (2017.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/457; 514/1.1; 514/11.7 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/0290328 A1 (PHASEBIO PHARMACEUTICALS, INC.) 15 October 2015 (15.10.2015) entire document	1-8, 10, 11, 15, 16, 32-36, 38-41, 101, 104, 106, 140, 141
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Y		9, 12, 17, 42, 60-67, 69-72, 74-79, 81, 82, 85-95, 102, 103, 105, 110-113, 116-119, 123-125, 129-131, 134-136
Y	US 5,428,014 A (LABROO et al) 27 June 1995 (27.06.1995) entire document	9, 12
Y	WALKER et al. "Cell Penetrating Peptides Fused to a Thermally Targeted Biopolymer Drug Carrier Improve the Delivery and Antitumor Efficacy of an Acid-sensitive Doxorubicin Derivative," Int J Pharm, 15 October 2012 (15.10.2012), Vol. 436, Pgs. 825-832. entire document	9, 12
Y	US 2011/0039776 A1 (CHILKOTI) 17 February 2011 (17.02.2011) entire document	17, 42, 69-72, 75, 76, 102, 103, 111, 112, 118, 119, 123-125, 130, 131, 135, 136
Y	WO 2004/081198 A2 (THE ARIZONA BOARD OF REGENTS ON BEHALF OF THE UNIVERSITY OF ARIZONA et al) 23 September 2004 (23.09.2004) entire document	60-67



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 August 2017

Date of mailing of the international search report

22 SEP 2017

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 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/030403

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2005/023863 A1 (AVENTIS PHARMA DEUTSCHLAND GMBH et al) 17 March 2005 (17.03.2005) entire document	65, 67
Y	US 2011/0245592 A1 (SCHOOLCRAFT et al) 06 October 2011 (06.10.2011) entire document	72
Y	US 2014/0187746 A1 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 03 July 2014 (03.07.2014) entire document	74-79, 81, 82
Y	WO 2000/053755 A2 (GENENTECH, INC. et al) 14 September 2000 (14.09.2000) entire document	77
Y	WO 2009/067639 A2 (BIOMARIN PHARMACEUTICAL INC. et al) 28 May 2009 (28.05.2009) entire document	78, 79
Y	US 2010/0316623 A1 (TURNER et al) 16 December 2010 (16.12.2010) entire document	81, 82
Y	US 2015/0005233 A1 (SENEB BIOSCIENCES, INC.) 01 January 2015 (01.01.2015) entire document	85-95
Y	WO 2009/099763 A1 (INDIANA UNIVERSITY RESEARCH AND TECHNOLOGY CORPORATION et al) 13 August 2009 (13.08.2009) entire document	87, 88
Y	US 2011/0245173 A1 (BACHOVCHIN et al) 06 October 2011 (06.10.2011) entire document	89
Y	WO 2000/066142 A2 (BIONEBRASKA, INC.) 09 November 2000 (09.11.2000) entire document	90
Y	WO 2012/006598 A2 (DANA-FARBER CANCER INSTITUTE, INC. et al) 12 January 2012 (12.01.2012) entire document	91, 92
Y	CN 102703459 A (CHINA AGRICULTURAL UNIVERSITY) 03 October 2012 (03.10.2012) entire document; see machine translation	93-95
Y	WO 1998/024813 A2 (AMGEN INC. et al) 11 June 1998 (11.06.1998) entire document	105
Y	US 2006/0019339 A1 (LAUTH et al) 26 January 2006 (26.01.2006) entire document	110-113
Y	US 2011/0020342 A1 (GLASS et al) 27 January 2011 (27.01.2011) entire document	116-119
Y	US 2004/0220111 A1 (KLEINMAN et al) 04 November 2004 (04.11.2004) entire document	129-131
Y	US 2012/0016033 A1 (DEGTEREV et al) 19 January 2012 (19.01.2012) entire document	134-136

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/030403

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 13, 14, 18-31, 37, 43-59, 100
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.