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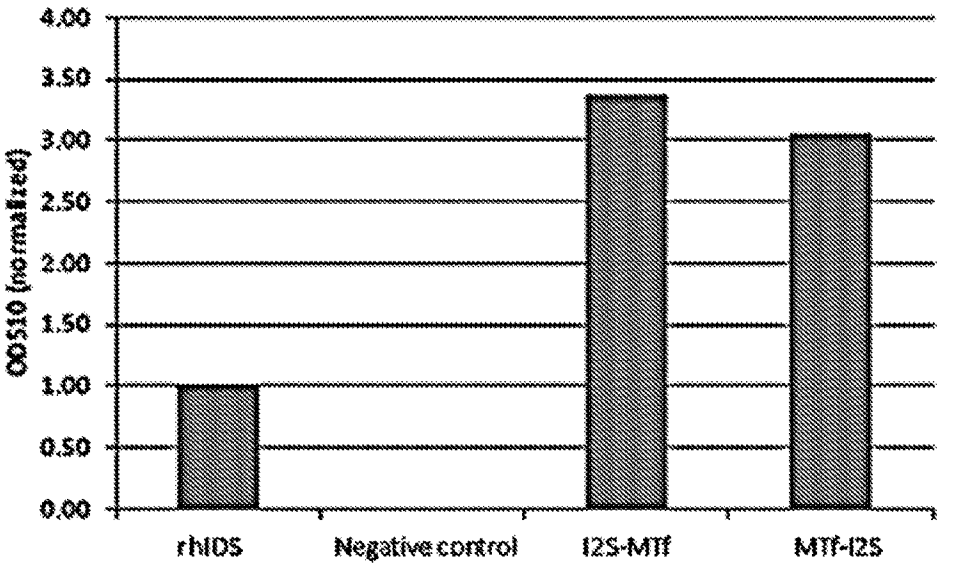
(54) **Titre : PROTEINES DE FUSION P97-IDS**
(54) **Title: P97-IDS FUSION PROTEINS**



(57) **Abrégé/Abstract:**
Provided are fusion proteins between p97 (melanotransferrin) and iduronate-2-sulfatase (IDS), and related compositions and methods of use thereof, for instance, to facilitate delivery of IDS across the blood-brain barrier (BBB) and/or improve its tissue penetration in CNS and/or peripheral tissues, and thereby treat and/or diagnose Hunter Syndrome (Mucopolysaccharidosis type II; MPS II) and related lysosomal storage disorders, including those having a central nervous system (CNS) component.

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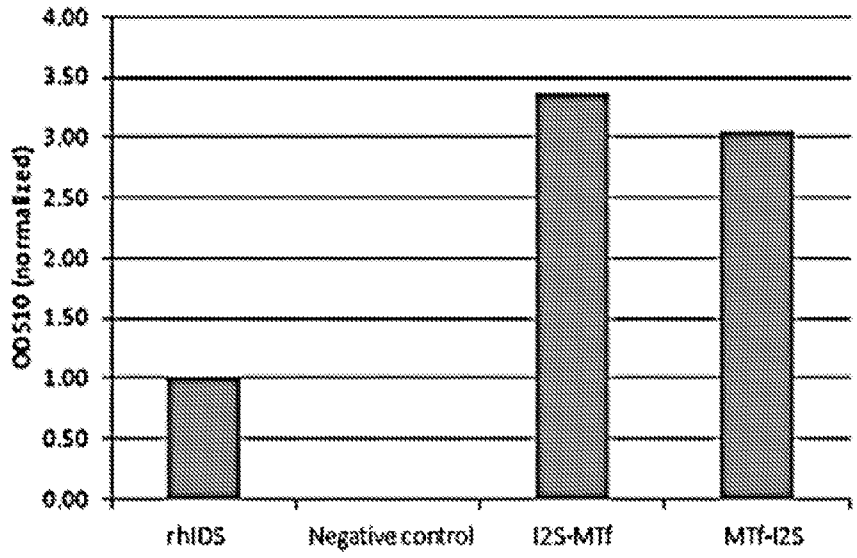

FIG. 2

(57) Abstract: Provided are fusion proteins between p97 (melanotransferrin) and iduronate-2-sulfatase (IDS), and related compositions and methods of use thereof, for instance, to facilitate delivery of IDS across the blood-brain barrier (BBB) and/or improve its tissue penetration in CNS and/or peripheral tissues, and thereby treat and/or diagnose Hunter Syndrome (Mucopolysaccharidosis type II; MPS II) and related lysosomal storage disorders, including those having a central nervous system (CNS) component.

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P97-IDS FUSION PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. Application No. 61/941,896, filed February 19, 2014.

SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text format in lieu of a paper copy. The name of the text file containing the Sequence Listing is BIOA_009_01WO_ST25.txt. The text file is about 153 KB, was created on February 9, 2015, and is being submitted electronically via EFS-Web.

BACKGROUND

Technical Field

The present invention relates to fusion proteins between p97 (melanotransferrin) and iduronate-2-sulfatase (IDS), and related compositions and methods of use thereof, for instance, to facilitate delivery of IDS across the blood-brain barrier (BBB) and/or improve its tissue penetration in CNS and/or peripheral tissues, and thereby treat and/or diagnose Hunter Syndrome (Mucopolysaccharidosis type II; MPS II) and related lysosomal storage disorders, including those having a central nervous system (CNS) component.

Description of the Related Art

Lysosomal storage diseases (LSDs) result from the absence or reduced activity of specific enzymes or proteins within the lysosomes of a cell. Within cells, the effect of the missing enzyme activity can be seen as an accumulation of un-degraded "storage material" within the intracellular lysosome. This build-up causes lysosomes to swell and malfunction, resulting in cellular and tissue damage. As lysosomal storage diseases typically have a genetic etiology, many tissues will lack the enzyme in question. However, different tissues suffer the absence of the same enzyme activity differently. How adversely a tissue will be affected is determined, to some extent, by the degree to which that tissue generates the substrate of the missing enzyme. The types of tissue most burdened by storage, in turn, dictate how the drug should be administered to the patient.

A large number of lysosomal storage disease enzymes have been identified and correlated with their respective diseases. Once the missing or deficient enzyme has been identified, treatment can focus on the problem of effectively delivering the replacement enzyme to a patient's affected tissues. Hunter Syndrome or Mucopolysaccharidosis type II (MPS II) is a lysosomal storage disorders

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(LSD) caused by a deficiency in iduronate-2-sulfatase (I2S or IDS). I2S is a lysosomal enzyme responsible for the metabolism of mucopolysaccharides. Deficiency in the enzyme activity leads a variety of pathologies ultimately and premature death. Enzyme replacement therapy (ERT) with recombinant I2S (Elaprase®) can treat peripheral symptoms but patients suffer eventually from dementia because the enzyme cannot cross the blood brain barrier (BBB).

Intravenous enzyme replacement therapy (ERT) can be beneficial for LSDs such as MPSII. However, means for enhancing the delivery of the therapeutic enzyme to the lysosome in such diseases would be advantageous in terms of reduced cost and increased therapeutic efficacy.

As one problem, the blood-brain barrier (BBB) blocks the free transfer of many agents from blood to brain. For this reason, LSDs that present with significant neurological aspect are not expected to be as responsive to intravenous ERT. For such diseases, methods of improving the delivery of the enzyme across the BBB and into the lysosomes of the affected cells would be highly desirable.

BRIEF SUMMARY

Embodiments of the present invention include p97 (melanotransferrin or MTf) fusion proteins, comprising an iduronate-2-sulfatase (IDS or I2S) polypeptide fused to a p97 polypeptide and an optional peptide linker (L) in between.

In some embodiments, the IDS polypeptide is fused to the N-terminus of the p97 polypeptide. In certain embodiments, the IDS polypeptide is fused to the C-terminus of the p97 polypeptide.

Certain fusion proteins comprise the peptide linker in between. In certain embodiments, the peptide linker is selected from one or more of a rigid linker, a flexible linker, and an enzymatically-cleavable linker. In certain embodiments, the peptide linker is a rigid linker, optionally comprising the sequence $(EAAAK)_{1-3}$ (SEQ ID NOS:36-38), such as EAAAKEAAAKEAAAK (SEQ ID NO:38). In some embodiments, the peptide linker is a flexible linker. In certain embodiments, the peptide linker is an enzymatically-cleavable linker.

In certain embodiments, the fusion protein comprises an N-terminal signal peptide (SP) sequence, optionally selected from **Table 4**. In some embodiments, the fusion protein comprises the structure: (a) SP-IDS-L-p97 or (b) SP-p97-L-IDS.

In particular embodiments, the SP comprises the sequence MEWSWVFLFFLSVTTGVHS (SEQ ID NO:149) and the p97 fusion protein comprises the structure: (a) SP-p97-IDS or (b) SP-p97-L-IDS.

In certain embodiments, the SP comprises the human p97 SP sequence MRGPSGALWLLLALRTVLG (SEQ ID NO:39) and the p97 fusion protein comprises the structure: (a) SP-p97-IDS or (b) SP-p97-L-IDS.

In certain embodiments, the SP comprises the human IDS SP sequence MPPPRTGRGLLWLGLVLSSVCVALG (SEQ ID NO:40) and the p97 fusion protein comprises the structure: (a) SP-IDS-p97 or (b) SP-IDS-L-p97.

In some embodiments, the fusion protein comprises a purification tag (TAG), optionally selected from **Table 5**. In certain embodiments, the fusion protein comprises the structure: (a) SP-TAG-IDS-L-p97 or (b) SP-TAG-p97-L-IDS. In certain embodiments, the tag comprises a poly-histidine tag, optionally a 10X poly-histidine tag. In some embodiments, the tag comprises a FLAG tag DYKDDDDK (SEQ ID NO:122). In specific embodiments, the tag comprises a poly-histidine tag, for example, a 10X poly-histidine tag, and a FLAG tag.

In certain embodiments, the fusion protein comprises a protease site (PS), optionally selected from **Table 6**. In particular embodiments, the fusion protein comprises the structure: (a) SP-TAG-PS-IDS-L-p97 or (b) SP-TAG-PS-p97-linker-IDS. In specific embodiments, the PS site comprises the TEV protease site ENLYFQG (SEQ ID NO:135).

In certain embodiments, the fusion protein comprises the structure (a) SP (MEWSWVFLFFLSVTTGVHS; SEQ ID NO:149)-HIS TAG-TEV PS-IDS-Rigid L-p97 or (b) SP (MEWSWVFLFFLSVTTGVHS; SEQ ID NO: 149)-HIS TAG-TEV PS-p97-Rigid L-IDS.

In specific embodiments, the fusion protein comprises the structure (a) SP (MEWSWVFLFFLSVTTGVHS; SEQ ID NO: 149)-HIS TAG-TEV PS-IDS-$(EAAAK)_3$-p97 or (b) SP (MEWSWVFLFFLSVTTGVHS; SEQ ID NO: 149)-HIS TAG-TEV PS-p97-$(EAAAK)_3$-IDS.

In certain embodiments, the fusion protein comprises the structure (a) IDS SP-HIS TAG-TEV PS-IDS-Rigid L-p97 or (b) p97 SP-HIS TAG-TEV PS-p97-Rigid L-IDS.

In particular embodiments, the fusion protein comprises the structure (a) IDS SP-10xHIS TAG-TEV PS-IDS-$(EAAAK)_3$-p97 (SEQ ID NO:29) or (b) p97 SP-10xHIS TAG-TEV PS-p97-$(EAAAK)_3$-IDS (SEQ ID NO:30).

In certain embodiments, the IDS polypeptide comprises, consists, or consists essentially of (a) an amino acid sequence set forth in SEQ ID NOs:31-35; (b) an amino acid sequence at least 90% identical to a sequence set forth in SEQ ID NOs:31-35; (c) or an amino acid sequence that differs from SEQ ID NOs:31-35 by addition, substitution, insertion, or deletion of about 1-50 amino acids. In some embodiments, the IDS polypeptide comprises, consists, or consists essentially of the amino acid sequence set forth in SEQ ID NO:32 or 33.

In certain embodiments, the p97 polypeptide comprises, consists, or consists essentially of (a) an amino acid sequence set forth in SEQ ID NOs:1-28; (b) an amino acid sequence at least 90% identical to a sequence set forth in SEQ ID NOs: 1-28; (c) or an amino acid sequence that differs from SEQ ID NOs: 1-28 by addition, substitution, insertion, or deletion of about 1-50 amino acids. In particular embodiments, the p97 polypeptide comprises, consists, or consists essentially of the amino acid sequence set forth in SEQ ID NO:2 (soluble human p97) or SEQ ID NO:14 or 148 (MTfpep).

In certain embodiments, the fusion protein comprises, consists, or consists essentially of (a) an amino acid sequence set forth in SEQ ID NO: 138-142 or 29-30; (b) an amino acid sequence at least 90% identical to a sequence set forth in SEQ ID NO: 138-142 or 29-30; (c) or an amino acid sequence that differs from SEQ ID NO: 138-142 or 29-30 by addition, substitution, insertion, or deletion of about 1-50 amino acids. In specific embodiments, the fusion protein comprises, consists, or consists essentially of an amino acid sequence set forth in SEQ ID NO: 138-142 or 29-30.

Also included are isolated polynucleotides which encodes a p97 fusion protein described herein. In some embodiments, the isolated polynucleotide is codon-optimized for expression in a host cell. In certain embodiments, the host cell is a mammalian cell, an insect cell, a yeast cell, or a bacterial cell. In particular embodiments, the polynucleotide comprises a sequence selected from SEQ ID NOs:143-147.

Some embodiments include recombinant host cells, comprising an isolated polynucleotide described herein, where the isolated polynucleotide is operably linked to one or more regulatory elements.

Also included are vectors, comprising an isolated polynucleotide that encodes a p97 fusion protein described herein, which is operably linked to one or more regulatory elements.

Also included are recombinant host cells, comprising a vector, isolated polynucleotide, and/or p97 fusion protein described herein. In certain embodiments, the host cell is a mammalian cell, an insect cell, a yeast cell, or a bacterial cell. In specific embodiments, the mammalian cell is a Chinese hamster ovary (CHO) cell, a HEK-293 cell, or a HT-1080 human fibrosarcoma cell.

Certain embodiments include pharmaceutical compositions, comprising a pharmaceutically-acceptable carrier and a p97 fusion protein described herein, where the pharmaceutical composition is sterile and non-pyrogenic.

Also included are methods for the treatment of a lysosomal storage disease in a subject in need thereof, comprising administering to the subject a p97 fusion protein or pharmaceutical composition described herein. In certain embodiments, the lysosomal storage disease is Hunter Syndrome (MPS II). In certain embodiments, the lysosomal storage disease has central nervous

system (CNS) involvement. In certain embodiments, the subject is at risk for developing CNS involvement of the lysosomal storage disease. In certain embodiments, the subject is a human male. In certain embodiments, the p97 fusion protein or pharmaceutical composition is administered by intravenous (IV) infusion or intraperitoneal (IP) injection.

In accordance with another aspect, there is a p97 fusion protein, comprising an iduronate-2-sulfatase (IDS) polypeptide fused to a p97 polypeptide and an optional peptide linker (L) in between.

In accordance with a further aspect, there is a p97 (melanotransferrin) fusion protein, comprising an iduronate-2-sulfatase (IDS) polypeptide fused to the N-terminus of a p97 polypeptide fragment, wherein the p97 polypeptide fragment consists of the amino acid sequence having at least 80% sequence identity to DSSHAFTLDELR (SEQ ID NO: 14) and having transport activity.

In accordance with another aspect, there is a p97 (melanotransferrin) fusion protein, comprising an iduronate-2-sulfatase (IDS) polypeptide fused to the C-terminus of a p97 polypeptide fragment, wherein the p97 polypeptide fragment consists of the amino acid sequence having at least 80% sequence identity to DSSHAFTLDELR (SEQ ID NO: 14) and having transport activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B illustrate the general structure of exemplary fusion proteins having a signal peptide (SP), purification or affinity tag (TAG), protease site (PS) for removal of the SP and TAG, p97 (melanotransferrin) polypeptide, a linker (L), and an iduronate-2-sulfatase (IDS) polypeptide.

Figure 2 shows the enzyme activity evaluation of I2S-MTf and MTf-I2S fusion proteins as measured by their ability to hydrolyze the substrate 4-Nitrocatechol Sulfate (PNCS) relative to recombinant human IDS and negative control (TZM-MTf fusion). 1ug of each sample was used in the enzyme activity assay, and data presented are normalized to rhIDS.

Figure 3 shows the enzyme activity evaluation of MTfpep-I2S and I2S-MTfpep (with I2S propeptide) fusion proteins as measured by their ability to hydrolyze the substrate PNCS relative to I2S-MTf fusion and negative control (TZM-MTf fusion). 1ug of each sample was used in the enzyme activity assay, and data presented are normalized to substrate blank.

Figure 4 shows a comparison of the enzyme activity of I2S-MTfpep (with I2S propeptide) and I2S-MTfpep (without I2S propeptide) fusion proteins as measured by their ability to hydrolyze the substrate PNCS. 1ug of each sample was used in the enzyme activity assay, and data presented are normalized to substrate blank.

Figure 5 shows quantification of the relative distribution of MTfpep-I2S (with propeptide) and I2S-MTf fusion proteins between capillaries (C) and parenchyma (P) in the brain, relative to the

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total (T) signal. Quantitative confocal microscopy imaging shows that both the MTfpep-I2S and I2S-MTf fusion proteins were strongly associated with parenchymal tissues of the CNS.

DETAILED DESCRIPTION

The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. *See, e.g.*, Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual* (3rd Edition, 2000); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *Oligonucleotide Synthesis: Methods and Applications*

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(P. Herdewijn, ed., 2004); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., 1985); *Nucleic Acid Hybridization: Modern Applications* (Buzdin and Lukyanov, eds., 2009); *Transcription and Translation* (B. Hames & S. Higgins, eds., 1984); *Animal Cell Culture* (R. Freshney, ed., 1986); Freshney, R.I. (2005) *Culture of Animal Cells, a Manual of Basic Technique*, 5th Ed. Hoboken NJ, John Wiley & Sons; B. Perbal, *A Practical Guide to Molecular Cloning* (3rd Edition 2010); Farrell, R., *RNA Methodologies: A Laboratory Guide for Isolation and Characterization* (3rd Edition 2005).

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, certain exemplary methods and materials are described herein. For the purposes of the present invention, the following terms are defined below.

The articles "**a**" and "**an**" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

By "**about**" is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

As used herein, the term "**amino acid**" is intended to mean both naturally occurring and non-naturally occurring amino acids as well as amino acid analogs and mimetics. Naturally occurring amino acids include the 20 (L)-amino acids utilized during protein biosynthesis as well as others such as 4-hydroxyproline, hydroxylysine, desmosine, isodesmosine, homocysteine, citrulline and ornithine, for example. Non-naturally occurring amino acids include, for example, (D)-amino acids, norleucine, norvaline, p-fluorophenylalanine, ethionine and the like, which are known to a person skilled in the art. Amino acid analogs include modified forms of naturally and non-naturally occurring amino acids. Such modifications can include, for example, substitution or replacement of chemical groups and moieties on the amino acid or by derivatization of the amino acid. Amino acid mimetics include, for example, organic structures which exhibit functionally similar properties such as charge and charge spacing characteristic of the reference amino acid. For example, an organic structure which mimics Arginine (Arg or R) would have a positive charge moiety located in similar molecular

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space and having the same degree of mobility as the e-amino group of the side chain of the naturally occurring Arg amino acid. Mimetics also include constrained structures so as to maintain optimal spacing and charge interactions of the amino acid or of the amino acid functional groups. Those skilled in the art know or can determine what structures constitute functionally equivalent amino acid analogs and amino acid mimetics.

Throughout this specification, unless the context requires otherwise, the words "**comprise**," "**comprises**," and "**comprising**" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. By "**consisting of**" is meant including, and limited to, whatever follows the phrase "consisting of." Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "**consisting essentially of**" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they materially affect the activity or action of the listed elements.

The term "**conjugate**" is intended to refer to the entity formed as a result of covalent or non-covalent attachment or linkage of an agent or other molecule, *e.g.*, a biologically active molecule, to a p97 polypeptide or p97 sequence. One example of a conjugate polypeptide is a "**fusion protein**" or "**fusion polypeptide**," that is, a polypeptide that is created through the joining of two or more coding sequences, which originally coded for separate polypeptides; translation of the joined coding sequences results in a single, fusion polypeptide, typically with functional properties derived from each of the separate polypeptides.

As used herein, the terms "**function**" and "**functional**" and the like refer to a biological, enzymatic, or therapeutic function.

"**Homology**" refers to the percentage number of amino acids that are identical or constitute conservative substitutions. Homology may be determined using sequence comparison programs such as GAP (Deveraux *et al.*, *Nucleic Acids Research*. 12, 387-395, 1984). In this way sequences of a similar or substantially different length to those cited herein could be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

By "**isolated**" is meant material that is substantially or essentially free from components that normally accompany it in its native state. For example, an "isolated peptide" or an "isolated polypeptide" and the like, as used herein, includes the *in vitro* isolation and/or purification of a

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peptide or polypeptide molecule from its natural cellular environment, and from association with other components of the cell; *i.e.*, it is not significantly associated with *in vivo* substances.

The term "**linkage**," "**linker**," "**linker moiety**," or "L" is used herein to refer to a linker that can be used to separate a p97 polypeptide from an agent of interest, or to separate a first agent from another agent, for instance where two or more agents are linked to form a p97 conjugate or fusion protein. The linker may be physiologically stable or may include a releasable linker such as an enzymatically degradable linker (*e.g.*, proteolytically cleavable linkers). In certain aspects, the linker may be a peptide linker, for instance, as part of a p97 fusion protein. In some aspects, the linker may be a non-peptide linker or non-proteinaceous linker. In some aspects, the linker may be particle, such as a nanoparticle.

The terms "**modulating**" and "**altering**" include "increasing," "enhancing" or "stimulating," as well as "decreasing" or "reducing," typically in a statistically significant or a physiologically significant amount or degree relative to a control. An "**increased,**" "**stimulated**" or "**enhanced**" amount is typically a "statistically significant" amount, and may include an increase that is 1.1, 1.2, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (*e.g.,* 500, 1000 times) (including all integers and decimal points in between and above 1, *e.g.,* 1.5, 1.6, 1.7. 1.8, etc.) the amount produced by no composition (*e.g.*, the absence of a fusion protein of the invention) or a control composition, sample or test subject. A "**decreased**" or "**reduced**" amount is typically a "**statistically significant**" amount, and may include a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18% , 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% decrease in the amount produced by no composition or a control composition, including all integers in between. As one non-limiting example, a control could compare the activity, such as the enzymatic activity, the amount or rate of transport/delivery across the blood brain barrier, the rate and/or levels of distribution to central nervous system tissue, and/or the C_{max} for plasma, central nervous system tissues, or any other systemic or peripheral non-central nervous system tissues, of a p97 fusion protein relative to the agent/protein alone. Other examples of comparisons and "statistically significant" amounts are described herein.

In certain embodiments, the "**purity**" of any given agent (*e.g.,* a p97 conjugate such as a fusion protein) in a composition may be specifically defined. For instance, certain compositions may comprise an agent that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% pure, including all decimals in between, as measured, for example and by no means limiting, by high pressure liquid chromatography (HPLC), a well-known form of column chromatography used frequently in biochemistry and analytical chemistry to separate, identify, and quantify compounds.

The terms "**polypeptide**" and "**protein**" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues are synthetic non-naturally occurring amino acids, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers. The polypeptides described herein are not limited to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide, and such terms may be used interchangeably herein unless specifically indicated otherwise. The polypeptides described herein may also comprise post-expression modifications, such as glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. A polypeptide may be an entire protein, or a subsequence, fragment, variant, or derivative thereof.

A "**physiologically cleavable**" or "**hydrolyzable**" or "**degradable**" bond is a bond that reacts with water (*i.e.*, is hydrolyzed) under physiological conditions. The tendency of a bond to hydrolyze in water will depend not only on the general type of linkage connecting two central atoms but also on the substituents attached to these central atoms. Appropriate hydrolytically unstable or weak linkages include, but are not limited to: carboxylate ester, phosphate ester, anhydride, acetal, ketal, acyloxyalkyl ether, imine, orthoester, thio ester, thiol ester, carbonate, and hydrazone, peptides and oligonucleotides.

A "**releasable linker**" includes, but is not limited to, a physiologically cleavable linker and an enzymatically degradable linker. Thus, a "releasable linker" is a linker that may undergo either spontaneous hydrolysis, or cleavage by some other mechanism (*e.g.*, enzyme-catalyzed, acid-catalyzed, base-catalyzed, and so forth) under physiological conditions. For example, a "releasable linker" can involve an elimination reaction that has a base abstraction of a proton, (*e.g.*, an ionizable hydrogen atom, Hα), as the driving force. For purposes herein, a "releasable linker" is synonymous with a "degradable linker." An "**enzymatically degradable linkage**" includes a linkage, *e.g.,* amino acid sequence that is subject to degradation by one or more enzymes, *e.g.,* peptidases or proteases. In particular embodiments, a releasable linker has a half life at pH 7.4, 25°C, *e.g.*, a physiological pH, human body temperature (*e.g.*, in vivo), of about 30 minutes, about 1 hour, about 2 hour, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 12 hours, about 18 hours, about 24 hours, about 36 hours, about 48 hours, about 72 hours, or about 96 hours or less.

The term "**reference sequence**" refers generally to a nucleic acid coding sequence, or amino acid sequence, to which another sequence is being compared. All polypeptide and polynucleotide sequences described herein are included as references sequences, including those described by name and those described in the Sequence Listing.

The terms "**sequence identity**" or, for example, comprising a "sequence 50% identical to," as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Included are nucleotides and polypeptides having at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% sequence identity to any of the reference sequences described herein (*see, e.g.,* Sequence Listing), typically where the polypeptide variant maintains at least one biological activity of the reference polypeptide.

Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity," and "substantial identity." A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (*i.e.*, only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (*i.e.*, gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.*, *Nucl. Acids Res.* 25:3389, 1997. A detailed

discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.*, "Current Protocols in Molecular Biology," John Wiley & Sons Inc, 1994-1998, Chapter 15.

By "**statistically significant**," it is meant that the result was unlikely to have occurred by chance. Statistical significance can be determined by any method known in the art. Commonly used measures of significance include the p-value, which is the frequency or probability with which the observed event would occur, if the null hypothesis were true. If the obtained p-value is smaller than the significance level, then the null hypothesis is rejected. In simple cases, the significance level is defined at a p-value of 0.05 or less.

The term "**solubility**" refers to the property of a protein to dissolve in a liquid solvent and form a homogeneous solution. Solubility is typically expressed as a concentration, either by mass of solute per unit volume of solvent (g of solute per kg of solvent, g per dL (100 mL), mg/ml, etc.), molarity, molality, mole fraction or other similar descriptions of concentration. The maximum equilibrium amount of solute that can dissolve per amount of solvent is the solubility of that solute in that solvent under the specified conditions, including temperature, pressure, pH, and the nature of the solvent. In certain embodiments, solubility is measured at physiological pH, or other pH, for example, at pH 5.0, pH 6.0, pH 7.0, or pH 7.4. In certain embodiments, solubility is measured in water or a physiological buffer such as PBS or NaCl (with or without NaP). In specific embodiments, solubility is measured at relatively lower pH (*e.g.,* pH 6.0) and relatively higher salt (*e.g.,* 500mM NaCl and 10mM NaP). In certain embodiments, solubility is measured in a biological fluid (solvent) such as blood or serum. In certain embodiments, the temperature can be about room temperature (*e.g.,* about 20, 21, 22, 23, 24, 25°C) or about body temperature (~37°C). In certain embodiments, a p97 polypeptide fusion protein has a solubility of at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 mg/ml at room temperature or at about 37°C.

A "**subject**," as used herein, includes any animal that exhibits a symptom, or is at risk for exhibiting a symptom, which can be treated or diagnosed with a p97 fusion protein of the invention. Suitable subjects (patients) include laboratory animals (such as mouse, rat, rabbit, or guinea pig), farm animals, and domestic animals or pets (such as a cat or dog). Non-human primates and, preferably, human patients, are included.

"**Substantially**" or "**essentially**" means nearly totally or completely, for instance, 95%, 96%, 97%, 98%, 99% or greater of some given quantity.

"**Substantially free**" refers to the nearly complete or complete absence of a given quantity for instance, less than about 10%, 5%, 4%, 3%, 2%, 1%, 0.5% or less of some given quantity. For

example, certain compositions may be "substantially free" of cell proteins, membranes, nucleic acids, endotoxins, or other contaminants.

"**Treatment**" or "**treating**," as used herein, includes any desirable effect on the symptoms or pathology of a disease or condition, and may include even minimal changes or improvements in one or more measurable markers of the disease or condition being treated. "Treatment" or "treating" does not necessarily indicate complete eradication or cure of the disease or condition, or associated symptoms thereof. The subject receiving this treatment is any subject in need thereof. Exemplary markers of clinical improvement will be apparent to persons skilled in the art.

The term "**wild-type**" refers to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally-occurring source. A wild type gene or gene product (*e.g.*, a polypeptide) is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene.

Fusion Proteins

Embodiments of the present invention relate generally to fusion proteins that comprise a human p97 (melanotransferrin; MTf) polypeptide sequence and a iduronate-2-sulfatase (IDS or I2S) polypeptide sequence, polynucleotides encoding the fusion proteins, host cells and methods of producing fusion proteins, and related compositions and methods of use thereof. Exemplary fusion proteins (e.g., Table 1), p97 polypeptide sequences (e.g., Table 2), and IDS polypeptide sequences (e.g., Table 3) are described herein. The terms "p97" and "MTf" are used interchangeably herein, as are the terms "IDS" and "I2S."

Also described are exemplary methods and components for coupling a p97 polypeptide sequence to an IDS sequence. In certain embodiments, the p97 fusion protein comprises one or more signal peptide sequences (SP), purification tags (TAG), protease cleavage sites (PS), and/or peptide linkers (L), including any combination of the foregoing, examples of which are provided herein. Variants and fragments of any of the foregoing are also described herein.

In certain embodiments, the p97 fusion protein comprises, consists, or consists essentially of at least one of the configurations illustrated below (N-terminus > C-terminus):

- IDS-p97
- p97-IDS
- IDS-L-p97
- p97-L-IDS
- SP-IDS-p97
- SP-p97-IDS

- SP-IDS-L-p97
- SP-P97-L-IDS
- SP-PS-IDS-p97
- SP-PS-P97-IDS
- SP-PS-IDS-L-p97
- SP-PS-p97-L-IDS
- SP-TAG-PS-IDS-p97
- SP-TAG-PS-p97-IDS
- SP-TAG-PS-IDS-L-p97
- SP-TAG-PS-p97-L-IDS
- TAG-IDS-p97
- TAG-p97-IDS
- TAG-IDS-L-p97
- TAG-p97-L-IDS
- TAG-PS-IDS-p97
- TAG-PS-p97-IDS
- TAG-PS-IDS-L-p97
- TAG-PS-p97-L-IDS
- IDS SP-HIS TAG-TEV PS-IDS-Rigid L-p97
- IDS SP-HIS TAG-TEV PS-IDS-$(EAAAK)_3$-p97
- p97 SP-HIS TAG-TEV PS-p97-Rigid L-IDS
- p97 SP-HIS TAG-TEV PS-p97-$(EAAAK)_3$-IDS

Fusion proteins of these and related configurations can be constructed using any of the IDS, p97, L, SP, TAG, or PS sequences described herein, including functional or active variants and fragments thereof.

Specific examples of p97 fusion proteins are illustrated in **Table 1** below.

Table 1: Exemplary p97 Fusion Proteins

Description	Sequence	SEQ ID NO:
IDS SP-10xHIS TAG-TEV PS-IDS-**Rigid L**-p97	MPPPRTGRGLLWLGLVLSSVCVALGHHHHHHHHHHENLYFQSETQANST TDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQA VCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTM SVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHA NLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPH IPFRYPKEFQKLYPLENITLAPDPEVPDGLPPVAYNPWMDIRQREDVQA LNISVPYGPIPVDFQRKIRQSYFASVSYLDTQVGRLLSALDDLQLANST IIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRTASLPEAGEK LFPYLDPFDSASQLMEPGRQSMDLVELVSLFPTLAGLAGLQVPPRCPVP SFHVELCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDIPQ WNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANFSDIHAGELY FVDSDPLQDHNMYNDSQGGDLFQLLMPEAAAKEAAAKEAAAKGMEVRWC	29

	ATSDPEQHKCGNMSEAFREAGIQPSLLCVRGTSADHCVQLIAAQEADAI TLDGGAIYEAGKEHGLKPVVGEVYDQEVGTSYYAVAVVRRSSHVTIDTL KGVKSCHTGINRTVGWNVPVGYLVESGRLSVMGCDVLKAVSDYFGGSCV PGAGETSYSESLCRLCRGDSSGEGVCDKSPLERYYDYSGAFRCLAEGAG DVAFVKHSTVLENTDGKTLPSWGQALLSQDFELLCRDGSRADVTEWRQC HLARVPAHAVVVRADTDGGLIFRLLNEGQRLFSHEGSSFQMFSSEAYGQ KDLLFKDSTSELVPIATQTYEAWLGHEYLHAMKGLLCDPNRLPPYLRWC VLSTPEIQKCGDMAVAFRRQRLKPEIQCVSAKSPQHCMERIQAEQVDAV TLSGEDIYTAGKTYGLVPAAGEHYAPEDSSNSYYVVAVVRRDSSHAFTL DELRGKRSCHAGFGSPAGWDVPVGALIQRGFIRPKDCDVLTAVSEFFNA SCVPVNNPKNYPSSLCALCVGDEQGRNKCVGNSQERYYGYRGAFRCLVE NAGDVAFVRHTTVFDNTNGHNSEPWAAELRSEDYELLCPNGARAEVSQF AACNLAQIPPHAVMVRPDTNIFTVYGLLDKAQDLFGDDHNKNGFKMFDS SNYHGQDLLFKDATVRAVPVGEKTTYRGWLGLDYVAALEGMSSQQCS	
P97 SP- 10xHIS TAG- TEV PS-p97- **Rigid L**-IDS	MRGPSGALWLLLALRTVLGHHHHHHHHHHHENLYFQGMEVRWCATSDPEQ HKCGNMSEAFREAGIQPSLLCVRGTSADHCVQLIAAQEADAITLDGGAI YEAGKEHGLKPVVGEVYDQEVGTSYYAVAVVRRSSHVTIDTLKGVKSCH TGINRTVGWNVPVGYLVESGRLSVMGCDVLKAVSDYFGGSCVPGAGETS YSESLCRLCRGDSSGEGVCDKSPLERYYDYSGAFRCLAEGAGDVAFVKH STVLENTDGKTLPSWGQALLSQDFELLCRDGSRADVTEWRQCHLARVPA HAVVVRADTDGGLIFRLLNEGQRLFSHEGSSFQMFSSEAYGQKDLLFKD STSELVPIATQTYEAWLGHEYLHAMKGLLCDPNRLPPYLRWCVLSTPEI QKCGDMAVAFRRQRLKPEIQCVSAKSPQHCMERIQAEQVDAVTLSGEDI YTAGKTYGLVPAAGEHYAPEDSSNSYYVVAVVRRDSSHAFTLDELRGKR SCHAGFGSPAGWDVPVGALIQRGFIRPKDCDVLTAVSEFFNASCVPVNN PKNYPSSLCALCVGDEQGRNKCVGNSQERYYGYRGAFRCLVENAGDVAF VRHTTVFDNTNGHNSEPWAAELRSEDYELLCPNGARAEVSQFAACNLAQ IPPHAVMVRPDTNIFTVYGLLDKAQDLFGDDHNKNGFKMFDSSNYHGQD LLFKDATVRAVPVGEKTTYRGWLGLDYVAALEGMSSQQCSEAAAKEAAA KEAAAKSETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLA SHSLLFQNAFAQQAVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFS TIPQYFKENGYVTMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYE NTKTCRGPDGELHANLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTS ASPFFLAVGYHKPHIPFRYPKEFQKLYPLENITLAPDPEVPDGLPPVAY NPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYLDTQVGR LLSALDDLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIFY VPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLVELVSLFPTLA GLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGNPREL IAYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDE FLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP	30
I2S-MTf (**SP** : Flag TAG and 10xHIS TAG : **TEV PS** : IDS : **Rigid L** : Soluble p97)	**MEWSWVFLFFLSVTTGVHS**DYKDDDDKEQKLISEEDLHHHHHHHHHHGG GG**ENLYFQ**GSETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRSPNID QLASHSLLFQNAFAQQAVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAG NFSTIPQYFKENGYVTMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSE KYENTKTCRGPDGELHANLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKM KTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLENITLAPDPEVPDGLPP VAYNPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYLDTQ VGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDVATHVPL IFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLVELVSLFP TLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGNP RELIAYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFN PDEFLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP**EAAAK** **EAAAKEAAAK**GMEVRWCATSDPEQHKCGNMSEAFREAGIQPSLLCVRGT SADHCVQLIAAQEADAITLDGGAIYEAGKEHGLKPVVGEVYDQEVGTSY YAVAVVRRSSHVTIDTLKGVKSCHTGINRTVGWNVPVGYLVESGRLSVM GCDVLKAVSDYFGGSCVPGAGETSYSESLCRLCRGDSSGEGVCDKSPLE RYYDYSGAFRCLAEGAGDVAFVKHSTVLENTDGKTLPSWGQALLSQDFE LLCRDGSRADVTEWRQCHLARVPAHAVVVRADTDGGLIFRLLNEGQRLF SHEGSSFQMFSSEAYGQKDLLFKDSTSELVPIATQTYEAWLGHEYLHAM KGLLCDPNRLPPYLRWCVLSTPEIQKCGDMAVAFRRQRLKPEIQCVSAK SPQHCMERIQAEQVDAVTLSGEDIYTAGKTYGLVPAAGEHYAPEDSSNS	138

	YYVVAVVRRDSSHAFTLDELRGKRSCHAGFGSPAGWDVPVGALIQRGFI RPKDCDVLTAVSEFFNASCVPVNNPKNYPSSLCALCVGDEQGRNKCVGN SQERYYGYRGAFRCLVENAGDVAFVRHTTVFDNTNGHNSEPWAAELRSE DYELLCPNGARAEVSQFAACNLAQIPPHAVMVRPDTNIFTVYGLLDKAQ DLFGDDHNKNGFKMFDSSNYHGQDLLFKDATVRAVPVGEKTTYRGWLGL DYVAALEGMSSQQCS	
MTf-I2S (**SP** : Flag TAG and 10xHIS TAG : **TEV PS** : Soluble p97 : **Rigid L** : IDS)	**MEWSWVFLFFLSVTTGVHS**DYKDDDDKEQKLISEEDLHHHHHHHHHHGG GG**ENLYFQG**GMEVRWCATSDPEQHKCGNMSEAFREAGIQPSLLCVRGTS ADHCVQLIAAQEADAITLDGGAIYEAGKEHGLKPVVGEVYDQEVGTSYY AVAVVRRSSHVTIDTLKGVKSCHTGINRTVGWNVPVGYLVESGRLSVMG CDVLKAVSDYFGGSCVPGAGETSYSESLCRLCRGDSSGEGVCDKSPLER YYDYSGAFRCLAEGAGDVAFVKHSTVLENTDGKTLPSWGQALLSQDFEL LCRDGSRADVTEWRQCHLARVPAHAVVVRADTDGGLIFRLLNEGQRLFS HEGSSFQMFSSEAYGQKDLLFKDSTSELVPIATQTYEAWLGHEYLHAMK GLLCDPNRLPPYLRWCVLSTPEIQKCGDMAVAFRRQRLKPEIQCVSAKS PQHCMERIQAEQVDAVTLSGEDIYTAGKTYGLVPAAGEHYAPEDSSNSY YVVAVVRRDSSHAFTLDELRGKRSCHAGFGSPAGWDVPVGALIQRGFIR PKDCDVLTAVSEFFNASCVPVNNPKNYPSSLCALCVGDEQGRNKCVGNS QERYYGYRGAFRCLVENAGDVAFVRHTTVFDNTNGHNSEPWAAELRSED YELLCPNGARAEVSQFAACNLAQIPPHAVMVRPDTNIFTVYGLLDKAQD LFGDDHNKNGFKMFDSSNYHGQDLLFKDATVRAVPVGEKTTYRGWLGLD YVAALEGMSSQQCS**EAAAKEAAAKEAAAK**SETQANSTTDALNVLLIIVD DLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQAVCAPSRVSFLTG RRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGISS NHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHANLLCPVDVLDVP EGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFRYPKEFQKL YPLENITLAPDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIPV DFQRKIRQSYFASVSYLDTQVGRLLSALDDLQLANSTIIAFTSDHGWAL GEHGEWAKYSNFDVATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSAS QLMEPGRQSMDLVELVSLFPTLAGLAGLQVPPRCPVPSFHVELCREGKN LLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDIPQWNSDKPSLKDIK IMGYSIRTIDYRYTVWVGFNPDEFLANFSDIHAGELYFVDSDPLQDHNM YNDSQGGDLFQLLMP	139
MTfpep-I2S (**SP** : Flag TAG and 10xHIS TAG : **TEV PS** : MTfpep w/C-terminal Y: **Rigid L** : I2S)	**MEWSWVFLFFLSVTTGVHS**DYKDDDDKEQKLISEEDLHHHHHHHHHHGG GG**ENLYFQG**DSSHAFTLDELRY**EAAAKEAAAKEAAAK**SETQANSTTDAL NVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQAVCAP SRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGK VFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHANLLC PVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFR YPKEFQKLYPLENITLAPDPEVPDGLPPVAYNPWMDIRQREDVQALNIS VPYGPIPVDFQRKIRQSYFASVSYLDTQVGRLLSALDDLQLANSTIIAF TSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRTASLPEAGEKLFPY LDPFDSASQLMEPGRQSMDLVELVSLFPTLAGLAGLQVPPRCPVPSFHV ELCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDIPQWNSD KPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANFSDIHAGELYFVDS DPLQDHNMYNDSQGGDLFQLLMP	140
I2S-MTfpep (**SP** : Flag TAG and 10xHIS TAG : **TEV PS** : I2S : **Rigid L** : MTfpep w/C-terminal Y)	**MEWSWVFLFFLSVTTGVHS**DYKDDDDKEQKLISEEDLHHHHHHHHHHGG GG**ENLYFQG**SETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRSPNID QLASHSLLFQNAFAQQAVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAG NFSTIPQYFKENGYVTMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSE KYENTKTCRGPDGELHANLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKM KTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLENITLAPDPEVPDGLPP VAYNPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYLDTQ VGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDVATHVPL IFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLVELVSLFP TLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGNP RELIAYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFN PDEFLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP**EAAAK** **EAAAKEAAAK**DSSHAFTLDELRY	141
I2S-MTfpep (without	**MEWSWVFLFFLSVTTGVHS**DYKDDDDKEQKLISEEDLHHHHHHHHHHGG GG**ENLYFQG**TDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLL	142

propep of I2S) **SP** : Flag TAG and 10xHIS TAG : **TEV PS** : I2S w/o propep : **Rigid L** : MTfpep w/C-terminal Y)	FQNAFAQQAVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQY FKENGYVTMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKTC RGPDGELHANLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFF LAVGYHKPHIPFRYPKEFQKLYPLENITLAPDPEVPDGLPPVAYNPWMD IRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYLDTQVGRLLSAL DDLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRT ASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLVELVSLFPTLAGLAGL QVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQ YPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANF SDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP**EAAAKEAAAKEAA** **AK**DSSHAFTLDELRY	

Thus, in some embodiments, the fusion protein comprises, consists, or consists essentially of an amino acid sequence from **Table 1**, or a variant and/or fragment thereof.

p97 Sequences. In certain embodiments, a p97 polypeptide sequence used in a composition and/or fusion protein of the invention comprises, consists essentially of, or consists of a human p97 reference sequence provided in Table 2 below. Also included are variants and fragments thereof.

Table 2: Exemplary p97 Sequences

Description	Sequence	SEQ ID NO:
FL Human p97	MRGPSGALWLLLALRTVLGGMEVRWCATSDPEQHKCGNMSEAFREAGIQ PSLLCVRGTSADHCVQLIAAQEADAITLDGGAIYEAGKEHGLKPVVGEV YDQEVGTSYYAVAVVRRSSHVTIDTLKGVKSCHTGINRTVGWNVPVGYL VESGRLSVMGCDVLKAVSDYFGGSCVPGAGETSYSESLCRLCRGDSSGE GVCDKSPLERYYDYSGAFRCLAEGAGDVAFVKHSTVLENTDGKTLPSWG QALLSQDFELLCRDGSRADVTEWRQCHLARVPAHAVVVRADTDGGLIFR LLNEGQRLFSHEGSSFQMFSSEAYGQKDLLFKDSTSELVPIATQTYEAW LGHEYLHAMKGLLCDPNRLPPYLRWCVLSTPEIQKCGDMAVAFRRQRLK PEIQCVSAKSPQHCMERIQAEQVDAVTLSGEDIYTAGKTYGLVPAAGEH YAPEDSSNSYYVVAVVRRDSSHAFTLDELRGKRSCHAGFGSPAGWDVPV GALIQRGFIRPKDCDVLTAVSEFFNASCVPVNNPKNYPSSLCALCVGDE QGRNKCVGNSQERYYGYRGAFRCLVENAGDVAFVRHTTVFDNTNGHNSE PWAAELRSEDYELLCPNGARAEVSQFAACNLAQIPPHAVMVRPDTNIFT VYGLLDKAQDLFGDDHNKNGFKMFDSSNYHGQDLLFKDATVRAVPVGEK TTYRGWLGLDYVAALEGMSSQQCSGAAAPAPGAPLLPLLLPALAARLLP PAL	1
Soluble Human p97	GMEVRWCATSDPEQHKCGNMSEAFREAGIQPSLLCVRGTSADHCVQLIA AQEADAITLDGGAIYEAGKEHGLKPVVGEVYDQEVGTSYYAVAVVRRSS HVTIDTLKGVKSCHTGINRTVGWNVPVGYLVESGRLSVMGCDVLKAVSD YFGGSCVPGAGETSYSESLCRLCRGDSSGEGVCDKSPLERYYDYSGAFR CLAEGAGDVAFVKHSTVLENTDGKTLPSWGQALLSQDFELLCRDGSRAD VTEWRQCHLARVPAHAVVVRADTDGGLIFRLLNEGQRLFSHEGSSFQMF SSEAYGQKDLLFKDSTSELVPIATQTYEAWLGHEYLHAMKGLLCDPNRL PPYLRWCVLSTPEIQKCGDMAVAFRRQRLKPEIQCVSAKSPQHCMERIQ AEQVDAVTLSGEDIYTAGKTYGLVPAAGEHYAPEDSSNSYYVVAVVRRD SSHAFTLDELRGKRSCHAGFGSPAGWDVPVGALIQRGFIRPKDCDVLTA VSEFFNASCVPVNNPKNYPSSLCALCVGDEQGRNKCVGNSQERYYGYRG AFRCLVENAGDVAFVRHTTVFDNTNGHNSEPWAAELRSEDYELLCPNGA RAEVSQFAACNLAQIPPHAVMVRPDTNIFTVYGLLDKAQDLFGDDHNKN GFKMFDSSNYHGQDLLFKDATVRAVPVGEKTTYRGWLGLDYVAALEGMS SQQCS	2
P97 fragment	WCATSDPEQHK	3
P97 fragment	RSSHVTIDTLK	4
P97 fragment	SSHVTIDTLKGVK	5
P97 fragment	LCRGDSSGEGVCDK	6
P97 fragment	GDSSGEGVCDKSPLER	7

P97 fragment	YYDYSGAFR	8
P97 fragment	ADVTEWR	9
P97 fragment	VPAHAVVVR	10
P97 fragment	ADTDGGLIFR	11
P97 fragment	CGDMAVAFR	12
P97 fragment	LKPEIQCVSAK	13
P97 fragment	DSSHAFTLDELR	14
P97 fragment	14	148
P97 fragment	SEDYELLCPNGAR	15
P97 fragment	AQDLFGDDHNKNGFK	16
P97 fragment	FSSEAYGQKDLLFKDSTSELVPIATQTYEAWLGHEYLHAM	17
P97 fragment	ERIQAEQVDAVTLSGEDIYTAGKTYGLVPAAGEHYAPEDSSNSYYVVAV VRRDSSHAFTLDELRGKRSCHAGFGSPAGWDVPVGALIQRGFIRPKDCD VLTAVSEFFNASCVPVNNPKNYPSSLCALCVGDEQGRNKCVGNSQERYY GYRGAFRCLVENAGDVAFVRHTTVFDNTNGHNSEPWAAELRSEDYELLC PNGARAEVSQFAACNLAQIPPHAVM	18
P97 fragment	VRPDTNIFTVYGLLDKAQDLFGDDHNKNGFKM	19
P97 fragment	GMEVRWCATSDPEQHKCGNMSEAFREAGIQPSLLCVRGTSADHCVQLIA AQEADAITLDGGAIYEAGKEHGLKPVVGEVYDQEVGTSYYAVAVVRRSS HVTIDTLKGVKSCHTGINRTVGWNVPVGYLVESGRLSVMGCDVLKAVSD YFGGSCVPGAGETSYSESLCRLCRGDSSGEGVCDKSPLERYYDYSGAFR CLAEGAGDVAFVKHSTVLENTDGKTLPSWGQALLSQDFELLCRDGSRAD VTEWRQCHLARVPAHAVVVRADTDGGLIFRLLNEGQRLFSHEGSSFQMF SSEAYGQKDLLFKDSTSELVPIATQTYEAWLGHEYLHAMKGLLCDPNRL PPYLRWCVLSTPEIQKCGDMAVAFRRQRLKPEIQCVSAKSPQHCMERIQ AEQVDAVTLSGEDIYTAGKTYGLVPAAGEHYAPEDSSNSYYVVAVVRRD SSHAFTLDELRGKRSCHAGFGSPAGWDVPVGALIQRGFIRPKDCDVLTA VSEFFNASCVPVNNPKNYPSSLCALCVGDEQGRNKCVGNSQERYYGYRG AFRCLVENAGDVAFVRHTTVFDNTN	20
P97 fragment	GHNSEPWAAELRSEDYELLCPN	21
P97 fragment	GARAEVSQFAACNLAQIPPHAVMVRPDTNIFTVYGLLDKAQDLFGDDHN KN	22
P97 fragment	GFKMFDSSNYHGQDLLFKDATVRAVPVGEKTTYRGWLGLDYVAALEGMS SQQC	23
P97 fragment	GMEVRWCATSDPEQHKCGNMSEAFREAGIQPSLLCVRGTSADHCVQLIA AQEADAITLDGGAIYEAGKEHGLKPVVGEVYDQEVGTSYYAVAVVRRSS HVTIDTLKGVKSCHTGINRTVGWNVPVGYLVESGRLSVMGCDVLKAVSD YFGGSCVPGAGETSYSESLCRLCRGDSSGEGVCDKSPLERYYDYSGAFR CLAEGAGDVAFVKHSTVLENTDGKTLPSWGQALLSQDFELLCRDGSRAD VTEWRQCHLARVPAHAVVVRADTDGGLIFRLLNEGQRLFSHEGSSFQMF SSEAYGQKDLLFKDSTSELVPIATQTYEAWLGHEYLHAMKGLLCDPNRL PPYLRWCVLSTPEIQKCGDMAVAFRRQRLKPEIQCVSAKSPQHCMERIQ AEQVDAVTLSGEDIYTAGKTYGLVPAAGEHYAPEDSSNSYYVVAVVRRD SSHAFTLDELRGKRSCHAGFGSPAGWDVPVGALIQRGFIRPKDCDVLTA VSEFFNASCVPVNNPKNYPSSLCALCVGDEQGRNKCVGNSQERYYGYRG AFRCLVENAGDVAFVRHTTVFDNTNGHNSEPWAAELRSEDYELLCPN	24
P97 fragment	GMEVRWCATSDPEQHKCGNMSEAFREAGIQPSLLCVRGTSADHCVQLIA AQEADAITLDGGAIYEAGKEHGLKPVVGEVYDQEVGTSYYAVAVVRRSS HVTIDTLKGVKSCHTGINRTVGWNVPVGYLVESGRLSVMGCDVLKAVSD YFGGSCVPGAGETSYSESLCRLCRGDSSGEGVCDKSPLERYYDYSGAFR CLAEGAGDVAFVKHSTVLENTDGKTLPSWGQALLSQDFELLCRDGSRAD VTEWRQCHLARVPAHAVVVRADTDGGLIFRLLNEGQRLFSHEGSSFQMF SSEAYGQKDLLFKDSTSELVPIATQTYEAWLGHEYLHAMKGLLCDPNRL PPYLRWCVLSTPEIQKCGDMAVAFRRQRLKPEIQCVSAKSPQHCMERIQ AEQVDAVTLSGEDIYTAGKTYGLVPAAGEHYAPEDSSNSYYVVAVVRRD SSHAFTLDELRGKRSCHAGFGSPAGWDVPVGALIQRGFIRPKDCDVLTA VSEFFNASCVPVNNPKNYPSSLCALCVGDEQGRNKCVGNSQERYYGYRG AFRCLVENAGDVAFVRHTTVFDNTNGHNSEPWAAELRSEDYELLCPNGA RAEVSQFAACNLAQIPPHAVMVRPDTNIFTVYGLLDKAQDLFGDDHNKN	25
P97 fragment	GHNSEPWAAELRSEDYELLCPNGARAEVSQFAACNLAQIPPHAVMVRPD	26

	TNIFTVYGLLDKAQDLFGDDHNKN	
P97 fragment	GHNSEPWAAELRSEDYELLCPNGARAEVSQFAACNLAQIPPHAVMVRPD TNIFTVYGLLDKAQDLFGDDHNKNGFKMFDSSNYHGQDLLFKDATVRAV PVGEKTTYRGWLGLDYVAALEGMSSQQC	27
P97 fragment	GARAEVSQFAACNLAQIPPHAVMVRPDTNIFTVYGLLDKAQDLFGDDHN KNGFKMFDSSNYHGQDLLFKDATVRAVPVGEKTTYRGWLGLDYVAALEG MSSQQC	28

In some embodiments, a p97 polypeptide sequence comprises a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity or homology, along its length, to a human p97 sequence in Table 2, or a fragment thereof.

In specific embodiments, the p97 polypeptide sequence comprises, consists, or consists essentially of SEQ ID NO:2 (soluble MTf) or SEQ ID NO:14 (MTfpep). In some embodiments, the MTfpep has a C-terminal tyrosine (Y) residue, as set forth in SEQ ID NO:148.

In particular embodiments, a p97 polypeptide sequence comprises a fragment of a human p97 sequence in Table 2. In certain embodiments, a p97 polypeptide fragment is about, at least about, or up to about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 700, 710, 720, 730 or more amino acids in length, including all integers and ranges in between, and which may comprise all or a portion of the sequence of a p97 reference sequence.

In certain embodiments, a p97 polypeptide fragment is about 5-700, 5-600, 5-500, 5-400, 5-300, 5-200, 5-100, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 10-700, 10-600, 10-500, 10-400, 10-300, 10-200, 10-100, 10-50, 10-40, 10-30, 10-25, 10-20, 10-15, 20-700, 20-600, 20-500, 20-400, 20-300, 20-200, 20-100, 20-50, 20-40, 20-30, 20-25, 30-700, 30-600, 30-500, 30-400, 30-300, 30-200, 30-100, 30-50, 30-40, 40-700, 40-600, 40-500, 40-400, 40-300, 40-200, 40-100, 40-50, 50-700, 50-600, 50-500, 50-400, 50-300, 50-200, 50-100, 60-700, 60-600, 60-500, 60-400, 60-300, 60-200, 60-100, 60-70, 70-700, 70-600, 70-500, 70-400, 70-300, 70-200, 70-100, 70-80, 80-700, 80-600, 80-500, 80-400, 80-300, 80-200, 80-100, 80-90, 90-700, 90-600, 90-500, 90-400, 90-300, 90-200, 90-100, 100-700, 100-600, 100-500, 100-400, 100-300, 100-250, 100-200, 100-150, 200-700, 200-600, 200-500, 200-400, 200-300, or 200-250 amino acids in length, and comprises all or a portion of a p97 reference sequence.

In certain embodiments, p97 polypeptide sequences of interest include p97 amino acid sequences, subsequences, and/or variants of p97 that are effective for transporting an agent of interest across the blood brain barrier and into the central nervous system (CNS). In particular embodiments, the variant or fragment comprises the N-lobe of human p97 (residues 20-361 of SEQ ID NO:1). In specific aspects, the variant or fragment comprises an intact and functional Fe^{3+}-binding site.

In some embodiments, a p97 polypeptide sequence is a soluble form of a p97 polypeptide (*see* Yang *et al.*, *Prot Exp Purif.* 34:28-48, 2004), or a fragment or variant thereof. In some aspects, the soluble p97 polypeptide has a deletion of the all or a portion of the hydrophobic domain (residues 710-738 of SEQ ID NO:1), alone or in combination with a deletion of all or a portion of the signal peptide (residues 1-19 of SEQ ID NO:1). In specific aspects, the soluble p97 polypeptide comprises or consists of SEQ ID NO:2 (~residues 20-710 or 20-711 of SEQ ID NO:1), including variants and fragments thereof.

In certain embodiments, for instance, those that employ liposomes, the p97 polypeptide sequence is a lipid soluble form of a p97 polypeptide. For instance, certain of these and related embodiments include a p97 polypeptide that comprises all or a portion of the hydrophobic domain, optionally with or without the signal peptide.

In certain other embodiments, the p97 fragment or variant is capable of specifically binding to a p97 receptor, an LRP1 receptor and/or an LRP1B receptor.

Variants and fragments of reference p97 polypeptides and other reference polypeptides are described in greater detail below.

Iduronate-2-Sulfatase Sequences. In certain embodiments, an IDS (or I2S) polypeptide sequence used in a fusion protein of the invention comprises, consists essentially of, or consists of one or more human IDS sequences illustrated in **Table 3** below.

Table 3: Exemplary IDS Sequences

Name	Sequence	SEQ ID NO:
Full-length human IDS (signal sequence underlined)	MPPPRTGRGLLWLGLVLSSVCVALGSETQANSTTDALNVLLIIVDDLRP SLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQAVCAPSRVSFLTGRRPD TTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGISSNHTD DSPYSWSFPPYHPSSEKYENTKTCRGPDGELHANLLCPVDVLDVPEGTL PDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLE NITLAPDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIPVDFQR KIRQSYFASVSYLDTQVGRLLSALDDLQLANSTIIAFTSDHGWALGEHG EWAKYSNFDVATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQLME PGRQSMDLVELVSLFPTLAGLAGLQVPPRCPVPSFHVELCREGKNLLKH FRFRDLEEDPYLPGNPRELIAYSQYPRPSDIPQWNSDKPSLKDIKIMGY SIRTIDYRYTVWVGFNPDEFLANFSDIHAGELYFVDSDPLQDHNMYNDS QGGDLFQLLMP	31
Human IDS with propeptide	SETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLF QNAFAQQAVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYF KENGYVTMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKTCR	32

sequence (underlined) but without signal sequence	GPDGELHANLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFL AVGYHKPHIPFRYPKEFQKLYPLENITLAPDPEVPDGLPPVAYNPWMDI RQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYLDTQVGRLLSALD DLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRTA SLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLVELVSLFPTLAGLAGLQ VPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQY PRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANFS DIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP	
Human IDS without propeptide or signal sequence	TDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQA VCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTM SVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHA NLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPH IPFRYPKEFQKLYPLENITLAPDPEVPDGLPPVAYNPWMDIRQREDVQA LNISVPYGPIPVDFQRKIRQSYFASVSYLDTQVGRLLSALDDLQLANST IIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRTASLPEAGEK LFPYLDPFDSASQLMEPGRQSMDLVELVSLFPTLAGLAGLQVPPRCPVP SFHVELCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDIPQ WNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANFSDIHAGELY FVDSDPLQDHNMYNDSQGGDLFQLLMP	33
Human IDS 42 kDa chain	TDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQA VCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTM SVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHA NLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPH IPFRYPKEFQKLYPLENITLAPDPEVPDGLPPVAYNPWMDIRQREDVQA LNISVPYGPIPVDFQRKIRQSYFASVSYLDTQVGRLLSALDDLQLANST IIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRTASLPEAGEK LFPYLDPFDSASQLMEPGRQSMDLVELVSLFPTLAGLAGLQVPPRCPVP SFHVELCREGKNLLKHFRFRDLEEDPYLPG	34
Human IDS 14 kDa chain	NPRELIAYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVG FNPDEFLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP	35

Also included are biologically active variants and fragments of the IDS sequences in Table 3 and the Sequence Listing. In certain aspects, a biologically active IDS polypeptide or variants/fragment thereof hydrolyzes the 2-sulfate groups of the L-iduronate 2-sulfate units of dermatan sulfate, heparan sulfate, and/or heparin, for example, at about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 200%, 300%, 400%, 500% or more of the activity of wild-type human IDS (e.g., SEQ ID NO:31).

Linkers. As noted above, certain fusion proteins may employ one or more linker groups, including peptide linkers. Such linkers can be rigid linkers, flexible linkers, stable linkers, or releasable linkers, such as enzymatically-cleavable linkers. *See, e.g.,* Chen et al., Adv. Drug. Deliv. Ref., 65:1357-69, 2012.

For instance, for polypeptide-polypeptide conjugates, peptide linkers can separate the components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence may be incorporated into the fusion protein using standard techniques described herein and well-known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a rigid or flexible extended conformation; (2) their inability to adopt a secondary structure that could interact with functional

epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea *et al.*, *Gene 40*:39-46, 1985; Murphy *et al.*, *Proc. Natl. Acad. Sci. USA 83*:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180.

In certain illustrative embodiments, a peptide linker is between about 1 to 5 amino acids, between 5 to 10 amino acids, between 5 to 25 amino acids, between 5 to 50 amino acids, between 10 to 25 amino acids, between 10 to 50 amino acids, between 10 to 100 amino acids, or any intervening range of amino acids. In other illustrative embodiments, a peptide linker comprises about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 or more amino acids in length. Particular linkers can have an overall amino acid length of about 1-200 amino acids, 1-150 amino acids, 1-100 amino acids, 1-90 amino acids, 1-80 amino acids, 1-70 amino acids, 1-60 amino acids, 1-50 amino acids, 1-40 amino acids, 1-30 amino acids, 1-20 amino acids, 1-10 amino acids, 1-5 amino acids, 1-4 amino acids, 1-3 amino acids, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 ,17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 70, 80, 90, 100 or more amino acids.

A peptide linker may employ any one or more naturally-occurring amino acids, non-naturally occurring amino acid(s), amino acid analogs, and/or amino acid mimetics as described elsewhere herein and known in the art. Certain amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea *et al.*, *Gene 40*:39-46, 1985; Murphy *et al.*, *PNAS USA. 83*:8258-8262, 1986; U.S. Pat. No. 4,935,233 and U.S. Pat. No. 4,751,180. Particular peptide linker sequences contain Gly, Ser, and/or Asn residues. Other near neutral amino acids, such as Thr and Ala may also be employed in the peptide linker sequence, if desired.

In particular embodiments, the linker is a rigid linker. Examples of rigid linkers include, without limitation, $(EAAAK)_x$ (SEQ ID NO:36)and $A(EAAAK)_x$ ALEA$(EAAAK)_x$A (SEQ ID NO:41), and $(Ala\text{-}Pro)_x$ where $_x$ is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more. Specific examples of rigid linkers include EAAAK (SEQ ID NO:36), $(EAAAK)_2$ (SEQ ID NO:37), $(EAAAK)_3$ (SEQ ID NO:38), $A(EAAAK)_4ALEA(EAAAK)_4A$ (SEQ ID NO:42), PAPAP (SEQ ID NO:43), and AEAAAKEAAAKA (SEQ ID NO:44).

In specific embodiments, the linker comprises, consists, or consists essentially of $(EAAAK)_3$ or EAAAKEAAAKEAAAK (SEQ ID NO:38)

In some embodiments, the linker is a flexible linker. In particular embodiments, the flexible linker is GGGGS (SEQ ID NO:45), $(GGGGS)_2$ (SEQ ID NO:46), $(GGGGS)_3$ (SEQ ID NO:47), or $Gly_{2\text{-}10}$ (SEQ ID NOS:48-54). Additional examples of flexible linkers are provided below.

Certain exemplary linkers include Gly, Ser and/or Asn-containing linkers, as follows: $[G]_x$, $[S]_x$, $[N]_x$, $[GS]_x$, $[GGS]_x$, $[GSS]_x$, $[GSGS]_x$ (SEQ ID NO:55), $[GGSG]_x$ (SEQ ID NO:56), $[GGGS]_x$ (SEQ ID NO: 57), $[GGGGS]_x$ (SEQ ID NO: 45), $[GN]_x$, $[GGN]_x$, $[GNN]_x$, $[GNGN]_x$ (SEQ ID NO: 58), $[GGNG]_x$ (SEQ ID NO: 59), $[GGGN]_x$ (SEQ ID NO: 60), $[GGGGN]_x$ (SEQ ID NO: 61) linkers, where $_x$ is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more. Other combinations of these and related amino acids will be apparent to persons skilled in the art. In specific embodiments, the linker comprises or consists of a $[GGGGS]_3$ (SEQ ID NO: 47) sequence, or GGGGSGGGGSGGGGS (SEQ ID NO: 47).

In specific embodiments, the linker sequence comprises a Gly3 linker sequence, which includes three glycine residues. In particular embodiments, flexible linkers can be rationally designed using a computer program capable of modeling both DNA-binding sites and the peptides themselves (Desjarlais & Berg, *PNAS.* 90:2256-2260, 1993; and *PNAS.* 91:11099-11103, 1994) or by phage display methods.

The peptide linkers may be physiologically stable or may include a releasable linker such as a physiologically degradable or enzymatically degradable linker (*e.g.,* proteolytically or enzymatically-cleavable linker). In certain embodiments, one or more releasable linkers can result in a shorter half-life and more rapid clearance of the fusion protein. These and related embodiments can be used, for example, to enhance the solubility and blood circulation lifetime of p97 fusion proteins in the bloodstream, while also delivering an agent into the bloodstream (or across the BBB) that, subsequent to linker degradation, is substantially free of the p97 sequence. These aspects are especially useful in those cases where polypeptides or other agents, when permanently fused to a p97 sequence, demonstrate reduced activity. By using the linkers as provided herein, such polypeptides can maintain their therapeutic activity when in conjugated or fused form. In these and other ways, the properties of the p97 fusion proteins can be more effectively tailored to balance the bioactivity and circulating half-life of the polypeptides over time.

Specific examples of enzymatically-cleavable linkers include, without limitation, a Factor XIa/FVIIa cleavable linker (VSQTSKLTR▼AETVFPDV) (SEQ ID NO:62), a matrix metalloprotease-1 cleavable linker (PLG▼LWA) (SEQ ID NO:63), an HIV protease cleavable linker (RVL▼AEA) (SEQ ID NO:64), a hepatitis C virus NS3 protease cleavable linker (EDVVCC▼SMSY) (SEQ ID NO:65), a Factor Xa cleavable linker (GGIEGR/GS) (SEQ ID NO:66), a Furin cleavable linker (TRHRQPR▼GWE or AGNRVRR▼SVG or RRRRRRR▼R▼R) (SEQ ID NOS:67-69), and a Cathepsin B cleavable linker (GFLG) (SEQ ID NO:70).

Enzymatically degradable linkages suitable for use in particular embodiments include, but are not limited to: an amino acid sequence cleaved by a serine protease such as thrombin, chymotrypsin, trypsin, elastase, kallikrein, or subtilisin. Illustrative examples of thrombin-cleavable

amino acid sequences include, but are not limited to: -Gly-Arg-Gly-Asp-(SEQ ID NO: 71), -Gly-Gly-Arg-, -Gly- Arg-Gly-Asp-Asn-Pro-(SEQ ID NO:72), -Gly-Arg-Gly-Asp-Ser-(SEQ ID NO: 73), -Gly-Arg-Gly-Asp-Ser-Pro-Lys-(SEQ ID NO: 74), -Gly-Pro- Arg-, -Val-Pro-Arg-, and -Phe- Val -Arg-. Illustrative examples of elastase-cleavable amino acid sequences include, but are not limited to: -Ala-Ala-Ala-, -Ala-Ala-Pro-Val-(SEQ ID NO:75), -Ala-Ala-Pro-Leu-(SEQ ID NO: 76), -Ala-Ala-Pro-Phe-(SEQ ID NO: 77), -Ala-Ala-Pro-Ala-(SEQ ID NO: 78), and -Ala-Tyr-Leu-Val-(SEQ ID NO: 79).

Enzymatically degradable linkages suitable for use in particular embodiments also include amino acid sequences that can be cleaved by a matrix metalloproteinase such as collagenase, stromelysin, and gelatinase. Illustrative examples of matrix metalloproteinase-cleavable amino acid sequences include, but are not limited to: -Gly-Pro-Y-Gly-Pro-Z-(SEQ ID NO: 80), -Gly-Pro-, Leu-Gly-Pro-Z-(SEQ ID NO: 81), -Gly-Pro-Ile-Gly-Pro-Z-(SEQ ID NO:82), and -Ala-Pro-Gly-Leu-Z-(SEQ ID NO: 83), where Y and Z are amino acids. Illustrative examples of collagenase-cleavable amino acid sequences include, but are not limited to: -Pro-Leu-Gly-Pro-D-Arg-Z-(SEQ ID NO: 84), -Pro- Leu-Gly-Leu-Leu-Gly-Z-(SEQ ID NO: 85), -Pro-Gln-Gly-Ile-Ala-Gly-Trp-(SEQ ID NO: 86), -Pro-Leu-Gly-Cys(Me)-His-(SEQ ID NO: 87), -Pro-Leu-Gly-Leu-Tyr-Ala-(SEQ ID NO:88), -Pro-Leu-Ala-Leu-Trp-Ala-Arg-(SEQ ID NO: 89), and -Pro-Leu-Ala-Tyr-Trp-Ala-Arg-(SEQ ID NO: 90), where Z is an amino acid. An illustrative example of a stromelysin-cleavable amino acid sequence is -Pro-Tyr-Ala-Tyr-Tyr-Met-Arg-(SEQ ID NO: 91); and an example of a gelatinase-cleavable amino acid sequence is -Pro-Leu-Gly-Met-Tyr- Ser-Arg-(SEQ ID NO: 92).

Enzymatically degradable linkages suitable for use in particular embodiments also include amino acid sequences that can be cleaved by an angiotensin converting enzyme, such as, for example, -Asp-Lys-Pro-, -Gly-Asp-Lys-Pro-(SEQ ID NO: 93), and -Gly-Ser-Asp-Lys-Pro-(SEQ ID NO: 94).

Enzymatically degradable linkages suitable for use in particular embodiments also include amino acid sequences that can be degraded by cathepsin B, such as, for example, -Val-Cit-, -Ala-Leu-Ala-Leu- (SEQ ID NO:95), -Gly-Phe-Leu-Gly- (SEQ ID NO:96) and -Phe-Lys-.

In certain embodiments, however, any one or more of the non-peptide or peptide linkers are optional. For instance, linker sequences may not be required in a fusion protein where the first and second polypeptides have non-essential N-terminal and/or C-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

Signal Peptide Sequences. In certain embodiments, a p97 fusion protein comprises one or more signal peptide sequences (SP). In particular embodiments, the signal peptide sequence is an N-terminal signal sequence, i.e., the most N-terminal portion of the fusion protein.

Specific examples of signal sequences are provided in **Table 4** below. *See* also Kober et al., Biotechnology and Bioengineering. 110:1164-73, 2013.

Table 4: Exemplary Signal Peptide Sequences (SP)

Protein	Signal Sequence	SEQ ID NO:
Human p97	MRGPSGALWLLLALRTVLG	39
Human IDS	MPPPRTGRGLLWLGLVLSSVCVALG	40
Ig Heavy Chain	MEWSWVFLFFLSVTTGVHS	149
Ig kappa light chain precursor	MDMRAPAGIFGFLLVLFPGYRS	97
Serum albumin preprotein	MKWVTFISLLFLFSSAYS	98
Ig heavy chain	MDWTWRVFCLLAVTPGAHP	99
Ig light chain	MAWSPLFLTLITHCAGSWA	100
Azurocidin preprotein	MTRLTVLALLAGLLASSRA	101
Cystatin-S precursor	MARPLCTLLLLMATLAGALA	102
Trypsinogen 2 precursor	MRSLVFVLLIGAAFA	103
Potassium channel blocker	MSRLFVFILIALFLSAIIDVMS	104
Alpha conotoxin	MGMRMMFIMFMLVVLATTVVS	105
Alfa-galactosidase (mutant m3)	MRAFLFLTACISLPGVFG	106
Cellulase	MKFQSTLLLAAAAGSALA	107
Aspartic proteinase nepenthesin-1	MASSLYSFLLALSIVYIFVAPTHS	108
Acid chitinase	MKTHYSSAILPILTLFVFLSINPSHG	109
K28 prepro-toxin	MESVSSLFNIFSTIMVNYKSLVLALLSVSNLKYARG	110
Killer toxin zygocin precursor	MKAAQILTASIVSLLPIYTSA	111
Cholera toxin	MIKLKFGVFFTVLLSSAYA	112

Thus, in some embodiments, the signal peptide comprises, consists, or consists essentially of at least one sequence from Table 4. In some embodiments, the signal peptide comprises SEQ ID NO:149.

In specific embodiments, the signal peptide sequence corresponds to the most N-terminal protein (p97 or IDS) of the fusion protein. That is, in some embodiments the N-terminal signal peptide sequence is the human p97 signal peptide sequence (SEQ ID NO:39) and the p97 fusion protein comprises the general structure: p97 SP-p97-IDS. In other embodiments, the N-terminal signal sequence is the human IDS signal peptide sequence (SEQ ID NO:40) and the p97 fusion protein comprises the general structure: IDS SP-IDS-p97. Optionally, the fusion protein can further comprise one or more purification tags and/or protease sites, for example, between the N-terminal signal sequence and the p97/IDS portions of the fusion protein, as described elsewhere herein. Here, the protease site is typically place at the C-terminus of the signal sequence or purification tag so that treatment with the corresponding protease removes the N-terminal signal sequence, purification tag, and most or the entire protease site from the fusion protein.

Purification Tags. In some embodiments, the fusion protein comprises one or more purification or affinity tags (TAG or TAGs). Non-limiting examples of purification tags include poly-

histidine tags (*e.g.,* 6xHis tags), avidin, FLAG tags, glutathione S-transferase (GST) tags, maltose-binding protein tags, chitin binding protein (CBP), and others. Also included are epitope tags, which bind to high-affinity antibodies, examples of which include V5-tags, Myc-tags, and HA-tags. In specific examples, the purification tag is a polyhistidine tag (H_{5-10}), for example, H_5, H_6, H_7, H_8, H_9, or H_{10} (SEQ ID NOS:113-118).

Non-limiting examples of purification tags are provided in **Table 5** below.

Table 5: Exemplary Purification Tags (TAG)

Name	Sequence	SEQ ID NO:
5X-HIS	HHHHH	113
6X-HIS	HHHHHH	114
7X-HIS	HHHHHHH	115
8X-HIS	HHHHHHHH	116
9X-HIS	HHHHHHHHH	117
10X-HIS	HHHHHHHHHH	118
AviTag	GLNDIFEAQKIEWHE	119
Calmodulin-tag	KRRWKKNFIAVSAANRFKKISSSGAL	120
Polyglutamate tag	EEEEEE	121
FLAG-tag	DYKDDDDK	122
HA-tag	YPYDVPDYA	123
MYC-tag	EQKLISEEDL	124
S-tag	KETAAAKFERQHMDS	125
SPB-tag	MDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP	126
Softag 1	SLAELLNAGLGGS	127
Softag 3	TQDPSRVG	128
V5 tag	GKPIPNPLLGLDST	129
Xpress tag	DLYDDDDK	130

Thus, in certain embodiments, the purification tag comprises, consists, or consists essentially of at least one sequence from Table 5. In specific embodiments, the tag comprises a FLAG tag and a HIS tag, for example, a 10X-HIS tag.

Protease Sites (PS). In some embodiments, the fusion protein comprises one or more protease sites. Optionally, the one or more protease sites are positioned at the C-terminus of the purification tag and/or signal peptide sequence (if either one or both are present) so that treatment with the corresponding protease removes the N-terminal signal sequence, purification tag, and/or most or all of the protease site from the fusion protein.

In particular embodiments, for instance, where the fusion protein comprises an enzymatically-cleavable linker, the protease site typically differs from that of the enzymatically-cleavable linker, so that treatment with the protease removes any terminal sequences (e.g., signal peptide sequence, purification tag) without cleaving the peptide linker between the p97 and IDS sequences.

Non-limiting examples of protease sites are provided in **Table 6** below.

Table 6: Exemplary Protease Sites (PS)

Protease	Sequence	SEQ ID NO:
Thrombin	LVPR▼GS	131
Enteropeptidase	DDDDK▼	132
Factor Xa	I(E/D)GR▼	133
Enterokinase	DDDDK▼	134
TEV Protease	ENLYFQ▼G	135
HRV 3C Protease	LEVLFQ▼GP	136
SUMO Protease (Ulp1)	GSLQDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEA FAKRQGKEMDSLTFLYDGIEIQADQTPEDLDMEDNDIIEAHREQIGG	137
▼ Denotes site of cleavage		

Thus, in certain embodiments, the protease site comprises, consists, or consists essentially of at least one sequence from Table 6. In specific embodiments, the protease site comprises the TEV protease site (SEQ ID NO:135).

Variant Sequences. Certain embodiments include variants of the reference polypeptide and polynucleotide sequences described herein, whether described by name or by reference to a sequence identifier, including p97 sequences, IDS sequences, linker sequences, signal peptide sequences, purification tags, and protease sites (see, e.g., Tables 1-6 and the Sequence Listing). The wild-type or most prevalent sequences of these polypeptides are known in the art, and can be used as a comparison for the variants and fragments described herein.

A "variant" sequence, as the term is used herein, refers to a polypeptide or polynucleotide sequence that differs from a reference sequence disclosed herein by one or more substitutions, deletions (e.g., truncations), additions, and/or insertions. Certain variants thus include fragments of a reference sequence described herein. Variant polypeptides are biologically active, that is, they continue to possess the enzymatic or binding activity of a reference polypeptide. Such variants may result from, for example, genetic polymorphism and/or from human manipulation.

In many instances, a biologically active variant will contain one or more conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. As described above, modifications may be made in the structure of the polynucleotides and polypeptides of the present invention and still obtain a functional molecule that encodes a variant or derivative polypeptide with desirable characteristics. When it is desired to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, variant or portion of a

polypeptide of the invention, one skilled in the art will typically change one or more of the codons of the encoding DNA sequence according to Table A below.

Table A

Amino Acids			Codons					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAA	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCA	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their utility.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte & Doolittle, 1982,). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte & Doolittle, 1982). These values are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (–0.4); threonine (–0.7); serine (–0.8); tryptophan (–0.9); tyrosine (–1.3); proline (–1.6); histidine (–3.2); glutamate (–3.5);

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glutamine (–3.5); aspartate (–3.5); asparagine (–3.5); lysine (–3.9); and arginine (–4.5). It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.*, still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101 states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (–0.4); proline (–0.5 ± 1); alanine (–0.5); histidine (–0.5); cysteine (–1.0); methionine (–1.3); valine (–1.5); leucine (–1.8); isoleucine (–1.8); tyrosine (–2.3); phenylalanine (–2.5); tryptophan (–3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

Amino acid substitutions may further be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

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A variant may also, or alternatively, contain non-conservative changes. In a preferred embodiment, variant polypeptides differ from a native or reference sequence by substitution, deletion or addition of fewer than about 10, 9, 8, 7, 6, 5, 4, 3, 2 amino acids, or even 1 amino acid. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure, enzymatic activity, and/or hydropathic nature of the polypeptide.

In certain embodiments, a polypeptide sequence is about, at least about, or up to about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700. 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000 or more contiguous amino acids in length, including all integers in between, and which may comprise all or a portion of a reference sequence (*see, e.g.,* Sequence Listing).

In other specific embodiments, a polypeptide sequence consists of about or no more than about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800. 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000 or more contiguous amino acids, including all integers in between, and which may comprise all or a portion of a reference sequence (*see, e.g.,* Sequence Listing).

In still other specific embodiments, a polypeptide sequence is about 10-1000, 10-900, 10-800, 10-700, 10-600, 10-500, 10-400, 10-300, 10-200, 10-100, 10-50, 10-40, 10-30, 10-20, 20-1000, 20-900, 20-800, 20-700, 20-600, 20-500, 20-400, 20-300, 20-200, 20-100, 20-50, 20-40, 20-30, 50-1000, 50-900, 50-800, 50-700, 50-600, 50-500, 50-400, 50-300, 50-200, 50-100, 100-1000, 100-900, 100-800, 100-700, 100-600, 100-500, 100-400, 100-300, 100-200, 200-1000, 200-900, 200-800, 200-700, 200-600, 200-500, 200-400, or 200-300 contiguous amino acids, including all ranges in between, and comprises all or a portion of a reference sequence. In certain embodiments, the C-terminal or N-terminal region of any reference polypeptide may be truncated by about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, or 800 or more amino acids, or by about 10-50, 20-50, 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800 or more amino acids, including all integers and ranges in between (*e.g.,* 101, 102, 103, 104, 105), so long as the truncated polypeptide retains the binding properties and/or activity of the reference polypeptide. Typically, the biologically-active fragment has no less than about 1%, about 5%, about 10%, about 25%, or about 50% of an activity of the biologically-active reference polypeptide from which it is derived.

In general, variants will display at least about 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% similarity or sequence identity or sequence homology to a reference polypeptide sequence. Moreover, sequences differing from the native or parent sequences by the addition (*e.g.,* C-terminal addition, N-terminal addition, both), deletion, truncation, insertion, or substitution (e.g., conservative substitution) of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids (including all integers and ranges in between) but which retain the properties or activities of a parent or reference polypeptide sequence are contemplated.

In some embodiments, variant polypeptides differ from reference sequence by at least one but by less than 50, 40, 30, 20, 15, 10, 8, 6, 5, 4, 3 or 2 amino acid residue(s). In other embodiments, variant polypeptides differ from a reference sequence by at least 1% but less than 20%, 15%, 10% or 5% of the residues. (If this comparison requires alignment, the sequences should be aligned for maximum similarity. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.)

Calculations of sequence similarity or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows. To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In certain embodiments, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or

nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position.

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch, (*J. Mol. Biol.* 48: 444-453, 1970) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used unless otherwise specified) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller (*Cabios.* 4:11-17, 1989) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.*, (1990, *J. Mol. Biol*, 215: 403-10). BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (*Nucleic Acids Res.* 25: 3389-3402, 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used.

In one embodiment, as noted above, polynucleotides and/or polypeptides can be evaluated using a BLAST alignment tool. A local alignment consists simply of a pair of sequence segments, one from each of the sequences being compared. A modification of Smith-Waterman or Sellers

algorithms will find all segment pairs whose scores cannot be improved by extension or trimming, called high-scoring segment pairs (HSPs). The results of the BLAST alignments include statistical measures to indicate the likelihood that the BLAST score can be expected from chance alone.

The raw score, S, is calculated from the number of gaps and substitutions associated with each aligned sequence wherein higher similarity scores indicate a more significant alignment. Substitution scores are given by a look-up table (*see* PAM, BLOSUM).

Gap scores are typically calculated as the sum of G, the gap opening penalty and L, the gap extension penalty. For a gap of length n, the gap cost would be G+Ln. The choice of gap costs, G and L is empirical, but it is customary to choose a high value for G (10-15), *e.g.,* 11, and a low value for L (1-2) *e.g.,* 1.

The bit score, S', is derived from the raw alignment score S in which the statistical properties of the scoring system used have been taken into account. Bit scores are normalized with respect to the scoring system, therefore they can be used to compare alignment scores from different searches. The terms "bit score" and "similarity score" are used interchangeably. The bit score gives an indication of how good the alignment is; the higher the score, the better the alignment.

The E-Value, or expected value, describes the likelihood that a sequence with a similar score will occur in the database by chance. It is a prediction of the number of different alignments with scores equivalent to or better than S that are expected to occur in a database search by chance. The smaller the E-Value, the more significant the alignment. For example, an alignment having an E value of e^{-117} means that a sequence with a similar score is very unlikely to occur simply by chance. Additionally, the expected score for aligning a random pair of amino acids is required to be negative, otherwise long alignments would tend to have high score independently of whether the segments aligned were related. Additionally, the BLAST algorithm uses an appropriate substitution matrix, nucleotide or amino acid and for gapped alignments uses gap creation and extension penalties. For example, BLAST alignment and comparison of polypeptide sequences are typically done using the BLOSUM62 matrix, a gap existence penalty of 11 and a gap extension penalty of 1.

In one embodiment, sequence similarity scores are reported from BLAST analyses done using the BLOSUM62 matrix, a gap existence penalty of 11 and a gap extension penalty of 1.

In a particular embodiment, sequence identity/similarity scores provided herein refer to the value obtained using GAP Version 10 (GCG, Accelrys, San Diego, Calif.) using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix (Henikoff and Henikoff, *PNAS USA*. 89:10915-10919, 1992). GAP uses the algorithm of Needleman

and Wunsch (*J Mol Biol*. 48:443-453, 1970) to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps.

In one particular embodiment, the variant polypeptide comprises an amino acid sequence that can be optimally aligned with a reference polypeptide sequence (*see, e.g.,* Sequence Listing) to generate a BLAST bit scores or sequence similarity scores of at least about 50, 60, 70, 80, 90, 100, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, or more, including all integers and ranges in between, wherein the BLAST alignment used the BLOSUM62 matrix, a gap existence penalty of 11, and a gap extension penalty of 1.

As noted above, a reference polypeptide may be altered in various ways including amino acid substitutions, deletions, truncations, additions, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of a reference polypeptide can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (*PNAS USA*. 82: 488-492, 1985); Kunkel *et al.*, (*Methods in Enzymol*. 154: 367-382, 1987), U.S. Pat. No. 4,873,192, Watson, J. D. *et al.*, ("Molecular Biology of the Gene," Fourth Edition, Benjamin/Cummings, Menlo Park, Calif., 1987) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.*, (1978) Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C.).

Methods for screening gene products of combinatorial libraries made by such modifications, and for screening cDNA libraries for gene products having a selected property are known in the art. Such methods are adaptable for rapid screening of the gene libraries generated by combinatorial mutagenesis of reference polypeptides. As one example, recursive ensemble mutagenesis (REM), a technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify polypeptide variants (Arkin and Yourvan, *PNAS USA* 89: 7811-7815, 1992; Delgrave *et al.*, *Protein Engineering*. 6: 327-331, 1993).

Polynucleotides, Host Cells, and Methods of Production. Certain embodiments relate to polynucleotides that encode the fusion proteins described herein, and vectors that comprise such polynucleotides, for example, where the polynucleotides are operably linked to one or more regulatory elements. Also included are recombinant host cells that comprise such polynucleotides, vectors, fusion proteins, and methods of recombinant production of the foregoing.

Fusion proteins may be prepared using standard techniques. Preferably, however, a fusion protein is expressed as a recombinant protein in an expression system, as described herein and known in the art. Fusion proteins can contain one or multiple copies of a p97 sequence and one or multiple copies of an IDS sequence, present in any desired arrangement.

Polynucleotides and fusion polynucleotides can contain one or multiple copies of a nucleic acid encoding a p97 polypeptide sequence, and/or may contain one or multiple copies of a nucleic acid encoding an IDS sequence.

For fusion proteins, DNA sequences encoding the p97 polypeptide sequence, the IDS sequence of interest, and optionally a peptide linker components may be assembled separately, and then ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component can be ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the other polypeptide component(s) so that the reading frames of the sequences are in frame. The ligated DNA sequences are operably linked to suitable transcriptional and/or translational regulatory elements. The regulatory elements responsible for expression of DNA are usually located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the most C-terminal polypeptide. This permits translation into a single fusion polypeptide that retains the biological activity of both component polypeptides.

Similar techniques, mainly the arrangement of regulatory elements such as promoters, stop codons, and transcription termination signals, can be applied to the recombinant production of non-fusion proteins.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral *e.g.* phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook *et al.*, 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Second Edition, Ausubel *et al.* eds., John Wiley & Sons, 1992, or subsequent updates thereto.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to

increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence. Such polynucleotides are commonly referred to as "codon-optimized." Any of the polynucleotides described herein may be utilized in a codon-optimized form. In certain embodiments, a polynucleotide can be codon optimized for use in specific bacteria such as *E. coli* or yeast such as *S. cerevisiae* (*see, e.g.,* Burgess-Brown *et al., Protein Expr Purif.* 59:94-102, 2008).

Exemplary polynucleotide sequences are provided in **Table 7** below.

Table 7. Exemplary polynucleotide sequences

Name	Polynucleotide Sequence	SEQ ID NO:
I2S-MTf	ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCAC TCCGACTACAAGGACGACGACGACAAAGAGCAGAAGCTGATCTCCGAAGAGGAC CTGCACCACCATCATCACCATCACCACCATCACGGAGGCGGTGGAGAGAACCTG TACTTTCAGGGCTCGGAAACTCAGGCCAACTCCACCACAGATGCACTCAACGTG CTGCTGATCATCGTAGATGACCTCCGACCTTCTCTGGGCTGTTACGGCGACAAG CTAGTACGGAGCCCAAACATCGACCAGCTCGCATCGCACTCTCTCCTATTCCAG AACGCATTCGCCCAGCAGGCTGTCTGTGCTCCCTCCCGAGTGTCCTTCCTCACG GGTCGGAGACCCGATACCACGAGGTTATATGACTTCAACTCATACTGGCGCGTG CATGCCGGTAACTTTTCTACTATACCCCAGTATTTTAAAGAAAATGGCTATGTT ACAATGTCCGTTGGCAAGGTATTTCATCCTGGTATTAGCAGCAACCACACAGAT GACTCTCCGTATAGCTGGTCATTCCCACCATACCACCCCTCCAGCGAAAAGTAC GAAAACACAAAGACTTGCCGGGGCCCAGATGGCGAACTGCACGCAAATCTGCTG TGCCCTGTAGATGTCTTGGACGTGCCCGAAGGTACTCTGCCCGACAAACAGTCC ACAGAACAGGCAATCCAACTCCTTGAAAAGATGAAAACGAGCGCGTCCCCCTTC TTCCTCGCCGTGGGCTACCACAAGCCCCACATCCCGTTTAGATACCCCAAGGAA TTTCAGAAACTGTACCCCCTGGAAAACATCACTCTCGCGCCCGACCCCGAAGTG CCAGACGGACTCCCTCCTGTTGCCTACAACCCTTGGATGGACATCAGACAACGT GAAGATGTGCAGGCCCTGAACATCTCAGTGCCTTACGGCCCCATTCCAGTTGAC TTCCAGAGGAAGATTCGGCAGTCCTACTTCGCCTCCGTTAGTTACCTGGACACC CAAGTGGGTAGACTCCTGAGCGCCTTGGACGATCTCCAGCTCGCAAACAGCACC ATCATTGCCTTCACCAGCGACCATGGTTGGGCGCTGGGTGAACATGGAGAATGG GCTAAATATTCAAATTTCGACGTTGCGACCCACGTCCCATTGATCTTCTACGTG CCTGGACGAACAGCCTCCTTGCCTGAAGCCGGGGAAAAGTTGTTTCCATATCTG GACCCTTTCGATTCTGCGAGCCAACTCATGGAACCTGGGCGACAGAGCATGGAC CTGGTGGAACTGGTCAGTTTATTTCCAACCCTGGCAGGCCTTGCAGGCCTCCAA GTTCCACCTCGGTGTCCCGTTCCCTCATTCCACGTCGAACTCTGTCGCGAAGGT AAAAACCTCCTCAAGCATTTTCGTTTTCGGGACCTCGAAGAAGACCCATACCTG CCAGGGAATCCAAGGGAACTGATTGCCTACAGCCAGTACCCTAGACCTAGCGAC ATCCCACAGTGGAACAGCGACAAGCCCTCCCTCAAGGACATTAAAATCATGGGT TATAGTATCCGGACTATTGACTACAGGTATACCGTGTGGGTGGGTTTCAACCCA GACGAATTTCTCGCCAATTTCTCCGACATCCACGCGGGCGAACTGTATTTCGTT GATTCCGATCCACTGCAAGATCATAATATGTACAACGATAGTCAAGGGGGTGAC CTCTTCCAGTTGCTAATGCCAGAAGCCGCCGCGAAAGAAGCCGCCGCAAAAGAA GCCGCTGCCAAAGGCATGGAAGTGCGTTGGTGCGCCACCTCTGACCCCGAGCAG CACAAGTGCGGCAACATGTCCGAGGCCTTCAGAGAGGCCGGCATCCAGCCTTCT CTGCTGTGTGTGCGGGGCACCTCTGCCGACCATTGCGTGCAGCTGATCGCCGCC CAGGAAGCCGACGCTATCACACTGGATGGCGGCGCTATCTACGAGGCTGGCAAA GAGCACGGCCTGAAGCCCGTCGTGGGCGAGGTGTACGATCAGGAAGTGGGCACC TCCTACTACGCCGTGGCTGTCGTGCGGAGATCCTCCCACGTGACCATCGACACC CTGAAGGGCGTGAAGTCCTGCCACACCGGCATCAACAGAACCGTGGGCTGGAAC GTGCCCGTGGGCTACCTGGTGGAATCCGGCAGACTGTCCGTGATGGGCTGCGAC GTGCTGAAGGCCGTGTCCGATTACTTCGGCGGCTCTTGTGTGCCTGGCGCTGGC GAGACATCCTACTCCGAGTCCCTGTGCAGACTGTGCAGGGGCGACTCTTCTGGC GAGGGCGTGTGCGACAAGTCCCCTCTGGAACGGTACTACGACTACTCCGGCGCC	143

	TTCAGATGCCTGGCTGAAGGTGCTGGCGACGTGGCCTTCGTGAAGCACTCCACC GTGCTGGAAAACACCGACGGCAAGACCCTGCCTTCTTGGGGCCAGGCACTGCTG TCCCAGGACTTCGAGCTGCTGTGCCGGGATGGCTCCAGAGCCGATGTGACAGAG TGGCGGCAGTGCCACCTGGCCAGAGTGCCTGCTCATGCTGTGGTCGTGCGCGCC GATACAGATGGCGGCCTGATCTTCCGGCTGCTGAACGAGGGCCAGCGGCTGTTC TCTCACGAGGGCTCCAGCTTCCAGATGTTCTCCAGCGAGGCCTACGGCCAGAAG GACCTGCTGTTCAAGGACTCCACCTCCGAGCTGGTGCCTATCGCCACCCAGACC TATGAGGCTTGGCTGGGCCACGAGTACCTGCACGCTATGAAGGGACTGCTGTGC GACCCCAACCGGCTGCCTCCTTATCTGAGGTGGTGCGTGCTGTCCACCCCCGAG ATCCAGAAATGCGGCGATATGGCCGTGGCCTTTCGGCGGCAGAGACTGAAGCCT GAGATCCAGTGCGTGTCCGCCAAGAGCCCTCAGCACTGCATGGAACGGATCCAG GCCGAACAGGTGGACGCCGTGACACTGTCCGGCGAGGATATCTACACCGCCGGA AAGACCTACGGCCTGGTGCCAGCTGCTGGCGAGCATTACGCCCCTGAGGACTCC TCCAACAGCTACTACGTGGTGGCAGTCGTGCGCCGGGACTCCTCTCACGCCTTT ACCCTGGATGAGCTGCGGGGCAAGAGAAGCTGTCACGCCGGCTTTGGAAGCCCT GCCGGATGGGATGTGCCTGTGGGCGCTCTGATCCAGCGGGGCTTCATCAGACCC AAGGACTGTGATGTGCTGACCGCCGTGTCTGAGTTCTTCAACGCCTCCTGTGTG CCCGTGAACAACCCCAAGAACTACCCCTCCAGCCTGTGCGCCCTGTGTGTGGGA GATGAGCAGGGCCGGAACAAATGCGTGGGCAACTCCCAGGAAAGATATTACGGC TACAGAGGCGCCTTCCGGTGTCTGGTGGAAAACGCCGGGGATGTGGCTTTTGTG CGGCACACCACCGTGTTCGACAACACCAATGGCCACAACTCCGAGCCTTGGGCC GCTGAGCTGAGATCCGAGGATTACGAACTGCTGTGTCCCAACGGCGCCAGGGCT GAGGTGTCCCAGTTTGCCGCCTGTAACCTGGCCCAGATCCCTCCCCACGCTGTG ATGGTGCGACCCGACACCAACATCTTCACCGTGTACGGCCTGCTGGACAAGGCC CAGGATCTGTTCGGCGACGACCACAACAAGAACGGGTTCAAGATGTTCGACTCC AGCAACTACCACGGACAGGATCTGCTGTTTAAAGATGCCACCGTGCGGGCCGTG CCAGTGGGCGAAAAGACCACCTACAGAGGATGGCTGGGACTGGACTACGTGGCC GCCCTGGAAGGCATGTCCTCCCAGCAGTGTTCCTGA	
MTf-I2S	ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCAC TCCGACTACAAGGACGACGACGACAAAGAGCAGAAGCTGATCTCCGAAGAGGAC CTGCACCACCATCATCACCATCACCACCATCACGGAGGCGGTGGAGAGAACCTG TACTTTCAGGGCGGCATGGAAGTGCGTTGGTGCGCCACCTCTGACCCCGAGCAG CACAAGTGCGGCAACATGTCCGAGGCCTTCAGAGAGGCCGGCATCCAGCCTTCT CTGCTGTGTGTGCGGGGCACCTCTGCCGACCATTGCGTGCAGCTGATCGCCGCC CAGGAAGCCGACGCTATCACACTGGATGGCGGCGCTATCTACGAGGCTGGCAAA GAGCACGGCCTGAAGCCCGTCGTGGGCGAGGTGTACGATCAGGAAGTGGGCACC TCCTACTACGCCGTGGCTGTCGTGCGGAGATCCTCCCACGTGACCATCGACACC CTGAAGGGCGTGAAGTCCTGCCACACCGGCATCAACAGAACCGTGGGCTGGAAC GTGCCCGTGGGCTACCTGGTGGAATCCGGCAGACTGTCCGTGATGGGCTGCGAC GTGCTGAAGGCCGTGTCCGATTACTTCGGCGGCTCTTGTGTGCCTGGCGCTGGC GAGACATCCTACTCCGAGTCCCTGTGCAGACTGTGCAGGGGCGACTCTTCTGGC GAGGGCGTGTGCGACAAGTCCCCTCTGGAACGGTACTACGACTACTCCGGCGCC TTCAGATGCCTGGCTGAAGGTGCTGGCGACGTGGCCTTCGTGAAGCACTCCACC GTGCTGGAAAACACCGACGGCAAGACCCTGCCTTCTTGGGGCCAGGCACTGCTG TCCCAGGACTTCGAGCTGCTGTGCCGGGATGGCTCCAGAGCCGATGTGACAGAG TGGCGGCAGTGCCACCTGGCCAGAGTGCCTGCTCATGCTGTGGTCGTGCGCGCC GATACAGATGGCGGCCTGATCTTCCGGCTGCTGAACGAGGGCCAGCGGCTGTTC TCTCACGAGGGCTCCAGCTTCCAGATGTTCTCCAGCGAGGCCTACGGCCAGAAG GACCTGCTGTTCAAGGACTCCACCTCCGAGCTGGTGCCTATCGCCACCCAGACC TATGAGGCTTGGCTGGGCCACGAGTACCTGCACGCTATGAAGGGACTGCTGTGC GACCCCAACCGGCTGCCTCCTTATCTGAGGTGGTGCGTGCTGTCCACCCCCGAG ATCCAGAAATGCGGCGATATGGCCGTGGCCTTTCGGCGGCAGAGACTGAAGCCT GAGATCCAGTGCGTGTCCGCCAAGAGCCCTCAGCACTGCATGGAACGGATCCAG GCCGAACAGGTGGACGCCGTGACACTGTCCGGCGAGGATATCTACACCGCCGGA AAGACCTACGGCCTGGTGCCAGCTGCTGGCGAGCATTACGCCCCTGAGGACTCC TCCAACAGCTACTACGTGGTGGCAGTCGTGCGCCGGGACTCCTCTCACGCCTTT ACCCTGGATGAGCTGCGGGGCAAGAGAAGCTGTCACGCCGGCTTTGGAAGCCCT GCCGGATGGGATGTGCCTGTGGGCGCTCTGATCCAGCGGGGCTTCATCAGACCC AAGGACTGTGATGTGCTGACCGCCGTGTCTGAGTTCTTCAACGCCTCCTGTGTG CCCGTGAACAACCCCAAGAACTACCCCTCCAGCCTGTGCGCCCTGTGTGTGGGA GATGAGCAGGGCCGGAACAAATGCGTGGGCAACTCCCAGGAAAGATATTACGGC	144

	TACAGAGGCGCCTTCCGGTGTCTGGTGGAAAACGCCGGGGATGTGGCTTTTGTG CGGCACACCACCGTGTTCGACAACACCAATGGCCACAACTCCGAGCCTTGGGCC GCTGAGCTGAGATCCGAGGATTACGAACTGCTGTGTCCCAACGGCGCCAGGGCT GAGGTGTCCCAGTTTGCCGCCTGTAACCTGGCCCAGATCCCTCCCCACGCTGTG ATGGTGCGACCCGACACCAACATCTTCACCGTGTACGGCCTGCTGGACAAGGCC CAGGATCTGTTCGGCGACGACCACAACAAGAACGGGTTCAAGATGTTCGACTCC AGCAACTACCACGGACAGGATCTGCTGTTTAAAGATGCCACCGTGCGGGCCGTG CCAGTGGGCGAAAAGACCACCTACAGAGGATGGCTGGGACTGGACTACGTGGCC GCCCTGGAAGGCATGTCCTCCCAGCAGTGTTCCGAAGCCGCCGCGAAAGAAGCC GCCGCAAAAGAAGCCGCTGCCAAATCGGAAACTCAGGCCAACTCCACCACAGAT GCACTCAACGTGCTGCTGATCATCGTAGATGACCTCCGACCTTCTCTGGGCTGT TACGGCGACAAGCTAGTACGGAGCCCAAACATCGACCAGCTCGCATCGCACTCT CTCCTATTCCAGAACGCATTCGCCCAGCAGGCTGTCTGTGCTCCCTCCCGAGTG TCCTTCCTCACGGGTCGGAGACCCGATACCACGAGGTTATATGACTTCAACTCA TACTGGCGCGTGCATGCCGGTAACTTTTCTACTATACCCCAGTATTTTAAAGAA AATGGCTATGTTACAATGTCCGTTGGCAAGGTATTTCATCCTGGTATTAGCAGC AACCACACAGATGACTCTCCGTATAGCTGGTCATTCCCACCATACCACCCCTCC AGCGAAAAGTACGAAAACACAAAGACTTGCCGGGGCCCAGATGGCGAACTGCAC GCAAATCTGCTGTGCCCTGTAGATGTCTTGGACGTGCCCGAAGGTACTCTGCCC GACAAACAGTCCACAGAACAGGCAATCCAACTCCTTGAAAAGATGAAAACGAGC GCGTCCCCCTTCTTCCTCGCCGTGGGCTACCACAAGCCCCACATCCCGTTTAGA TACCCCAAGGAATTTCAGAAACTGTACCCCCTGGAAAACATCACTCTCGCGCCC GACCCCGAAGTGCCAGACGGACTCCCTCCTGTTGCCTACAACCCTTGGATGGAC ATCAGACAACGTGAAGATGTGCAGGCCCTGAACATCTCAGTGCCTTACGGCCCC ATTCCAGTTGACTTCCAGAGGAAGATTCGGCAGTCCTACTTCGCCTCCGTTAGT TACCTGGACACCCAAGTGGGTAGACTCCTGAGCGCCTTGGACGATCTCCAGCTC GCAAACAGCACCATCATTGCCTTCACCAGCGACCATGGTTGGGCGCTGGGTGAA CATGGAGAATGGGCTAAATATTCAAATTTCGACGTTGCGACCCACGTCCCATTG ATCTTCTACGTGCCTGGACGAACAGCCTCCTTGCCTGAAGCCGGGGAAAAGTTG TTTCCATATCTGGACCCTTTCGATTCTGCGAGCCAACTCATGGAACCTGGGCGA CAGAGCATGGACCTGGTGGAACTGGTCAGTTTATTTCCAACCCTGGCAGGCCTT GCAGGCCTCCAAGTTCCACCTCGGTGTCCCGTTCCCTCATTCCACGTCGAACTC TGTCGCGAAGGTAAAAACCTCCTCAAGCATTTTCGTTTTCGGGACCTCGAAGAA GACCCATACCTGCCAGGGAATCCAAGGGAACTGATTGCCTACAGCCAGTACCCT AGACCTAGCGACATCCCACAGTGGAACAGCGACAAGCCCTCCCTCAAGGACATT AAAATCATGGGTTATAGTATCCGGACTATTGACTACAGGTATACCGTGTGGGTG GGTTTCAACCCAGACGAATTTCTCGCCAATTTCTCCGACATCCACGCGGGCGAA CTGTATTTCGTTGATTCCGATCCACTGCAAGATCATAATATGTACAACGATAGT CAAGGGGGTGACCTCTTCCAGTTGCTAATGCCATGA	
MTfpep-I2S	ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCAC TCCGACTACAAGGACGACGACGACAAAGAGCAGAAGCTGATCTCCGAAGAGGAC CTGCACCACCATCATCACCATCACCACCATCACGGAGGCGGTGGAGAGAACCTG TACTTTCAGGGCGACTCCTCTCACGCCTTCACCCTGGACGAGCTGCGGTACGAA GCCGCCGCGAAAGAAGCCGCCGCAAAAGAAGCCGCTGCCAAATCGGAAACTCAG GCCAACTCCACCACAGATGCACTCAACGTGCTGCTGATCATCGTAGATGACCTC CGACCTTCTCTGGGCTGTTACGGCGACAAGCTAGTACGGAGCCCAAACATCGAC CAGCTCGCATCGCACTCTCTCCTATTCCAGAACGCATTCGCCCAGCAGGCTGTC TGTGCTCCCTCCCGAGTGTCCTTCCTCACGGGTCGGAGACCCGATACCACGAGG TTATATGACTTCAACTCATACTGGCGCGTGCATGCCGGTAACTTTTCTACTATA CCCCAGTATTTTAAAGAAAATGGCTATGTTACAATGTCCGTTGGCAAGGTATTT CATCCTGGTATTAGCAGCAACCACACAGATGACTCTCCGTATAGCTGGTCATTC CCACCATACCACCCCTCCAGCGAAAAGTACGAAAACACAAAGACTTGCCGGGGC CCAGATGGCGAACTGCACGCAAATCTGCTGTGCCCTGTAGATGTCTTGGACGTG CCCGAAGGTACTCTGCCCGACAAACAGTCCACAGAACAGGCAATCCAACTCCTT GAAAAGATGAAAACGAGCGCGTCCCCCTTCTTCCTCGCCGTGGGCTACCACAAG CCCCACATCCCGTTTAGATACCCCAAGGAATTTCAGAAACTGTACCCCCTGGAA AACATCACTCTCGCGCCCGACCCCGAAGTGCCAGACGGACTCCCTCCTGTTGCC TACAACCCTTGGATGGACATCAGACAACGTGAAGATGTGCAGGCCCTGAACATC TCAGTGCCTTACGGCCCCATTCCAGTTGACTTCCAGAGGAAGATTCGGCAGTCC TACTTCGCCTCCGTTAGTTACCTGGACACCCAAGTGGGTAGACTCCTGAGCGCC TTGGACGATCTCCAGCTCGCAAACAGCACCATCATTGCCTTCACCAGCGACCAT	145

	GGTTGGGCGCTGGGTGAACATGGAGAATGGGCTAAATATTCAAATTTCGACGTT GCGACCCACGTCCCATTGATCTTCTACGTGCCTGGACGAACAGCCTCCTTGCCT GAAGCCGGGGAAAAGTTGTTTCCATATCTGGACCCTTTCGATTCTGCGAGCCAA CTCATGGAACCTGGGCGACAGAGCATGGACCTGGTGGAACTGGTCAGTTTATTT CCAACCCTGGCAGGCCTTGCAGGCCTCCAAGTTCCACCTCGGTGTCCCGTTCCC TCATTCCACGTCGAACTCTGTCGCGAAGGTAAAAACCTCCTCAAGCATTTTCGT TTTCGGGACCTCGAAGAAGACCCATACCTGCCAGGGAATCCAAGGGAACTGATT GCCTACAGCCAGTACCCTAGACCTAGCGACATCCCACAGTGGAACAGCGACAAG CCCTCCCTCAAGGACATTAAAATCATGGGTTATAGTATCCGGACTATTGACTAC AGGTATACCGTGTGGGTGGGTTTCAACCCAGACGAATTTCTCGCCAATTTCTCC GACATCCACGCGGGCGAACTGTATTTCGTTGATTCCGATCCACTGCAAGATCAT AATATGTACAACGATAGTCAAGGGGGTGACCTCTTCCAGTTGCTAATGCCATGA	
I2S-MTfpep	ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCAC TCCGACTACAAGGACGACGACGACAAAGAGCAGAAGCTGATCTCCGAAGAGGAC CTGCACCACCATCATCACCATCACCACCATCACGGAGGCGGTGGAGAGAACCTG TACTTTCAGGGCTCGGAAACTCAGGCCAACTCCACCACAGATGCACTCAACGTG CTGCTGATCATCGTAGATGACCTCCGACCTTCTCTGGGCTGTTACGGCGACAAG CTAGTACGGAGCCCAAACATCGACCAGCTCGCATCGCACTCTCTCCTATTCCAG AACGCATTCGCCCAGCAGGCTGTCTGTGCTCCCTCCCGAGTGTCCTTCCTCACG GGTCGGAGACCCGATACCACGAGGTTATATGACTTCAACTCATACTGGCGCGTG CATGCCGGTAACTTTTCTACTATACCCCAGTATTTTAAAGAAAATGGCTATGTT ACAATGTCCGTTGGCAAGGTATTTCATCCTGGTATTAGCAGCAACCACACAGAT GACTCTCCGTATAGCTGGTCATTCCCACCATACCACCCCTCCAGCGAAAAGTAC GAAAACACAAAGACTTGCCGGGGCCCAGATGGCGAACTGCACGCAAATCTGCTG TGCCCTGTAGATGTCTTGGACGTGCCCGAAGGTACTCTGCCCGACAAACAGTCC ACAGAACAGGCAATCCAACTCCTTGAAAAGATGAAAACGAGCGCGTCCCCCTTC TTCCTCGCCGTGGGCTACCACAAGCCCCACATCCCGTTTAGATACCCCAAGGAA TTTCAGAAACTGTACCCCCTGGAAAACATCACTCTCGCGCCCGACCCCGAAGTG CCAGACGGACTCCCTCCTGTTGCCTACAACCCTTGGATGGACATCAGACAACGT GAAGATGTGCAGGCCCTGAACATCTCAGTGCCTTACGGCCCCATTCCAGTTGAC TTCCAGAGGAAGATTCGGCAGTCCTACTTCGCCTCCGTTAGTTACCTGGACACC CAAGTGGGTAGACTCCTGAGCGCCTTGGACGATCTCCAGCTCGCAAACAGCACC ATCATTGCCTTCACCAGCGACCATGGTTGGGCGCTGGGTGAACATGGAGAATGG GCTAAATATTCAAATTTCGACGTTGCGACCCACGTCCCATTGATCTTCTACGTG CCTGGACGAACAGCCTCCTTGCCTGAAGCCGGGGAAAAGTTGTTTCCATATCTG GACCCTTTCGATTCTGCGAGCCAACTCATGGAACCTGGGCGACAGAGCATGGAC CTGGTGGAACTGGTCAGTTTATTTCCAACCCTGGCAGGCCTTGCAGGCCTCCAA GTTCCACCTCGGTGTCCCGTTCCCTCATTCCACGTCGAACTCTGTCGCGAAGGT AAAAACCTCCTCAAGCATTTTCGTTTTCGGGACCTCGAAGAAGACCCATACCTG CCAGGGAATCCAAGGGAACTGATTGCCTACAGCCAGTACCCTAGACCTAGCGAC ATCCCACAGTGGAACAGCGACAAGCCCTCCCTCAAGGACATTAAAATCATGGGT TATAGTATCCGGACTATTGACTACAGGTATACCGTGTGGGTGGGTTTCAACCCA GACGAATTTCTCGCCAATTTCTCCGACATCCACGCGGGCGAACTGTATTTCGTT GATTCCGATCCACTGCAAGATCATAATATGTACAACGATAGTCAAGGGGGTGAC CTCTTCCAGTTGCTAATGCCAGAGGCCGCTGCTAAAGAGGCTGCCGCCAAAGAA GCCGCCGCTAAGGACTCCTCTCACGCCTTCACCCTGGACGAGCTGCGGTACTAA	146
I2S-MTfpep (without propep of I2S)	ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCAC TCCGACTACAAGGACGACGACGACAAAGAGCAGAAGCTGATCTCCGAAGAGGAC CTGCACCACCATCATCACCATCACCACCATCACGGAGGCGGTGGAGAGAACCTG TACTTTCAGGGCACAGATGCACTCAACGTGCTGCTGATCATCGTAGATGACCTC CGACCTTCTCTGGGCTGTTACGGCGACAAGCTAGTACGGAGCCCAAACATCGAC CAGCTCGCATCGCACTCTCTCCTATTCCAGAACGCATTCGCCCAGCAGGCTGTC TGTGCTCCCTCCCGAGTGTCCTTCCTCACGGGTCGGAGACCCGATACCACGAGG TTATATGACTTCAACTCATACTGGCGCGTGCATGCCGGTAACTTTTCTACTATA CCCCAGTATTTTAAAGAAAATGGCTATGTTACAATGTCCGTTGGCAAGGTATTT CATCCTGGTATTAGCAGCAACCACACAGATGACTCTCCGTATAGCTGGTCATTC CCACCATACCACCCCTCCAGCGAAAAGTACGAAAACACAAAGACTTGCCGGGGC CCAGATGGCGAACTGCACGCAAATCTGCTGTGCCCTGTAGATGTCTTGGACGTG CCCGAAGGTACTCTGCCCGACAAACAGTCCACAGAACAGGCAATCCAACTCCTT GAAAAGATGAAAACGAGCGCGTCCCCCTTCTTCCTCGCCGTGGGCTACCACAAG CCCCACATCCCGTTTAGATACCCCAAGGAATTTCAGAAACTGTACCCCCTGGAA	147

	AACATCACTCTCGCGCCCGACCCCGAAGTGCCAGACGGACTCCCTCCTGTTGCC TACAACCCTTGGATGGACATCAGACAACGTGAAGATGTGCAGGCCCTGAACATC TCAGTGCCTTACGGCCCCATTCCAGTTGACTTCCAGAGGAAGATTCGGCAGTCC TACTTCGCCTCCGTTAGTTACCTGGACACCCAAGTGGGTAGACTCCTGAGCGCC TTGGACGATCTCCAGCTCGCAAACAGCACCATCATTGCCTTCACCAGCGACCAT GGTTGGGCGCTGGGTGAACATGGAGAATGGGCTAAATATTCAAATTTCGACGTT GCGACCCACGTCCCATTGATCTTCTACGTGCCTGGACGAACAGCCTCCTTGCCT GAAGCCGGGGAAAAGTTGTTTCCATATCTGGACCCTTTCGATTCTGCGAGCCAA CTCATGGAACCTGGGCGACAGAGCATGGACCTGGTGGAACTGGTCAGTTTATTT CCAACCCTGGCAGGCCTTGCAGGCCTCCAAGTTCCACCTCGGTGTCCCGTTCCC TCATTCCACGTCGAACTCTGTCGCGAAGGTAAAAACCTCCTCAAGCATTTTCGT TTTCGGGACCTCGAAGAAGACCCATACCTGCCAGGGAATCCAAGGGAACTGATT GCCTACAGCCAGTACCCTAGACCTAGCGACATCCCACAGTGGAACAGCGACAAG CCCTCCCTCAAGGACATTAAAATCATGGGTTATAGTATCCGGACTATTGACTAC AGGTATACCGTGTGGGTGGGTTTCAACCCAGACGAATTTCTCGCCAATTTCTCC GACATCCACGCGGGCGAACTGTATTTCGTTGATTCCGATCCACTGCAAGATCAT AATATGTACAACGATAGTCAAGGGGGTGACCTCTTCCAGTTGCTAATGCCAGAG GCCGCTGCTAAAGAGGCTGCCGCCAAAGAAGCCGCCGCTAAGGACTCCTCTCAC GCCTTCACCCTGGACGAGCTGCGGTACTAA	

Thus, in certain embodiments, a polynucleotide that encodes a fusion protein or antibody fusion described herein, or a portion thereof, comprises one or more polynucleotide sequences from Table 7 (e.g., SEQ ID NOS:143-147), or a fragment/variant thereof.

In some embodiments, a nucleic acids or vectors encoding a subject p97 polypeptide, an IDS polypeptide, and/or a p97-IDS fusion are introduced directly into a host cell, and the cell is incubated under conditions sufficient to induce expression of the encoded polypeptide(s). Therefore, according to certain related embodiments, there is provided a recombinant host cell which comprises a polynucleotide or a fusion polynucleotide that encodes one or more fusion proteins described herein, and which optionally comprises additional exogenous polynucleotides.

Expression of a fusion protein in the host cell may be achieved by culturing the recombinant host cells (containing the polynucleotide(s)) under appropriate conditions. Following production by expression, the polypeptide(s) and/or fusion proteins, may be isolated and/or purified using any suitable technique, and then used as desired. The term "host cell" is used to refer to a cell into which has been introduced, or which is capable of having introduced into it, a nucleic acid sequence encoding one or more of the polypeptides described herein, and which further expresses or is capable of expressing a selected gene of interest, such as a gene encoding any herein described polypeptide. The term includes the progeny of the parent cell, whether or not the progeny are identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present. Host cells may be chosen for certain characteristics, for instance, the expression of aminoacyl tRNA synthetase(s) that can incorporate unnatural amino acids into the polypeptide.

Systems for cloning and expression of a protein in a variety of different host cells are well known. Suitable host cells include mammalian cells, bacteria, yeast, and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include

Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney cells, HEK-293 cells, human fibrosarcoma cell line HT-1080 (*see, e.g.,* Moran, Nat. Biotechnol. 28:1139-40, 2010), NSO mouse melanoma cells and many others. Additional examples of useful mammalian host cell lines include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells sub-cloned for growth in suspension culture, Graham *et al., J. Gen Virol*. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, *Biol. Reprod*. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather *et al., Annals N.Y. Acad. Sci*. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells (Urlaub *et al.*, PNAS USA 77:4216 (1980)); and myeloma cell lines such as NSO and Sp2/0. For a review of certain mammalian host cell lines suitable for polypeptide production, *see, e.g.*, Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B. K.C Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 255-268. Certain preferred mammalian cell expression systems include CHO and HEK293-cell based expression systems. Mammalian expression systems can utilize attached cell lines, for example, in T-flasks, roller bottles, or cell factories, or suspension cultures, for example, in 1L and 5L spinners, 5L, 14L, 40L, 100L and 200L stir tank bioreactors, or 20/50L and 100/200L WAVE bioreactors, among others known in the art.

A common, preferred bacterial host is *E. coli*. The expression of proteins in prokaryotic cells such as *E. coli* is well established in the art. For a review, see for example Pluckthun, *A. Bio/Technology*. 9:545-551 (1991). Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for recombinant production of polypeptides (*see* Ref, *Curr. Opinion Biotech*. 4:573-576, 1993; and Trill *et al., Curr. Opinion Biotech.* 6:553-560, 1995). In specific embodiments, protein expression may be controlled by a T7 RNA polymerase (*e.g.,* pET vector series). These and related embodiments may utilize the expression host strain BL21(DE3), a λDE3 lysogen of BL21 that supports T7-mediated expression and is deficient in lon and ompT proteases for improved target protein stability. Also included are expression host strains carrying plasmids encoding tRNAs rarely used in *E. coli*, such as Rosetta™ (DE3) and Rosetta 2 (DE3) strains. Cell lysis and sample handling may also be improved using reagents such as Benzonase® nuclease and BugBuster® Protein Extraction Reagent. For cell culture, auto-inducing media can improve the efficiency of many expression systems, including high-throughput expression systems. Media of this type (*e.g.,* Overnight Express™ Autoinduction System) gradually elicit protein expression through

metabolic shift without the addition of artificial inducing agents such as IPTG. Particular embodiments employ hexahistidine tags (such as His•Tag® fusions), followed by immobilized metal affinity chromatography (IMAC) purification, or related techniques. In certain aspects, however, clinical grade proteins can be isolated from *E. coli* inclusion bodies, without or without the use of affinity tags (*see, e.g.,* Shimp *et al., Protein Expr Purif.* 50:58-67, 2006). As a further example, certain embodiments may employ a cold-shock induced *E. coli* high-yield production system, because over-expression of proteins in *Escherichia coli* at low temperature improves their solubility and stability (*see, e.g.,* Qing *et al., Nature Biotechnology.* 22:877-882, 2004).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, post-translational modifications such as acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing, which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as yeast, CHO, HeLa, MDCK, HEK293, and W138, in addition to bacterial cells, which have or even lack specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the fusion protein of interest.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines that stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which, successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type. Transient production, such as by transient transfection or infection, can also be employed. Exemplary mammalian expression systems that are suitable for transient production include HEK293 and CHO-based systems.

Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. Certain specific embodiments utilize serum free cell expression systems. Examples include HEK293 cells and CHO cells that can grow on serum free medium (*see, e.g.,* Rosser *et al., Protein Expr. Purif.* 40:237–43, 2005; and U.S. Patent number 6,210,922).

The protein(s) produced by a recombinant cell can be purified and characterized according to a variety of techniques known in the art. Exemplary systems for performing protein purification and analyzing protein purity include fast protein liquid chromatography (FPLC) (*e.g.,* AKTA and Bio-Rad FPLC systems), high-pressure liquid chromatography (HPLC) (*e.g.,* Beckman and Waters HPLC). Exemplary chemistries for purification include ion exchange chromatography (*e.g.,* Q, S), size exclusion chromatography, salt gradients, affinity purification (*e.g.,* Ni, Co, FLAG, maltose, glutathione, protein A/G), gel filtration, reverse-phase, ceramic HyperD® ion exchange chromatography, and hydrophobic interaction columns (HIC), among others known in the art. Also included are analytical methods such as SDS-PAGE (*e.g.,* coomassie, silver stain), immunoblot, Bradford, and ELISA, which may be utilized during any step of the production or purification process, typically to measure the purity of the protein composition.

Also included are methods of concentrating recombinantly produced proteins, e.g., fusion proteins. Examples include lyophilization, which is typically employed when the solution contains few soluble components other than the protein of interest. Lyophilization is often performed after HPLC run, and can remove most or all volatile components from the mixture. Also included are ultrafiltration techniques, which typically employ one or more selective permeable membranes to concentrate a protein solution. The membrane allows water and small molecules to pass through and retains the protein; the solution can be forced against the membrane by mechanical pump, gas pressure, or centrifugation, among other techniques.

In certain embodiments, the fusion proteins have a purity of at least about 90%, as measured according to routine techniques in the art. In certain embodiments, such as diagnostic compositions or certain therapeutic compositions, the fusion proteins have a purity of at least about 95%. In specific embodiments, such as therapeutic or pharmaceutical compositions, the fusion proteins have a purity of at least about 97% or 98% or 99%. In other embodiments, such as when being used as reference or research reagents, fusion proteins can be of lesser purity, and may have a purity of at least about 50%, 60%, 70%, or 80%. Purity can be measured overall or in relation to selected components, such as other proteins, *e.g.,* purity on a protein basis.

In certain embodiments, as noted above, the compositions described here are about substantially endotoxin free, including, for example, about 95% endotoxin free, preferably about 99% endotoxin free, and more preferably about 99.99% endotoxin free. The presence of endotoxins can be detected according to routine techniques in the art, as described herein. In specific embodiments, the fusion proteins are made from a eukaryotic cell such as a mammalian or human cell in substantially serum free media.

Methods of Use and Pharmaceutical Compositions

Certain embodiments of the present invention relate to methods of using the p97 fusion proteins described herein. Examples of such methods include methods of treatment and methods of diagnosis, including for instance, the use of p97 fusion proteins for medical imaging of certain organs/tissues, such as those of the nervous system. Some embodiments include methods of diagnosing and/or treating disorders or conditions of the central nervous system (CNS), or disorders or conditions having a CNS component. Particular aspects include methods of treating a lysosomal storage disorder (LSD), including those having a CNS component.

Accordingly, certain embodiments include methods of treating a subject in need thereof, comprising administering a p97 fusion protein described herein. Also included are methods of delivering an IDS enzyme to the nervous system (*e.g.*, central nervous system tissues) of a subject, comprising administering a composition that comprises a p97 fusion protein described herein. In certain of these and related embodiments, the methods increase the rate of delivery of the agent to the central nervous system tissues, relative, for example, to delivery by a composition that comprises a non-fusion IDS enzyme.

In some instances, the subject has or is at risk for having a lysosomal storage disease. Certain methods thus relate to the treatment of lysosomal storage diseases in a subject in need thereof, optionally those lysosomal storage diseases associated with the central nervous system, or having CNS involvement. Exemplary lysosomal storage diseases include mucopolysaccharidosis type II (Hunter Syndrome). Hunter Syndrome is an X-linked multisystem disorder characterized by glycosaminoglycans (GAG) accumulation. The vast majority of affected individuals are male; on rare occasion carrier females manifest findings. Age of onset, disease severity, and rate of progression may vary significantly.

In those with severe disease, CNS involvement (manifest primarily by progressive cognitive deterioration), progressive airway disease, and cardiac disease usually result in death in the first or second decade of life. Certain embodiments therefore include the treatment of Hunter Syndrome with CNS involvement.

In those with attenuated disease, the CNS is not (or is minimally) affected, although the effect of GAG accumulation on other organ systems may be just as severe as in those who have progressive cognitive decline. Survival into the early adult years with normal intelligence is common in the attenuated form of the disease. However, subjects with attenuated disease can still benefit from administration of a p97-IDS fusion protein having improved penetration into CNS tissues, for instance, to reduce the risk of progression from attenuated Hunter Syndrome to that with CNS involvement.

Additional findings in both forms of Hunter Syndrome include: short stature; macrocephaly with or without communicating hydrocephalus; macroglossia; hoarse voice; conductive and sensorineural hearing loss; hepatomegaly and/or splenomegaly; dysostosis multiplex and joint contractures including ankylosis of the temporomandibular joint; spinal stenosis; and carpal tunnel syndrome. Subjects undergoing treatment with fusion proteins described herein may thus have one or more of these findings of Hunter Syndrome.

Urine GAGs and skeletal surveys can establish the presence of an MPS condition but are not specific to MPS II. The gold standard for diagnosis of MPS II in a male proband is deficient iduronate sulfatase (IDS) enzyme activity in white cells, fibroblasts or plasma in the presence of normal activity of at least one other sulfatase. Molecular genetic testing of *IDS*, the only gene in which mutation is known to be associated with Hunter Syndrome, can be used to confirm the diagnosis in a male proband with an unusual phenotype or a phenotype that does not match the results of GAG testing.

Common treatments for Hunter Syndrome include developmental, occupational, and physical therapy; shunting for hydrocephalus; tonsillectomy and adenoidectomy; positive pressure ventilation (CPAP or tracheostomy); carpal tunnel release; cardiac valve replacement; inguinal hernia repair. Hence, in certain aspects, a subject for treatment by the fusion proteins described herein may be about to undergo, is undergoing, or has undergone one or more of these treatments.

Disease monitoring can depend on organ system involvement and disease severity, and usually includes annual cardiac evaluation and echocardiograms; pulmonary evaluations including pulmonary function testing; audiograms; eye examinations; developmental assessments; and neurologic examinations. Additional studies may include sleep studies for obstructive apnea; nerve conduction velocity (NCV) to assess for carpal tunnel syndrome; evaluations for hydrocephalus; orthopedic evaluations to monitor hip disease. Thus, in some aspects, a subject for treatment by the fusion proteins described herein may be about to undergo, is undergoing, or has undergone one or more of these disease monitoring protocols.

For *in vivo* use, for instance, for the treatment of human disease, medical imaging, or testing, the p97 fusion proteins described herein are generally incorporated into a pharmaceutical composition prior to administration. A pharmaceutical composition comprises one or more of the p97 fusion proteins described herein in combination with a physiologically acceptable carrier or excipient.

To prepare a pharmaceutical composition, an effective or desired amount of one or more fusion proteins is mixed with any pharmaceutical carrier(s) or excipient known to those skilled in the art to be suitable for the particular mode of administration. A pharmaceutical carrier may be liquid, semi-liquid or solid. Solutions or suspensions used for parenteral, intradermal, subcutaneous or

topical application may include, for example, a sterile diluent (such as water), saline solution (*e.g.,* phosphate buffered saline; PBS), fixed oil, polyethylene glycol, glycerin, propylene glycol or other synthetic solvent; antimicrobial agents (such as benzyl alcohol and methyl parabens); antioxidants (such as ascorbic acid and sodium bisulfite) and chelating agents (such as ethylenediaminetetraacetic acid (EDTA)); buffers (such as acetates, citrates and phosphates). If administered intravenously (e.g., by IV infusion), suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, polypropylene glycol and mixtures thereof.

Administration of fusion proteins described herein, in pure form or in an appropriate pharmaceutical composition, can be carried out via any of the accepted modes of administration of agents for serving similar utilities. The pharmaceutical compositions can be prepared by combining a fusion protein-containing composition with an appropriate physiologically acceptable carrier, diluent or excipient, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. In addition, other pharmaceutically active ingredients (including other small molecules as described elsewhere herein) and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the composition.

Administration may be achieved by a variety of different routes, including oral, parenteral, nasal, intravenous, intradermal, subcutaneous or topical. Preferred modes of administration depend upon the nature of the condition to be treated or prevented. Particular embodiments include administration by IV infusion. Some embodiments include administration by intraperitoneal (IP) injection. Also included are combinations thereof.

Carriers can include, for example, pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as polysorbate 20 (TWEEN™) polyethylene glycol (PEG), and poloxamers (PLURONICS™), and the like.

In certain aspects, a fusion protein is bound to or encapsulated within a particle, *e.g.*, a nanoparticle, bead, lipid formulation, lipid particle, or liposome, *e.g.*, immunoliposome. The fusion proteins may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate)microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980). The particle(s) or liposomes may further comprise other therapeutic or diagnostic agents.

The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Controlled clinical trials may also be performed. Dosages may also vary with the severity of the condition to be alleviated. A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. The composition may be administered one time, or may be divided into a number of smaller doses to be administered at intervals of time. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

Typical routes of administering these and related pharmaceutical compositions thus include, without limitation, oral, topical, transdermal, inhalation, parenteral, sublingual, buccal, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Pharmaceutical compositions according to certain embodiments of the present invention are formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a subject or patient may take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of a herein described conjugate in aerosol form may hold a plurality of dosage units. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington: The Science and Practice of Pharmacy*, 20th Edition (Philadelphia College of Pharmacy and Science, 2000). The composition to be administered will typically contain a therapeutically effective amount of a fusion protein described herein, for treatment of a disease or condition of interest.

A pharmaceutical composition may be in the form of a solid or liquid. In one embodiment, the carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form.

The carrier(s) may be liquid, with the compositions being, for example, an oral oil, injectable liquid or an aerosol, which is useful in, for example, inhalatory administration. When intended for oral administration, the pharmaceutical composition is preferably in either solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the pharmaceutical composition may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like. Such a solid composition will typically contain one or more inert diluents or edible carriers. In addition, one or more of the following may be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent. When the pharmaceutical composition is in the form of a capsule, for example, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or oil.

The pharmaceutical composition may be in the form of a liquid, for example, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred composition contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

The liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following adjuvants: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

A liquid pharmaceutical composition intended for either parenteral or oral administration should contain an amount of a fusion protein such that a suitable dosage will be obtained. Typically, this amount is at least 0.01% of the agent of interest in the composition. When intended for oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition. Certain oral pharmaceutical compositions contain between about 4% and about 75% of the agent of interest. In certain embodiments, pharmaceutical compositions and preparations according to the present invention are prepared so that a parenteral dosage unit contains between 0.01 to 10% by weight of the agent of interest prior to dilution.

The pharmaceutical composition may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device.

The pharmaceutical composition may be intended for rectal administration, in the form, for example, of a suppository, which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter, and polyethylene glycol.

The pharmaceutical composition may include various materials, which modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients may be encased in a gelatin capsule. The pharmaceutical composition in solid or liquid form may include an agent that binds to the conjugate or agent and thereby assists in the delivery of the compound. Suitable agents that may act in this capacity include monoclonal or polyclonal antibodies, one or more proteins or a liposome.

The pharmaceutical composition may consist essentially of dosage units that can be administered as an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols may be delivered in single phase, bi-phasic, or tri-phasic systems in order to deliver the active ingredient(s). Delivery of the aerosol includes the necessary container, activators, valves,

subcontainers, and the like, which together may form a kit. One of ordinary skill in the art, without undue experimentation may determine preferred aerosols.

The compositions described herein may be prepared with carriers that protect the fusion proteins against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art.

The pharmaceutical compositions may be prepared by methodology well known in the pharmaceutical art. For example, a pharmaceutical composition intended to be administered by injection may comprise one or more of salts, buffers and/or stabilizers, with sterile, distilled water so as to form a solution. A surfactant may be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non-covalently interact with the conjugate so as to facilitate dissolution or homogeneous suspension of the conjugate in the aqueous delivery system.

The compositions may be administered in a therapeutically effective amount, which will vary depending upon a variety of factors including the activity of the specific compound employed; the metabolic stability and length of action of the compound; the age, body weight, general health, sex, and diet of the patient; the mode and time of administration; the rate of excretion; the drug combination; the severity of the particular disorder or condition; and the subject undergoing therapy. Generally, a therapeutically effective daily dose is (for a 70 kg mammal) from about 0.001 mg/kg (*i.e.*, ~ 0.07 mg) to about 100 mg/kg (*i.e.*, ~ 7.0 g); preferably a therapeutically effective dose is (for a 70 kg mammal) from about 0.01 mg/kg (*i.e.*, ~ 0.7 mg) to about 50 mg/kg (*i.e.*, ~ 3.5 g); more preferably a therapeutically effective dose is (for a 70 kg mammal) from about 1 mg/kg (*i.e.*, ~ 70 mg) to about 25 mg/kg (*i.e.*, ~ 1.75 g).

Compositions described herein may also be administered simultaneously with, prior to, or after administration of one or more other therapeutic agents, as described herein. For instance, in one embodiment, the conjugate is administered with an anti-inflammatory agent. Anti-inflammatory agents or drugs include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDS) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate.

Such combination therapy may include administration of a single pharmaceutical dosage formulation, which contains a compound of the invention (i.e., fusion protein) and one or more additional active agents, as well as administration of compositions comprising conjugates of the invention and each active agent in its own separate pharmaceutical dosage formulation. For example, a fusion protein as described herein and the other active agent can be administered to the patient together in a single oral dosage composition such as a tablet or capsule, or each agent administered in separate oral dosage formulations. Similarly, a fusion protein as described herein and the other active agent can be administered to the patient together in a single parenteral dosage composition such as in a saline solution or other physiologically acceptable solution, or each agent administered in separate parenteral dosage formulations. Where separate dosage formulations are used, the compositions comprising fusion proteins and one or more additional active agents can be administered at essentially the same time, *i.e.,* concurrently, or at separately staggered times, i.e., sequentially and in any order; combination therapy is understood to include all these regimens.

The various embodiments described herein can be combined to provide further embodiments. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, application and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

EXAMPLES

Example 1

In Vitro Activity of Fusion Proteins

Fusion proteins of human p97 (melanotransferrin; MTf) and human duronate-2-sulfatase (IDS) were prepared and tested for enzymatic activity in vitro. **Table E1** provides the amino acid sequences and **Table E2** provides the corresponding polynucleotide coding sequences of the fusion proteins that were prepared and tested.

Table E1. Polypeptide Sequences of Fusion Proteins		
Name	Sequence	SEQ

Date Reçue/Date Received 2020-06-18

		ID NO:
I2S-MTf (**SP** : TAG : **PS** : I2S : **Linker** : Soluble MTf)	**MEWSWVFLFFLSVTTGVHS**DYKDDDDKEQKLISEEDLHHHHHHHHHHGGGG**ENL YFQ**GSETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQ NAFAQQAVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYV TMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHANLL CPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFRYPKE FQKLYPLENITLAPDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIPVD FQRKIRQSYFASVSYLDTQVGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEW AKYSNFDVATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMD LVELVSLFPTLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYL PGNPRELIAYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNP DEFLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP**EAAAKEAAAKE AAAK**GMEVRWCATSDPEQHKCGNMSEAFREAGIQPSLLCVRGTSADHCVQLIAA QEADAITLDGGAIYEAGKEHGLKPVVGEVYDQEVGTSYYAVAVVRRSSHVTIDT LKGVKSCHTGINRTVGWNVPVGYLVESGRLSVMGCDVLKAVSDYFGGSCVPGAG ETSYSESLCRLCRGDSSGEGVCDKSPLERYYDYSGAFRCLAEGAGDVAFVKHST VLENTDGKTLPSWGQALLSQDFELLCRDGSRADVTEWRQCHLARVPAHAVVVRA DTDGGLIFRLLNEGQRLFSHEGSSFQMFSSEAYGQKDLLFKDSTSELVPIATQT YEAWLGHEYLHAMKGLLCDPNRLPPYLRWCVLSTPEIQKCGDMAVAFRRQRLKP EIQCVSAKSPQHCMERIQAEQVDAVTLSGEDIYTAGKTYGLVPAAGEHYAPEDS SNSYYVVAVVRRDSSHAFTLDELRGKRSCHAGFGSPAGWDVPVGALIQRGFIRP KDCDVLTAVSEFFNASCVPVNNPKNYPSSLCALCVGDEQGRNKCVGNSQERYYG YRGAFRCLVENAGDVAFVRHTTVFDNTNGHNSEPWAAELRSEDYELLCPNGARA EVSQFAACNLAQIPPHAVMVRPDTNIFTVYGLLDKAQDLFGDDHNKNGFKMFDS SNYHGQDLLFKDATVRAVPVGEKTTYRGWLGLDYVAALEGMSSQQCS	138
MTf-I2S (**SP** : TAG : **PS** : Soluble MTf : **Linker** : I2S)	**MEWSWVFLFFLSVTTGVHS**DYKDDDDKEQKLISEEDLHHHHHHHHHHGGGG**ENL YFQG**GMEVRWCATSDPEQHKCGNMSEAFREAGIQPSLLCVRGTSADHCVQLIAA QEADAITLDGGAIYEAGKEHGLKPVVGEVYDQEVGTSYYAVAVVRRSSHVTIDT LKGVKSCHTGINRTVGWNVPVGYLVESGRLSVMGCDVLKAVSDYFGGSCVPGAG ETSYSESLCRLCRGDSSGEGVCDKSPLERYYDYSGAFRCLAEGAGDVAFVKHST VLENTDGKTLPSWGQALLSQDFELLCRDGSRADVTEWRQCHLARVPAHAVVVRA DTDGGLIFRLLNEGQRLFSHEGSSFQMFSSEAYGQKDLLFKDSTSELVPIATQT YEAWLGHEYLHAMKGLLCDPNRLPPYLRWCVLSTPEIQKCGDMAVAFRRQRLKP EIQCVSAKSPQHCMERIQAEQVDAVTLSGEDIYTAGKTYGLVPAAGEHYAPEDS SNSYYVVAVVRRDSSHAFTLDELRGKRSCHAGFGSPAGWDVPVGALIQRGFIRP KDCDVLTAVSEFFNASCVPVNNPKNYPSSLCALCVGDEQGRNKCVGNSQERYYG YRGAFRCLVENAGDVAFVRHTTVFDNTNGHNSEPWAAELRSEDYELLCPNGARA EVSQFAACNLAQIPPHAVMVRPDTNIFTVYGLLDKAQDLFGDDHNKNGFKMFDS SNYHGQDLLFKDATVRAVPVGEKTTYRGWLGLDYVAALEGMSSQQCS**EAAAKEA AAKEAAAK**SETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHS LLFQNAFAQQAVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKE NGYVTMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELH ANLLCPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFR YPKEFQKLYPLENITLAPDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGP IPVDFQRKIRQSYFASVSYLDTQVGRLLSALDDLQLANSTIIAFTSDHGWALGE HGEWAKYSNFDVATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGR QSMDLVELVSLFPTLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEE DPYLPGNPRELIAYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWV GFNPDEFLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP	139
MTfpep-I2S (**SP** : TAG : **PS** : MTfpep : **Linker** : I2S)	**MEWSWVFLFFLSVTTGVHS**DYKDDDDKEQKLISEEDLHHHHHHHHHHGGGG**ENL YFQG**DSSHAFTLDELRY**EAAAKEAAAKEAAAK**SETQANSTTDALNVLLIIVDDL RPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQAVCAPSRVSFLTGRRPDTTR LYDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVFHPGISSNHTDDSPYSWSF PPYHPSSEKYENTKTCRGPDGELHANLLCPVDVLDVPEGTLPDKQSTEQAIQLL EKMKTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLENITLAPDPEVPDGLPPVA YNPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQSYFASVSYLDTQVGRLLSA LDDLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDVATHVPLIFYVPGRTASLP EAGEKLFPYLDPFDSASQLMEPGRQSMDLVELVSLFPTLAGLAGLQVPPRCPVP SFHVELCREGKNLLKHFRFRDLEEDPYLPGNPRELIAYSQYPRPSDIPQWNSDK PSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANFSDIHAGELYFVDSDPLQDH	140

	NMYNDSQGGDLFQLLMP	
I2S-MTfpep (**SP** : TAG : **PS** : I2S : **Linker** : MTfpep)	**MEWSWVFLFFLSVTTGVHS**DYKDDDDKEQKLISEEDLHHHHHHHHHHGGGG**ENL YFQG**SETQANSTTDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQ NAFAQQAVCAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYV TMSVGKVFHPGISSNHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHANLL CPVDVLDVPEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFRYPKE FQKLYPLENITLAPDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIPVD FQRKIRQSYFASVSYLDTQVGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEW AKYSNFDVATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMD LVELVSLFPTLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYL PGNPRELIAYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNP DEFLANFSDIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP**EAAAKEAAAKE AAAK**DSSHAFTLDELRY	141
I2S-MTfpep (without propep of I2S) **SP** : TAG : **PS** : I2S w/o propep : **Linker** : MTfpep)	**MEWSWVFLFFLSVTTGVHS**DYKDDDDKEQKLISEEDLHHHHHHHHHHGGGG**ENL YFQG**TDALNVLLIIVDDLRPSLGCYGDKLVRSPNIDQLASHSLLFQNAFAQQAV CAPSRVSFLTGRRPDTTRLYDFNSYWRVHAGNFSTIPQYFKENGYVTMSVGKVF HPGISSNHTDDSPYSWSFPPYHPSSEKYENTKTCRGPDGELHANLLCPVDVLDV PEGTLPDKQSTEQAIQLLEKMKTSASPFFLAVGYHKPHIPFRYPKEFQKLYPLE NITLAPDPEVPDGLPPVAYNPWMDIRQREDVQALNISVPYGPIPVDFQRKIRQS YFASVSYLDTQVGRLLSALDDLQLANSTIIAFTSDHGWALGEHGEWAKYSNFDV ATHVPLIFYVPGRTASLPEAGEKLFPYLDPFDSASQLMEPGRQSMDLVELVSLF PTLAGLAGLQVPPRCPVPSFHVELCREGKNLLKHFRFRDLEEDPYLPGNPRELI AYSQYPRPSDIPQWNSDKPSLKDIKIMGYSIRTIDYRYTVWVGFNPDEFLANFS DIHAGELYFVDSDPLQDHNMYNDSQGGDLFQLLMP**EAAAKEAAAKEAAAK**DSSH AFTLDELRY	142

Table E2. Polynucleotide Coding Sequences of Fusion Constructs

Name	Polynucleotide Sequence	SEQ ID NO:
I2S-MTf	ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCAC TCCGACTACAAGGACGACGACGACAAAGAGCAGAAGCTGATCTCCGAAGAGGAC CTGCACCACCATCATCACCATCACCACCATCACGGAGGCGGTGGAGAGAACCTG TACTTTCAGGGCTCGGAAACTCAGGCCAACTCCACCACAGATGCACTCAACGTG CTGCTGATCATCGTAGATGACCTCCGACCTTCTCTGGGCTGTTACGGCGACAAG CTAGTACGGAGCCCAAACATCGACCAGCTCGCATCGCACTCTCTCCTATTCCAG AACGCATTCGCCCAGCAGGCTGTCTGTGCTCCCTCCCGAGTGTCCTTCCTCACG GGTCGGAGACCCGATACCACGAGGTTATATGACTTCAACTCATACTGGCGCGTG CATGCCGGTAACTTTTCTACTATACCCCAGTATTTTAAAGAAAATGGCTATGTT ACAATGTCCGTTGGCAAGGTATTTCATCCTGGTATTAGCAGCAACCACACAGAT GACTCTCCGTATAGCTGGTCATTCCCACCATACCACCCCTCCAGCGAAAAGTAC GAAAACACAAAGACTTGCCGGGGCCCAGATGGCGAACTGCACGCAAATCTGCTG TGCCCTGTAGATGTCTTGGACGTGCCCGAAGGTACTCTGCCCGACAAACAGTCC ACAGAACAGGCAATCCAACTCCTTGAAAAGATGAAAACGAGCGCGTCCCCCTTC TTCCTCGCCGTGGGCTACCACAAGCCCCACATCCCGTTTAGATACCCCAAGGAA TTTCAGAAACTGTACCCCCTGGAAAACATCACTCTCGCGCCCGACCCCGAAGTG CCAGACGGACTCCCTCCTGTTGCCTACAACCCTTGGATGGACATCAGACAACGT GAAGATGTGCAGGCCCTGAACATCTCAGTGCCTTACGGCCCCATTCCAGTTGAC TTCCAGAGGAAGATTCGGCAGTCCTACTTCGCCTCCGTTAGTTACCTGGACACC CAAGTGGGTAGACTCCTGAGCGCCTTGGACGATCTCCAGCTCGCAAACAGCACC ATCATTGCCTTCACCAGCGACCATGGTTGGGCGCTGGGTGAACATGGAGAATGG GCTAAATATTCAAATTTCGACGTTGCGACCCACGTCCCATTGATCTTCTACGTG CCTGGACGAACAGCCTCCTTGCCTGAAGCCGGGGAAAAGTTGTTTCCATATCTG GACCCTTTCGATTCTGCGAGCCAACTCATGGAACCTGGGCGACAGAGCATGGAC CTGGTGGAACTGGTCAGTTTATTTCCAACCCTGGCAGGCCTTGCAGGCCTCCAA GTTCCACCTCGGTGTCCCGTTCCCTCATTCCACGTCGAACTCTGTCGCGAAGGT AAAAACCTCCTCAAGCATTTTCGTTTTCGGGACCTCGAAGAAGACCCATACCTG CCAGGGAATCCAAGGGAACTGATTGCCTACAGCCAGTACCCTAGACCTAGCGAC ATCCCACAGTGGAACAGCGACAAGCCCTCCCTCAAGGACATTAAAATCATGGGT TATAGTATCCGGACTATTGACTACAGGTATACCGTGTGGGTGGGTTTCAACCCA	143

	GACGAATTTCTCGCCAATTTCTCCGACATCCACGCGGGCGAACTGTATTTCGTT GATTCCGATCCACTGCAAGATCATAATATGTACAACGATAGTCAAGGGGGTGAC CTCTTCCAGTTGCTAATGCCAGAAGCCGCCGCGAAAGAAGCCGCCGCAAAAGAA GCCGCTGCCAAAGGCATGGAAGTGCGTTGGTGCGCCACCTCTGACCCCGAGCAG CACAAGTGCGGCAACATGTCCGAGGCCTTCAGAGAGGCCGGCATCCAGCCTTCT CTGCTGTGTGTGCGGGGCACCTCTGCCGACCATTGCGTGCAGCTGATCGCCGCC CAGGAAGCCGACGCTATCACACTGGATGGCGGCGCTATCTACGAGGCTGGCAAA GAGCACGGCCTGAAGCCCGTCGTGGGCGAGGTGTACGATCAGGAAGTGGGCACC TCCTACTACGCCGTGGCTGTCGTGCGGAGATCCTCCCACGTGACCATCGACACC CTGAAGGGCGTGAAGTCCTGCCACACCGGCATCAACAGAACCGTGGGCTGGAAC GTGCCCGTGGGCTACCTGGTGGAATCCGGCAGACTGTCCGTGATGGGCTGCGAC GTGCTGAAGGCCGTGTCCGATTACTTCGGCGGCTCTTGTGTGCCTGGCGCTGGC GAGACATCCTACTCCGAGTCCCTGTGCAGACTGTGCAGGGGCGACTCTTCTGGC GAGGGCGTGTGCGACAAGTCCCCTCTGGAACGGTACTACGACTACTCCGGCGCC TTCAGATGCCTGGCTGAAGGTGCTGGCGACGTGGCCTTCGTGAAGCACTCCACC GTGCTGGAAAACACCGACGGCAAGACCCTGCCTTCTTGGGGCCAGGCACTGCTG TCCCAGGACTTCGAGCTGCTGTGCCGGGATGGCTCCAGAGCCGATGTGACAGAG TGGCGGCAGTGCCACCTGGCCAGAGTGCCTGCTCATGCTGTGGTCGTGCGCGCC GATACAGATGGCGGCCTGATCTTCCGGCTGCTGAACGAGGGCCAGCGGCTGTTC TCTCACGAGGGCTCCAGCTTCCAGATGTTCTCCAGCGAGGCCTACGGCCAGAAG GACCTGCTGTTCAAGGACTCCACCTCCGAGCTGGTGCCTATCGCCACCCAGACC TATGAGGCTTGGCTGGGCCACGAGTACCTGCACGCTATGAAGGGACTGCTGTGC GACCCCAACCGGCTGCCTCCTTATCTGAGGTGGTGCGTGCTGTCCACCCCCGAG ATCCAGAAATGCGGCGATATGGCCGTGGCCTTTCGGCGGCAGAGACTGAAGCCT GAGATCCAGTGCGTGTCCGCCAAGAGCCCTCAGCACTGCATGGAACGGATCCAG GCCGAACAGGTGGACGCCGTGACACTGTCCGGCGAGGATATCTACACCGCCGGA AAGACCTACGGCCTGGTGCCAGCTGCTGGCGAGCATTACGCCCCTGAGGACTCC TCCAACAGCTACTACGTGGTGGCAGTCGTGCGCCGGGACTCCTCTCACGCCTTT ACCCTGGATGAGCTGCGGGGCAAGAGAAGCTGTCACGCCGGCTTTGGAAGCCCT GCCGGATGGGATGTGCCTGTGGGCGCTCTGATCCAGCGGGGCTTCATCAGACCC AAGGACTGTGATGTGCTGACCGCCGTGTCTGAGTTCTTCAACGCCTCCTGTGTG CCCGTGAACAACCCCAAGAACTACCCCTCCAGCCTGTGCGCCCTGTGTGTGGGA GATGAGCAGGGCCGGAACAAATGCGTGGGCAACTCCCAGGAAAGATATTACGGC TACAGAGGCGCCTTCCGGTGTCTGGTGGAAAACGCCGGGGATGTGGCTTTTGTG CGGCACACCACCGTGTTCGACAACACCAATGGCCACAACTCCGAGCCTTGGGCC GCTGAGCTGAGATCCGAGGATTACGAACTGCTGTGTCCCAACGGCGCCAGGGCT GAGGTGTCCCAGTTTGCCGCCTGTAACCTGGCCCAGATCCCTCCCCACGCTGTG ATGGTGCGACCCGACACCAACATCTTCACCGTGTACGGCCTGCTGGACAAGGCC CAGGATCTGTTCGGCGACGACCACAACAAGAACGGGTTCAAGATGTTCGACTCC AGCAACTACCACGGACAGGATCTGCTGTTTAAAGATGCCACCGTGCGGGCCGTG CCAGTGGGCGAAAAGACCACCTACAGAGGATGGCTGGGACTGGACTACGTGGCC GCCCTGGAAGGCATGTCCTCCCAGCAGTGTTCCTGA	
MTf-I2S	ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCAC TCCGACTACAAGGACGACGACGACAAAGAGCAGAAGCTGATCTCCGAAGAGGAC CTGCACCACCATCATCACCATCACCACCATCACGGAGGCGGTGGAGAGAACCTG TACTTTCAGGGCGGCATGGAAGTGCGTTGGTGCGCCACCTCTGACCCCGAGCAG CACAAGTGCGGCAACATGTCCGAGGCCTTCAGAGAGGCCGGCATCCAGCCTTCT CTGCTGTGTGTGCGGGGCACCTCTGCCGACCATTGCGTGCAGCTGATCGCCGCC CAGGAAGCCGACGCTATCACACTGGATGGCGGCGCTATCTACGAGGCTGGCAAA GAGCACGGCCTGAAGCCCGTCGTGGGCGAGGTGTACGATCAGGAAGTGGGCACC TCCTACTACGCCGTGGCTGTCGTGCGGAGATCCTCCCACGTGACCATCGACACC CTGAAGGGCGTGAAGTCCTGCCACACCGGCATCAACAGAACCGTGGGCTGGAAC GTGCCCGTGGGCTACCTGGTGGAATCCGGCAGACTGTCCGTGATGGGCTGCGAC GTGCTGAAGGCCGTGTCCGATTACTTCGGCGGCTCTTGTGTGCCTGGCGCTGGC GAGACATCCTACTCCGAGTCCCTGTGCAGACTGTGCAGGGGCGACTCTTCTGGC GAGGGCGTGTGCGACAAGTCCCCTCTGGAACGGTACTACGACTACTCCGGCGCC TTCAGATGCCTGGCTGAAGGTGCTGGCGACGTGGCCTTCGTGAAGCACTCCACC GTGCTGGAAAACACCGACGGCAAGACCCTGCCTTCTTGGGGCCAGGCACTGCTG TCCCAGGACTTCGAGCTGCTGTGCCGGGATGGCTCCAGAGCCGATGTGACAGAG TGGCGGCAGTGCCACCTGGCCAGAGTGCCTGCTCATGCTGTGGTCGTGCGCGCC GATACAGATGGCGGCCTGATCTTCCGGCTGCTGAACGAGGGCCAGCGGCTGTTC	144

	TCTCACGAGGGCTCCAGCTTCCAGATGTTCTCCAGCGAGGCCTACGGCCAGAAG GACCTGCTGTTCAAGGACTCCACCTCCGAGCTGGTGCCTATCGCCACCCAGACC TATGAGGCTTGGCTGGGCCACGAGTACCTGCACGCTATGAAGGGACTGCTGTGC GACCCCAACCGGCTGCCTCCTTATCTGAGGTGGTGCGTGCTGTCCACCCCCGAG ATCCAGAAATGCGGCGATATGGCCGTGGCCTTTCGGCGGCAGAGACTGAAGCCT GAGATCCAGTGCGTGTCCGCCAAGAGCCCTCAGCACTGCATGGAACGGATCCAG GCCGAACAGGTGGACGCCGTGACACTGTCCGGCGAGGATATCTACACCGCCGGA AAGACCTACGGCCTGGTGCCAGCTGCTGGCGAGCATTACGCCCCTGAGGACTCC TCCAACAGCTACTACGTGGTGGCAGTCGTGCGCCGGGACTCCTCTCACGCCTTT ACCCTGGATGAGCTGCGGGGCAAGAGAAGCTGTCACGCCGGCTTTGGAAGCCCT GCCGGATGGGATGTGCCTGTGGGCGCTCTGATCCAGCGGGGCTTCATCAGACCC AAGGACTGTGATGTGCTGACCGCCGTGTCTGAGTTCTTCAACGCCTCCTGTGTG CCCGTGAACAACCCCAAGAACTACCCCTCCAGCCTGTGCGCCCTGTGTGTGGGA GATGAGCAGGGCCGGAACAAATGCGTGGGCAACTCCCAGGAAAGATATTACGGC TACAGAGGCGCCTTCCGGTGTCTGGTGGAAAACGCCGGGGATGTGGCTTTTGTG CGGCACACCACCGTGTTCGACAACACCAATGGCCACAACTCCGAGCCTTGGGCC GCTGAGCTGAGATCCGAGGATTACGAACTGCTGTGTCCCAACGGCGCCAGGGCT GAGGTGTCCCAGTTTGCCGCCTGTAACCTGGCCCAGATCCCTCCCCACGCTGTG ATGGTGCGACCCGACACCAACATCTTCACCGTGTACGGCCTGCTGGACAAGGCC CAGGATCTGTTCGGCGACGACCACAACAAGAACGGGTTCAAGATGTTCGACTCC AGCAACTACCACGGACAGGATCTGCTGTTTAAAGATGCCACCGTGCGGGCCGTG CCAGTGGGCGAAAAGACCACCTACAGAGGATGGCTGGGACTGGACTACGTGGCC GCCCTGGAAGGCATGTCCTCCCAGCAGTGTTCCGAAGCCGCCGCGAAAGAAGCC GCCGCAAAAGAAGCCGCTGCCAAATCGGAAACTCAGGCCAACTCCACCACAGAT GCACTCAACGTGCTGCTGATCATCGTAGATGACCTCCGACCTTCTCTGGGCTGT TACGGCGACAAGCTAGTACGGAGCCCAAACATCGACCAGCTCGCATCGCACTCT CTCCTATTCCAGAACGCATTCGCCCAGCAGGCTGTCTGTGCTCCCTCCCGAGTG TCCTTCCTCACGGGTCGGAGACCCGATACCACGAGGTTATATGACTTCAACTCA TACTGGCGCGTGCATGCCGGTAACTTTTCTACTATACCCCAGTATTTTAAAGAA AATGGCTATGTTACAATGTCCGTTGGCAAGGTATTTCATCCTGGTATTAGCAGC AACCACACAGATGACTCTCCGTATAGCTGGTCATTCCCACCATACCACCCCTCC AGCGAAAAGTACGAAAACACAAAGACTTGCCGGGGCCCAGATGGCGAACTGCAC GCAAATCTGCTGTGCCCTGTAGATGTCTTGGACGTGCCCGAAGGTACTCTGCCC GACAAACAGTCCACAGAACAGGCAATCCAACTCCTTGAAAAGATGAAAACGAGC GCGTCCCCCTTCTTCCTCGCCGTGGGCTACCACAAGCCCCACATCCCGTTTAGA TACCCCAAGGAATTTCAGAAACTGTACCCCCTGGAAAACATCACTCTCGCGCCC GACCCCGAAGTGCCAGACGGACTCCCTCCTGTTGCCTACAACCCTTGGATGGAC ATCAGACAACGTGAAGATGTGCAGGCCCTGAACATCTCAGTGCCTTACGGCCCC ATTCCAGTTGACTTCCAGAGGAAGATTCGGCAGTCCTACTTCGCCTCCGTTAGT TACCTGGACACCCAAGTGGGTAGACTCCTGAGCGCCTTGGACGATCTCCAGCTC GCAAACAGCACCATCATTGCCTTCACCAGCGACCATGGTTGGGCGCTGGGTGAA CATGGAGAATGGGCTAAATATTCAAATTTCGACGTTGCGACCCACGTCCCATTG ATCTTCTACGTGCCTGGACGAACAGCCTCCTTGCCTGAAGCCGGGGAAAAGTTG TTTCCATATCTGGACCCTTTCGATTCTGCGAGCCAACTCATGGAACCTGGGCGA CAGAGCATGGACCTGGTGGAACTGGTCAGTTTATTTCCAACCCTGGCAGGCCTT GCAGGCCTCCAAGTTCCACCTCGGTGTCCCGTTCCCTCATTCCACGTCGAACTC TGTCGCGAAGGTAAAAACCTCCTCAAGCATTTTCGTTTTCGGGACCTCGAAGAA GACCCATACCTGCCAGGGAATCCAAGGGAACTGATTGCCTACAGCCAGTACCCT AGACCTAGCGACATCCCACAGTGGAACAGCGACAAGCCCTCCCTCAAGGACATT AAAATCATGGGTTATAGTATCCGGACTATTGACTACAGGTATACCGTGTGGGTG GGTTTCAACCCAGACGAATTTCTCGCCAATTTCTCCGACATCCACGCGGGCGAA CTGTATTTCGTTGATTCCGATCCACTGCAAGATCATAATATGTACAACGATAGT CAAGGGGGTGACCTCTTCCAGTTGCTAATGCCATGA	
MTfpep-I2S	ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCAC TCCGACTACAAGGACGACGACGACAAAGAGCAGAAGCTGATCTCCGAAGAGGAC CTGCACCACCATCATCACCATCACCACCATCACGGAGGCGGTGGAGAGAACCTG TACTTTCAGGGCGACTCCTCTCACGCCTTCACCCTGGACGAGCTGCGGTACGAA GCCGCCGCGAAAGAAGCCGCCGCAAAAGAAGCCGCTGCCAAATCGGAAACTCAG GCCAACTCCACCACAGATGCACTCAACGTGCTGCTGATCATCGTAGATGACCTC CGACCTTCTCTGGGCTGTTACGGCGACAAGCTAGTACGGAGCCCAAACATCGAC CAGCTCGCATCGCACTCTCTCCTATTCCAGAACGCATTCGCCCAGCAGGCTGTC	145

	TGTGCTCCCTCCCGAGTGTCCTTCCTCACGGGTCGGAGACCCGATACCACGAGG TTATATGACTTCAACTCATACTGGCGCGTGCATGCCGGTAACTTTTCTACTATA CCCCAGTATTTTAAAGAAAATGGCTATGTTACAATGTCCGTTGGCAAGGTATTT CATCCTGGTATTAGCAGCAACCACACAGATGACTCTCCGTATAGCTGGTCATTC CCACCATACCACCCCTCCAGCGAAAAGTACGAAAACACAAAGACTTGCCGGGGC CCAGATGGCGAACTGCACGCAAATCTGCTGTGCCCTGTAGATGTCTTGGACGTG CCCGAAGGTACTCTGCCCGACAAACAGTCCACAGAACAGGCAATCCAACTCCTT GAAAAGATGAAAACGAGCGCGTCCCCCTTCTTCCTCGCCGTGGGCTACCACAAG CCCCACATCCCGTTTAGATACCCCAAGGAATTTCAGAAACTGTACCCCCTGGAA AACATCACTCTCGCGCCCGACCCCGAAGTGCCAGACGGACTCCCTCCTGTTGCC TACAACCCTTGGATGGACATCAGACAACGTGAAGATGTGCAGGCCCTGAACATC TCAGTGCCTTACGGCCCCATTCCAGTTGACTTCCAGAGGAAGATTCGGCAGTCC TACTTCGCCTCCGTTAGTTACCTGGACACCCAAGTGGGTAGACTCCTGAGCGCC TTGGACGATCTCCAGCTCGCAAACAGCACCATCATTGCCTTCACCAGCGACCAT GGTTGGGCGCTGGGTGAACATGGAGAATGGGCTAAATATTCAAATTTCGACGTT GCGACCCACGTCCCATTGATCTTCTACGTGCCTGGACGAACAGCCTCCTTGCCT GAAGCCGGGGAAAAGTTGTTTCCATATCTGGACCCTTTCGATTCTGCGAGCCAA CTCATGGAACCTGGGCGACAGAGCATGGACCTGGTGGAACTGGTCAGTTTATTT CCAACCCTGGCAGGCCTTGCAGGCCTCCAAGTTCCACCTCGGTGTCCCGTTCCC TCATTCCACGTCGAACTCTGTCGCGAAGGTAAAAACCTCCTCAAGCATTTTCGT TTTCGGGACCTCGAAGAAGACCCATACCTGCCAGGGAATCCAAGGGAACTGATT GCCTACAGCCAGTACCCTAGACCTAGCGACATCCCACAGTGGAACAGCGACAAG CCCTCCCTCAAGGACATTAAAATCATGGGTTATAGTATCCGGACTATTGACTAC AGGTATACCGTGTGGGTGGGTTTCAACCCAGACGAATTTCTCGCCAATTTCTCC GACATCCACGCGGGCGAACTGTATTTCGTTGATTCCGATCCACTGCAAGATCAT AATATGTACAACGATAGTCAAGGGGGTGACCTCTTCCAGTTGCTAATGCCATGA	
I2S- MTfpep	ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCAC TCCGACTACAAGGACGACGACGACAAAGAGCAGAAGCTGATCTCCGAAGAGGAC CTGCACCACCATCATCACCATCACCACCATCACGGAGGCGGTGGAGAGAACCTG TACTTTCAGGGCTCGGAAACTCAGGCCAACTCCACCACAGATGCACTCAACGTG CTGCTGATCATCGTAGATGACCTCCGACCTTCTCTGGGCTGTTACGGCGACAAG CTAGTACGGAGCCCAAACATCGACCAGCTCGCATCGCACTCTCTCCTATTCCAG AACGCATTCGCCCAGCAGGCTGTCTGTGCTCCCTCCCGAGTGTCCTTCCTCACG GGTCGGAGACCCGATACCACGAGGTTATATGACTTCAACTCATACTGGCGCGTG CATGCCGGTAACTTTTCTACTATACCCCAGTATTTTAAAGAAAATGGCTATGTT ACAATGTCCGTTGGCAAGGTATTTCATCCTGGTATTAGCAGCAACCACACAGAT GACTCTCCGTATAGCTGGTCATTCCCACCATACCACCCCTCCAGCGAAAAGTAC GAAAACACAAAGACTTGCCGGGGCCCAGATGGCGAACTGCACGCAAATCTGCTG TGCCCTGTAGATGTCTTGGACGTGCCCGAAGGTACTCTGCCCGACAAACAGTCC ACAGAACAGGCAATCCAACTCCTTGAAAAGATGAAAACGAGCGCGTCCCCCTTC TTCCTCGCCGTGGGCTACCACAAGCCCCACATCCCGTTTAGATACCCCAAGGAA TTTCAGAAACTGTACCCCCTGGAAAACATCACTCTCGCGCCCGACCCCGAAGTG CCAGACGGACTCCCTCCTGTTGCCTACAACCCTTGGATGGACATCAGACAACGT GAAGATGTGCAGGCCCTGAACATCTCAGTGCCTTACGGCCCCATTCCAGTTGAC TTCCAGAGGAAGATTCGGCAGTCCTACTTCGCCTCCGTTAGTTACCTGGACACC CAAGTGGGTAGACTCCTGAGCGCCTTGGACGATCTCCAGCTCGCAAACAGCACC ATCATTGCCTTCACCAGCGACCATGGTTGGGCGCTGGGTGAACATGGAGAATGG GCTAAATATTCAAATTTCGACGTTGCGACCCACGTCCCATTGATCTTCTACGTG CCTGGACGAACAGCCTCCTTGCCTGAAGCCGGGGAAAAGTTGTTTCCATATCTG GACCCTTTCGATTCTGCGAGCCAACTCATGGAACCTGGGCGACAGAGCATGGAC CTGGTGGAACTGGTCAGTTTATTTCCAACCCTGGCAGGCCTTGCAGGCCTCCAA GTTCCACCTCGGTGTCCCGTTCCCTCATTCCACGTCGAACTCTGTCGCGAAGGT AAAAACCTCCTCAAGCATTTTCGTTTTCGGGACCTCGAAGAAGACCCATACCTG CCAGGGAATCCAAGGGAACTGATTGCCTACAGCCAGTACCCTAGACCTAGCGAC ATCCCACAGTGGAACAGCGACAAGCCCTCCCTCAAGGACATTAAAATCATGGGT TATAGTATCCGGACTATTGACTACAGGTATACCGTGTGGGTGGGTTTCAACCCA GACGAATTTCTCGCCAATTTCTCCGACATCCACGCGGGCGAACTGTATTTCGTT GATTCCGATCCACTGCAAGATCATAATATGTACAACGATAGTCAAGGGGGTGAC CTCTTCCAGTTGCTAATGCCAGAGGCCGCTGCTAAAGAGGCTGCCGCCAAAGAA GCCGCCGCTAAGGACTCCTCTCACGCCTTCACCCTGGACGAGCTGCGGTACTAA	146
I2S-	ATGGAATGGAGCTGGGTCTTTCTCTTCTTCCTGTCAGTAACGACTGGTGTCCAC	147

MTfpep (without propep of I2S)	TCCGACTACAAGGACGACGACGACAAAGAGCAGAAGCTGATCTCCGAAGAGGAC CTGCACCACCATCATCACCATCACCACCATCACGGAGGCGGTGGAGAGAACCTG TACTTTCAGGGCACAGATGCACTCAACGTGCTGCTGATCATCGTAGATGACCTC CGACCTTCTCTGGGCTGTTACGGCGACAAGCTAGTACGGAGCCCAAACATCGAC CAGCTCGCATCGCACTCTCTCCTATTCCAGAACGCATTCGCCCAGCAGGCTGTC TGTGCTCCCTCCCGAGTGTCCTTCCTCACGGGTCGGAGACCCGATACCACGAGG TTATATGACTTCAACTCATACTGGCGCGTGCATGCCGGTAACTTTTCTACTATA CCCCAGTATTTTAAAGAAAATGGCTATGTTACAATGTCCGTTGGCAAGGTATTT CATCCTGGTATTAGCAGCAACCACACAGATGACTCTCCGTATAGCTGGTCATTC CCACCATACCACCCCTCCAGCGAAAAGTACGAAAACACAAAGACTTGCCGGGGC CCAGATGGCGAACTGCACGCAAATCTGCTGTGCCCTGTAGATGTCTTGGACGTG CCCGAAGGTACTCTGCCCGACAAACAGTCCACAGAACAGGCAATCCAACTCCTT GAAAAGATGAAAACGAGCGCGTCCCCCTTCTTCCTCGCCGTGGGCTACCACAAG CCCCACATCCCGTTTAGATACCCCAAGGAATTTCAGAAACTGTACCCCCTGGAA AACATCACTCTCGCGCCCGACCCCGAAGTGCCAGACGGACTCCCTCCTGTTGCC TACAACCCTTGGATGGACATCAGACAACGTGAAGATGTGCAGGCCCTGAACATC TCAGTGCCTTACGGCCCCATTCCAGTTGACTTCCAGAGGAAGATTCGGCAGTCC TACTTCGCCTCCGTTAGTTACCTGGACACCCAAGTGGGTAGACTCCTGAGCGCC TTGGACGATCTCCAGCTCGCAAACAGCACCATCATTGCCTTCACCAGCGACCAT GGTTGGGCGCTGGGTGAACATGGAGAATGGGCTAAATATTCAAATTTCGACGTT GCGACCCACGTCCCATTGATCTTCTACGTGCCTGGACGAACAGCCTCCTTGCCT GAAGCCGGGGAAAAGTTGTTTCCATATCTGGACCCTTTCGATTCTGCGAGCCAA CTCATGGAACCTGGGCGACAGAGCATGGACCTGGTGGAACTGGTCAGTTTATTT CCAACCCTGGCAGGCCTTGCAGGCCTCCAAGTTCCACCTCGGTGTCCCGTTCCC TCATTCCACGTCGAACTCTGTCGCGAAGGTAAAAACCTCCTCAAGCATTTTCGT TTTCGGGACCTCGAAGAAGACCCATACCTGCCAGGGAATCCAAGGGAACTGATT GCCTACAGCCAGTACCCTAGACCTAGCGACATCCCACAGTGGAACAGCGACAAG CCCTCCCTCAAGGACATTAAAATCATGGGTTATAGTATCCGGACTATTGACTAC AGGTATACCGTGTGGGTGGGTTTCAACCCAGACGAATTTCTCGCCAATTTCTCC GACATCCACGCGGGCGAACTGTATTTCGTTGATTCCGATCCACTGCAAGATCAT AATATGTACAACGATAGTCAAGGGGGTGACCTCTTCCAGTTGCTAATGCCAGAG GCCGCTGCTAAAGAGGCTGCCGCCAAAGAAGCCGCCGCTAAGGACTCCTCTCAC GCCTTCACCCTGGACGAGCTGCGGTACTAA	

Recombinant proteins were prepared and tested for enzymatic activity against the substrate 4-Nitrocatechol Sulfate (PNCS) relative to recombinant human IDS and a negative control (trastuzumab-MTf fusion). The results are shown in Figures 2-4. One µg of each sample was used in the enzyme activity assay, and the data presented are normalized to substrate blank.

Figure 2 shows the enzyme activity evaluation of I2S-MTf and MTf-I2S fusion proteins as measured by their ability to hydrolyze the substrate 4-Nitrocatechol Sulfate (PNCS) relative to recombinant human IDS and negative control (TZM-MTf fusion). These data show that the I2S-MTf and MTf-I2S fusion proteins not only had significant enzymatic activity, but also had increased enzymatic activity relative to wild-type (non-fusion) human IDS.

Figure 3 shows the enzyme activity evaluation of MTfpep-I2S and I2S-MTfpep (with I2S propeptide) fusion proteins as measured by their ability to hydrolyze the substrate PNCS relative to I2S-MTf fusion and negative control (TZM-MTf fusion). These data show that the MTfpep-I2S and I2S-MTfpep fusion proteins not only had significant enzymatic activity, but also had increased enzymatic activity relative to the significantly active I2S-MTf fusion protein (from Figure 2), and thus increased enzymatic activity relative to wild-type (non-fusion) human IDS.

Figure 4 shows a comparison of the enzyme activity of I2S-MTfpep (with I2S propeptide) and I2S-MTfpep (without I2S propeptide) fusion proteins as measured by their ability to hydrolyze the substrate PNCS. These data show that the MTfpep-I2S and I2S-MTfpep fusion proteins not only had significant enzymatic activity, but also had increased enzymatic activity relatively to wild-type (non-fusion) human IDS.

Example 2

In Vivo Distribution of I2S-MTf and MTfpep-I2S Fusions in the Brain

The brain biodistribution of the I2S-MTf and MTfpep-I2S fusion proteins in mice was evaluated by quantitative confocal microscopy imaging. Therapeutic dose equivalents of I2S-MTf and MTfpep-I2S were administered in 100 µL volume to mice via tail vein injection. Prior to euthanasia, mice were injected (i.v.) with Tomato Lectin-FITC (40 µg) for 10 min to stain the brain vasculature. Blood was cleared by intracardiac perfusion of 10ml heparinised saline at a rate of 1ml per minute. Brains were excised and frozen in OCT and stored at -80°C. Brains were mounted in Tissue Tek and sectioned with a cryostat at -20°C. Sections were mounted on Superfrost Plus microscope slides, fixed in cold Acetone/MeOH (1:1) for 10 minutes at room temperature, and washed with PBS. Glass coverslips were mounted on sections using Prolong Gold antifade reagent with DAPI (molecular probes, P36931). Three-dimensional (3D) confocal microscopy and quantitative analysis was performed.

Figure 5 shows quantification of the relative distribution of MTfpep-I2S (with propeptide) and I2S-MTf fusion proteins between capillaries (C) and parenchyma (P) in the brain, relative to the total (T) signal. The significant staining of parenchymal tissues relative to capillaries confirms that the MTfpep-I2S and I2S-MTf fusion proteins were both able to cross the blood brain barrier (BBB) and accumulate in tissues of the central nervous system.

In summary, the data from Examples 1 and 2 show that the MTfpep-I2S (with propeptide) and I2S-MTf fusion proteins are not only able to cross the BBB and accumulate in tissues of the CNS, but also have significantly increased enzymatic activity relative to wild-type (non-fusion) recombinant human IDS.

Example 3

In Vivo Activity of I2S-MTf and MTfpep-I2S Fusions in Mouse Model of MPS II

The therapeutic efficacy of the I2S-MTf and MTfpep-I2S fusion proteins is evaluated in a mouse model of Hunter Syndrome or Mucopolysaccharidosis type II (MPS II) relative to Idursulfase (Elaprase®), which is indicated for the treatment of Hunter Syndrome. These studies are designed to

evaluate the effect of intravenous (IV) and intraperitoneal (IP) administration of the fusion proteins on brain pathology in a knock-out mouse model of Mucopolysaccharidosis II (MPSII).

Hunter Syndrome. As noted above, Hunter Syndrome is an X-linked recessive disease caused by insufficient levels of the lysosomal enzyme iduronate 2-sulfatase (IDS). This enzyme cleaves the terminal 2-O-sulfate moieties from the glycosaminoglycans (GAG) dermatan-sulfate and heparan-sulfate. Due to the missing or defective IDS enzyme activity in patients with Hunter syndrome, GAG accumulate progressively in the lysosomes of a variety of cell types. This leads to cellular engorgement, organomegaly, tissue destruction, and organ system dysfunction.

Mouse Model. IDS-KO mice have little or no tissue IDS activity and exhibit many of the cellular and clinical effects observed in Hunter's syndrome including increased tissue vacuolization, GAG levels, and urinary excretion of GAG. Due to the X-linked recessive nature of Hunter syndrome, all pharmacology studies are performed in male mice. Animal breeding is performed as described by Garcia et al, 2007 (3). Briefly, carrier females are bred with wild type male mice of the C57Bl/6 background strain, producing heterogenous females and hemizygous male knock-out mice, as well as wild-type (WT) males and females. IDS-KO male mice are alternatively obtained by breeding carrier females with IDS-KO male mice. The genotype of all mice used in these experiments is confirmed by polymerase chain reaction of DNA obtained from tail snip. All IDS-KO mice are hemizygous IKO (-/0) male and between 12-13 weeks old at the beginning of treatment initiation (mice younger than 12 weeks are not used in this study). A group of untreated WT littermate (+/0) males are used as controls.

Idursulfase (Elaprase®). Idursulfase is a drug used to treat Hunter syndrome (also called MPS-II) (see Garcia et al., Mol Genet Metab. 91:183-90, 2007). It is a purified form of the lysosomal enzyme iduronate-2-sulfatase and is produced by recombinant DNA technology in a human cell line

Study Design. The study design is outlined in **Table E3** below.

Table E3

Group	Animal	Mice/ Group	Dose level (mg/kg)	Dose volume (mL/kg)	Treatment regimen	Sacrifice
Vehicle (control)	WT	5	0	5-6	IV, once per week for 6wks	24h after last injection
Vehicle (control)	IDS-KO	5	0	5-6	IV, once per week for 6wks	24h after last injection
IDS (Elaprase) (high dose)	IDS-KO	5	6 mg/kg	5-6	IV, bi-weekly for 6wks	24h after last injection
hMTf	IDS-KO	3-5	Molar equivalent to hMTf-IDS dose	5-6	IV, once per week for 6wks	24h after last injection
IDS-hMTf	IDS-KO	5	Activity	5-6	IV, once per	24h after last

			equivalent to IDS (high dose)		week for 6wks	injection
hMTfpep-IDS	IDS-KO	5	Activity equivalent to IDS (high dose)	5-6	IV, once per week for 6wks	24h after last injection

All test articles and vehicle controls are administered by two slow bolus (one IV and one IP injection), to be performed once a week for a total of 6 weeks.

Body weights are determined at randomization on the first day of treatment and weekly thereafter. Clinical observations are performed daily. The animals are sacrificed approximately 24 hours after the last treatment.

Selected organs (brain, liver, kidney and heart) are collected and their weights recorded. The brains are preserved for histopathology and immunostaining analysis. The other tissues are divided with one half or one paired organ and preserved for histopathology and immunostaining in a manner similar to the brain. The other half or paired organ is frozen in liquid nitrogen and stored at -80°C until assayed for GAG.

Study end points: The primary endpoints are as follows:

- Histological evaluation: Hematoxylin and eosin staining of brain sections. This method is used to evaluate whether treatment has an effect on reducing the number/size of cellular storage vacuoles observed in IDS-KO mice; and
- Immunohistochemical evaluation of lysosomal associated membrane protein-1 (LAMP-1) in brain sections: This method is used to determine if treatment has effect on reducing the elevated LAMP-1 immunoreactivity that is observed in IDS-KO mice.

If feasible, qualitative or semi-qualitative methods are also employed for analysis of the end points 1-2 (such as scoring, area measurements, section scans, etc.). The histopathologist performing this analysis is blinded with regard to slide allocation to the study groups. Lysosome surface area is quantified by scanning areas stained for LAMP1 (IHC) and compared between experimental groups.

The secondary endpoints are as follows:

- GAG levels in selected tissues (liver, kidney, and heart);
- H&E staining of selected tissues and detection of cellular storage vacuoles; and
- Immunohistochemical evaluation of LAMP-1 levels in selected organs/tissues.

Histopathology (H&Estain). Tissues are collected and fixed in 10% neutral buffered formalin, then processed and embedded in paraffin. 5 µm paraffin sections are prepared and stained with hematoxylin and eosin (H&E) using standard procedures.

Immunohistochemistry (LAMP-1). Deparaffinized slides are incubated overnight with rat anti-LAMP-1 IgG (Santa CruzBiotechnology) as the primary antibody or rat IgG2a as a control antibody (AbDSerotec, Raleigh, NC). Following overnight incubation at 2-8°C, biotinylated rabbit anti-rat IgG (H&L)mouse adsorbed (Vector Laboratories) is added. Following 30minutes of incubation at 37°C, samples are washed and then treated with avidin-biotin-peroxidase complex (Vector Laboratories) for 30 minutes. Labeled protein is localized by incubation with 3,39-diaminobenzidine.The area of LAMP-1–positive cells is analyzed with Image-Pro Plus software (Media Cybernetics, Inc., Bethesda, MD).

GAG measurements. Tissue extracts are prepared by homogenizing tissue in a lysis buffer (10 mM Tris, 5 mM EDTA, 0.1% Igepal CA-630, 2 mM Pefabloc SC) using a glass grinder (Kontes Glass Company, Vineland, NJ) or a motorized tissue homogenizer (PowerGen Model 125, Omni International, Warrenton, VA). Homogenates re then subjected to 5 freeze-thaw cycles using an ethanol/dry ice bath and a 37°Cwater bath. Tissue debris is pelleted twice by room temperature centrifugation at 2000g for 12 minutes, and supernatants are collected and assayed for total protein concentration (mg/mL) using the bicinchonic acid (BCA) assay (Pierce, Rockford, IL).

GAG concentration in urine and tissue extracts is quantified by acolorimetric assay using 1,9-dimethylmethylene blue (DMB) dye and a standard curve (1.56–25 μg/mL) prepared from dermatan sulfate (MP Biomedicals, Aurora, OH). Urine samples are run at dilutions of1/10, 1/20, and 1/40. To avoid assay interference from protein, tissue extract samples are diluted to protein concentrations of <200 μg/mL. GAG concentrations in urine is adjusted for creatinine concentrations measured with a commercially available kit (Sigma, St. Louis, MO, part no. 555A) to compensate for differences in kidney function and expressed as μg GAG/mg creatinine. GAG levels in tissue extracts are adjusted for protein concentration (μg GAG/mg protein) or gram tissue.

WE CLAIM:

1. A p97 (melanotransferrin) fusion protein, comprising an iduronate-2-sulfatase (IDS) polypeptide fused to the N-terminus of a p97 polypeptide fragment, wherein the p97 polypeptide fragment consists of the amino acid sequence having at least 80% sequence identity to DSSHAFTLDELR (SEQ ID NO: 14) and having transport activity.

2. A p97 (melanotransferrin) fusion protein, comprising an iduronate-2-sulfatase (IDS) polypeptide fused to the C-terminus of a p97 polypeptide fragment, wherein the p97 polypeptide fragment consists of the amino acid sequence having at least 80% sequence identity to DSSHAFTLDELR (SEQ ID NO: 14) and having transport activity.

3. The p97 (melanotransferrin) fusion protein of claim 1 or 2, further comprising a heterologous peptide linker (L) in between the IDS polypeptide and the p97 polypeptide fragment.

4. The p97 (melanotransferrin) fusion protein of claim 3, wherein the peptide linker is selected from one or more of a rigid linker, a flexible linker, and an enzymatically-cleavable linker.

5. The p97 (melanotransferrin) fusion protein of claim 4, wherein the peptide linker comprises a rigid linker.

6. The p97 (melanotransferrin) fusion protein of claim 5, wherein the rigid linker is amino acid $(EAAAK)_1$ (SEQ ID NO:36),$(EAAAK)_2$ (SEQ ID NO:37), or $(EAAAK)_3$ (SEQ ID NO:38).

7. The p97 (melanotransferrin) fusion protein of claim 3 or 4, wherein the peptide linker comprises a flexible linker.

8. The p97 (melanotransferrin) fusion protein of claim 3 or 4, wherein the peptide linker comprises an enzymatically-cleavable linker.

9. The p97 (melanotransferrin) fusion protein of claim 1 or 2, wherein the fusion protein comprises an N-terminal signal peptide (SP) sequence.

10. The p97 (melanotransferrin) fusion protein of any one of claims 3 to 8, wherein the fusion protein comprises an N-terminal signal peptide (SP) sequence.

11. The p97 (melanotransferrin) fusion protein of claim 10, wherein the fusion protein comprises the structure: (a) SP-IDS-L-p97 or (b) SP-p97-L-IDS.

12. The p97 (melanotransferrin) fusion protein of claim 9, wherein the SP comprises the sequence MEWSWVFLFFLSVTTGVHS (SEQ ID NO:149) and wherein the fusion protein comprises the structure SP-p97-IDS.

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13. The p97 (melanotransferrin) fusion protein of claim 10 or 11, wherein the SP comprises the sequence MEWSWVFLFFLSVTTGVHS (SEQ ID NO:149) and wherein the fusion protein comprises the structure SP-p97-L-IDS.

14. The p97 (melanotransferrin) fusion protein of claim 9, wherein the SP comprises the human p97 SP sequence or MRGPSGALWLLLALRTVLG (SEQ ID NO:39) and wherein the fusion protein comprises the structure SP-p97-IDS.

15. The p97 (melanotransferrin) fusion protein of claim 10 or 11, wherein the SP comprises the human p97 SP sequence or MRGPSGALWLLLALRTVLG (SEQ ID NO:39) and wherein the fusion protein comprises the structure SP-p97-L-IDS.

16. The p97 (melanotransferrin) fusion protein of claim 9, wherein the SP comprises the human IDS SP sequence MPPPRTGRGLLWLGLVLSSVCVALG (SEQ ID NO:40) and wherein the fusion protein comprises the structure: (a) SP-IDS-p97.

17. The p97 (melanotransferrin) fusion protein of claim 10 or 11, wherein the SP comprises the human IDS SP sequence MPPPRTGRGLLWLGLVLSSVCVALG (SEQ ID NO:40) and wherein the fusion protein comprises the structure SP-IDS-L-p97.

18. The p97 (melanotransferrin) fusion protein of any one of claims 1-2, 9, 12, 14, and 16, wherein the fusion protein comprises a purification tag (TAG).

19. The p97 (melanotransferrin) fusion protein of any one of claims 3 to 10, 11, 13, 15, and 17, wherein the fusion protein comprises a purification tag (TAG).

20. The p97 fusion protein of claim 19, wherein the fusion protein comprises the structure: (a) SP-TAG-IDS-L-p97 or (b) SP-TAG-p97-L-IDS.

21. The p97 (melanotransferrin) fusion protein of claim 18, wherein the purification tag is a poly-histidine tag, a 10X poly-histidine tag, AviTag, Calmodulin-tag, polyglutamate tag, FLAG-tag, Human influenza hemagglutinin (HA)-tag, Myc-tag, S-tag, Streptavidin-Binding Peptide (SPB)-tag, Softag 1, Softag 3, V5 tag or Xpress tag (SEQ ID NOs:113-130).

22. The p97 (melanotransferrin) fusion protein of claim 19 or 20, wherein the purification tag is a poly-histidine tag, a 10X poly-histidine tag, AviTag, Calmodulin-tag, polyglutamate tag, FLAG-tag, Human influenza hemagglutinin (HA)-tag, Myc-tag, S-tag, Streptavidin-Binding Peptide (SPB)-tag, Softag 1, Softag 3, V5 tag or Xpress tag (SEQ ID NOs:113-130).

23. The p97 (melanotransferrin) fusion protein of claim 18 or 21, wherein the purification tag comprises a poly-histidine tag.

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24. The p97 (melanotransferrin) fusion protein of any one of claims 19, 20 and 22, wherein the purification tag comprises a poly-histidine tag.

25. The p97 (melanotransferrin) fusion protein of any one of claim 18 or 21, wherein the purification tag comprises a 10X poly-histidine tag (SEQ ID NO: 118).

26. The p97 (melanotransferrin) fusion protein of any one of claims 19, 20 and 22, wherein the purification tag comprises a 10X poly-histidine tag (SEQ ID NO: 118).

27. The p97 (melanotransferrin) fusion protein of claim 18 or 21, wherein the purification tag comprises a FLAG-tag DYKDDDDK (SEQ ID NO:122).

28. The p97 (melanotransferrin) fusion protein of any one of claims 19, 20 and 22, wherein the purification tag comprises a FLAG-tag DYKDDDDK (SEQ ID NO:122).

29. The p97 (melanotransferrin) fusion protein of any one of claims 1-2, 9, 12, 14, 16, 18, 21, 23, 25, and 27, wherein the fusion protein comprises a protease site (PS).

30. The p97 (melanotransferrin) fusion protein of any one of claims 10, 11, 13, 15, 17, 19, 22, 24, 26 and 28 wherein the fusion protein comprises a protease site (PS).

31. The p97 (melanotransferrin) fusion protein of claim 30, wherein the fusion protein comprises the structure: (a) SP-TAG-PS-IDS-L-p97 or (b) SP-TAG-PS-p97-L-IDS.

32. The p97 (melanotransferrin) fusion protein of claim 29, wherein the protease site (PS) is a Tobacco Etch Virus (TEV) protease site of SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136 or SEQ ID NO:137.

33. The p97 (melanotransferrin) fusion protein of claim 30 or 31, wherein the protease site (PS) is a Tobacco Etch Virus (TEV) protease site of SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136 or SEQ ID NO:137.

34. The p97 (melanotransferrin) fusion protein of claim 29 or 32, wherein the protease site (PS) is the Tobacco Etch Virus (TEV) protease site ENLYFQG (SEQ ID NO:135).

35. The p97 (melanotransferrin) fusion protein of any one of claims 30, 31 and 33, wherein the protease site (PS) is the Tobacco Etch Virus (TEV) protease site ENLYFQG (SEQ ID NO:135).

36. The p97 (melanotransferrin) fusion protein of any one of claims 30, 31, 33 and 35, wherein the fusion protein comprises the structure (a) signal peptide (SP) (MEWSWVFLFFLSVTTGVHS; SEQ ID NO:149)-histidine tag (HIS TAG)- Tobacco Etch Virus protease site (TEV PS)-IDS-L-p97 or (b) signal peptide (SP) (MEWSWVFLFFLSVTTGVHS; SEQ ID NO: 149)-histidine tag (HIS TAG)- Tobacco Etch Virus protease site (TEV PS)-p97-L-IDS.

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37. The p97 (melanotransferrin) fusion protein of claim 36, wherein the linker (L) is the rigid linker.

38. The p97 (melanotransferrin) fusion protein of one of claims 30, 31, 33 and 35, wherein the fusion protein comprises the structure (a) signal peptide (SP) (MEWSWVFLFFLSVTTGVHS; SEQ ID NO: 149)-histidine tag (HIS TAG)- Tobacco Etch Virus protease site (TEV PS)-IDS-L-p97 or (b) signal peptide (SP) (MEWSWVFLFFLSVTTGVHS; SEQ ID NO: 149)- histidine tag (HIS TAG)- Tobacco Etch Virus protease site (TEV PS)-p97-L-IDS.

39. The p97 (melanotransferrin) fusion protein of claim 38, wherein the linker (L) is the rigid linker and has the amino acid sequence $(EAAAK)_3$.

40. The p97 (melanotransferrin) fusion protein of any one of claims 30, 31, 33 and 35, wherein the fusion protein comprises the structure (a) SP-IDS -HIS TAG-TEV PS-IDS-L-p97 or (b) SP-p97-histidine tag (HIS TAG)- Tobacco Etch Virus protease site (TEV PS)-p97-L-IDS.

41. The p97 (melanotransferrin) fusion protein of claim 40, wherein the linker (L) is the rigid linker.

42. The p97 (melanotransferrin) fusion protein of any one of claims 30, 31, 33 and 35, wherein the fusion protein comprises the structure (a) SP-IDS- histidine tag containing 10 consecutive histidines (10xHIS TAG)- Tobacco Etch Virus protease site (TEV PS)-IDS-L-p97 or (b)SP-p97- histidine tag containing 10 consecutive histidines (10xHIS TAG)- Tobacco Etch Virus protease site (TEV PS)-p97-L-IDS.

43. The p97 (melanotransferrin) fusion protein of claim 42, wherein the linker (L) is the rigid linker and has the amino acid sequence $(EAAAK)_3$.

44. The p97 (melanotransferrin) fusion protein of any one of claims 1 to 43, wherein the IDS polypeptide comprises the amino acid sequence set forth in SEQ ID NO:32 or 33.

45. The p97 (melanotransferrin) fusion protein of any one of claims 1 to 44, comprising an amino acid sequence set forth in SEQ ID NO: 29, 30, 138, 139, 140, 141 or 142.

46. An isolated polynucleotide which encodes the p97 (melanotransferrin) fusion protein of any one of claims 1 to 45.

47. The isolated polynucleotide of claim 46, wherein the polynucleotide is SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, or SEQ ID NO:147.

48. The isolated polynucleotide of claim 46 or 47, wherein the polynucleotide is codon-optimized for expression in a host cell.

49. The isolated polynucleotide of claim 48, wherein the host cell is a mammalian cell, an insect cell, a yeast cell, or a bacterial cell.

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50. A recombinant host cell, comprising the isolated polynucleotide of any one of claims 46 to 49, wherein the isolated polynucleotide is operably linked to one or more regulatory elements.

51. A vector, comprising the isolated polynucleotide of any one of claims 46 to 49, wherein the polynucleotide is operably linked to one or more regulatory elements.

52. A recombinant host cell, comprising the vector of claim 51.

53. A recombinant host cell, comprising the p97 (melanotransferrin) fusion protein of any one of claims 1 to 45.

54. The recombinant host cell of claims 52 or 53, wherein the host cell is a mammalian cell, an insect cell, a yeast cell, or a bacterial cell.

55. The recombinant host cell of claim 54, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell, a HEK-293 cell, or a HT-1080 human fibrosarcoma cell.

56. A pharmaceutical composition, comprising a pharmaceutically-acceptable carrier and the p97 (melanotransferrin) fusion protein of any one of claims 1 to 45, wherein the pharmaceutical composition is sterile and non-pyrogenic.

57. Use of the p97 (melanotransferrin) fusion protein of any one of claims 1 to 45, or the pharmaceutical composition of claim 56, for the treatment of a lysosomal storage disease in a subject in need thereof.

58. The use of claim 57, wherein the lysosomal storage disease is Hunter Syndrome (MPS II).

59. The use of claim 57 or 58, wherein the lysosomal storage disease has central nervous system (CNS) involvement.

60. The use of any one of claims 57 to 59, wherein the subject is at risk for developing CNS involvement of the lysosomal storage disease.

61. The use of any one of claims 57 to 60, wherein the subject is a human male.

62. The use of any one of claims 57 to 61, wherein the p97 (melanotransferrin) fusion protein or the pharmaceutical composition is administrable by intravenous (IV) infusion or intraperitoneal (IP) injection.

63. The use of the pharmaceutical composition of claim 56 in the manufacture of a medicament for the treatment of a lysosomal storage disease in a subject in need thereof.

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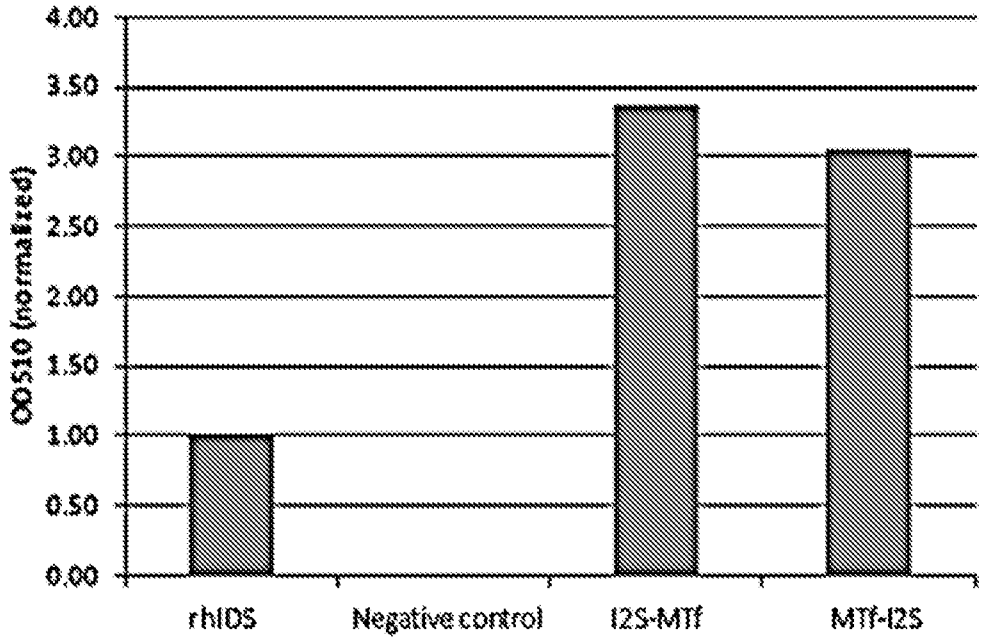
A

B

FIG. 1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2.00
1.50
1.00
0.50
0.00
OD510 (normalized)
rhIDS
Negative control
I2S-MTf
MTf-I2S

FIG. 2

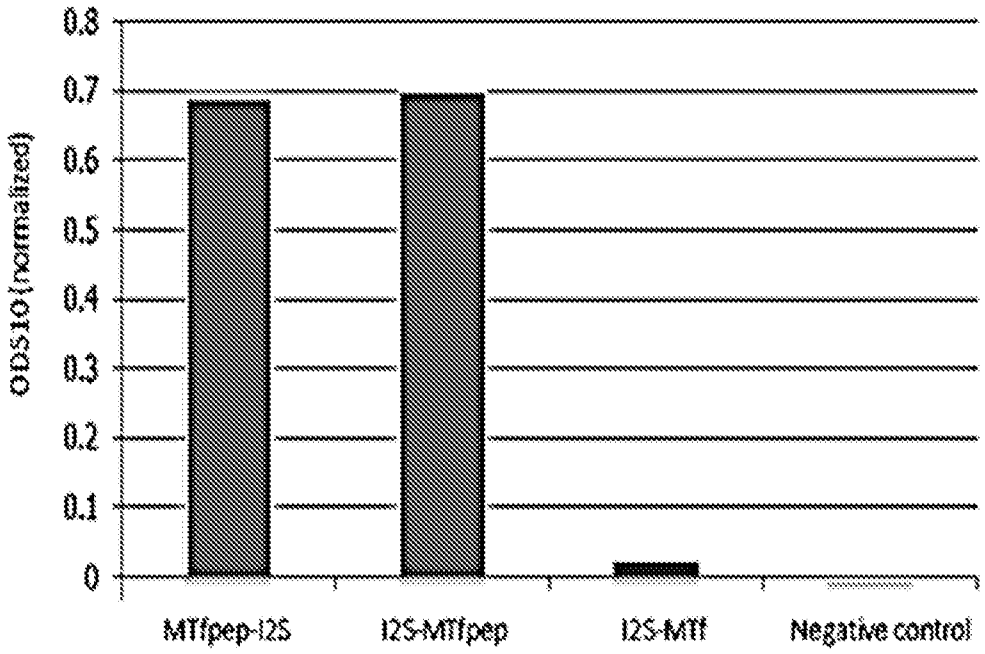

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD510 (normalized)
MTfpep-I2S
I2S-MTfpep
I2S-MTf
Negative control

FIG. 3

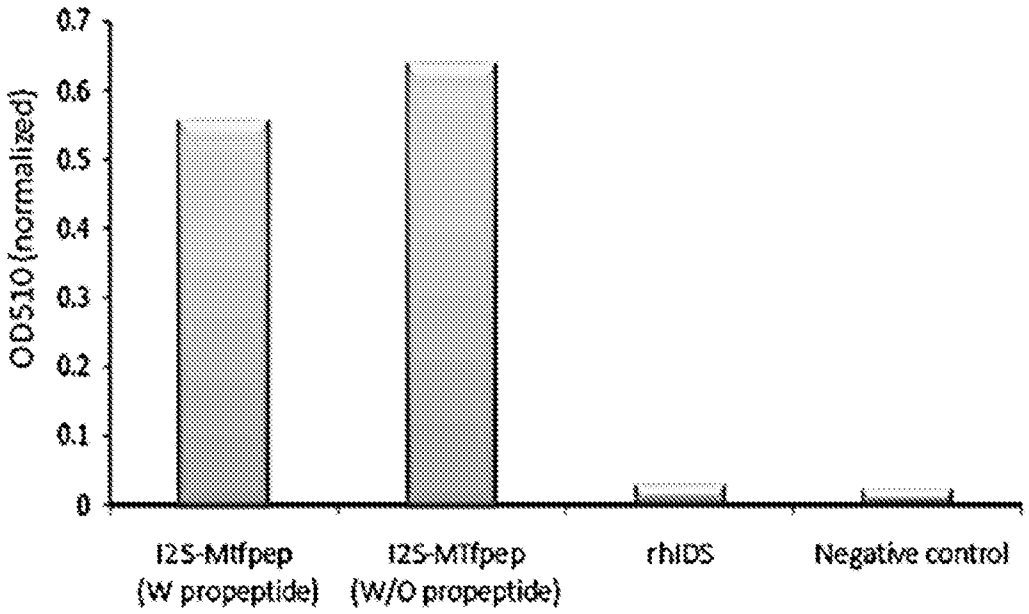

OD510 (normalized)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
I2S-MTfpep (W propeptide)
I2S-MTfpep (W/O propeptide)
rhIDS
Negative control

FIG. 4

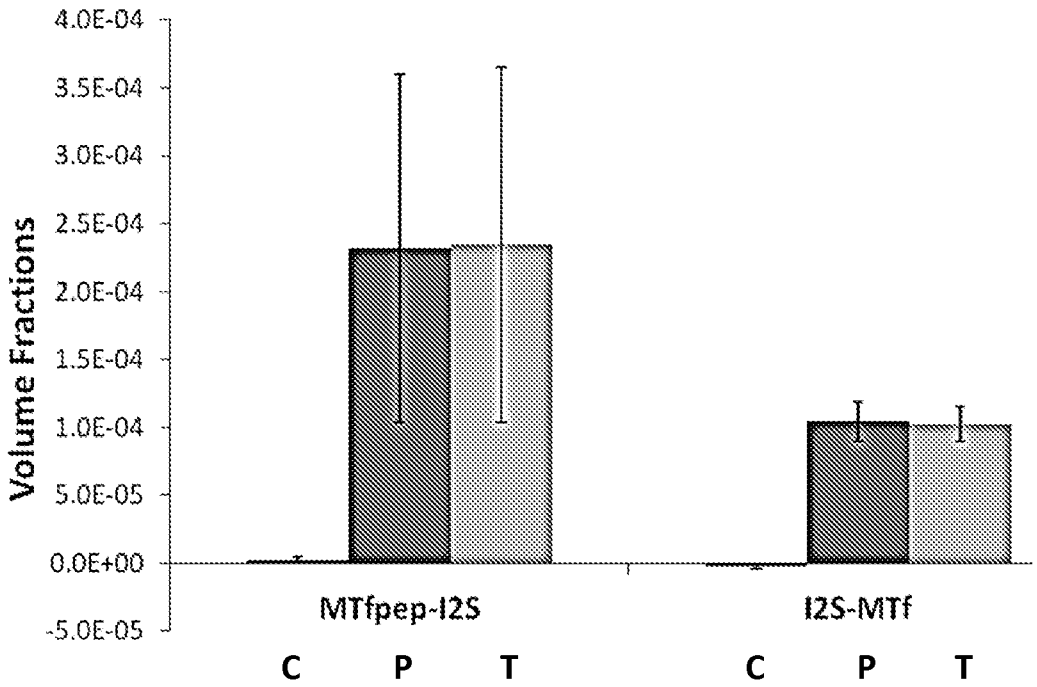
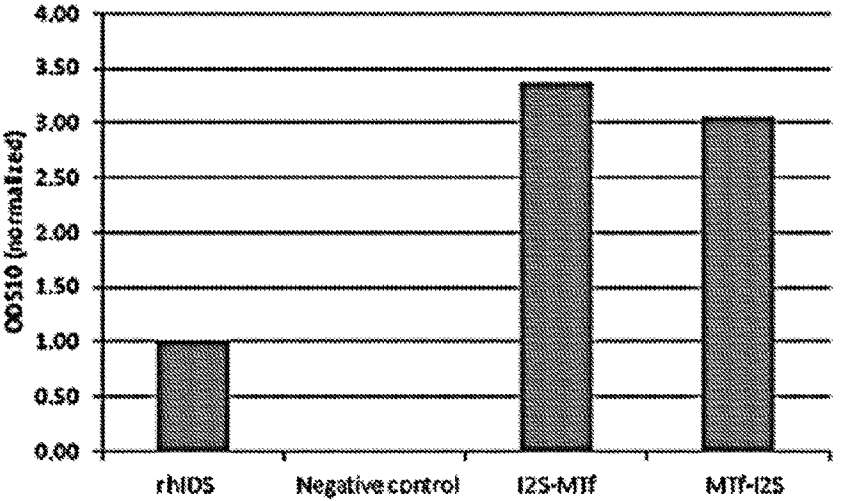

4.0E-04
3.5E-04
3.0E-04
2.5E-04
2.0E-04
1.5E-04
1.0E-04
5.0E-05
0.0E+00
-5.0E-05
Volume Fractions
MTfpep-I2S
I2S-MTf
C
P
T
C
P
T

FIG. 5


4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
OD510 (normalized)
rhIDS
Negative control
I2S-MTf
MTf-I2S