

COMPOSITIONS AND METHODS FOR PROPHYLAXIS AND/OR TREATMENT OF
ALPHA SYNUCLEIN PROTEINOPENIA IN PARKINSON'S AND OTHER DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

5 [0001] This application claims priority to, and the benefit of, U.S. Provisional Application No. 63/520,545 filed August 18, 2023, which is incorporated by reference in its entirety. This application is related to international patent application No. PCT/EP2022/064374 filed 26 May 2022, which claims priority to U.S. Provisional Application No. 63/193,258, filed on of 26 May 2021, which is incorporated by reference herein in their entirety.

TECHNICAL FIELD

10 [0002] The present disclosure pertains to compositions for preventing and/or treating aggregation of proteins in proteinopenia.

BACKGROUND

15 [0003] Alpha synuclein (α -syn) is a 140-amino acid protein that is evolutionarily conserved and highly expressed in the central nervous system (CNS), being found predominantly in presynaptic terminals. It is expressed from the SNCA gene. Alpha synuclein is an important protein with well-documented roles in synaptic function through the regulation of synaptic vesicle trafficking and neurotransmitter release. The physiological concentration of α -syn in human cerebrospinal fluid (CSF) is in the picogram/ml range (1600-2000 pg/ml). The levels of soluble α -syn in the CSF are below normal (proteinopenia) in Parkinson's disease (PD) and other diseases including dementia with Lewi bodies (DLB) and multiple system atrophy (MSA). The depletion of the functional monomer diseases results either from aggregation and amyloid plaque formation, which sequesters the soluble monomer, or from heritable mutations in familial forms of disease, some of which also lead to increased aggregation. So far, most of the therapeutic approaches for PD and other diseases have focused on removing 20 the aggregated forms of α -syn or further reducing the α -syn monomer. Other approaches are directed to interfere with aggregation process and prevent plaque accumulation, which can maintain the available levels of soluble α -syn but cannot restore the normal concentration of the protein when sequestered in aggregates and depleted below normal levels.

SUMMARY OF INVENTION

30 [0004] In one aspect, a protein analogue for the treatment or prevention of α -syn proteinopenia includes an N-terminal domain having at least 85% identity to positions 1-60 of SEQ ID NO:1, a C-terminal domain having at least 85% identity to positions 96-140 of

SEQ ID NO:1, and a modified middle domain corresponding to positions 61-95 of SEQ ID NO:1, where the modified middle domain includes deletion of 1 to 35 amino acids or one or more substitutions including an amino acid, an amino acid analogue, or both. In some variations, the N-terminal domain includes at least 90% or at least 95% identity to positions 1-60 of SEQ ID NO 1. In some variations, the C-terminal domain includes at least 90% identity or at least 95% identity to positions 96-140 of SEQ ID NO 1.

[0005] The protein analogue may also include where the one or more substitutions comprise an amino acid, such as a charged amino acid, a polar amino acid, or both. In various variations, the amino acid is selected from proline, glycine, lysine, arginine, histidine, glutamic acid, aspartic acid, serine, threonine, asparagine, glutamine, and cysteine. The protein analogue may also include where the one or more substitutions comprise an amino acid analogue. In other variations, the protein analogue may alternatively include where the modified middle domain includes a consecutive amino acid deletion that includes at least a valine deletion at position 95.

[0006] The protein analogue may also include where the C-terminus of the protein analogue includes a modification configured to enhance at least one of circulation time, protease resistance and CSF half-life of the protein analogue as compared to wild type α -syn protein. The protein analogue may also include where the C-terminus of the protein analogue is modified with a functional group selected from an amide, an ester, an alcohol, a hydrazide, an aldehyde, and a hydroxamic acid. In particular variation, the C-terminus of the protein analogue is modified to an amide.

[0007] The protein analogue may also include at least one of: a decreased beta-sheet-forming propensity, enhanced solubility, improved stability, improved circulation time, improved brain penetrance, and increased CSF half-life. In some variations, the half-life is at least about 10 min, at least about 20 min, at least about 30 min, at least about 40 min, at least about 50 min, at least about 1 hours, at least about 5 hours, at least about 10 hours, at least about 1 day, at least about 10 days, at least about 60 days, at least about 10 weeks, or at least about 50 weeks. In some variations, the solubility is at least about 1 mg/ml, more preferably at least about 5 mg/ml and most preferably at least about 50 mg/ml. The protein analogue may also perform a function of wild type α -syn protein to at least 50% of the level performed by wild type α -syn protein.

[0008] In one aspect, nucleic acids encoding the protein analogues as described herein are provided.

[0009] In one aspect, a method for treatment and/or prevention of α -syn proteinopenia in a subject includes administering to the subject a therapeutically effective amount of a protein analogue as described herein. The therapeutically effective amount can be lower than that for wild type α -syn protein. The method may also include a prior step of identifying the subject
5 as a candidate for treatment by determining a concentration of α -syn protein in the CSF of the subject. A method of treating or preventing α -syn proteinopenia in a subject, includes administering to the subject a nucleic acid encoding the protein analogue as defined in any one of claims 1 to 33.

[0010] In one aspect, a composition for treating or preventing α -syn proteinopenia includes
10 a therapeutically effective amount of a protein analogue as described herein or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable vehicle. The composition may be formulated for parenteral administration, or more particularly, intrathecal administration.

[0011] Other technical features may be readily apparent to one skilled in the art from the
15 following figures, descriptions, and claims.

REFERENCE TO A SEQUENCE LISTING

The application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy,
20 created on August 19, 2024, is named PD_PCT_SequenceListing_20240818.xml and is 1348 kilobytes in size.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIGS. 1A-1EE show exemplary protein analogues having various modifications, particularly to the middle domain of the α -syn protein.
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TERMS AND DEFINITIONS

[0013] Throughout this specification, including the claims which follow, unless the context requires otherwise, the word “comprise,” and variations such as “comprises” and “comprising,” will be understood to imply the inclusion of a stated integer or step or group
30 of integers or steps but not the exclusion of any other integer or step or group of integers or steps. 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 no other elements are present that materially affect the activity or action of the listed elements.

[0014] It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a pharmaceutical carrier” includes mixtures of two or more such carriers, and the like.

[0015] The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives. The term “and/or” should be understood to mean either one, or both of the alternatives.

[0016] As used herein, the term “about” or “approximately” refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 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. In one embodiment, the term “about” or “approximately” refers a range of quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length $\pm 15\%$, $\pm 10\%$, $\pm 9\%$, $\pm 8\%$, $\pm 7\%$, $\pm 6\%$, $\pm 5\%$, $\pm 4\%$, $\pm 3\%$, $\pm 2\%$, or $\pm 1\%$ about a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[0017] Reference throughout this specification to “one embodiment,” “an embodiment,” “a particular embodiment,” “a related embodiment,” “a certain embodiment,” “an additional embodiment,” or “a further embodiment” or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. It is also understood that the positive recitation of a feature in one embodiment, serves as a basis for excluding the feature in a particular embodiment.

[0018] “Amino acid identity,” “residue identity,” “identity,” and the like, as used herein refers to the structure of the functional group (R group) on the polypeptide backbone at a given position. Amino acid identities are (name/3-letter code/one-letter code): alanine/Ala/A;

arginine/Arg/R; asparagine/Asn/N; aspartic acid/Asp/D; cysteine/Cys/C; glutamine/Gln/Q; glutamic acid/Glu/E; glycine/Gly/G; histidine/His/H; isoleucine/Ile/I; leucine/Leu/L; lysine/Lys/K; methionine/Met/M; phenylalanine/Phe/F; proline/Pro/P; pyrrollysine/Pyl/O; serine/Ser/S; threonine/Thr/T; tryptophan/Trp/W; tyrosine/Tyr/Y; and valine/Val/V. Amino

5 acids can be grouped according to similarities in the properties of their side chains. Such properties include size, charge, polarity, hydrophobicity, and chain rigidity/orientation. Accordingly, amino acids may be designated herein with reference to a property of their side chains e.g., an amino acid having a hydrophobic, charged, or polar side chain may be referred to as a “hydrophobic amino acid”, “charged amino acid”, or a “polar

10 amino acid” respectively.

[0019] “Engineered” or “modified” as used herein to describe a peptide or nucleic acid refers to the aspect of having been manipulated by human intervention. Disclosed herein are engineered peptides, polypeptides, proteins, genes, etc. In one example, a protein is considered to be “engineered” when at least one aspect of the polypeptide, e.g., its sequence,

15 has been intentionally manipulated by human intervention (directly or indirectly) to differ from the aspect as it exists in a patient/subject or in nature. Artificial manipulation may be accomplished by chemical synthesis or by the editing (insertion, deletion, mutation, etc.) of isolated segments of nucleic acids, e.g., by genetic engineering techniques. In contrast, “native” or “wild-type” as used herein refers to un-engineered and/or un-modified genes,

20 peptides, proteins, nucleic acid sequences, amino acid sequences, and portions thereof.

[0020] An amino acid within a molecule may be substituted to create an engineered peptide or peptide analogue. The amino acid residue can be replaced by a residue having similar physiochemical characteristics, that is a ‘conservative substitution’ – e.g., substituting one aliphatic residue for another or substitution of one polar residue for another. Alternatively,

25 “non-conservative substitution” can entail replacing an amino acid residue with another residue having different or dissimilar physiochemical characteristics.

[0021] Similarity between amino acid or peptide sequences is expressed in terms of the homology of two sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (percentage of identical residues for

30 peptides or bases for nucleic acids; or similarity or homology); the higher the percentage, the more similar the two sequences are. Complete identity is 100% identical over a given sequence, for example 50, 100, 150, or 200 bases or residues.

[0022] “Variant,” as used herein refers to a polypeptide, gene, sequence, or molecule that is substantially homologous to a naturally occurring or reference member, but which is different

from that of the native or reference member because of one or a plurality of deletions, insertions, substitutions, molecules, expression levels, etc.. Variant polypeptide-encoding DNA sequences encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to a native or reference DNA sequence, but that
5 encode a variant protein or fragment thereof. A wide variety of cloning, PCR-based site-specific mutagenesis, and genomic editing approaches are known in the art, and can be applied by the ordinarily skilled artisan.

[0023] Variant amino acid or nucleic acid sequences can be at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%,
10 at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, identical to a native or reference sequence. The degree of homology (percent identity) between a native and variant sequence can be determined, for example, by comparing the two sequences using freely available computer programs commonly employed for this purpose on the world wide web (e.g., BLASTp or BLASTn with default settings).

15 [0024] The terms “treat,” “treating,” and “treatment” refer to eliminating, reducing, suppressing, or ameliorating, either temporarily or permanently, either partially or completely, a clinical symptom, manifestation or progression of an event, disease or condition associated with proteinopenia and diseases described herein. As is recognized in the pertinent field, methods and compositions employed as therapies may reduce the severity of a given
20 disease state but need not abolish every manifestation of the disease to be regarded as useful. Similarly, a prophylactically administered treatment need not be completely effective in preventing the onset of a condition to constitute a viable prophylactic method or agent. Simply reducing the impact of a disease and/or reducing the number or severity of associated symptoms, or by increasing the effectiveness of another treatment, or by producing another
25 beneficial effect, or reducing the likelihood that the disease will occur or worsen in a subject, is sufficient.

[0025] “Therapeutically effective amount” means an amount of a drug, composition, compound, treatment, or therapy of the present disclosure that alone, or in combination with other therapies, (i) treats the particular disease, condition, or disorder, (ii) attenuates,
30 ameliorates, or eliminates one or more symptoms of the particular disease, condition, or disorder, or (iii) prevents or delays, the onset of one or more symptoms of the particular disease, condition, or disorder described herein.

[0026] The following embodiments and aspects thereof are described and illustrated in conjunction with systems, tools, and methods which are meant to be exemplary and illustrative, not limiting in scope.

DESCRIPTION OF EMBODIMENTS

5 [0027] The following embodiments and aspects thereof are described and illustrated in conjunction with systems, tools, and methods which are meant to be exemplary and illustrative, not limiting in scope.

[0028] This disclosure includes information that may be useful in understanding the present disclosure. It is not an admission that any of the information provided herein is prior art or
10 relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0029] A number of publications are cited herein in order to more fully describe and disclose the subject matter and the state of the art to which the subject matter pertains. Each of these references is incorporated herein by reference in its entirety into the present disclosure, to the
15 same extent as if each individual reference was specifically and individually indicated to be incorporated by reference.

[0030] The present disclosure relates generally to proteinopenia conditions, and more specifically to methods, compositions and methods for the prophylaxis and/or treatment of α -syn proteinopenia in Parkinson's disease and other diseases.

20 [0031] Alpha synuclein has documented roles in synaptic function, which in turn is critical to nervous system function. It is postulated that the pathogenesis in PD and other diseases where α -syn is depleted below its normal physiological levels is due to the loss of the important neuronal functions that require adequate concentrations of soluble α -syn. This understanding is in contrast with the prevailing view of Parkinson's and other diseases, where
25 instead of viewing toxicity as emanating directly from the aggregates/plaques (toxic gain-of-function), pathogenesis is seen as more dependent on the loss of the critical neuronal functions that require adequate levels of soluble α -syn (toxic loss-of-function). Accordingly, instead of viewing PD and related diseases as analogous to cancer (i.e., a toxic mass), they can be viewed as analogous to diabetes mellitus (depletion of a crucial peptide), where replacement
30 therapy has been very successful. Therapeutic approaches to restore the normal concentration and function of α -syn using non-aggregating/non-amyloid forming peptide analogues were previously described in prior patent application (PCT/EP2022/064374). In being non-aggregating or non-amyloid forming, the mechanism of action of the analogues to restore function is not the result of inhibiting propagation of beta-sheet structure of the

amyloid fibers, or otherwise to bind to or be incorporated into a beta-sheet structure. Thus, in some variations, the α -syn protein analogue may have a lower binding affinity or constant to wildtype α -syn protein or the analogues of α -syn protein, than that of wildtype α -syn protein. The present disclosure provides new methods and compositions that further builds on the proteinopenia concept as the underlying pathogenic mechanism in PD and other neurodegenerative diseases where the α -syn protein is known to be depleted.

[0032] The wild-type sequence of native α -syn protein is as set forth in SEQ ID NO:1. It is hypothesized that a non/low amyloid-forming protein analogue of the α -syn protein may be used to correct α -syn depletion (proteinopenia) associated with diseases such as Parkinson's Disease, with or without dementia, Lewy Body Dementia (LBD) and Multiple System Atrophy (MSA). In some embodiments, the protein analogue can comprise a substantially intact α -syn N-terminal domain (positions 1-60), which is involved in interaction with synaptic vesicles. This domain is highly conserved among synucleins, and is subject to multiple familial mutations. The protein analogue may further comprise a substantially intact C-terminal domain (positions 96-140), which is highly negatively charged and involved with protein solubility in the native α -syn protein. A modified middle domain (positions 61-95), which in the native α -syn protein is responsible for amyloid formation, aggregation, and low solubility, may include one or more substitutions, and/or an amino acid deletion or deletion sequence. In some embodiments, the modified middle domain includes a substitution of one or more of the amino acids with amino acids or amino acid analogues that interfere with beta-sheet formation. Potential amino acid analogues include, without limitation: 3-hydroxyproline, 4-hydroxyproline, selenocysteine, pyroglutamic acid, carboxyglutamic acid, octenyl alanine, pyrrollysine, palmitoyl aspartate, D-amino acids including D-proline, β -amino acids, γ -amino acids, homo-amino acids, β -homo-amino acids, α -methyl amino acids, N-methyl amino acids, N-ethyl amino acids, N-alkylated amino acid derivatives (preferably with 1, 2 or 3 carbons in the alkyl moiety), peptoid substituents, pyruvic acid derivatives, branched-chain amino acid derivatives, nitro amino acid derivatives, halogenated amino acid derivatives, ring-substituted amino acid derivatives, aromatic amino acid derivatives, linear core amino acids, hydroxylated amino acid derivatives, cyclic amino acids, bicyclic amino acids, 3-amino-3-aryl-propionic acids, 3-amino-4-aryl-butyric acids, amino acids with aromatic spacers, alicyclic amino acids, α -phenylglycine derivatives, or the like.

[0033] In some variations, the modified middle domain includes a substitution of one or more of the amino acids at positions 61-95 (including any position therebetween, e.g. positions 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93 or 95) with an amino acid that interferes with beta-sheet

formation such as P, G, K, R, H, E, D, S, T, N, Q, C. In some variations, the deletion sequence is consecutive and includes the valine deletion at position 95 of the wild type protein.

[0034] As described herein, the analogue can include modifications to the middle domain that decrease the analogue's propensity to form beta sheets. Modifications to the middle domain may be selected to prevent or decrease the formation of secondary structures or intrapeptide interactions that may contribute to aggregation and amyloid formation. In some embodiments, a non/low amyloid-forming peptide analogue of the α -syn protein can comprise a middle domain wherein at least one position from 61 to 95 is replaced to prevent or decrease the formation of backbone hydrogen bonds, and/or to alter hydrophobicity in that domain. In a non-limiting example, such a substitution may involve replacing a hydrophobic residue with a charged or polar amino acid or amino acid analogue. In some embodiments, a substitution is selected that alters the conformation of the peptide backbone. In a non-limiting example, such a substitution may involve replacing a residue at a selected position with a proline, a D-amino acid, or amino acid analogue.

[0035] In various aspects, said modifications may impart other useful enhancements to the peptide analogue as compared to the native α -syn protein. Such enhancements include, but are not limited to, one or more of: increased solubility in a body fluid or other medium; increased stability e.g., in a body fluid or other medium; increased circulation time after administration to a subject; increased penetrance/uptake in the brain of a subject; increased half-life in CNS tissue and/or CSF; and decrease in the amount administered to a subject in order to achieve a given therapeutic effect. In some embodiments, these modifications may also facilitate synthesis and purification.

[0036] In some aspects, a non/low amyloid-forming peptide analogue of the α -syn peptide can exhibit enhanced solubility in a body fluid and/or a pharmaceutically acceptable liquid medium, wherein the solubility is at least about 1 mg/ml, more preferably at least about 5 mg/ml and most preferably at least about 50 mg/ml. In various embodiments, the solubility is at least about 1 mg/ml, at least about 2 mg/ml, at least about 3 mg/ml, at least about 4 mg/ml, at least about 5 mg/ml, at least about 6 mg/ml, at least about 7 mg/ml, at least about 8 mg/ml, at least about 9 mg/ml, at least about 10 mg/ml, at least about 11 mg/ml, at least about 12 mg/ml, at least about 13 mg/ml, at least about 14 mg/ml, at least about 15 mg/ml, at least about 16 mg/ml, at least about 17 mg/ml, at least about 18 mg/ml, at least about 19 mg/ml, at least about 20 mg/ml, at least about 25 mg/ml, at least about 30 mg/ml, at least about 35 mg/ml, at least about 40 mg/ml, at least about 45 mg/ml, or at least about 50 mg/ml.

[0037] In some aspects, a non/low amyloid-forming peptide analogue of the α -syn protein can exhibit increased stability e.g. shelf life, wherein the peptide remains $\geq 95\%$ intact for at least about 2 years at about -20°C , more preferably remains $\geq 95\%$ intact for at least about 2 years at about 4°C and most preferably, remains $\geq 95\%$ intact for at least about 2 years at room temperature.

[0038] In some aspects, a non/low amyloid-forming peptide analogue of the α -syn protein can exhibit enhanced circulation time and half-life in CNS tissue and/or CSF, wherein the half-life in CSF is at least about 10 min, at least about 20 min, at least about 30 min, at least about 40 min, or at least about 50 min. More preferably the half-life is at least about 1 hours, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, at least about 6 hours, at least about 7 hours, at least about 8 hours, at least about 9 hours, or at least about 10 hours. Still more preferably the half-life is at least about 1 day, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 20 days, at least about 30 days, at least about 40 days, at least about 50 days, or at least about 60 days. Most preferably the half-life is at least about 10 weeks, at least about 20 weeks, at least about 30 weeks, at least about 40 weeks, or at least about 50 weeks.

[0039] In some aspects, a non/low amyloid-forming peptide analogue of the α -syn protein can exhibit enhanced brain penetrance, wherein the percentage uptake in the brain as measured at no more than about 60 minutes after administration is at least about 0.1%, more preferably at least about 1% and most preferably at least about 10%. In various embodiments, the percentage uptake in the brain as measured at no more than about 60 minutes after administration is at least about 0.1%, at least about 0.2%, at least about 0.3%, at least about 0.4%, at least about 0.5%, at least about 0.6%, at least about 0.7%, at least about 0.8%, at least about 0.9%, at least about 1.0%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, or at least about 10%.

[0040] In some aspects, a non/low amyloid-forming peptide analogue of the α -syn protein can exhibit enhanced synthesis yield and better purity, wherein the percent yield is at least 50% and percent purity is at least 90%, more preferably at least 95% and most preferably at least 98%. In various embodiments, the yield is at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 92%, at least about 94%, at least about 96%, or at least about 98%, where in any of said embodiments, the purity is at least about 90%, at least about 92%, at least about 94%, at least about 96%, or at least about 98%.

[0041] In various embodiments, the peptide analogue can comprise an N-terminal domain having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to positions 1-60 of SEQ ID NO:1 without significant alteration in the associated function of that region. In various embodiments, the peptide analogue can comprise an C-terminal domain having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to positions 96-140 of SEQ ID NO:1 without significant alteration in the associated function of that region.

[0042] Accordingly, the analogue may exhibit enhancements such as described above while substantially retaining one or more of the native α -syn protein's functions. For example, the analogue is able to perform the native function(s) at at least 50% of the level of the native soluble peptide (including e.g. at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the level of the native soluble peptide). In particular aspects, the native function can comprise modulation of synaptic vesicle trafficking.

[0043] In accordance with the present disclosure, a non/low amyloid-forming peptide analogue of the α -syn protein can comprise a modification to the C-terminus selected to impart useful enhancements to the peptide analogue as compared to the native α -syn protein. These enhancements can include, without limitation, one or more of increased circulation time, enhanced protease resistance and increased CSF half-life. It will be understood that these properties may be interrelated, i.e., decreased susceptibility to proteolysis can result in the analogue remaining functionally intact in the CSF for a longer period of time after administration, and therefore circulating in the CSF for a longer duration. Furthermore, these benefits can be amplified by enhancements resulting from other modifications, such as decreased beta-sheet formation, which results in reduced sequestration of functional peptide/analogue into amyloid structures. In some aspects, these enhancements can reduce the amount of analogue needed to bring about a desired therapeutic effect. Accordingly, the analogues described herein can provide for a reduced therapeutically effective amount as compared to the native α -syn protein. It will be further understood that increased solubility resulting from other modifications can provide for a higher concentration of analogue in a solvent-based therapeutic composition. As a result, a lower volume of the composition can be administered to a subject to produce a therapeutic effect.

[0044] In some embodiments, the C-terminus of a non/low amyloid-forming peptide analogue of the α -syn protein is modified into an amide. The present disclosure also encompasses C-terminus modifications with other moieties that can provide increased

circulation time, enhanced protease resistance and/or increased CSF half-life. Exemplary groups for C-terminus modifications include, without limitation: amides, including N-alkyl amides; esters, including alkyl esters; alcohols; hydrazides; aldehydes; and hydroxamic acids. Specific examples include, without limitation, amide, N-methyl amide, cysteamide, methyl ester, aldehyde, hydrazide and hydroxamic acid. In various embodiments, modifications to the C-terminus may be combined with modifications to the middle domain that, either alone or through combination with the modification to the C-terminus, impart useful properties to the peptide analogue. For example, the C-terminus may be modified into an amide to enhance circulation time, protease resistance and/or CSF half-life together with a proline substitution in the middle domain (positions 61-95) to decrease the propensity for beta-sheet stacking and amyloid formation. In other variations, the C-terminus may be modified to an amide to enhance circulation time, protease resistance and/or CSF half-life together with deletion of amino acids in the middle domain (positions 61-95) to decrease propensity for beta-sheet stacking and amyloid formation and to enhance peptide solubility and to reduce therapeutically effective amount and/or administration volume. In still other variations, the C-terminus may be modified to an amide to enhance circulation time, protease resistance and/or CSF half-life together with one or more charged or polar amino acid substitution(s) in the middle domain (positions 61-95) to decrease propensity for beta-sheet stacking and amyloid formation and to enhance peptide solubility and to reduce therapeutically effective amount and/or administration volume. In some embodiments, middle domain modifications and C-terminus modifications can act together in an additive or synergistic fashion in producing an enhancement.

[0045] Sequences of α -syn protein and analogues thereof according to some embodiments are presented in FIGS 1A-1EE. The wild-type sequence of the α -syn protein is as set forth in SEQ ID NO:1 and comprises an N-terminal domain, a middle domain and a C-terminal domain as shown. The middle domains in SEQ ID NOS:2-876 comprise modifications, which in SEQ ID NOS:2-36 comprise deletions of 1 to 35 amino acids respectively, and in SEQ ID NOS:37-438 comprise substitutions at one of positions 61-95. As also shown in FIGS 1A-1EE, in some embodiments the C-terminus is modified into amide to enhance circulation time, protease resistance, and CSF half-life compared to wild type protein. For example, in one embodiment the C-terminus of SEQ ID NO:1 is modified into amide to enhance protease resistance, circulation time, and brain half-life, resulting in the analogue SEQ ID NO:439.

[0046] In other embodiments, the C-terminus may be modified into an amide together with a deletion in the middle domain. For example, in an embodiment α -syn protein analogue SEQ ID NO:2 comprises a deletion at α -syn position 95. In another embodiment, the C-terminus

of SEQ ID NO:2 is modified into amide, resulting in the analogue SEQ ID NO:440. In another example, α -syn protein analogue SEQ ID NO:3 comprises a deletion at α -syn positions 94 and 95. In another embodiment, the C-terminus of SEQ ID NO:3 is modified into amide, resulting in the analogue SEQ ID NO:441. As discussed above, the deletions may confer on the modified analogues one or more enhancements selected from decreased propensity for beta-sheet stacking and amyloid formation, increased stability, and enhanced solubility. The C-terminus modification may confer on the modified analogues one or more enhancements selected from enhanced circulation time, protease resistance and CSF half-life. As a result of these enhancements, the therapeutically effective amount of the analogues may be reduced.

5 [0047] In other embodiments, the C-terminus may be modified into an amide together with one or more charged or polar amino acid substitutions in the middle domain. For example, in an embodiment α -syn protein analogue SEQ ID NO:438 comprises a substitution of cysteine for valine at α -syn position 95. In another embodiment, the C-terminus of SEQ ID NO:438 is modified into amide, resulting in the analogue SEQ ID NO:876. In another example, in an embodiment α -syn protein analogue SEQ ID NO:429 comprises a substitution of lysine for valine at α -syn position 95. In another embodiment, the C-terminus of SEQ ID NO:429 is modified into amide, resulting in the analogue SEQ ID NO:867. As discussed above, these substitutions may confer on the modified analogues one or more enhancements selected from decreased propensity for beta-sheet stacking and amyloid formation, increased stability, and enhanced solubility. The C-terminus modification may confer on the modified analogues one or more enhancements selected from enhanced circulation time, protease resistance and CSF half-life. As a result of these enhancements, the therapeutically effective amount of the analogues may be reduced.

15 [0048] In other embodiments, the C-terminus may be modified into an amide together with one or more proline substitutions in the middle domain. For example, in an embodiment α -syn protein analogue SEQ ID NO:37 comprises a substitution of proline for glutamic acid at α -syn position 61. In another embodiment, the C-terminus of SEQ ID NO:37 is modified into amide, resulting in the analogue SEQ ID NO:475. In another example, in an embodiment α -syn protein analogue SEQ ID NO:48 comprises a substitution of proline for glutamine at α -syn position 62. In another embodiment, the C-terminus of SEQ ID NO:48 is modified into amide, resulting in the analogue SEQ ID NO:486. As discussed above, these substitutions may confer on the modified analogues one or more enhancements selected from decreased propensity for beta-sheet stacking and amyloid formation, increased stability, and enhanced solubility. The C-terminus modification may confer on the modified analogues one or more enhancements selected from enhanced circulation time, protease resistance and CSF half-

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life. As a result of these enhancements, the therapeutically effective amount of the analogues may be reduced.

[0049] Non/low amyloid-forming analogues of α -syn protein as described herein can be manufactured using standard solution phase methodology. Suitable synthesis methodologies include solid phase peptide synthesis, liquid phase peptide synthesis, microwave-assisted peptide synthesis, In an exemplary method, the analogue is generated using a solid phase peptide synthesis (SPPS) method. SPPS is a well-established methodology (see for example: Solid-phase peptide synthesis: from standard procedures to the synthesis of difficult sequences. Coin I. et al., Nature Protocols, 2007 or Methods and protocols of modern solid phase peptide synthesis. Amblard M. et al., Molecular Biotechnology, 2006). SPPS is initiated by attaching the carboxy group of an N-terminally protected amino acid to an inert solid support carrying a cleavable linker. The two standard protecting groups for α -amino functions of the coupled amino acids are 9-fluorenylmethyloxycarbonyl group (Fmoc) and t-butyloxycarbonyl (Boc). The solid support can be any polymer that allows coupling of the initial amino acid, e.g., a trityl resin, a chlorotrityl resin, a Wang resin or a Rink resin in which the linkage of the carboxy group (or carboxamide for Rink resin) to the resin is sensitive to acid (when Fmoc strategy is used). The polymer support must be stable under the conditions used to deprotect the α -amino group during the peptide synthesis. After the first amino acid has been coupled to the solid support, the α -amino protecting group of this amino acid is removed. The remaining protected amino acids are then coupled one after the other in the order represented by the peptide sequence using appropriate amide coupling reagents, for example BOP (benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate), HBTU (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), HATU (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) or DIC (N,N'-diisopropylcarbodiimide)/HOBT (1-hydroxybenzotriazol), wherein BOP, HBTU and HATU are used with tertiary amine bases. Alternatively, the liberated N-terminus can be functionalized with groups other than amino acids, for example carboxylic acids, etc. Usually, reactive side-chain groups of the amino acids are protected with suitable blocking groups. These protecting groups are removed after the desired peptides have been assembled. They are removed concomitantly with the cleavage of the desired product from the resin under the same conditions. Protecting groups and the procedures to introduce protecting groups can be found in Protecting Groups in Peptide Synthesis. Conda-Sheridan M. et al., Methods Mol Biol, 2020.

[0050] C-terminus amidation can be performed using benzhydrylamine resins as solid support, and other C-terminus modifications can be performed using established protocols

(see for example: Recent advances in the synthesis of C-terminally modified peptides, Arbour C. et al., Org Biomol Chem. 2020). The synthesized peptide is cleaved from the resin, which can be achieved by using King's cocktail (consisting of 82.5% trifluoroacetic acid (TFA), 5% phenol, 5% water, 5% thioanisole, 2.5% ethanedithiol). The raw material can then be purified
5 by chromatography, e.g., preparative RP-HPLC, if necessary. Peptides are then analyzed by analytical HPLC and checked for the correct mass by mass spectrometry. Ion exchange of the residual TFA ions to chloride or acetate can sometimes be performed.

[0051] In some embodiments, a nucleic acid encoding the peptide analogues is provided. In particular embodiments the nucleic acid encodes a sequence selected from SEQ ID NOS:190
10 and 201. The nucleic acid can be e.g., DNA or RNA (such as mRNA) that can be transcribed and/or translated by cellular machinery to produce the desired engineered protein *in vivo*. Any suitable method of genetic engineering (e.g. transfection of cells obtained from and then reintroduced into the body, CRISPR-Cas gene editing, introduction of a suitable viral vector into target cells, or the like) and/or nucleic acid delivery system (e.g. lipid nanoparticles,
15 which can be used to deliver CRISPR-Cas gene editing systems, mRNA or viral vectors to target cells) can be used to supply the nucleic acid encoding the engineered peptide to the desired target cells. Any methods now known or developed in future for causing desired cells to express a desired peptide could be used in various embodiments to cause cells to express the desired non-aggregating engineered protein. In some embodiments, including
20 embodiments in which the engineered peptide incorporates a non-naturally occurring amino acid analogue, the engineered peptide is chemically modified.

[0052] A pharmaceutical composition for treating or preventing α -syn proteinopathy can comprise a therapeutically effective amount of a non/low amyloid-forming analogue of α -syn protein as described herein or a pharmaceutically acceptable salt thereof. In some
25 embodiments, the analogue may be in the form of a prodrug configured to yield the analogue by solvolysis or under physiological conditions. In some embodiments, the pharmaceutical composition can be formulated for parenteral administration such as by intrathecal, intravenous, intramuscular, or subcutaneous injection or infusion. For example, the composition can be formulated with a pharmaceutically acceptable vehicle or diluent.

[0053] Exemplary compositions for parenteral administration can contain, for example,
30 suitable non-toxic, parenterally acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution, an isotonic sodium chloride solution, or other suitable dispersing or wetting and suspending agents. Pharmaceutically acceptable compositions may include one or more co-solvents including, but not limited to ethanol, N,N dimethylacetamide,
35 propylene glycol, glycerol and polyethylene glycols, e.g., polyethylene glycol 300 and/or

polyethylene glycol 400. Surfactants may be used including, without limitation, d- α -Tocopheryl polyethylene glycol 1000 succinate (TPGS), Solutol HS 15, polysorbate 80, polysorbate 20, poloxamer, pyrrolidones such as N-alkylpyrrolidone (e.g., N-methylpyrrolidone) and/or polyvinylpyrrolidone. The formulation may also comprise use of
5 one or more buffers including, without limitation, sodium phosphate, sodium citrate, diethanolamine, triethanolamine, L-arginine, L-lysine, L-histidine, L-alanine, glycine, sodium carbonate, tromethamine (a/k/a tris[hydroxymethyl]aminomethane or Tris), and/or mixtures thereof.

[0054] Methods of treating or preventing α -syn proteinopenia in a subject are encompassed
10 by the present disclosure. In some embodiments, a method of treating or preventing α -syn proteinopenia in a subject can comprise administering to the subject a therapeutically effective amount of a non/low amyloid-forming analogue of α -syn protein as described herein. In some embodiments, such a method can comprise directly administering to the subject a therapeutically effective amount of a composition comprising the analogue or a
15 pharmaceutically acceptable salt thereof. In some embodiments, the subject is a mammalian subject. In more particular embodiments, the subject is a human.

[0055] In some embodiments, treatment may follow screening the subject to determine whether the subject is a candidate for treatment and/or prophylaxis of a proteinopathy using a non/low amyloid-forming analogue of α -syn
20 protein are encompassed by the present disclosure. In some embodiments, the method comprises determining a concentration of α -syn in the CSF of the subject. If the measured concentration of α -syn in the CSF is less than about 1000 pM, less than about 800 pM, less than about 600 pM, or less than about 400 pM, including e.g. less than about 300, 250, 200, 150 or 100 pM, then the subject is identified as a candidate for treatment or prophylaxis
25 via the administration of a non/low amyloid-forming analogue of α -syn protein as described herein. In some such embodiments, the concentration of α -syn in the cerebrospinal fluid of the subject is determined using an immunoassay or liquid chromatography-tandem mass spectrometry.

[0056] In various embodiments, therapeutic compositions comprising engineered peptides
30 that are non/low amyloid-forming analogues of the α -syn protein or nucleic acids encoding such peptides as described herein are administered in any suitable manner now known or developed in future, including direct administration, genetic engineering techniques, liposome-mediated delivery including lipid nanoparticle delivery, viral vectors or the like. Modes of direct administration can include subcutaneous, intravenous,
35 intracerebroventricular, intracerebral, intrathecal, intraperitoneal, intramuscular or

intravenous injection, infusion, or topical, nasal, oral (including sublingual or buccal), rectal, ocular or otic, or other form of delivery, including pumping or direct injection into the brain of a subject. Modes of liposome-mediated delivery can include direct delivery of the engineered peptide or a nucleic acid (e.g., mRNA) encoding the engineered peptide for expression by a cell, or DNA encoding the engineered peptide together with suitable mechanisms (e.g. CRISPR-Cas gene editing systems) to integrate the DNA into the genome of the cell to facilitate expression of the engineered peptide by the cell, or using a viral vector as an expression module for the desired peptide.

[0057] In some embodiments, the amount of protein analogue to be administered or caused to be expressed can be determined by a person skilled in the art dependent on the condition to be treated and the mode of administration. In some embodiments, the interval of administration of the analogue can be determined by a person skilled in the art dependent on the condition to be treated and the mode of administration. In some embodiments the amount of analogue to be administered or caused to be expressed is sufficient to provide a concentration of the analogue in the CSF of a subject of about 400 to about 2000 pg/mL, including any value or subrange therebetween, e.g. 600, 800, 1000, 1200, 1400, 1600 or 1800 pg/mL. In some embodiments, the amount of analogue to be administered or caused to be expressed is less than an amount of native α -syn protein that would be administered to achieve the same therapeutic effect.

EXAMPLES

[0058] Synthesis of α -syn protein analogue RTPD496-Leucine (SEQ ID NO:478):

1. Expression: RTPD496-Leucine gene fragment ATG GAT GTG TTT ATG AAG GGT TTG TCA AAA GCG AAG GAG GGG GTC GTT GCT GCA GCC GAA AAG ACT AAG CAG GGG GTT GCG GAA GCT GCA GGT AAG ACT AAA GAG GGA GTG CTG TAT GTA GGT TCA AAG ACA AAG GAG GGA GTT GTT CAC GGT GTT GCT ACA GTT GCA GAA AAG ACC AAG CGT CAA GTA ACT AAT GTT GGC GGG GCC GTG GTG ACA GGT GTT ACA GCG GTG GCG CAG AAA ACA GTC GAA GGC GCG GGC TCC ATC GCA GCG GCG ACG GGG TTC GTT AAA AAG GAC CAA TTA GGA AAG AAC GAA GAG GGA GCT CCT CAA GAA GGA ATT TTA GAA GAC ATG CCC GTC GAT CCA GAT AAC GAA GCG TAT GAG ATG CCA AGC GAA GAA GGC TAT CAG GAT TAT GAG CCG GAG GCA CTG (SEQ ID NO:877) is cloned into an *Escherichia coli* expression vector (e.g., pAED4) then overexpressed in *Escherichia coli* BL21(DE3).

2. Protein purification: bacterial cells are harvested then resuspended in 10 mM tris(hydroxymethyl)aminomethane (Tris) (pH 7.5), 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethanesulfonyl fluoride (PMSF), and 1 mM dithiothreitol (DTT) (1/10 culture volume). Cells are then lysed by freezing in liquid nitrogen followed by
5 thawing and then passed through a French cell press (2x). The cell lysate is then treated with streptomycin sulfate and ammonium sulfate. The α -syn protein analogue, RTPD496-Leucine, is then resuspended in a minimal volume of 10 mM Tris (pH 7.5) and 0.5 M NaCl and boiled for 20 min. The supernatant is saved, concentrated, and exchanged into 10 mM Tris (pH 7.5). The protein is then loaded onto a Sephacryl® S-300 size-exclusion
10 column and eluted in 100 mM NH_4HCO_3 and lyophilized.
3. C-terminus amidation: H-Arg- $\text{NH}_2 \cdot 2\text{HCl}$ reagent is dissolved in 5 mM EDTA, then RTPD496-Leucine is added to a final concentration of 40 mM in 5 % acetic acid, and then mixed with carboxypeptidase Y to a final concentration of 1.0 μM . The reaction mixture is incubated at 30 °C for 2 h. Then the analogue is purified and reaction efficiency
15 determined by RP-HPLC.

CLAIMS

What is claimed is:

1. A protein analogue for the treatment or prevention of α -syn proteinopenia, comprising:
an N-terminal domain having at least 85% identity to positions 1-60 of SEQ ID NO:1;
5 a C-terminal domain having at least 85% identity to positions 96-140 of SEQ ID NO:1;
and
a modified middle domain corresponding to positions 61-95 of SEQ ID NO:1 and
comprising:
deletion of 1 to 35 amino acids; or
10 one or more substitutions comprising an amino acid, an amino acid analogue,
or both.
2. The protein analogue of claim 1, wherein the N-terminal domain comprises at least 90%
identity to positions 1-60 of SEQ ID NO:1.
3. The protein analogue of claim 1, wherein the N-terminal domain comprises at least 95%
15 identity to positions 1-60 of SEQ ID NO:1.
4. The protein analogue of claim 1, wherein the C-terminal domain comprises at least 90%
identity to positions 96-140 of SEQ ID NO:1.
5. The protein analogue of claim 1, wherein the C-terminal domain comprises at least 90%
identity to positions 96-140 of SEQ ID NO:1.
- 20 6. The protein analogue of any one of claims 1 to 5, wherein the one or more substitutions
comprise a charged amino acid, a polar amino acid, or both.
7. The protein analogue of any one of claims 1 to 6, wherein the one or more substitutions
comprise an amino acid selected from the group consisting of: proline, glycine, lysine,
arginine, histidine, glutamic acid, aspartic acid, serine, threonine, asparagine, glutamine, and
25 cysteine.
8. The protein analogue of claim 7, wherein the one or more substitutions comprise proline.
9. The protein analogue of claim 7, wherein the protein analogue is selected from SEQ ID
NOS:37-438.

10. The protein analogue of any one of claims 1 to 5, wherein the modified middle domain comprises a consecutive amino acid deletion that includes at least a valine deletion at position 95.
11. The protein analogue of claim 10, wherein the protein analogue is selected from SEQ ID
5 NOS:2-36.
12. The protein analogue of any one of claims 1 to 8, wherein the one or more substitutions comprise an amino acid analogue.
13. The protein analogue of claim 12, wherein the amino acid analogue is selected from 3-hydroxyproline, 4-hydroxyproline, selenocysteine, pyroglutamic acid, carboxyglutamic acid,
10 octenyl alanine, pyrrolysine, palmitoyl aspartate, D-amino acids including D-proline, β -amino acids, γ -amino acids, homo-amino acids, β -homo-amino acids, α -methyl amino acids, N-methyl amino acids, N-ethyl amino acids, N-alkylated amino acid derivatives (preferably with 1, 2 or 3 carbons in the alkyl moiety), peptoid substituents, pyruvic acid derivatives,
15 branched-chain amino acid derivatives, nitro amino acid derivatives, halogenated amino acid derivatives, ring-substituted amino acid derivatives, aromatic amino acid derivatives, linear core amino acids, hydroxylated amino acid derivatives, cyclic amino acids, bicyclic amino acids, 3-amino-3-aryl-propionic acids, 3-amino-4-aryl-butyric acids, amino acids with aromatic spacers, alicyclic amino acids and α -phenylglycine derivatives.
14. The protein analogue of any one of claims 1 to 13, wherein the C-terminus of the protein
20 analogue includes a modification configured to enhance at least one of circulation time, protease resistance and CSF half-life of the protein analogue as compared to wild type α -syn protein.
15. The protein analogue of any one of claims 1 to 5, wherein the C-terminus of the protein analogue is modified with a functional group selected from an amide, an ester, an alcohol, a
25 hydrazide, an aldehyde; and a hydroxamic acid.
16. The protein analogue of claim 15, wherein the C-terminus of the protein analogue is modified to an amide.
17. The protein analogue of claim 16, wherein the protein analogue is SEQ ID NO:439.
18. The protein analogue of claim 16, wherein the modified middle domain comprises at least
30 one consecutive amino acid deletion that includes at least a valine deletion at position 95.

19. The protein analogue of claim 18, wherein the protein analogue is selected from SEQ ID NOS:440-474.
20. The protein analogue of claim 16, wherein the one or more substitutions comprise an amino acid selected from the group consisting of: proline, glycine, lysine, arginine, histidine,
5 glutamic acid, aspartic acid, serine, threonine, asparagine, glutamine, and cysteine.
21. The protein analogue of claim 20, wherein the protein analogue is selected from SEQ ID NOS:475-876.
22. The protein analogue of claim 1, wherein the protein analogue is selected from a group consisting of SEQ ID NOS:2-876.
- 10 23. The protein analogue of claim 1, wherein the protein analogue comprises, relative to wild type α -syn protein, at least one of: a decreased beta-sheet-forming propensity, enhanced solubility, improved stability, improved circulation time, improved brain penetrance, and increased CSF half-life.
24. The protein analogue of claim 23, wherein the half-life is at least about 10 min, at least
15 about 20 min, at least about 30 min, at least about 40 min, or at least about 50 min.
25. The protein analogue of claim 23, wherein the half-life is at least about 1 hours, at least about 5 hours, or at least about 10 hours.
26. The protein analogue of claim 23, wherein the half-life is at least about 1 day, at least about 10 days, at least about 60 days, at least about 10 weeks, or at least about 50 weeks.
- 20 27. The protein analogue of claim 23, wherein the protein analogue comprises an increased protease resistance relative to wild type α -syn protein.
28. The protein analogue of claim 23, wherein the protein analogue has a decreased propensity for beta-sheet stacking and amyloid formation relative to wild type α -syn protein.
29. The protein analogue of claim 23, wherein the protein analogue comprises increased
25 solubility in a liquid medium relative to wild type α -syn protein.
30. The protein analogue of claim 29, wherein the medium is a pharmaceutically acceptable medium.

31. The protein analogue of claim 29, wherein the solubility is at least about 1 mg/ml, more preferably at least about 5 mg/ml and most preferably at least about 50 mg/ml.
32. The protein analogue of claim 1, wherein the protein analogue performs a function of wild type α -syn protein to at least 50% of the level performed by wild type α -syn protein.
- 5 33. A nucleic acid encoding the protein analogue as defined in any one of claims 1 to 32.
34. A method for treatment and/or prevention of α -syn proteinopenia in a subject, comprising administering to the subject a therapeutically effective amount of the protein analogue of any one of claims 1 to 32.
35. The method of claim 34, wherein the therapeutically effective amount is sufficient to
10 provide a concentration of the analogue in the CSF of a subject of about 400 to about 2000 pg/mL..
36. The method of claim 34, wherein the therapeutically effective amount is lower than that for wild type α -syn protein.
37. The method of claim 34, comprising a prior step of identifying the subject as a candidate
15 for treatment by determining a concentration of α -syn protein in the CSF of the subject.
38. A method of treating or preventing α -syn proteinopenia in a subject, comprising administering to the subject a nucleic acid encoding the protein analogue as defined in any one of claims 1 to 32.
39. A composition for treating or preventing α -syn proteinopenia, comprising:
20 a therapeutically effective amount of the protein analogue as defined in any one of claims 1 to 32 or a pharmaceutically acceptable salt thereof; and
a pharmaceutically acceptable vehicle.
40. The composition of claim 39, wherein the composition is formulated for parenteral administration.
- 25 41. The composition of claim 40, wherein the composition is formulated for intrathecal administration.