

COMPOSITIONS AND METHODS FOR TREATMENT AND/OR PROPHYLAXIS OF PROTEINOPATHIES

Cross-Reference to Related Applications

- 5 **[0001]** This application claims priority to, and the benefit of, United States provisional patent application No. 63/193258 filed 26 May 2021, which is incorporated by reference herein in its entirety.

Technical Field

- 10 **[0002]** Some aspects relate to compositions for preventing aggregation of proteins in proteinopathies. Some aspects relate to compositions for supplying the native function of a protein while limiting and/or preventing aggregation of said protein in vivo. Some aspects relate to compositions and/or methods useful for the treatment and/or prophylaxis of proteinopathies.

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Background

- [0003]** Amyloids are fibrillary protein aggregates formed when certain proteins transition from a natively folded conformation to a cross-beta conformation. In this conformation, the protein molecules are arranged in the form of two oppositely stacked beta-sheets that
- 20 exclude water molecules between them and have interdigitating generally hydrophobic side chains forming a dry steric zipper. This elongated cross-beta conformation constitutes the basic amyloid fibrillary subunit, the protofilament. The cross-beta architecture can be provided by one folded molecule or two separate molecules, and the beta-sheets can stack in parallel, anti-parallel, face-to-face, or face-to-back orientations.
- 25 **[0004]** The protofilaments once formed can associate in a number of different ways to produce different superstructural polymorphs associated with various disorders, including flat fibrillary structures with varied numbers of horizontally stacked protofilaments. These flat fibrillary structures can evolve to amyloid crystals or different twisted ribbon structures of single or multiple intertwined protofilaments, which can further evolve into nanotubes.

- [0005]** A number of disorders have been determined to be amyloid-associated diseases or amyloidopathies, which can be more generally referred to as proteinopathies, including Alzheimer's disease (AD), Parkinson's disease, Lewy body disease, Pick's disease, transmissible spongiform encephalopathies (TSEs) caused by prions such as Creutzfeldt-Jakob disease or Kuru, Huntington's disease, type II diabetes, thyroid medullary carcinoma, pulmonary alveolar proteinosis and atrial amyloidosis resulting from the amyloid accumulation of calcitonin, surfactant protein C and atrial natriuretic factor, amyotrophic lateral sclerosis, Down syndrome, multiple system atrophy, neuronal degeneration with brain iron accumulation type I (Hallervorden-Spatz disease), mild cognitive impairment (MCI), cerebral amyloid angiopathy (CAA), and the like. Examples of proteinopathies include tauopathies, synucleinopathies, prionopathies, TDP-43, and the like. Examples of tauopathies include: Pick's disease, progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, globular glial tauopathies, aging-related tau astrogliopathy, chronic traumatic encephalopathy, primary age-related tauopathy (PART) (or tangle dominant dementia), Parkinsonism-Dementia complex of Guam, postencephalitic Parkinsonism, atypical Parkinsonism of Guadeloupe, diffuse neurofilament tangles with calcification, frontotemporal dementia, Parkinsonism linked to Chromosome tauopathy, and the like. Examples of synucleinopathies include: Parkinson's disease with dementia, pure autonomic failure (PAF), multiple systemic atrophy (MSA), and the like. Examples of prionopathies include fatal familial insomnia (FFI), Gerstmann-Straussler-Scheinker (GSS), and the like. Examples of TDP-43 include: frontotemporal lobar degeneration (FTLD) with TDP43 inclusion, FTLD with moto-neurons disease (FTLD-MND), hippocampal sclerosis, amyotrophic lateral sclerosis, frontotemporal dementia, Parkinsonism linked to Chromosome 17 3R, 4R, or 3R+4R tauopathy, or the like.
- [0006]** Exemplary proteins that are known to be involved in proteinopathies include amyloid-beta protein (Alzheimer's disease), alpha-synuclein (Parkinson's disease), islet amyloid polypeptide (IAPP, type II diabetes), tau (a microtubule-associated protein implicated in frontal temporal dementia with Parkinsonism and Pick's disease), p53 (a tumor suppressor transcription factor, implicated in many cancers), huntingtin protein in Huntington's disease, prion protein in Creutzfeldt-Jakob disease, and the like. Table 1 below lists exemplary proteinopathies and the proteins known to be implicated or involved in such proteinopathies.

Table 1. Known proteinopathies and the corresponding protein implicated in the proteinopathy.

Alzheimer's disease	amyloid-beta
Hereditary cerebral hemorrhage with amyloidosis	amyloid-beta
Parkinson's disease	alpha-synuclein
Lewy body dementia	alpha-synuclein
Parkinson's disease with dementia	alpha-synuclein
Multiple system atrophy	alpha-synuclein
Pick's disease	tau protein
Progressive supranuclear palsy	tau protein
Corticobasal degeneration	tau protein
Frontotemporal dementia with parkinsonism linked to chr 17	tau protein
Argyrophilic grain disease	tau protein
Tangle predominant dementia	tau protein
Guam Parkinson dementia complex	tau protein
Frontotemporal lobar degeneration	tau protein
Chronic traumatic encephalopathy	tau protein
Ganglioglioma	tau protein
Meningioangiomas	tau protein
Subacute sclerosing panencephalitis	tau protein
Lead encephalopathy	tau protein
Tuberous sclerosis	tau protein
Hallervorden-Spatz disease	tau protein
Lipofuscinosis	tau protein
Spongiform encephalopathy including Creutzfeldt-Jakob disease	prion protein (PrP)
Fatal insomnia	prion protein (PrP)
Gerstmann-Sträussler-Scheinker disease	prion protein (PrP)
Huntington disease-like 1	prion protein (PrP)
Spongiform encephalopathy with neuropsychiatric features	prion protein (PrP)
New variant Creutzfeldt-Jakob disease	prion protein (PrP)
Kuru	prion protein (PrP)
Hereditary sensory and autonomic neuropathy	prion protein (PrP)
Type 2 diabetes	Fragments of islet amyloid polypeptide
Insulinoma	Islet amyloid polypeptide
Primary systemic amyloidosis	Immunoglobulin light chain or fragments thereof
Secondary systemic amyloidosis	Fragments of serum amyloid-A
Senile systemic amyloidosis or familial amyloid polyneuropathy	Transthyretin and fragments thereof
Hemodialysis-related amyloidosis	Beta2-microglobulin
Hereditary cerebral amyloid	Cystatin C

angiopathy, Icelandic type	
Familial amyloidosis, Finnish type	Gelsolin fragments
Familial amyloid polyneuropathy	Fragments of apolipoprotein A-1
Medullar carcinoma of the thyroid	Fragments of calcitonin
Atrial amyloidosis	Atrial natriuretic factor
Hereditary non-neuropathic systemic amyloidosis	Lysozyme or fragments thereof
Hereditary renal amyloidosis	Fibrinogen fragments
Amyloidosis in senescence	Apolipoprotein A-II
Huntingtin disease	Huntingtin
Familial British dementia	ABri peptide
Familial Danish dementia	ADan peptide
Light-chain amyloidosis	Fragments of immunoglobulin light chains
Heavy-chain amyloidosis, including renal	Fragments of immunoglobulin heavy chains
AA amyloidosis	Full or N-terminal fragments of serum amyloid A protein (SAA)
Senile systemic amyloidosis	Transthyretin (TTR)
Familial amyloidotic polyneuropathy	Transthyretin (TTR)
Familial amyloid cardiomyopathy	Transthyretin (TTR)
Leptomeningeal amyloidosis	Transthyretin (TTR)
Dialysis-related amyloidosis	β_2 -microglobulin
Hereditary visceral amyloidosis	β_2 -microglobulin
ApoAI amyloidosis, including in various organs	N-terminal fragments of apolipoprotein A-I (ApoAI)
ApoAII amyloidosis, including renal	C-terminal extended apolipoprotein A-II (ApoAII)
ApoAIV amyloidosis, including in various organs	N-terminal fragments of apolipoprotein A-IV (ApoAIV)
ApoCII amyloidosis, including renal	Apolipoprotein C-II (ApoCII)
ApoCIII amyloidosis, including renal	Apolipoprotein C-III (ApoCIII)
Lysozyme amyloidosis, including visceral	Lysozyme
Fibrinogen amyloidosis, including renal	Fragments of fibrinogen alpha-chain
Hereditary cerebral hemorrhage with amyloidosis, Icelandic type	N-terminal truncated cystatin C
Pituitary prolactinoma	N-terminal fragments of prolactin
Aortic medial amyloidosis	Medin
Gelatinous drop-like corneal dystrophy	Lactotransferrin (lactoferrin)
Calcifying epithelial odontogenic tumors	Odontogenic ameloblast-associated protein (ODAM)
Pulmonary alveolar proteinosis	Pulmonary surfactant-associated protein C (SP-C)
Renal amyloidosis	Leukocyte cell-derived chemotaxin-2 (LECT-2)
Lichen amyloidosis	Galectin 7 (Gal-7)

Macular amyloidosis	Galectin 7 (Gal-7)
Hypotrichosis simplex of the scalp	Corneodesmosin (CDSN)
Lattice corneal dystrophy, type 1	C-terminal fragments of kerato-epithelin
Lattice corneal dystrophy, type 3A	C-terminal fragments of kerato-epithelin
Lattice corneal dystrophy, Avellino type	C-terminal fragments of kerato-epithelin
Seminal vesicle amyloidosis	Semenogelin-1 (SGI)
Prostate cancer	Proteins S100A8/A9
Injection-localized amyloidosis	Enfuvirtide
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)	Neurogenic locus notch homolog protein 3 (Notch 3) ectodomain
Heavy-chain deposition disease, including renal	Immunoglobulin heavy chains
Light-chain deposition disease, including renal	Immunoglobulin light chains
Myeloma cast nephropathy, renal	Immunoglobulin light chains
Fanconi syndrome, renal	Immunoglobulin light chains
FN glomerulopathy	Fibronectin (FN)
Frontotemporal lobar degeneration with ubiquitin-positive inclusions	TAR DNA-binding protein 43 (TDP-43)
Amyotrophic lateral sclerosis	TAR DNA-binding protein 43 (TDP-43)
Frontotemporal lobar degeneration with ubiquitin-positive inclusions	RNA-binding protein FUS (FUS)
Amyotrophic lateral sclerosis	RNA-binding protein FUS (FUS)
Amyotrophic lateral sclerosis	[Cu-Zn] superoxide dismutase (SOD1)
Amyotrophic lateral sclerosis	C9ORF72 protein
C1q nephropathy	Complement C1q subcomponent
IgA nephropathy (Berger disease)	Immunoglobulin A (IgA)
Henoch-Schönlein purpura	Immunoglobulin A (IgA)
Primary hyperoxaluria type 1	Alanine:glyoxylate aminotransferase (AGT)
Multiple myeloma/plasmacytoma (Russell bodies)	Immunoglobulin G (IgG)
Medullary cystic kidney disease 2	Uromodulin, or Tamm-Horsfall urinary glycoprotein (THP)
Familial juvenile hyperuricemic nephropathy	Uromodulin, or Tamm-Horsfall urinary glycoprotein (THP)
Glomerulocystic kidney disease	Uromodulin, or Tamm-Horsfall urinary glycoprotein (THP)
Spinocerebellar ataxia 1	Ataxin-1
Sickle cell anemia	Hemoglobin
Heinz body anemia	Hemoglobin
Inclusion body β -thalassemia	Hemoglobin
α 1-antitrypsin deficiency	α 1-antitrypsin
Hereditary hyperferritinemia cataract syndrome	Ferritin light chain

Alzheimer's disease	Actin
Frontotemporal dementia	Actin
Cancer	Cellular tumor antigen p53

[0007] Amyloid-beta peptide is a 39-43 amino acid peptide that is derived by proteolysis from amyloid precursor protein (APP). Amyloid precursor protein (APP) is cleaved by a β -secretase to produce a 99-residue transmembrane fragment C99, which then undergoes

5 further cleavages by γ -secretase to generate the amyloid-beta peptide. The predominant circulating form of amyloid-beta protein is A β -40, although A β -42 and A β -43 are also found in amyloid-beta plaques. The function of amyloid-beta protein is not well understood, but it does appear to play a role in normal synaptic plasticity and memory. A growing body of literature demonstrates the role of A β peptides in memory and synaptic plasticity via alpha-7

10 nicotinic acetylcholine receptor signaling.

[0008] Considering Alzheimer's disease as an example proteinopathy, there are numerous different types and causes of Alzheimer's disease. Alzheimer's disease can include Alzheimer's disease arising from any cause or of any other type, including familial Alzheimer's disease (also called autosomal dominant Alzheimer's disease), sporadic

15 Alzheimer's disease, or early-onset sporadic Alzheimer's disease. Apart from being a condition on its own right, mild cognitive impairment may be regarded as an early stage of Alzheimer's disease. Thus, the emergence of mild cognitive impairment may signal the need for initiating therapy to avoid progression to the more profound cognitive impairment typical for Alzheimer's disease. In other words, subjects with mild cognitive impairment are

20 at increased risk of Alzheimer's disease.

[0009] A subset of familial Alzheimer's disease cases are caused by specific genetic defects in the presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*) or amyloid- β protein precursor (*APP*) genes. *PSEN1* functions as the catalytic subunit of γ -secretase while mutations in *PSEN2* may increase γ -secretase activity. It has been established by numerous studies

25 that the level of soluble A β 42 in various types of familial Alzheimer's disease, including that caused by mutations in *PSEN1*, *PSEN2* or *APP*, begins to decline many years before disease onset. Thus, a decrease in soluble levels of A β 42 is associated with Alzheimer's disease, and occurs long before the disease develops.

[0010] A subset of familial Alzheimer's disease is caused by mutations in the A β -42 peptide, particularly at residues 19-24 of the peptide. Further, it is believed that the YEVHHQ domain at residues 10-15 of the A β 42 peptide is also important for native function of the peptide.

5 **[0011]** In addition to Alzheimer's disease, a decrease in soluble A β 42 peptide has also been identified in other disorders, including Amyotrophic lateral sclerosis (ALS), frontotemporal dementia, Parkinson's disease, Parkinson's disease dementia, progressive supranuclear palsy, corticobasal degeneration, dementia with Lewy bodies, multiple system atrophy, and neurodegenerative dementias. See e.g. Mllenbauer et al., *J. Neurochem.* (2016)
10 **139** (Suppl. 1), 290-317, which is incorporated by reference herein in its entirety.

[0012] Alpha-synuclein is a protein that is abundant in the brain, and which is found mainly at the tips of neurons in presynaptic terminals. The function of alpha-synuclein is not well understood, but it is believed to play a role in synaptic vesicle recycling and it may also help to regulate the release of dopamine. Patients with Parkinson's disease or Lewy body
15 dementia generally develop Lewy bodies, which are aggregations of alpha-synuclein, in their brains.

[0013] Prion protein is a cellular glycoprotein that has been implicated in Creutzfeldt-Jakob disease. Soluble prion protein in its native conformation is believed to be involved in myelin maintenance and cellular proliferation processes.

20 **[0014]** Conventional wisdom suggests that many of the disease states caused by proteinopathies are caused by a toxic gain of function of the proteins involved as they aggregate. Most conventional therapies are aimed at minimizing protein aggregation or removing the aggregated proteins (e.g. by reducing protein expression, or by techniques such as immunotherapy aimed at clearing aggregated proteins from cells). However, such
25 therapies have not been particularly successful, and in some cases halting expression of the protein involved in the proteinopathy (for example by knocking out or knocking down the relevant protein) can actually result in a disease phenotype even in the absence of the relevant protein and its aggregated forms. Further, for example in Alzheimer's disease, it has been shown that there is not always a correlation between plaque load and disease
30 severity.

[0015] There is emerging evidence that the disease states caused by proteinopathies may in fact be caused by the loss of function of the native proteins that become aggregated in such diseases, particularly in earlier stages of disease. Most amyloid-forming proteins are known or suspected to perform various functions in their native folded state. As such
5 proteins unfold into a cross-beta conformation and form fibrils, the native function of the protein may be lost. Such loss of function of the protein can have deleterious effects on cells that are at least part of the disease process.

[0016] There is a general desire for improved therapeutics for proteinopathies. There is a desire for improved therapeutics for disorders involving proteinopathies that can address
10 loss-of-function of the native protein.

[0017] The foregoing examples of the related art and limitations related thereto are intended to be illustrative and not exclusive. Other limitations of the related art will become apparent to those of skill in the art upon a reading of the specification and a study of the drawings.

15 **Summary**

[0018] 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. In various embodiments, one or more of the above-described problems have been reduced or eliminated, while other embodiments are
20 directed to other improvements.

[0019] Some aspects provide a non-aggregating peptide analogue of the A β 42 peptide. The A β 42 peptide has an N-terminal domain corresponding to positions 1-28 of SEQ ID NO:1, and a beta-sheet aggregation domain corresponding to positions 29-42 of SEQ ID NO:1. The non-aggregating peptide analogue has a beta-sheet destabilizing modification in
25 the beta sheet aggregation domain, and substantially retains the native function of the A β 42 peptide except for being non-aggregating. In some aspects, the non-aggregating peptide analogue is for use in the treatment and/or prophylaxis of Alzheimer's disease or mild cognitive impairment or another disorder associated with a decreased level of soluble A β 42 peptide. In some aspects, a method of screening a subject to determine if the subject is a
30 candidate for the treatment and/or prophylaxis of Alzheimer's disease or another disorder associated with a decreased level of soluble A β 42 peptide using a non-aggregating peptide

analogue as disclosed herein is provided. The concentration of A β 42 in a sample of cerebrospinal fluid of the subject is determined, and if the measured concentration of A β 42 in the cerebrospinal fluid sample is less than about 500 pM, preferably less than about 400 pM, preferably less than about 300 pM, more preferably less than 200 pM, the
5 subject is identified as a candidate for treatment and/or prophylaxis via the administration of a non-aggregating peptide analogue as described herein. In some aspects, the method further comprises administering the non-aggregating peptide analogue to the subject.

[0020] In addition to the exemplary aspects and embodiments described above, further aspects and embodiments will become apparent by reference to the drawings and by study
10 of the following detailed descriptions.

Brief Description of the Drawings

[0021] Exemplary embodiments are illustrated in referenced figures of the drawings. It is intended that the embodiments and figures disclosed herein are to be considered illustrative
15 rather than restrictive.

[0022] FIG. 1, having panels 1.1, 1.2, 1.3, 1.4, 1.5 and 1.6, shows exemplary engineered proteins having a beta-sheet destabilizing mutation in the beta-sheet aggregation domain of the amyloid-beta protein. The presence of a blank shaded block in the table indicates a deletion mutation.

20 **[0023]** FIG. 2 shows an example embodiment of a method for designing a non-aggregating analogue of a protein implicated in a proteinopathy.

[0024] FIG. 3 shows an example embodiment of an engineered protein that is a non-aggregating analogue of a protein implicated in a proteinopathy.

25 **[0025]** FIG. 4A shows the restoration of phenotype in the Alzheimer's disease animal model 5XFAD demonstrating that administration of an engineered peptide intravenously restored wild type behaviour when administered for five weeks. FIGs. 4B and 4C show parallel results from additional experiments.

[0026] FIG. 5A shows the relative plaque load in the cortices of vehicle treated (left side) 5XFAD mice as compared with 5XFAD mice treated for five weeks with an engineered

peptide that is a non-aggregating analogue of A β 42. FIG. 5B shows the corresponding level of plaque accumulation in the hippocampi of the mice.

[0027] FIG. 6A shows the aggregation propensity of A β 42 peptide and two non-aggregating analogues thereof, as measured using a thioflavin-T kinetics assay. FIG. 6B shows the aggregation propensity of A β 42 peptide and two additional exemplary non-aggregating analogues thereof incorporating a non-naturally occurring amino acid substitution.

[0028] FIG. 7A and FIG. 7B show results of assays on SH-SY5Y neuronal cell-line expressing amyloid precursor protein gene with the Swedish mutations. FIG. 7A shows cell proliferation relative to untreated cells, while FIG. 7B shows cell proliferation relative to wild type cells. FIG. 7C and 7D show data from additional similar experiments showing cell proliferation over untreated cells plotted along a shorter y-axis to better show comparisons.

[0029] FIG. 8 shows results of assays on SH-SY5Y neuronal cells that were treated with 10 μ M of γ -secretase inhibitor.

[0030] FIGs. 9A and 9B show the levels of soluble A β 42 that differentiate healthy individuals (cognitively normal or CN) from those with mild cognitive impairment (MCI) or Alzheimer's disease (AD).

[0031] FIG. 10 shows adjusted prediction of CDR non-progression with baseline CSF A β 42 levels.

[0032] FIG. 11A-11D shows the percentage of facilitation of α 7 nicotinic acetylcholine receptors in cortical neurons in comparison with pre- and post-control responses for an example non-aggregating peptide analogue.

[0033] FIG. 12A-12D and 13 show the percentage of facilitation of α 7 nicotinic acetylcholine receptors in cortical neurons in comparison with pre- and post-control responses for additional example non-aggregating peptide analogues.

[0034] FIG. 14 shows the percentage facilitation of α 7 nicotinic acetylcholine receptors in cortical neurons in comparison with pre- and post-control responses for A β 42.

[0035] FIGs 15A-15D show the percentage of facilitation of α 7 nicotinic acetylcholine receptors in cortical neurons in comparison with pre- and post-control responses for a control scrambled peptide.

[0036] FIG. 16 shows that RT88 induces downstream ERK1/2 phosphorylation at pM concentrations.

[0037] FIG. 17 shows docking simulations showing that an exemplary non-aggregating peptide analogue of A β 42 binds to the α 7 nicotinic acetylcholine receptor via its N-terminal domain.

[0038] FIG. 18 shows a model of the formation of intermolecular beta-sheets, with the position occupied by the methionine at position 35 circled.

Description

[0039] Throughout the following description specific details are set forth in order to provide a more thorough understanding to persons skilled in the art. However, well known elements may not have been shown or described in detail to avoid unnecessarily obscuring the disclosure. Accordingly, the description and drawings are to be regarded in an illustrative, rather than a restrictive, sense.

[0040] The inventors have now determined that administration of engineered peptides/proteins that provide the beneficial wild type function of a peptide/protein implicated in a proteinopathy but which have been modified to avoid or minimize the formation of a cross-beta sheet structure can be therapeutic and/or prophylactic for the proteinopathy. Without being bound by theory, the administration of such proteins is believed to provide equivalent or similar function to the native protein, to address the loss of function of such protein that is believed to be caused by the proteinopathy, without contributing further to aggregation of the protein.

[0041] As used herein, the term "engineered peptide" may be used interchangeably with the term "engineered protein", it being understood by those skilled in the art that both peptides and proteins are made up of amino acids joined by peptide bonds, with proteins being longer chains of amino acids than peptides. The protein involved in a proteinopathy may sometimes be a peptide, and so a reference to protein involved in a proteinopathy also encompasses a peptide involved in a proteinopathy.

[0042] As used herein, amino acid sequences for peptides are provided, including a numbering of the positions of each amino acid residue within that sequence, for example

positions 1-42 of the A β 42 peptide (SEQ ID NO: 1). It will be recognized by those skilled in the art that for corresponding peptide analogues, if one or more amino acid residues are deleted, the notional numbering of the positions of the peptide will change, for example if the first residue of the A β 42 peptide is deleted in a particular peptide analogue, that
5 particular analogue will have only residues 1-41. As used herein, the term “corresponding” when referring to the numbering of the position of an amino acid within a peptide sequence refers to the numbering of the consensus sequence of the peptide as provided herein, and so refers to the corresponding numbering of the amino acid residue relative to the reference sequence when the two peptide sequences are aligned. Thus, the particular analogue in
10 which the first residue of the A β 42 peptide mentioned above will contain amino acid residues corresponding to residues 2-42 if the A β 42 peptide as described herein.

[0043] As used herein, the term “beta-sheet aggregation domain” refers to a domain of a peptide or protein that is involved in the formation of a cross-beta conformation of the protein that is implicated in a proteinopathy. While the beta-sheet aggregation domain may
15 not in healthy subjects be associated with the formation of a beta-sheet structure or with aggregation, in individuals suffering from or developing proteinopathies, the collective intra- and inter-molecular interactions of the beta-sheet aggregation domain result in the formation of cross-beta sheets that contribute to the formation of amyloid deposits of the protein or peptide. For the A β 42 peptide, the beta-sheet aggregation domain is located at
20 positions corresponding to residues 29-42 of SEQ ID NO:1.

[0044] The terms “treat”, “treating” and “treatment” refer to an approach for obtaining desired clinical results. Desired clinical results can include, but are not limited to, reduction or alleviation of at least one symptom of a disease. For example, treatment can be
25 diminishment of at least one symptom of disease, diminishment of extent of disease, stabilization of disease state, prevention of spread of disease, delay or slowing of disease progression, palliation of disease, diminishment of disease reoccurrence, remission of disease, prolonging survival with disease, or complete eradication of disease. The term “prophylaxis” includes an approach for preventing disease from occurring or developing from an early stage to a later stage. The term “prevention” in the present context refers to
30 preventive measures resulting in any degree of reduction in the likelihood of developing the condition to be prevented, including a minor, substantial or major reduction in likelihood of

developing the condition as well as total prevention. Preferably, the degree of likelihood reduction is at least a minor reduction.

[0045] As used herein, the term proteinopathy is used to refer to a disease or disorder caused by the aggregation of a protein or peptide into a cross-beta sheet structure that facilitates the formation of fibrillary aggregates of the protein or peptide. Examples of proteinopathies include amyloidopathies, synucleopathies, tauopathies, and the like.

[0046] As used herein, the term “naturally occurring amino acid residue” includes not only the wild type amino acid residue typically found at a particular position within a protein or peptide, but also a mutant amino acid that may occur at that particular position in particular disorders or in an individual subject with a genetic mutation that causes a difference in amino acid sequence at that particular position. An “amino acid analogue” is any non-naturally occurring analogue of a naturally occurring amino acid residue, whether produced synthetically or in vivo via post-translational modification. A non-naturally occurring amino acid analogue may be an analogue that comprises an amino acid. Alternatively, an amino acid analogue may also be devoid of an amino acid moiety, i.e. the non-naturally occurring analogue may comprise any chemical moiety that can be incorporated into an engineered peptide as long as the engineered peptide maintains its native function with the exception of being non-aggregating.

[0047] As used herein, amino acid residues are represented with their one-letter code as follows: alanine (A), arginine (R), asparagine (N), aspartic acid (D), cysteine (C), glutamic acid (E), glutamine (Q), glycine (G), histidine (H), isoleucine (I), leucine (L), lysine (K), methionine (M), phenylalanine (F), proline (P), serine (S), threonine (T), tryptophan (W), tyrosine (Y), valine (V). Unless further specified, all references to amino acid residues herein are to the L-amino acids normally produced by eukaryotes. In some alternative embodiments, the corresponding D-amino acids could be used in place of some or all of the corresponding L-amino acids, including within the beta-sheet aggregation domain and/or as the beta-sheet destabilizing mutation. In alternative embodiments, other non-naturally occurring amino acids or amino acid analogues could be used, including e.g. N-alkyl analogues of the amino acids. Peptides that contain D-amino acids or other non-naturally occurring amino acid residues may have a longer half-life in vivo than peptides that contain only naturally occurring amino acids, for example as a result of serum protein binding and/or enhanced stability against proteases encountered in vivo.

[0048] As used herein, the term “conservative amino acid substitution” means an amino acid sequence that differs from a reference sequence by one or more conservative substitutions of one or more amino acid residues relative to a reference molecule, regardless of where such amino acid substitution occurs (i.e. regardless of whether such substitution is within the beta-sheet aggregation domain described herein or outside such domain). Amino acid substitutions that are considered to be “conservative” include any of:

- Interchanging any one of A, V, L, or I, or an amino acid analogue substantially similar thereto;
- Interchanging any one of S, C, U, T, or M, or an amino acid analogue substantially similar thereto;
- Interchanging any one of F, Y, or W, or an amino acid analogue substantially similar thereto;
- Interchanging any one of D or E or an amino acid analogue substantially similar thereto;
- Interchanging any one of N or Q or an amino acid analogue substantially similar thereto; and/or
- Interchanging any one of H, K or R or an amino acid analogue substantially similar thereto.

[0049] In some embodiments, an engineered protein that is a non-aggregating analogue of a protein implicated in a proteinopathy that can be used as a therapeutic and/or prophylactic agent for the proteinopathy is provided. In some embodiments, the engineered protein includes one or more beta-sheet destabilizing modifications in the beta-sheet aggregation domain. A beta-sheet destabilizing modification is a modification that interferes with and/or disrupts the formation of a beta-sheet structure, thereby interfering with or preventing the formation of the cross-beta sheet structure that can contribute to the proteinopathy. For example, in some embodiments, a beta-sheet destabilizing modification may disrupt one or more hydrogen bonds that are important to forming the stacked beta-sheet structure and/or the cross-beta sheet structure that can contribute to the proteinopathy. In some embodiments, a beta-sheet destabilizing modification may alter a conformation of the beta-sheet aggregation domain (e.g. by kinking the peptide backbone and/or causing interactions with other residues) to interfere with formation of the stacked beta-sheet structure and/or the cross-beta sheet structure that can contribute to the proteinopathy. In some

embodiments, the beta-sheet destabilizing modification does not interfere with, or interferes only minimally with, the native function of the protein, so that the engineered protein retains or substantially retains the wild type activity of the protein.

[0050] In some embodiments, the engineered non-aggregating analogue of the protein involved in a proteinopathy is considered to be non-aggregating when the beta-sheet destabilizing modification results in at least a 2-fold, at least a 3-fold or at least a 4-fold lower steady state level of aggregation in a thioflavin-T assay as compared with the wild type protein. In some embodiments, the engineered non-aggregating analogue is an analogue of A β 42 peptide and has at least a 2-fold, at least a 3-fold or at least a 4-fold lower steady state level of aggregation in a thioflavin-T assay as compared with wild-type A β 42 having the amino acid sequence of SEQ ID NO:1.

[0051] Examples of beta-sheet destabilizing modifications include:

- Substitution of a naturally occurring amino acid with a charged or hydrophilic amino acid within the beta-sheet aggregation domain (e.g. K, R, H, E, D, S, T, N, Q or C);
- Substitution of a naturally occurring amino acid with a proline (P) residue within the beta-sheet aggregation domain;
- Substitution of a naturally occurring amino acid with a glycine (G) residue within the beta-sheet aggregation domain;
- Substitution of a naturally occurring amino acid with an amino acid analogue that interferes with beta-sheet formation within the beta-sheet aggregation domain, examples of said amino acid analogues that interfere with beta-sheet formation include 3-hydroxyproline, 4-hydroxyproline, selenocysteine, pyroglutamic acid, carboxyglutamic acid, 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, 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, peptoid derivatives, 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.

- Deletion of one or more naturally occurring amino acid residues within the beta-sheet aggregation domain, optionally including deletion of up to and including the entirety of the beta-sheet aggregation domain; and/or
 - Insertions of one or more amino acid residues within the beta-sheet aggregation domain, e.g. insertion of 1, 2, 3, 4 or 5 amino acid residues within the beta-sheet aggregation domain. In some embodiments, one or more of the inserted amino acid residues is a proline (P) residue. In some embodiments, one or more of the inserted amino acid residues is a glycine (G) residue. In some embodiments, one or more of the inserted amino acid residues is a charged and/or hydrophilic amino acid (e.g. K, R, H, E, D, S, T, N, Q or C). In some embodiments, one or more of the inserted amino acid residues is an amino acid analogue that interferes with beta-sheet formation as described above, examples of said amino acid analogues that interfere with beta-sheet formation include 3-hydroxyproline, 4-hydroxyproline, selenocysteine, pyroglutamic acid, carboxyglutamic acid, octenyl alanine, pyrrolisine, 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), 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, peptoid derivatives, 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.
- 25 **[0052]** Without being bound by theory, it is believed that substitution of such amino acid residues or deletion or insertion of at least one amino acid residue within the beta-sheet aggregation domain of the protein involved in a proteinopathy interferes with the effective formation of a cross-beta sheet structure, thereby limiting or preventing aggregation of the protein while leaving the protein soluble and available to perform its wild-type function.
- 30 Thus, the engineered protein can be used to restore the loss-of-function defects that may be associated with the proteinopathy, while not aggravating or increasing aggregation of the protein involved in the proteinopathy. To achieve this, in some embodiments, the engineered protein incorporating one or more beta-sheet destabilizing modifications within a

beta-sheet aggregation domain fully or substantially retains its native function, e.g. the engineered protein is able to perform its native function at at least 50% of the level of the native soluble protein (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 protein).

[0053] In some embodiments, the non-aggregating peptide analogue is an analogue of A β 42, and the native function of the A β 42 is to enhance or induce α 7 nicotinic acetylcholine receptor (α 7nAChR) mediated Ca^{2+} influx. Typically, the α 7nAChR mediated Ca^{2+} influx can be triggered using an agonist (e.g. a small molecule agonist such as PNU 282987), but the influx is increased in the presence of an enhancing molecule such as the non-aggregating peptide analogue of the A β 42 peptide described herein. The peptide described herein can also induce the influx on its own. In some embodiments, enhancing α 7nAChR mediated Ca^{2+} influx means that a level of the α 7nAChR mediated Ca^{2+} influx in the presence of the peptide is at least 130%, at least 150%, or at least 170% of a pre-control level of Ca^{2+} influx. In some such embodiments, the non-aggregating peptide analogue is able to perform the native function of enhancing α 7nAChR mediated Ca^{2+} influx at a level of at least 50% of the level of the native soluble A β 42 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 A β 42 peptide). In some embodiments, the non-aggregating analogue of A β 42 is able to enhance α 7nAChR-mediated Ca^{2+} influx when present in cortical neurons at a concentration of ≤ 3000 pM, including e.g. ≤ 300 pM, including e.g. ≤ 30 pM, including e.g. ≤ 3 pM. In some embodiments, the non-aggregating analogue of A β 42 is able to enhance α 7nAChR-mediated Ca^{2+} influx when present in neuronal cell-lines such as N2a and SH-SY5Y cells at a concentration of ≤ 3000 pM, including e.g. ≤ 300 pM, including e.g. ≤ 30 pM, including e.g. ≤ 3 pM. In some embodiments, the non-aggregating peptide analogue of A β 42 is able to enhance α 7nAChR mediated Ca^{2+} influx to a greater extent than wild-type A β 42 peptide having the amino acid sequence of SEQ ID NO:1, e.g. The α 7nAChR mediated Ca^{2+} influx can also be indirectly detected or quantitated by measuring a downstream event, such as ERK1/2 phosphorylation, cell proliferation or neurogenesis. ERK1/2 phosphorylation occurs within minutes of α 7nAChR mediated Ca^{2+} influx and can be measured using ELISA. Yet another alternative way of detecting or quantitating native function is to assess rescue of neuronal

cells after pharmacological depletion of A β 42 using γ -secretase inhibitor. Still another alternative way of detecting or quantitating native function is to assess proliferation of neuronal cells which express a non-functional mutant amyloid precursor protein (APP).

[0054] In some embodiments, portions of the protein other than the beta-sheet aggregation domain may be important for the native function of the protein. For example, in the case of A β 42 peptide, without being bound by theory, it is believed that the YEVHHQ domain at residues 10-15 of the peptide and the residues at positions 19-24 of the peptide are important for native protein function, for example for proper binding to the α 7 nicotinic acetylcholine receptor (α 7nAChR). Thus, in some embodiments, the portions of A β 42 located N-terminal to the beta-sheet aggregation domain, i.e. positions 1-28 of the peptide, are believed to be important for native peptide function. In this description, these residues at or corresponding to positions 1-28 of the A β 42 peptide are referred to as the "N-terminal domain".

[0055] The N-terminal domain of the non-aggregating peptide analogue may comprise an amino-acid sequence differing from residues 1-28 of SEQ ID NO:1 by no more than 6, (preferably 5, more preferably 4, even more preferably 3, yet more preferably 2, still more preferably 1, most preferably 0) deletions, insertions and/or substitutions, preferably conservative substitutions. In some embodiments, the engineered peptide that is a non-aggregating analogue of A β 42 retains the wild type amino acid sequence of the N-terminal domain, or contains only conservative amino acid substitutions within the N-terminal domain. In some embodiments, the peptide that is a non-aggregating analogue of A β 42 has zero or at most one, at most two, at most three, at most four, at most five, at most six, at most seven, at most eight, or at most nine conservative amino acid substitutions in the N-terminal domain. The beta-sheet aggregation domain of the non-aggregating analogue comprises an amino-acid sequence differing from positions corresponding to positions residues 29-42 of SEQ ID NO:1 by no more than 6, (preferably 5, more preferably 4, even more preferably 3, yet more preferably 2, still more preferably 1, most preferably 0) deletions, insertions and/or substitutions, preferably conservative substitutions. Preferably, the non-aggregating peptide analogue as defined comprises, in addition to the beta-sheet destabilizing modification, at most three (preferably two, more preferably one) conservative amino acid substitutions in the beta-sheet aggregation domain relative to SEQ ID NO:1.

[0056] In some embodiments, the disease or disorder that is the proteinopathy is Alzheimer's disease, Parkinson's disease, Lewy body disease or Lewy body dementia, Pick's disease, Creutzfeld-Jakob disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), Down syndrome, neuronal degeneration with brain iron accumulation type I (Hallervorde-Spatz disease), Kuru or other transmissible spongiform encephalopathy (TSE), mild cognitive impairment (MCI), cerebral amyloid angiopathy (CAA), vascular dementias, any neurodegenerative disease characterized by abnormal amyloid deposition, any other disease or disorder listed in Table 1, or the like.

[0057] In some embodiments, the engineered protein is a non-aggregating analogue of a protein implicated in a proteinopathy, including without limitation amyloid precursor protein (APP), amyloid beta protein (including A β -39, A β -40, A β -42 or A β -43 isoforms), alpha-synuclein, prion protein, Huntingtin protein, p53, any protein or peptide listed in Table 1, or the like.

[0058] In some embodiments, a nucleic acid encoding the engineered peptide is provided. 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 expression vector into target cells, or the like) and/or nucleic acid delivery system (e.g. lipid nanoparticles, which can be used to deliver CRISPR-Cas gene editing systems, mRNA or expression vectors to target cells) can be used to supply the nucleic acid encoding the engineered protein to the desired target cells. Any methods now known or developed in future for causing desired cells to express a desired protein could be used in various embodiments to cause cells to express the desired non-aggregating engineered peptide. In some embodiments, including embodiments in which the engineered peptide incorporates a non-naturally occurring amino acid analogue, the engineered peptide is chemically synthesized.

[0059] In some embodiments, therapeutic compositions comprising engineered peptides that are non-aggregating analogues 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, or the like. Modes of direct administration can include

subcutaneous, intravenous, 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
5 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 protein by the cell, or any other mechanism of using a DNA vector as an expression module
10 for the desired peptide.

[0060] In some embodiments, the amount of engineered peptide to be administered or caused to be expressed can be determined by the 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 engineered peptide can be determined by the person skilled
15 in the art dependent on the condition to be treated and the mode of administration. In some embodiments in which the engineered peptide is a non-aggregating analogue of A β 42, the amount of engineered peptide to be administered or caused to be expressed is sufficient to provide a concentration of the engineered peptide in the cerebrospinal fluid of a subject of between about 200 and 600 pg/mL, including any value or subrange therebetween, e.g.
20 250, 300, 350, 400, 450, 500 or 550 pg/mL. The concentration of A β 42 and/or the engineered peptide in the cerebrospinal fluid of the subject may be determined using liquid chromatography-tandem mass spectrometry or an immunoassay, preferably an ELISA immunoassay (e.g. Elecsys, AlzBio3). The precise target concentration of engineered peptide may vary depending on the method of measurement, the specific engineered
25 peptide and the specific condition, but can be established by routine experimentation involving samples from diseased/at risk individuals compared to healthy controls (see Example 4).

[0061] In some embodiments, a method of screening a subject to determine whether the subject is a candidate for treatment and/or prophylaxis of a proteinopathy using an
30 engineered peptide that is a non-aggregating analogue of a protein involved in the proteinopathy is provided. In some embodiments, the proteinopathy is Alzheimer's disease and the protein involved in the proteinopathy is the A β 42 peptide, or the proteinopathy is

another disease or disorder associated with a decreased level of soluble A β 42 peptide. In some embodiments, the method of screening the subject to determine whether the subject is a candidate for treatment and/or prophylaxis of Alzheimer's disease involves determining a concentration of A β 42 in the cerebrospinal fluid of the subject. If the measured

5 concentration of A β 42 in the cerebrospinal fluid is less than about 500 pM, less than about 400 pM, less than about 300 pM, or less than about 200 pM, including e.g. less than about 175, 150, 125 or 100 pM, then the subject is identified as a candidate for treatment and/or prophylaxis of Alzheimer's disease via the administration of a non-aggregating analogue of the A β 42 peptide as described herein. In some such embodiments, the concentration of

10 A β 42 in the cerebrospinal fluid of the subject is determined using liquid chromatography-tandem mass spectrometry or an immunoassay (e.g. Elecsys, AlzBio3).

[0062] In some embodiments, the subject has familial Alzheimer's disease, including familial Alzheimer's disease caused by mutations in *PSEN1*, *PSEN2*, or *A β PP*. Without being bound by theory, it is believed that the mutations associated with familial Alzheimer's

15 disease may reduce the level of soluble A β 42 peptide and/or interfere with the normal function of the peptide. Thus, supplementing the level of functional A β 42 peptide in the subject via administration of an engineered peptide that is a non-aggregating analogue of A β 42 as described herein may be particularly beneficial for such subjects.

[0063] In some embodiments, the subject is a mammalian subject. In some embodiments,

20 the subject is a human subject.

[0064] With specific reference to the example of the amyloid-beta peptide implicated in Alzheimer's disease and other disorders, the wild type sequence of human A β -42 is as set forth in SEQ ID NO:1, and this is shown in FIG. 1. The beta-sheet aggregation domain of A β -42 is believed to be the carboxy-terminal residues 29-42 thereof. Accordingly, in some

25 embodiments, the engineered peptide is a form of amyloid-beta peptide having a beta-sheet destabilizing modification at one or more of positions 29-42 thereof. The beta-sheet destabilizing modification may be a substitution of one or more of the amino acids at positions 29-42 (including any position therebetween, e.g. positions 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41 or 42) with an amino acid that interferes with beta-sheet formation

30 such as P, G, K, R, H, E, D, S, T, N, Q, C or an amino acid analogue that interferes with beta-sheet formation including e.g. 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), 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, peptoid derivatives, 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. The beta-sheet destabilizing modification may be a deletion of the naturally occurring amino acid residue at that position, or insertion of between 1 and 5 amino acid residues at that position (optionally where at least one of the inserted amino acid residues is P, G, K, R, H, E, D, S, T, N, Q, or C or an amino acid analogue that interferes with beta-sheet formation as set forth above). Examples of proteins having beta-sheet destabilizing modifications include the amino acid sequences of SEQ ID NO:2-193 shown in FIG. 1 or SEQ ID NOs:194-207 or SEQ ID NOs: 712-742 (wherein X indicates the position of a beta-sheet destabilizing modification) or any combination thereof. Additional examples include any sequence that is at least 90% identical to any of SEQ ID NO: 2-207, including at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.9% identical thereto. In some embodiments, the engineered protein does not include a peptide having the sequence of SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:15. In the illustrated preferred embodiments of FIG. 1, the X at position 36 of SEQ ID NO:187 represents octenyl alanine; the X at position 35 of SEQ ID NO: 188 represents D-proline, the X at position 35 of SEQ ID NO:189 represents pyroglutamic acid; the X at position 35 of SEQ ID NO:190 represents N-methyl methionine, the X at position 35 of SEQ ID NO:191 represents carboxyglutamic acid, the X at position 35 of SEQ ID NO:192 represents pyrrolysine, and the X at position 1 of SEQ ID NO:193 represents palmitoyl-aspartate. Peptides analogues according to SEQ ID NO: 190 or 192 are the most preferred.

[0065] In some embodiments, the engineered peptide is A β -43 having any of the foregoing sequences described for A β -42, with an additional T residue provided at the carboxy-terminal end of the peptide to provide the A β -43 isoform.

[0066] In some embodiments, the engineered peptide is A β -40 having any of the foregoing sequences described for A β -42, with the two carboxy-terminal amino acids removed to provide the A β -40 isoform.

[0067] In some embodiments, the engineered peptide is A β -39 having any of the foregoing sequences described for A β -42, with the three carboxy-terminal amino acids removed to provide the A β -39 isoform.

[0068] With specific reference to the example of the alpha-synuclein protein implicated in Parkinson's disease, the wild type sequence of human alpha-synuclein is as set forth in SEQ ID NO:208. The beta-sheet aggregation domain of alpha-synuclein is believed to be residues 61-95 thereof. Accordingly, in some embodiments, the engineered protein is a form of alpha-synuclein having a beta-sheet destabilizing modification at one or more of positions 61-95 thereof. In some embodiments, the beta-sheet destabilizing modification is 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, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94 or 95) with an amino acid that interferes with beta-sheet formation or with an amino acid analogue that interferes with beta-sheet formation as described above, or by deletion of the naturally occurring amino acid residue at that position, or by insertion of between 1 and 5 amino acid residues at that position (optionally wherein at least one of the inserted amino acid residues is P, G, K, R, H, E, D, S, T, N, Q, or C or an amino acid analogue that interferes with beta-sheet formation), including for example a protein having any of the sequences of SEQ ID NO:209-663 or any of the sequences of SEQ ID NO:664-698 (wherein X indicates the position of a beta-sheet destabilizing modification) or any combination thereof, or any sequence that is at least 90% identical thereto, including at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.9% identical thereto.

[0069] With specific reference to the example of prion protein implicated in Creutzfeld-Jakob disease, the wild type sequence of human prion protein is as set forth in SEQ ID NO:699. The beta-sheet aggregation domain of the human prion protein is believed to be residues 109-121 thereof. Accordingly, in some embodiments, the engineered protein is a form of prion protein having a beta-sheet destabilizing modification at one or more of positions 109-121 thereof. In some embodiments, the beta-sheet destabilizing modification is a substitution of one or more of the amino acids at positions 109-121 thereof (including

any position therebetween, e.g. positions 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120 or 121) with an amino acid that interferes with beta-sheet formation or with an amino acid analogue that interferes with beta-sheet formation as described above, or by deletion of the naturally occurring amino acid residue at that position, or by insertion of
5 between 1 and 5 amino acid residues at that position (optionally wherein at least one of the inserted amino acid residues is P, G, K, R, H, E, D, S, T, N, Q, or C or an amino acid analogue that interferes with beta-sheet formation), including for example a protein having the amino acid sequence of any one of SEQ ID NO:700-710 or any combination thereof, or any sequence that is at least 90% identical thereto, including at least 91%, 92%, 93%, 94%,
10 95%, 96%, 97%, 98%, 99% or 99.9% identical thereto, wherein the X denotes the position of the beta-sheet destabilizing modification.

[0070] With reference to FIG. 2, in some embodiments a method **100** of designing a non-aggregating analogue of a protein implicated in a proteinopathy is described. At **102**, the target protein that is implicated in the proteinopathy is selected. For example, where the
15 proteinopathy is Alzheimer's disease, amyloid-beta could be selected as the target protein (or peptide) that is implicated in the proteinopathy based on available scientific literature. Where the proteinopathy is Parkinson's disease, alpha-synuclein could be selected as the target protein that is implicated in the proteinopathy based on available scientific literature. Where the proteinopathy is Creutzfeld-Jakob disease, prion protein could be selected as the
20 target protein that is implicated in the proteinopathy based on available scientific literature. In other embodiments where the protein that is implicated in the proteinopathy is not known, suitable experiments could be conducted to determine the identity of a protein that has aggregated in a proteinopathy to select the relevant protein for further steps.

[0071] At **104**, the beta-sheet aggregation domain of the selected protein is identified. For
25 example, structural biology studies (e.g. X-ray crystallography or other studies) or a literature review of such studies can be carried out to evaluate the amyloid structure of the selected protein, to identify the domain(s) involved in aggregation.

[0072] At **106**, one or more beta-sheet destabilizing modifications that may prevent the formation of a cross-beta sheet structure by the selected protein are identified. The beta-
30 sheet destabilizing modification(s) are made in some embodiments by modifying and/or deleting certain naturally occurring amino acid residues within the beta-sheet aggregation domain. For example, one or more naturally occurring amino acid residues within the beta-

sheet aggregation domain may be selected for deletion, or for substitution with G, P, K, R, H, E, D, S, T, N, Q or C or an amino acid analogue that disrupts beta-sheet formation as described above, or the like.

[0073] Once the beta-sheet destabilizing modification(s) have been identified by applying method **100**, then a suitable construct for administration in vivo (e.g. an engineered peptide or a nucleic acid encoding such a peptide) can be made and administered to a subject suffering from or at risk of developing the proteinopathy as described above.

[0074] FIG. 3 shows an example embodiment of an engineered protein **200** that is a non-aggregating analogue of a protein involved in a proteinopathy. Engineered protein **200** has an N-terminus **202**, a C-terminus **204**, and a beta-sheet aggregation domain **206**. Within beta-sheet aggregation domain **206**, a beta-sheet destabilizing modification is engineered into the peptide sequence, so that engineered protein **200** will be a non-aggregating analogue of a protein involved in a proteinopathy.

[0075] With specific reference to engineered peptides useful for the treatment of Alzheimer's Disease, in some embodiments, one or more of the amino acids at positions 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41 or 42 of A β 42 is substituted with a non-naturally occurring amino acid that disrupts beta-sheet formation. In some embodiments, the non-naturally occurring amino acid that disrupts beta-sheet formation is an N-alkyl analogue of the naturally occurring amino acid, for example an N-methyl analogue or an N-ethyl analogue of the naturally occurring amino acid. Without being bound by theory, it is believed that substitution of an N-alkyl analogue of the naturally occurring amino acid within the beta-sheet aggregation domain can disrupt the formation of intermolecular hydrogen bonds between aggregating peptides, while preserving the amino acid side chain and therefore preserving the normal function of the peptide.

[0076] In some specific embodiments, one or more of the following substitutions of a non-naturally occurring amino acid that disrupts beta-sheet formation is made in the A β 42 peptide to provide an engineered non-aggregating peptide analogue: G29 is N-methyl glycine or N-ethyl glycine, A30 is N-methyl alanine or N-ethyl alanine, I31 is N-methyl isoleucine or N-ethyl isoleucine, I32 is N-methyl isoleucine or N-ethyl isoleucine, G33 is N-methyl glycine or N-ethyl glycine, L34 is N-methyl leucine or N-ethyl leucine, M35 is N-methyl methionine or N-ethyl methionine, V36 is N-methyl valine or N-ethyl valine, G37 is N-

5 methyl glycine or N-ethyl glycine, G38 is N-methyl glycine or N-ethyl glycine, V39 is N-methyl valine or N-ethyl valine, V40 is N-methyl valine or N-ethyl valine, I41 is N-methyl isoleucine or N-ethyl isoleucine, and/or A42 is N-methyl alanine or N-ethyl alanine. In some embodiments, the non-aggregating peptide analogue maintains or substantially maintains the wild type function of A β 42.

10 **[0077]** In some specific embodiments, the substitution of a non-naturally occurring amino acid in the engineered non-aggregating analogue of the A β 42 peptide is made at one or both of positions 35 and 36. Without being bound by theory, it is believed that M35 lies in a strategic position in the cross- β amyloid conformation of A β 42, so that this residue and adjacent residues may represent particularly desirable target sites for modification with a non-naturally occurring amino acid to help strategically disrupt intermolecular hydrogen bonding, thereby decreasing the aggregation propensity of the peptide. In some embodiments, the engineered non-aggregating analogue of the A β 42 peptide has the non-naturally occurring amino acid N-methyl methionine or N-ethyl methionine substituted for
15 M35. In some embodiments, the engineered non-aggregating analogue of the A β 42 peptide has the non-naturally occurring amino acid N-methyl valine or N-ethyl valine substituted for V36.

Examples

20 **[0078]** Specific embodiments are further described with reference to the following examples, which are intended to be illustrative and not limiting in nature.

Mouse Study Methods

25 **[0079]** Female wild type and 5xFAD transgenic mice (Jackson laboratories) were used for the mouse behavioural experiment. Mice were individually housed in controlled humidity, temperature and light conditions, and had ad libitum access to food and water. Randomization to treatment groups was carried out by using GraphPad QuickCalcs (GraphPad Software, San Diego, CA, USA), and all data analysis was performed blinded to the experimental groups. All the animal experiments followed the Council of Europe
30 Legislation and Regulation for Animal Protection and are approved by the National Animal

Experiment Board of Finland. Every effort was made to minimize the harm and suffering of the animals. The mice were treated with the vehicle or peptide preparations once a week i.v. starting at the age of 1 month until 3 months of age. Behavioral testing: the 5xFAD mice have deficits in nest building test starting at 3 months of age. Briefly, the soft bedding material and plastic shelter tube are moved to the one end of the cage and soft tissue paper (17cmx17cm) is placed in the other end of the cage. After 24 hours, a picture of the cage is taken, and the points given according to the set scale. 0 points are given if tissue paper remains untouched, 5 points are given when animal incorporates tissue into nest. Sacrifice and sample processing: At the time of sacrifice, the mice were anesthetized using 250 mg/kg Avertin® (Sigma-Aldrich, St. Louis, MO, USA) and perfused transcardially with saline containing 2500 IU/L heparin (Heparin LEO 5000 IU/ml, Leo Pharma A/S, Ballerup, Denmark). Terminal blood samples were taken at the time of sacrifice (approximately 300 µl of blood) using 1:10 dilution of 3.8% sodium citrate as anticoagulant. The blood samples were centrifuged first 1500g for 6 minutes, after which the supernatant was removed to a new tube and further centrifuged for 12 000g for 3 minutes. The resulting plasma was stored in two separate aliquots at -80 degrees for further analysis. The brains were dissected out and cut mid-sagittally into left and right hemispheres. The left hemispheres were post-fixed in 4% paraformaldehyde (PFA, Sigma-Aldrich, St. Louis, MO, USA) for 20 hours, cryoprotected in 30% sucrose in PB for 2 days and frozen on liquid nitrogen. The cortices and hippocampi of the right hemispheres were freshly frozen.

[0080] Immunohistochemistry: The brains were post-fixed in 4% paraformaldehyde (PFA, Sigma-Aldrich, St. Louis, MO, USA) for 20 hours, followed by cryoprotection in 30% sucrose for 48 hours. The brains were frozen on liquid nitrogen and cut coronally into 20 µm thick sections with a cryostat (Leica Microsystems, Wetzlar, Germany) in anti-freeze solution. The immunohistochemical staining for WO2, GFAP and Iba1 was carried out on six consecutive sections at 400 µm intervals. The brain sections were incubated overnight at room temperature with primary antibody (Iba-1 1:250 dilution, Wako Chemicals, Tokyo, Japan). Secondary antibody was applied on sections after three washes in 0.05% Tween®20 (Sigma-Aldrich, St. Louis, MO, USA) in PBS. Fluorescent Alexa 488 or 568-conjugated secondary antibodies (1:200 dilution, Abcam, Cambridge, UK) was used for visualization of the immunoreactivities. For quantification of the immunoreactivities, hippocampal areas were imaged using 4 or 10x magnification on an AX70 microscope

(Olympus corporation, Tokyo, Japan) with an adjacent digital camera (Color View 12 or F-View, Soft imaging system, Muenster, Germany) running AnalySis software (Soft Imaging System). Quantification of the immunoreactivities was performed using ImagePro Plus software (Media Cybernetics Inc., Rockville, MD, USA) at a predefined range and presented as relative immunoreactive area. All analyses will be performed blinded to the study groups.

[0081] THT method. ThT (Sigma-Aldrich) was prepared at 4 mM in MQ water. 50 μ L of 500 μ g/ml peptide (in normal saline) were incubated with 250 μ L of 2.4 mM ThT solution (in water). ThT fluorescence was measured at 440 nm excitation and 480 nm emission in a black, clear-bottom 96-well plates (Corning, USA) at 10–15 min intervals (from bottom with periodic shaking) over 12–24 h on SpectraMax i3 microplate reader (Molecular Devices, USA). Curves were fitted using GraphPad Prism software.

[0082] In-vitro method. WT SH-SY5Y neuroblastoma cell line and SH-SY5Y cells stably expressing amyloid-precursor protein with the Swedish mutations (APP- SH-SY5Y) were grown in in growth medium Dulbecco's modified Eagle's medium (DMEM) with 5% FBS (Fetal Bovine Serum) ThermoFisher, USA), 1% Penicillin/Streptomycin (ThermoFisher, USA) at 37 °C and 5% CO₂. The day before treatments, cells were seeded in 96-well cell culture plate in DMEM medium without FBS. On the day of treatment, WT SH-SY5Y were treated with 10 μ M γ -secretase inhibitor (aldehyde 2-naphthoyl-VF-CHO) together with peptides at different concentrations for 48 hours. APP- SH-SY5Y were treated only with peptides for 72 hours. Cell proliferation was then measured using the WST-1 assay (Sigma-Aldrich) according to manufacturer's protocol.

[0083] Clinical method. To find the minimum required level of A β 42 required for normal cognition, the inventors studied data from the Alzheimer's Disease Neuroimaging Initiative (ADNI), which is a study of over 2700 participants aged between 55 and 90 years (Weiner et al., *Alzheimers Dement.* 2013 Sep;9(5):e111-94). The inventors studied the levels of cerebrospinal fluid (CSF) A β 42 within a sample of ADNI participants that were all positive for amyloid plaques based on positron emission tomography tests. The inventors found that amyloid positive individuals with normal cognition (CN) have an average CSF A β 42 level of 900 pg/ml, which was significantly (asterisks representing significant differences, **P < 0.01, and ***P < 0.001) higher than CSF A β 42 levels in individuals with mild cognitive impairment (MCI) and Alzheimer's disease (AD). The inventors conclude that reaching a CSF

concentration of 900 pg/ml or equivalent in a subject's cerebrospinal fluid using active A β 42 analogues is a reasonable therapeutic target for replacement therapy.

Example 1 – In Vivo Demonstration of Replacement Therapy

- 5 **[0084]** The results shown in FIGs. 4A, 4B and 4C demonstrate that replacement therapy using the engineered version of A β 42 (A β 40) having SEQ ID NO:3 with a beta-sheet destabilizing modification by virtue of the deletion of residues 41 and 42 of the beta-sheet aggregation domain leads to phenotype restoration in a well-established AD animal model (5XFAD mice).
- 10 **[0085]** Briefly, mice were administered a peptide having the sequence of SEQ ID NO:3 (A β -40) intravenously at a dosage of 5 mg/kg/day for five weeks.
- 15 **[0086]** As can be seen from the results shown in FIG. 4A, wild type mice exhibited a behavioral score of approximately 5/5, whereas the untreated 5XFAD mice exhibited an average behavioral score of 4/5. Administration of the engineered peptide A β -40 that is a non-aggregating analogue of A β -42 by virtue of the deletion of residues 41 and 42 of the beta-sheet aggregation domain for a period of five weeks restored mice to a behavioral score of approximately 5/5 (far right). Parallel results for wild type mice administered a scrambled peptide (having the sequence of SEQ ID NO:711) and 5XFAD mice administered the scrambled peptide as controls, together with the phenotype rescue demonstrated by
- 20 administration of non-aggregating peptides RT3 (having the sequence of SEQ ID NO:3 (A β -40)) and RT88 (having the sequence of SEQ ID NO:88) are shown in FIGs. 4B and 4C, respectively.
- 25 **[0087]** As can be seen from FIG. 5A, 5XFAD mice that were treated with the engineered peptide that is a non-aggregating analogue of A β 42 (SEQ ID NO:3) also exhibited reduced plaque load in the cortex as compared with vehicle treated animals. FIG. 5B shows a corresponding decreased level of plaque accumulation in the hippocampi of the treated animals as well.

Example 2 – Evaluation of Aggregation Propensity of A β 42 Analogues

[0088] A thioflavin-T kinetics study was carried out on peptides having five different sequences, SEQ ID NO:88 having a proline (P) substitution in place of methionine (M) at position 35, wild type A β 42 (SEQ ID NO:1), A β 40 (SEQ ID NO:3), which has the last two C-terminal residues in the beta-sheet aggregation domain of A β 42 deleted, SEQ ID NO:190, 5 which has a non-naturally occurring amino acid N-methyl methionine substituted for the naturally occurring methionine at position 35, and SEQ ID NO:192, which has a non-naturally occurring amino acid pyrrolysine substituted for the naturally occurring methionine at position 35. As shown in FIG. 6A, the peptide having SEQ ID NO:88 has a lower aggregation propensity than the peptide having SEQ ID NO:3, which has a lower 10 aggregation propensity than wild type A β 42 having SEQ ID NO:1. As shown in FIG. 6B, the peptides having SEQ ID NO:190 and SEQ ID NO:192 also had lower aggregation propensity than wild type A β 42 (SEQ ID NO:1).

Example 3 – Evaluation of Cell Proliferation in SH-SY5Y Neuronal Cell Line

15 **[0089]** Experiments were conducted to demonstrate that non-aggregating analogues of A β 42 can enhance proliferation in neuronal cells which express mutant amyloid precursor protein (APP) and rescue neuronal cells after pharmacological depletion of A β 42 using γ -secretase inhibitor.

[0090] FIG. 7A shows the percentage cell proliferation over untreated cells in assays on 20 SH-SY5Y neuronal cell-line expressing amyloid precursor protein gene with the Swedish mutations (APP-SH-SY5Y). Cells were treated with different concentrations of A β 42 or non-aggregating analogues thereof or a scrambled control peptide (YHAGVDKEVVFDEGAGAEHGLAQKIVRGFGVSDVSMIHINLF, SEQ ID NO:711) for 72h in serum-free conditions, then cell proliferation was measured using the WST-1 assay.

25 Results represent the mean of two experiments in quadruplets, with asterisks representing significant differences (significant differences in comparison untreated APP-SH-SY5Y cells were assessed using one-way ANOVA with Dunnetts's multiple comparison test and are indicated by *P < 0.05, **P < 0.01, and ***P < 0.001, ns: non significant.). 0001 Peptide is wild type A β 42 having SEQ ID NO:1. 0003 Peptide is A β 40 peptide having SEQ ID NO:3. 30 0088 Peptide is an A β 42 analogue having SEQ ID NO:88 (M35P modification), and 0100 Peptide is an A β 42 analogue having SEQ ID NO:100 (V36P).

[0091] FIG. 7B shows the percentage cell proliferation over wild type cells in assays on SH-SY5Y neuronal cell-line expressing amyloid precursor protein gene with the Swedish mutations (APP- SH-SY5Y). Cells were treated with different concentrations of A β 42 or non-aggregating analogues thereof or a scrambled control peptide having SEQ ID NO:711 for 72h in serum-free conditions, then cell proliferation was measured using the WST-1 assay. Results represent the mean of two experiments in quadruplets, with asterisks representing significant differences (significant differences in comparison untreated wild type SH-SY5Y cells were assessed using one-way ANOVA with Dunnett's multiple comparison test and are indicated by *P < 0.05, **P < 0.01, and ***P < 0.001, ns: non significant.). 0001 Peptide is wild type A β 42 having SEQ ID NO:1. 0003 Peptide is A β 40 peptide having SEQ ID NO:3. 0088 Peptide is an A β 42 analogue having SEQ ID NO:88 (M35P modification), and 0100 Peptide is an A β 42 analogue having SEQ ID NO:100 (V36P).

[0092] FIG. 7C and 7D show data from additional similar experiments showing cell proliferation over untreated cells plotted along a shorter y-axis to better show comparisons.

[0093] FIG. 8 shows SH-SY5Y neuronal cells that were treated with 10 μ M of γ -secretase inhibitor (aldehyde 2-naphthoyl-VF-CHO) then treated with different concentrations of A β 42 or non-aggregating analogues thereof or a scrambled control peptide peptides (sequences as above) for 48 hours in serum-free conditions, then cell proliferation was measured using the WST-1 assay. Results represent the mean of quadruplet treatments, with asterisks representing significant differences compared to γ -secretase inhibitor treated cells. Significant differences were assessed using one-way ANOVA with Dunnett's multiple comparison test and are indicated by *P < 0.05, **P < 0.01, and ***P < 0.001, ns: non significant.

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Example 4 – Determination of Importance of Soluble A β 42 for Cognitive Function

[0094] FIG. 9A and FIG. 9B show that decreasing levels of soluble A β 42 peptide in cerebrospinal fluid is associated with decreasing cognitive function, even across patients with increasing plaque load (FIG. 9B). In this study, levels of A β 42 in cerebrospinal fluid (CSF) were determined using an ELISA immunoassay (Elecsys) according to data provided by the Alzheimer's Disease Neuroimaging Initiative (ADNI). Soluble A β 42 across

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all subjects in each diagnostic category is shown in FIG. 9A, while FIG. 9B shows soluble A β 42 levels in each diagnostic category across CL tertiles, where CL tertiles were determined by quantifying standard uptake value ratio (SUVR) for a PET scan for amyloidosis, quantifying SUVR across cortical gray matter, normalized by the whole cerebellum and dividing into SUVR tertiles; SUVR levels were converted in centiloids (CL) using the specific equation for each tracer as provided by ADNI. Lower levels of soluble A β 42 were associated with mild cognitive impairment, with even lower levels associated with the presence of Alzheimer's disease. See also *EClinical Medicine* (2021) Vol. 38 100988, the entirety of which is incorporated by reference herein. Thus, decreasing levels of soluble A β 42 are associated with decreasing levels of cognitive function.

[0095] FIG. 10 shows a comparison of cerebrospinal fluid A β 42 levels between non-progressors and progressors to a clinical dementia rating (CDR) in a PiB-PET positive cohort (i.e. patients exhibiting amyloid plaques) in a retrospective longitudinal study among mutation carriers participating in the Dominantly Inherited Alzheimer Network (DIAN) cohort study. In this study, CSF levels of A β 42 were evaluated using the AlzBio3 assay from Fujirebio, Malvern, PA. Non-CDR progressors had a higher value of A β 42 in CSF (297.73 ± 13.66) vs. CDR progressors (218.73 ± 17.22); overall cohort: non-CDR progressors (380.83 ± 14.5) vs. CDR progressors (313.35 ± 26.46). Error bar represents the standard error of mean. CDR progression was defined as any increase in CDR over the follow-up period of the study. Patients were defined as amyloid PiB-PET-positive if their standard uptake (SUVR) of Pittsburgh compound B PET (PiB-PET) was greater than or equal to 1.42. Different assays may produce different absolute numbers for the level of soluble A β 42 in CSF, but the overall trend of lower levels of soluble A β 42 being associated with poorer outcomes is consistent.

[0096] Replacement therapy in various embodiments will aim to restore the levels of soluble functional protein to this level using non aggregating analogues.

Example 5 – Effect of Non-Aggregating Peptide Analogues on the α 7 Nicotinic Acetylcholine Receptors in Cortical Neurons

[0097] Live cell calcium imaging of cultured WT mouse cortical neurons with PC-driven fast application of combination of the α 7 agonist PNU 282987 (1 μ M) and positive allosteric modulator (PAM) of α 7 PAM PNU 120596 (10 μ M) was carried out. This combination

uncovers the full activity range of $\alpha 7$ nAChR. The specificity of $\alpha 7$ mediated effects was validated with $\alpha 7$ nAChR blockers MLA and α -BungTX. As discussed below, it was determined that treatment with a non-aggregating analogue of A β 42 enhances $\alpha 7$ nAChR mediated Ca^{+2} influx, showing that co-application of the non-aggregating analogs of A β 42 at pM concentration promotes/facilitates/enhances the ability of PAM PNU 120596 to activate $\alpha 7$ receptor, which suggests that the non-aggregating analogue of A β 42 functions as a positive modulators of the receptor activity. The middle bar in each figure (PNU4) represents the percentage of enhancement in Ca^{+2} influx mediated by the non-aggregating peptide analogue compared to the baseline signal before (PNU3) and after (PNU5) application.

[0098] FIGs. 11A-11D show the percentage of facilitation of $\alpha 7$ nicotinic acetylcholine receptors in cortical neurons in comparison with pre- and post-control responses for an example non-aggregating peptide analogue having the amino acid sequence of SEQ ID NO:88 at the indicated concentrations. FIG. 12A-12D and 13 show the percentage of facilitation of $\alpha 7$ nicotinic acetylcholine receptors in cortical neurons in comparison with pre- and post-control responses for additional example non-aggregating peptide analogues having the amino acid sequences of SEQ ID NO:3 and SEQ ID NO:190, respectively, at the indicated concentrations. FIG. 14 shows the percentage facilitation of $\alpha 7$ nicotinic acetylcholine receptors in cortical neurons in comparison with pre- and post-control responses for A β 42 having SEQ ID NO:1, and FIGs. 15A-15D shows the percentage of facilitation of $\alpha 7$ nicotinic acetylcholine receptors in cortical neurons in comparison with pre- and post-control responses for a control scrambled peptide having the amino acid sequence of SEQ ID NO:711.

[0099] These results show that co-application of non-aggregating analogues at pM concentration promotes the ability of PAM PNU 120596 to activate $\alpha 7$ receptor, which without being bound by theory suggests that the analogue functions as a positive modulator of the receptor activity.

[0100] FIG. 16 shows that a non-aggregating peptide analogue having the amino acid sequence of SEQ ID NO:88 or SEQ ID NO:190 induces downstream ERK1/2 phosphorylation in SHSY-5Y cells after 5 minutes of treatment with the peptide at pM concentrations. Phosphorylation of ERK is important for cell proliferation, neurogenesis and synaptic plasticity, and is downstream of calcium influx through the $\alpha 7$ receptor. To obtain

the data shown in FIG. 16, SH-SY5Y cells were treated with 30 pM peptide for 5 minutes, then lysed and ERK phosphorylation quantified via a commercial ELISA kit. The results shown in FIG. 16 demonstrate that the tested non-aggregating analogues of A β 42 activate the neurogenic signaling pathways mediated by α 7 binding and Ca²⁺ influx. Although A β 42 was not used as a control in FIG. 16, parallel experiments comparing the activity of A β 42 against the non-aggregating peptide analogues showed higher activity on the part of the non-aggregating peptide analogues.

Example 6 – Structural Studies

10 **[0101]** FIG. 17 shows the results of docking simulations showing that a peptide having the amino acid sequence of SEQ ID NO:88 binds to the α 7 nicotinic acetylcholine receptor via the N-terminal domain of the peptide analogue. The peptide is visible on the right-hand side of the figure where the N-terminal portion of the peptide is received within a binding pocket formed within the extracellular domain of the α 7 receptor. Without being bound by theory, 15 the fact that binding to the α 7 receptor occurs via the N-terminal domain of the peptide analogue, whereas the C-terminal domain is the beta-sheet aggregation domain, is believed to support that making modifications at the C-terminal portion of the peptide is less likely to interfere with the normal biological function of the A β 42 peptide.

[0102] FIG. 18 shows a structural representation of the oppositely stacked beta-sheets of the A β 42 peptide that are believed to form the protofilaments that form amyloid deposits in Alzheimer's disease. The position occupied by methionine 35 in each of the oppositely stacked beta-sheets is circled and indicated by an arrow to show the position of this residue in stabilizing the cross- β amyloid conformation.

[0103] The foregoing examples demonstrate that administration of a non-aggregating analogue of A β 42 can be used to ameliorate symptoms of Alzheimer's disease in an accepted animal model for the disease. Based on these results, it can be soundly predicted that the use of non-aggregating analogues of other proteins implicated in various proteinopathies can be used in the treatment and/or prophylaxis of such proteinopathies.

[0104] While a number of exemplary aspects and embodiments have been discussed above, those of skill in the art will recognize certain modifications, permutations, additions 30 and sub-combinations thereof. It is therefore intended that the following appended claims

and claims hereafter introduced are interpreted to include all such modifications, permutations, additions and sub-combinations as are consistent with the broadest interpretation of the specification as a whole.

5 References

[0105] The following references are of potential interest with respect to the subject matter described herein. Each of the following references is incorporated by reference herein in its entirety.

- Kanaan and Manfredsson, Journal of Parkinson's Disease 2:249-267 (2012).
 - 10 • Malmberg et al., Frontiers in Neuroscience, 14:256 (2020).
 - Wood et al., Biochemistry, 34(3):724-30 (1995).
 - Chiti and Dobson, Annu. Rev. Biochem. 86:26-68 (2017).
 - US 2008/0063636 to Mori et al.
 - US 2010/0081613 to Arancio et al.
 - 15 • US 6689753 to Soto-Jara
 - US 7342091 to Kapurniotu et al.
 - WO 03/045128 to Frangione et al.
 - US 5686411 to Gaeta et al.
- 20 **[0106]** Certain embodiments are further defined with reference to the following aspects, which are illustrative and not limiting in scope.
- A. A non-aggregating protein analogue of a protein involved in a proteinopathy, the protein having a beta-sheet aggregation domain and the non-aggregating protein analogue comprising a beta-sheet destabilizing mutation in the beta-sheet
 - 25 aggregation domain, the non-aggregating protein analogue retaining substantially the wild type function of the protein.
 - B. A non-aggregating protein analogue as defined in the preceding aspect, comprising two or more beta-sheet destabilizing mutations in the beta-sheet aggregation domain.
 - 30 C. A non-aggregating protein analogue as defined in any one of the preceding aspects, wherein the beta-sheet destabilizing mutation comprises substitution of a naturally

occurring amino acid residue of the protein involved in the proteinopathy with a charged or polar amino acid residue, a glycine residue, a proline residue, or a beta-sheet destabilizing analogue of an amino acid.

- 5 D. A non-aggregating protein analogue as defined in the preceding aspect, wherein the charged or polar amino acid residue or analogue thereof comprises K, R, H, E, D, S, T, N, Q, C, or with an amino acid analogue that interferes with beta-sheet formation within the beta-sheet aggregation domain, wherein said amino acid analogues that interfere with beta-sheet formation are optionally 3-hydroxyproline, 4-hydroxyproline, selenocysteine, pyroglutamic acid, carboxyglutamic acid, octenyl alanine,
 - 10 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), pyruvic acid derivatives, branched-chain amino acid derivatives, nitro amino acid derivatives, halogenated
 - 15 amino acid derivatives, ring-substituted amino acid derivatives, aromatic amino acid derivatives, linear core amino acids, peptoid derivatives, 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, or α -phenylglycine derivatives.
 - 20 E. A non-aggregating protein analogue as defined in any one of the preceding aspects, wherein the beta-sheet destabilizing mutation comprises deletion of one or more naturally occurring amino acid residues of the protein involved in the proteinopathy, optionally including the deletion of up to or including all of the naturally occurring amino acid residues of the beta-sheet aggregation domain.
 - 25 F. A non-aggregating protein analogue as defined in any one of the preceding aspects, wherein the beta-sheet destabilizing mutation comprises insertion of between one and five amino acid residues.
 - G. A non-aggregating protein analogue as defined in the preceding claim, wherein at least one of the inserted amino acid residues comprises K, R, H, E, D, S, T, N, Q, C,
 - 30 P or G, or an amino acid analogue that interferes with beta-sheet formation.
 - H. A non-aggregating protein analogue as defined in any one of the preceding aspects, wherein the protein involved in the proteinopathy comprises amyloid precursor protein (APP), amyloid beta protein (including A β -39, A β -40, A β -42 or A β -43

isoforms), alpha-synuclein, prion protein, Huntingtin protein, p53, or any of the proteins or peptides listed in Table 1.

- I. A non-aggregating protein analogue as defined in any one of the preceding aspects, wherein the protein involved in the proteinopathy comprises amyloid beta, wherein optionally:

the beta-sheet aggregation domain comprises positions 29-42 of the amyloid beta;

the beta-sheet destabilizing mutation comprises a deletion or substitution of one of the following amino acid residues for the naturally occurring amino acid residue: G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation;

the beta-sheet destabilizing mutation comprises an insertion of between one and five amino acid residues adjacent to the naturally occurring amino acid, optionally wherein at least one of the inserted amino acid residues comprises G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation;

the non-aggregating protein analogue comprises a protein having the amino acid sequence set forth in any one of SEQ ID NO:2-193; and/or

the non-aggregating protein analogue comprises a protein having the amino acid sequence set forth in any one of SEQ ID NOs:194-207 or SEQ ID NOs:712-742, wherein the X denotes a deletion, insertion of between one and five amino acid residues, optionally G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation, or substitution of one of the following amino acid residues for the naturally occurring amino acid: G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation.

- J. A non-aggregating protein analogue as defined in any one of the preceding aspects, wherein the protein involved in the proteinopathy comprises alpha-synuclein, wherein optionally:

the beta-sheet aggregation domain comprises positions 61-95 of the alpha-synuclein;

the beta-sheet destabilizing mutation comprises a deletion or substitution of one of the following amino acid residues for the naturally occurring amino acid

- residue: G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation;
- the beta-sheet destabilizing mutation comprises an insertion of between one and five amino acid residues adjacent to the naturally occurring amino acid,
- 5 optionally wherein at least one of the inserted amino acid residues comprises G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation;
- the non-aggregating protein analogue comprises a protein having the amino acid sequence set forth in any one of SEQ ID NO:209-698, wherein the X denotes a
- 10 deletion, insertion of between one and five amino acid residues, optionally G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation, or substitution of one of the following amino acid residues for the naturally occurring amino acid: G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation.
- 15 K. A non-aggregating protein analogue as defined in any one of the preceding aspects, wherein the protein involved in the proteinopathy comprises prion protein, wherein optionally:
- the beta-sheet aggregation domain comprises positions 109-121 of the prion protein;
- 20 the beta-sheet destabilizing mutation comprises a deletion or substitution of one of the following amino acid residues for the naturally occurring amino acid residue: G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation;
- the beta-sheet destabilizing mutation comprises an insertion of between one and five amino acid residues adjacent to the naturally occurring amino acid,
- 25 optionally wherein at least one of the inserted amino acid residues comprises G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation; and/or
- the non-aggregating protein analogue comprises a protein having the amino acid sequence set forth in any one of SEQ ID NO:700-710, wherein the X denotes a
- 30 deletion, insertion of between one and five amino acid residues, optionally G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation, or substitution of one of the following amino acid residues for

the naturally occurring amino acid: G, P, K, R, H, E, D, S, T, N, Q, C, or an amino acid analogue that interferes with beta-sheet formation.

- L. A nucleic acid encoding the non-aggregating protein analogue as defined in any one of the preceding aspects.
- 5 M. A nucleic acid as defined in the preceding aspect, comprising mRNA.
- N. A liposome-based particle comprising a nucleic acid as defined in any one of the preceding aspects, the liposome-based particle being designed for delivery of the nucleic acid to a target cell to cause expression of the non-aggregating protein analogue.
- 10 O. A method for treatment and/or prophylaxis of a proteinopathy comprising administering to a subject having or at risk of developing the proteinopathy a peptide or nucleic acid as defined in any one of the preceding aspects.
- P. A method as defined in the preceding claim, wherein the proteinopathy comprises Alzheimer's disease, Parkinson's disease, Lewy body disease or Lewy body
- 15 dementia, Pick's disease, Creutzfeld-Jakob disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), Down syndrome, neuronal degeneration with brain iron accumulation type I (Hallervorde-Spatz disease), Kuru or other transmissible spongiform encephalopathy (TSE), mild cognitive impairment (MCI), cerebral amyloid angiopathy (CAA), vascular dementias, or any neurodegenerative
- 20 disease characterized by abnormal amyloid deposition, or any of the diseases or disorders listed in Table 1.
- Q. A method as defined in any one of the preceding aspects, wherein administration is by intravenous, intracerebroventricular, intracerebral, intrathecal, intraperitoneal, intramuscular or intravenous injection, infusion, or topical, nasal, oral (including
- 25 sublingual or buccal), anal, ocular or otic delivery; by liposome-mediated delivery including by direct delivery of the non-aggregating protein analogue as defined in any one of the preceding claims, direct delivery of a mRNA encoding the non-aggregating protein analogue as defined in any one of the preceding claims, or delivery of DNA encoding the non-aggregating protein analogue as defined in any
- 30 one of the preceding claims 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 non-aggregating protein analogue by the cell.

- R. A method of designing a non-aggregating analogue of a protein implicated in a proteinopathy comprising the steps of:
- selecting the protein implicated in the proteinopathy;
 - identifying a beta-sheet aggregation domain of the protein implicated in the proteinopathy; and
 - identifying one or more beta-sheet destabilizing mutations within the beta-sheet aggregation domain.
- S. A method as defined in the preceding aspect, wherein the step of identifying a beta-sheet aggregation domain of the protein implicated in the proteinopathy comprises conducting structural biology studies.
- T. A method as defined in either one of the preceding aspects, wherein the step of identifying one or more beta-sheet destabilizing mutations within the beta-sheet aggregation domain comprises one or more of:
- deleting a naturally occurring amino acid residue from the beta-sheet aggregation domain and/or;
 - replacing a naturally occurring amino acid residue in the beta-sheet aggregation domain with an amino acid residue that impairs formation of a cross-beta sheet structure.
- U. A method as defined in the preceding aspect, wherein replacing the naturally occurring amino acid residue in the beta-sheet aggregation domain with an amino acid residue that impairs formation of a cross-beta sheet structure comprises replacing the naturally occurring amino acid residue with one of G, P, K, R, H, E, D, S, T, N, Q, or C, with an amino acid analogue that interferes with beta-sheet formation.
- V. A non-aggregating protein analogue or method as defined in any one of the preceding aspects, wherein said amino acid analogues that interfere with beta-sheet formation are optionally 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), pyruvic acid derivatives, branched-chain amino acid derivatives, nitro amino acid derivatives, halogenated amino acid derivatives, ring-

5 substituted amino acid derivatives, aromatic amino acid derivatives, linear core amino acids, peptoid derivatives, 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, or α -phenylglycine derivatives.

CLAIMS:

1. A non-aggregating peptide analogue of the A β 42 peptide, the A β 42 peptide having an N-terminal domain corresponding to positions 1-28 of SEQ ID NO:1 and a beta-sheet aggregation domain corresponding to positions 29-42 of SEQ ID NO:1,
5 the non-aggregating peptide analogue comprising a beta-sheet destabilizing modification in the beta sheet aggregation domain; and
the non-aggregating peptide analogue substantially retaining native function of the A β 42 peptide except for being non-aggregating.
2. The non-aggregating peptide analogue as defined in the preceding claim, wherein
10 the retained native peptide function comprises capability of enhancing or inducing α 7 nicotinic acetylcholine receptor (α 7nAChR) mediated Ca²⁺ influx.
3. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the retained native peptide function is the capability of enhancing or inducing α 7nAChR-mediated Ca²⁺ influx in cortical neurons at ≤ 3000 pM
15 concentration, preferably at ≤ 300 pM, more preferably at ≤ 30 pM, most preferably at ≤ 3 pM.
4. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the peptide analogue is able to perform the retained native function of A β 42 at least at 90% of a level of the native soluble A β 42 peptide.
- 20 5. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet destabilizing modification results in at least 2-fold (preferably at least 3-fold, most preferably at least 4-fold) lower steady-state level of aggregation in a thioflavin-T assay for the analogue compared to wild-type A β 42 (having the amino acid sequence of SEQ ID NO:1).
- 25 6. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the N-terminal domain of the analogue comprises an amino-acid sequence differing from residues 1-28 of SEQ ID NO:1 by no more than 3 (preferably 2, more preferably 1, most preferably 0) deletions, insertions and/or substitutions, preferably conservative substitutions.

7. The non-aggregating peptide analogue as defined in any one of the preceding claims, comprising at most three (preferably two, more preferably one) conservative amino acid substitutions in the N-terminal domain relative to SEQ ID NO:1.
- 5 8. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet aggregation domain of the analogue comprises an amino-acid sequence differing from residues 29-42 of SEQ ID NO:1 by no more than 5 (preferably 3, more preferably 1, most preferably 0) deletions, insertions and/or substitutions, preferably conservative substitutions.
- 10 9. The non-aggregating peptide analogue as defined in any one of the preceding claims comprising, in addition to the beta-sheet destabilizing modification, at most three (preferably two, more preferably one) conservative amino acid substitutions in the beta-sheet aggregation domain relative to SEQ ID NO:1.
- 15 10. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet destabilizing modification comprises substitution of a naturally occurring amino acid residue of the peptide with an amino acid analogue.
11. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet destabilizing modification comprises substitution of at most three naturally occurring amino acid residues at three separate positions relative to SEQ ID NO:1 with an amino acid analogue.
- 20 12. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the amino acid analogue is one of: 3-hydroxyproline, 4-hydroxyproline, selenocysteine, pyroglutamic acid, carboxyglutamic acid, octenyl alanine, pyrrollysine, palmitoyl aspartate, D-amino acids, β -amino acids, γ -amino acids, Homo-amino acids, β -Homo-amino acids, α -methyl amino acids, N-alkylated amino acid derivatives, 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, peptoid derivatives, hydroxylated amino acid derivatives, cyclic amino acids, bicyclic amino acids, 3-amino-3-aryl-propionic acids, 3-amino-4-aryl-butyric
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acids, amino acids with aromatic spacers, alicyclic amino acids, α -phenylglycine derivatives.

13. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the amino acid analogue is one of: N-alkylated amino acid derivatives (N-methyl, N-ethyl, N-propyl), β -amino acids, γ -amino acids, linear core amino acids or cyclic amino acids.
14. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the amino acid analogue comprises an N-alkylated amino acid analogue, preferably with 1-3 carbons in the alkyl moiety.
15. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the amino acid analogue comprises an N-methyl or an N-ethyl analogue of the naturally occurring amino acid residue of the peptide.
16. The non-aggregating peptide analogue as defined in claim 1, wherein the non-aggregating peptide analogue comprises a peptide having the amino acid sequence set forth in any one of SEQ ID NO:2-193.
17. The non-aggregating peptide analogue as defined in claim 1, wherein the non-aggregating peptide analogue comprises a peptide having the amino acid sequence set forth in any one of SEQ ID NOs:194-207 or SEQ ID NOs: 712-742, wherein X is a substitution with a non-naturally occurring amino acid.
18. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet destabilizing modification comprises a substitution of M35, preferably with an amino acid analogue.
19. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet destabilizing modification comprises a substitution of V36, preferably with an amino-acid analogue.
20. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet destabilizing modification comprises a substitution of M35 to proline, N-methyl methionine or pyrrollysine, preferably N-methyl methionine.

21. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet destabilizing modification comprises a substitution of V36 to proline, N-methyl valine or pyrrolysine, preferably pyrrolysine.
22. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet destabilizing modification comprises one or more of:
 5 replacement of G29 with methyl glycine or N-ethyl glycine;
 replacement of A30 with N-methyl alanine or N-ethyl alanine;
 replacement of I31 is N-methyl isoleucine or N-ethyl isoleucine;
 replacement of I32 with N-methyl isoleucine or N-ethyl isoleucine;
 10 replacement of G33 with N-methyl glycine or N-ethyl glycine;
 replacement of L34 with N-methyl leucine or N-ethyl leucine;
 replacement of M35 with N-methyl methionine or N-ethyl methionine;
 replacement of V36 with N-methyl valine or N-ethyl valine;
 replacement of G37 with N-methyl glycine or N-ethyl glycine;
 15 replacement of G38 with N-methyl glycine or N-ethyl glycine
 replacement of V39 with N-methyl valine or N-ethyl valine;
 replacement of V40 with N-methyl valine or N-ethyl valine;
 replacement of I41 with N-methyl isoleucine or N-ethyl isoleucine; and/or
 replacement of A42 with N-methyl alanine or N-ethyl alanine.
23. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet destabilizing modification comprises a replacement of M35 with N-methyl methionine or N-ethyl methionine.
24. The non-aggregating peptide analogue as defined in any one of the preceding claims, wherein the beta-sheet destabilizing modification is a replacement of V36
 25 with N-methyl valine or N-ethyl valine.
25. The non-aggregating peptide analogue as defined in any one of the preceding claims, having the amino acid sequence of one of SEQ ID NO:3, SEQ ID NO:88, SEQ ID NO:100, SEQ ID NO:190 or SEQ ID NO:192 with at most three conservative amino acid substitutions in either or both of the N-terminal domain and the beta-sheet aggregation domain.
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26. The non-aggregating peptide analogue as defined in any one of the preceding claims, having the amino acid sequence of one of SEQ ID NO:88, SEQ ID NO:100, SEQ ID NO:190 or SEQ ID NO:192.
- 5 27. The non-aggregating peptide analogue according to any one of the preceding claims, for use in therapy.
28. The non-aggregating peptide analogue according to any one of the preceding claims, for use in the treatment and/or prophylaxis of Alzheimer's disease or mild cognitive impairment.
- 10 29. The non-aggregating peptide analogue according to any one of the preceding claims, for use in the treatment and/or prophylaxis of autosomal dominant Alzheimer's disease, sporadic Alzheimer's disease, or early-onset sporadic Alzheimer's disease.
- 15 30. The non-aggregating peptide analogue according to any one of the preceding claims, for use in the treatment and/or prophylaxis of autosomal dominant Alzheimer's disease.
31. The non-aggregating peptide analogue according to any one of the preceding claims, for use in the treatment and/or prophylaxis of autosomal dominant Alzheimer's disease caused by mutations in *PSEN1*, *PSEN2*, or *APP*.
- 20 32. The non-aggregating peptide analogue according to any one of the preceding claims, for use in the treatment and/or prophylaxis of Amyotrophic lateral sclerosis (ALS), frontotemporal dementia, Parkinson's disease, Parkinson's disease dementia, progressive supranuclear palsy, corticobasal degeneration, hereditary cerebral hemorrhage with amyloidosis, dementia with Lewy bodies, or multiple system atrophy.
- 25 33. The non-aggregating peptide analogue for use according to any one of claims 27-32, wherein the peptide analogue is administered to a subject having an A β 42 level in the cerebrospinal fluid of less than about 500 pM, preferably less than about 400 pM, preferably less than about 300 pM, more preferably less than 200 pM.

34. The non-aggregating peptide analogue according for use according to any one of claims 27-33, wherein the peptide is administered by subcutaneous, intravenous, intracerebroventricular, intracerebral, intrathecal, intraperitoneal or intramuscular injection or infusion; or topical, nasal, oral (including sublingual or buccal), rectal, ocular or otic delivery.
35. A method for treatment and/or prophylaxis of Alzheimer's disease comprising administering to a subject having or at risk of developing Alzheimer's disease a non-aggregating peptide analogue as defined in any one of claims 1-26.
36. A method for treatment and/or prophylaxis of Amyotrophic lateral sclerosis (ALS), frontotemporal dementia, Parkinson's disease, Parkinson's disease dementia, progressive supranuclear palsy, corticobasal degeneration, hereditary cerebral hemorrhage with amyloidosis, dementia with Lewy bodies, or multiple system atrophy in a subject, the method comprising administering to the subject a non-aggregating peptide analogue as defined in any one of claims 1-26.
37. A method of screening a subject to determine if the subject is a candidate for treatment and/or prophylaxis of Alzheimer's disease or another disorder associated with a decreased level of soluble A β 42 peptide using a non-aggregating peptide analogue as defined in any one of the preceding claims, the method comprising: determining a concentration of A β 42 in a sample of cerebrospinal fluid of the subject; and if the measured concentration of A β 42 in the cerebrospinal fluid sample is less than about 500 pM, preferably less than about 400 pM, preferably less than about 300 pM, more preferably less than 200 pM, identifying the subject as a candidate for treatment and/or prophylaxis of Alzheimer's disease via the administration of the peptide as defined in any one of the preceding claims; wherein the method optionally further comprises administering the non-aggregating peptide analogue to the subject.
38. The method according to claim 37, wherein the concentration of A β 42 in the cerebrospinal fluid sample is determined using liquid chromatography-tandem mass spectrometry or an immunoassay (e.g. Elecsys, AlzBio3.)