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- (71) Applicant: **NEUROPHAGE PHARMACEUTICALS, INC.** [US/US]; 222 Third Street, Suite 3120, Cambridge, MA 02142 (US).
- (72) Inventors: **KRISHNAN, Rajaraman**; 281 Winter Street, Ashland, MA 01721 (US). **ASP, Eva**; 31 Bow Road, Newton, MA 02459 (US). **PROSCHITSKY, Ming**; 98 Wildwood Street, Winchester, MA 10890 (US). **FISHER, Richard**; 98 Wildwood Street, Winchester, MA 10890 (US). **CARR, Francis, J.**; Birchlea, The Holdings, Balmedie, Aberdeen AB23 8WU (GB). **HOLGATE, Robert, G.E.**; 22 Stamford Avenue, Royston, Hertfordshire SG8 7DD (GB). **JONES, Timothy, D.**; 27 Brick Row, Babraham, Cambridge CB22 3AJ (GB).
- (74) Agents: **MCDONELL, Leslie** et al.; Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., 901 New York Avenue, NW, Washington, DC 20001-4413 (US).

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(57) Abstract: The invention relates to polypeptides that comprise a portion of filamentous bacteriophage gene 3 protein (g3p) sufficient to bind to and/or disaggregate amyloid, e.g., the N1-N2 portion of g3p and mutants and fragments thereof, wherein that g3p amino acid sequence has been modified through amino acid deletion, insertion or substitution to remove a putative glycosylation signal. The invention further relates to such polypeptides that are also modified through additional amino acid substitution to be substantially less immunogenic than the corresponding wild-type g3p amino acid sequence when used in vivo. The polypeptides of the invention retain their ability to bind and/or disaggregate amyloid. The invention further relates to the use of these g3p-modified polypeptides in the treatment and/or prevention of diseases associated with misfolding or aggregation of amyloid.



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POLYPEPTIDES COMPRISING A MODIFIED BACTERIOPHAGE G3P AMINO ACID SEQUENCE LACKING A GLYCOSYLATION SIGNAL

[0001] This application claims the benefit of U.S. Provisional Application No. 62/087,052, filed December 3, 2014, which is incorporated here by reference in its entirety to provide continuity of disclosure.

[0002] The invention relates to polypeptides that comprise a portion of filamentous bacteriophage gene 3 protein (g3p) sufficient to bind to and/or disaggregate amyloid, i.e., the N1-N2 portion of g3p and mutants and fragments thereof, wherein that g3p amino acid sequence has been modified through amino acid deletion, insertion or substitution to remove a putative glycosylation signal. The invention further relates to such polypeptides that are also modified through additional amino acid substitution to be substantially less immunogenic than the corresponding wild-type g3p amino acid sequence when used in vivo. The polypeptides of the invention retain their ability to bind and/or disaggregate amyloid. The invention further relates to the use of these g3p-modified polypeptides in the treatment and/or prevention of diseases associated with misfolding or aggregation of amyloid.

[0003] Filamentous bacteriophage g3p protein, and in particular the polypeptide portion thereof comprising the N1-N2 region of g3p, has been demonstrated to bind to and disaggregate various amyloids, such as β -amyloid, tau protein, and prion proteins. See co-pending PCT application PCT/US2012/066793, and US provisional applications US 61/801,349, and US 61/801,849, the disclosure of each of which is incorporated herein by reference. See also, R. Krishnan et al., *J. Mol. Biol.* (2014). Despite that efficacy, it is expected that production of such polypeptides in recombinant mammalian cell systems could be deleteriously affected by glycosylation at a putative asparagine-linked glycosylation signal in the g3p sequence. In addition, systemic administration of polypeptides comprising g3p or the N1-N2 region thereof to humans could cause a deleterious immune response. None of these prior art teachings identify any potential problems relating to putative glycosylation.

[0004] The efficacy of many recombinant or otherwise non-native therapeutic proteins or polypeptides may be limited by unwanted immune reactions of patients to the therapeutic protein or polypeptide. A principal factor in the induction of an immune response by a protein is the presence of T-cell epitopes within the protein, i.e., amino acid sequences that can stimulate the activity of T-cells via presentation on major histocompatibility complex (MHC) class II molecules. T-cell epitopes are commonly defined as any amino acid sequences with the ability to bind to MHC class II molecules. When bound to MHC molecules, T-cell epitopes can be recognized by a T-cell receptor (TCR), and can cause the activation of T-cells by engaging a T-cell receptor to promote a T-cell response. It is, however, generally understood that certain T-cell epitopes which bind to MHC class II molecules do not stimulate T-cell response, because these peptides are recognized as "self" within the organism to which the protein is administered.

[0005] Some T-cell epitopes may be released as peptides during the degradation of the therapeutic protein or polypeptide within cells and then presented by molecules of the MHC to trigger the activation of T-cells. For peptides presented by MHC class II molecules, such activation of T-cells can then give rise, for example, to an antibody response by direct stimulation of B-cells to produce such antibodies.

[0006] MHC class II molecules are a group of highly polymorphic proteins which play a central role in helper T-cell selection and activation. The human leukocyte antigen group DR (HLA-DR) are the predominant isotype of this group of proteins. However, isotypes HLA-DQ and HLA-DP perform similar functions. In humans approximately 70 different allotypes of the DR isotype are known, for DQ there are 30 different allotypes and for DP 47 different allotypes are known. Each individual bears two to four DR alleles, two DQ and two DP alleles.

[0007] The immune response to a protein or polypeptide in an individual is heavily influenced by T-cell epitope recognition which is a function of the peptide binding specificity of that individual's HLA-DR allotype. In order to identify T-cell epitopes within a protein or polypeptide in the context of a global population, it is desirable to consider the binding properties of as diverse a set of HLA-DR allotypes as possible, thus covering as high a percentage of the world population as possible.

[0008] T-cell epitope identification is the first step to epitope elimination. Methods enabling the detection of T-cell epitopes are known in the art and are disclosed in WO 98/52976, WO 00/34317, US2007/0269435; US 7,208,147, Kern et al., *Nature Medicine* 4:975-978 (1998); and Kwok et al., *Trends in Immunology* 22:583-588 (2001). In these approaches, predicted or identified T-cell epitopes are removed by the use of judicious amino acid substitutions within the primary sequence of the therapeutic protein or polypeptide. Although these references enable putative identification of T-cell epitopes, the selection of amino acid substitutions that avoid negative impact on biological activity cannot be reasonably predicted. That can only be determined by testing each of the modified polypeptides for such activity.

[0009] Thus, it would be desirable to examine and reduce the glycosylation, either alone or together with reducing the immunogenicity of the N1-N2 portion of g3p without destroying its amyloid-binding/disaggregation properties. This would allow the polypeptide comprising the N1-N2 portion of g3p to be made in mammalian cells without the manufacturing difficulties associated with glycosylation. In addition, reduced immunogenicity will allow a polypeptide comprising that N1-N2 portion to be chronically administered systemically for therapeutic and/or diagnostic purposes. The present invention meets this need, by identifying the putative glycosylation signal in the N1-N2 portion of g3p and providing modifications thereof that prevent glycosylation while preserving activity of g3p polypeptides. as described in PCT/US13/62862 (WO 2014/055515) or of g3p polypeptides that have been modified to reduce or eliminate immunogenicity as described in PCT/US2014/039760, both of which are incorporated herein by reference. Thus, certain g3p polypeptides of the invention are not only modified to prevent glycosylation, but also comprise specific amino acids substitutions within these potential T-cell epitopes to produce a variant N1-N2 sequence that will reduce or eliminate the

immunogenicity of that T-cell epitope without destroying the ability of the variant N1-N2 to bind to amyloid, prevent amyloid aggregation, and/or effect disaggregation of amyloid plaques.

[0010] In certain embodiments of the invention, the polypeptides comprise g3p or an amyloid binding fragment thereof that has been modified to remove a glycosylation signal. In one embodiment, the invention also provides polypeptides comprising a variant of an N1-N2 amino acid sequence, or a mutant or fragment thereof, having reduced immunogenicity due to one or more amino acid substitutions within one or more of the identified T-cell epitopes and lacking a glycosylation signal. In one aspect, the invention provides fusion proteins comprising the variant N1-N2 sequence fused to a human immunoglobulin Fc region.

[0011] In another embodiment, the invention provides pharmaceutical compositions comprising the polypeptides of the invention and methods of treating or preventing diseases associated with misfolded and/or aggregated amyloid proteins by administering such pharmaceutical compositions to a subject suffering from or susceptible to such disease.

[0012] In a further embodiment, the invention provides nucleic acid molecules which code for the polypeptides of the invention, as well as vectors comprising those nucleic acid molecules and cells harboring such vectors.

[0013] In another embodiment, the invention provides methods for producing the polypeptides of the invention. In particular, such methods employ the nucleic acid molecules and/or cells harboring a vector that comprises such nucleic acid molecules.

BRIEF DESCRIPTION OF THE FIGURES

[0014] FIG. 1 presents the amino acid sequence of an N1-N2-hIgG1-Fc fusion protein (SEQ ID NO:1) with five T-cell epitopes identified by bold and underline and the putative glycosylation signal italicized, bolded and underlined. Amino acids 1-217 constitute the N1-N2 portion of the wild-type g3p sequence. Amino acids 218-256 represent a linker region consisting of the wild-type g3p glycine-rich N2-C-terminal linker present in M13 bacteriophage. This region is identified by shading. Amino acids 257-261 represent amino acids encoded by the multiple cloning site used to construct a nucleic acid molecule encoding the fusion protein. The IgG-Fc portion of the protein begins at amino acid 262.

[0015] FIG. 2 presents the amino acid sequence of another g3p-hIgG1-Fc fusion protein (SEQ ID NO:2) with three T-cell epitopes identified by bold and underline and the putative glycosylation signal italicized, bolded and underlined. The fourth T-cell epitope has been eliminated by substitution of V215A and G220E as compared to SEQ ID NO:1 and the fifth T-cell epitope has been eliminated by deletion of amino acids corresponding to amino acids 258 and 259 of SEQ ID NO:1.

[0016] FIG. 3 presents a DNA sequence (SEQ ID NO:3) encoding the g3p-hIgG1-Fc fusion protein of SEQ ID NO:1 with a N-terminal mammalian signal sequence.

[0017] FIG. 4 presents a DNA sequence (SEQ ID NO:4) encoding the g3p-hIgG1-Fc fusion protein of SEQ ID NO:2 with a N-terminal mammalian signal sequence.

[0018] FIG. 5 provides a comparison of the frequency of donor allotypes expressed in the study described in Example 1.

[0019] FIG. 6 presents the amino acid sequence (SEQ ID NO:5) of a polypeptide of the invention that is a g3p-hIgG1-Fc fusion protein with amino acid changes in the putative glycosylation signal (T41G), epitope 1 (T56H), and epitope 3 (K174R), as compared to SEQ ID NO:2. The substituted amino acids are bolded, italicized, underlined and by gray highlighting.

[0020] FIG. 7 presents a DNA sequence (SEQ ID NO:6) of a plasmid encoding Polypeptide 86 with a N-terminal mammalian signal sequence. The coding sequence, including the signal sequence is indicated by bolding. The codon changes as compared to the DNA encoding SEQ ID NO:1 are indicated by underlining.

[0021] FIG. 8 presents a DNA sequence (SEQ ID NO:7) of a plasmid encoding Polypeptide 86 T41G with a N-terminal mammalian signal sequence. The coding sequence, including the signal sequence is indicated by bolding. The codon changes as compared to the DNA encoding SEQ ID NO:1 are indicated by underlining.

[0022] FIG. 9 depicts a SDS-PAGE analysis comparing the polypeptide of SEQ ID NO:1 with Polypeptide 86 and Polypeptide 86 T41G.

[0023] FIG. 10 depicts the results a filter retardation assay comparing the polypeptide of SEQ ID NO:1 with Polypeptide 86 and Polypeptide 86 T41G.

[0024] FIG. 11 depicts comparative binding of a polypeptide of SEQ ID NO:1 with Polypeptide 86 T41G for three different types of fibers as measured by ELISA.

DETAILED DESCRIPTION OF THE INVENTION

[0025] In the present application the term “variant” (and its cognates) with respect to a reference (unmodified) amino acid or nucleic acid sequence refers to a sequence that contains one or more amino acids substitutions, deletions or insertions, or corresponding substitution, deletion or insertion of codons. The reference sequence is also referred to as a “starting amino acid sequence” or “starting sequence.” A variant does not necessarily require physical manipulation of the reference sequence. As long as a sequence contains a different amino acid as compared to a reference sequence it will be considered a “variant” regardless of how it was synthesized.

[0026] As used herein, the term “mutant” (and its cognates) refers to a starting sequence that has been modified as compared to a specific sequence set forth in the application (e.g., SEQ ID NO:1, SEQ ID NO:2, etc).

[0027] As used herein, the term “modified” (and its cognates) refers to change in a reference amino acid sequence or nucleic acid sequence. When a starting sequence is modified the resulting sequence is a variant. A modification includes one or more amino acids substitutions, deletions or insertions, or corresponding substitution, deletion or insertion of codons.

[0028] The term “corresponding substitution” as used herein means a substitution in a mutant or fragment of amino acids 1-217 of SEQ ID NO:1 that corresponds to the equivalent amino acid substitution in Table 1, Table 2, Table 6 or Table 7 when such mutant or fragment is aligned with amino acids 1-217 of SEQ ID NO:1.

[0029] An example of a fragment of amino acids 1-217 of SEQ ID NO:1 that binds to and/or disaggregates amyloid includes, but is not limited to, any fragment that comprises amino acids 1-67 of SEQ ID NO:1. Example of mutants of amino acids 1-217 of SEQ ID NO:1 that bind to and/or disaggregate amyloid include, but are not limited to: (1) amino acids 1-217 of SEQ ID NO:2; (2) amino acids 1-217 of SEQ ID NO:1, amino acids 1-217 of SEQ ID NO:2, or amino acids 1-217 of SEQ ID NO:5 bearing substitution of VVV at amino acids 43-45 with AAA; (3) amino acids 1-217 of SEQ ID NO:1, amino acids 1-217 of SEQ ID NO:2, or amino acids 1-217 of SEQ ID NO:5 having the substitution C53W; (4) amino acids 1-217 of SEQ ID NO:1, amino acids 1-217 of SEQ ID NO:2, or amino acids 1-217 of SEQ ID NO:5 having a deletion of amino acids 96-103; (5) amino acids 1-217 of SEQ ID NO:1, amino acids 1-217 of SEQ ID NO:2, or amino acids 1-217 of SEQ ID NO:5 bearing the substitution of QPP at amino acids 212-214 with AGA; (6) amino acids 1-217 of SEQ ID NO:1, amino acids 1-217 of SEQ ID NO:2, or amino acids 1-217 of SEQ ID NO:5 having the substitutions W181A, F190A and F194A; (7) other active mutants and fragments disclosed in PCT/US2012/066793; (8) amino acids 1-217 of SEQ ID NO:5; (9) amino acids 2-217 of SEQ ID NO:1, amino acids 2-217 of SEQ ID NO:2, or amino acids 2-217 of SEQ ID NO:5; (10) amino acids 3-217 of SEQ ID NO:1, amino acids 3-217 of SEQ ID NO:2, or amino acids 3-217 of SEQ ID NO:5; (11) any one of amino acids 1-217 of SEQ ID NO:1, amino acids 1-217 of SEQ ID NO:2, or amino acids 1-217 of SEQ ID NO:5 containing an additional N-terminal methionine residue.

[0030] The N1-N2 portion of filamentous bacteriophage g3p protein has previously been shown to possess amyloid binding and disaggregation properties (see PCT/US2012/066793). The N1-N2 portion of native M13 phage is represented by amino acids 1-217 of SEQ ID NO:1. The same N1-N2 amino acid sequence is also present in fd and f1 filamentous bacteriophage. It should be understood that amino acids 218-256 of SEQ ID NO:1 are also part of the native g3p sequence and are typically referred to as the glycine-rich linker connecting the N2 region of g3p to the C-terminal domain of g3p (CT), also known as the N3 domain. Amino acids 257-261 of SEQ ID NO:1 represent amino acids encoded by the multiple cloning site used to construct a nucleic acid molecule encoding the fusion protein of SEQ ID NO:1.

Polypeptides

[0031] Thus, in one embodiment, the invention provides a polypeptide comprising a variant of a starting amino acid sequence, wherein the starting amino acid sequence is selected from: amino acids 1-217 of SEQ ID NO:1 or amino acids 1-217 of SEQ ID NO:2 and mutants of any of the foregoing having one or more of the following modifications: substitution of VVV at amino acids 43-45 with AAA;

substitution C53W; deletion of amino acids 96-103; substitution of QPP at amino acids 212-214 with AGA; substitutions W181A, F190A and F194A; deletion of amino acid 1; deletion of amino acids 1 and 2; and addition of a N-terminal methionine residue, wherein:

- (a) the starting amino acid sequence is modified to remove the putative glycosylation signal at amino acids 39-41; and
- (b) the polypeptide binds to and/or disaggregates amyloid.

[0032] In one aspect of this embodiment, the starting amino acid sequence is selected from: amino acids 1-217 of SEQ ID NO:1, and amino acids 1-217 of SEQ ID NO:2 .

[0033] Elimination of the putative glycosylation signal is achieved by amino acid substitution of one or more of N39, A40 and/or T41; deletion of one or more of N39, A40 and/or T41; insertion of one or more amino acids between N39 and A40; and insertion of one or more amino acids between A40 and T41 insofar as such substitution, deletion or insertion does not regenerate a glycosylation signal. The putative glycosylation sequence is Asn-X-Thr/Ser, wherein X is any amino acid other than Pro or Cys. Thus, only certain substitutions for A40 will eliminate the glycosylation sequence. In one aspect of these embodiments elimination of the putative glycosylation signal is achieved by amino acid substitution of one or more of N39, A40 and/or T41. In a more specific aspect of these embodiments elimination of the putative glycosylation signal is achieved by amino acid substitution of T41. In an even more specific aspect of these embodiments elimination of the putative glycosylation signal is achieved by any of the following substitutions: T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, or T41A. In the most specific aspect of these embodiments, elimination of the putative glycosylation signal is achieved by a T41G substitution.

[0034] In another embodiment of the invention the polypeptide that has been modified to remove the putative glycosylation signal at amino acids 39-41 additionally has reduced immunogenicity as compared to a corresponding polypeptide comprising the starting amino acid sequence; and the variant has from 1 to 9 amino acid substitutions (in addition to any substitutions that have eliminated the glycosylation signal) as compared to the starting amino acid sequence, wherein each amino acid substitution is selected from the group of amino acid substitutions set forth in Table 1 and Table 2. The term "corresponding polypeptide comprising the starting amino acid sequence" as used herein means a

[0035] polypeptide which, except for the modification of the glycosylation signal and the additional substitution(s), has the same amino acid sequence as the polypeptide comprising the starting amino acid sequence.

Table 1. Deimmunizing Amino Acid Substitutions to Amino Acids 1-217 of SEQ ID NO:1 or SEQ ID NO:3.

Epitope	Amino Acid #	Amino Acid present at the indicated Amino Acid # of SEQ ID NO: 1*	Substitution
1	48	G	H, K, R, S, T
1	51	T	G, H, K, R, P, Q, N
1	54	Y	G, H, K, R, P
1	56	T	G, H, K, R, P
2	135	M	A, D, G, K, N, T, H, R
2	140	R	D, E, H, Q, A, G
2	141	F	D, E
2	143	N	A, G
3	173	S	G, P, K
3	174	K	R
3	176	M	G, H, K, N, R
3	178	D	G, N, Q, S, T
3	181	W	G, H, K, R

Table 2. Alternate or Additional De-Immunizing Amino Acid Substitutions to Amino Acids 1-217 of SEQ ID NO:1, or SEQ ID NO:3.

Epitope	Amino Acid #	Amino Acid present at the indicated Amino Acid # of SEQ ID NO: 1*	Substitution
1	48	G	D, P
1	50	E	G, H, K, P, R
1	51	T	W
1	53	C	F, H, K, N, Q, R, W, Y
2	135	M	C, E, P, Q, S
2	137	Q	D, E
2	138	N	D, E, G, H, P, Q, S, T
2	140	R	M, N, P, S, Y
2	141	F	G, N, P, Q, Y
3	173	S	D, H, R, T
3	175	A	G, H, K, P, R
3	176	M	P, Q, W
3	178	D	F, H, K, R, W, Y
3	179	A	H, K, P, R
3	181	W	P

*In Tables 1 and 2, each of the indicated amino acids is the same in SEQ ID NOS: 1 and 3.

[0036] The amino acid substitutions set forth in Tables 1 and 2 were derived by identifying the T-cell epitopes present completely within the N1-N2 amino acid sequence. This was done by incubating different overlapping peptide portions of the N1-N2 sequence against the peripheral blood mononuclear cells (PBMC) from a cohort of community blood donors best representing the world population of HLA-DR allotypes to identify the potential T-cell epitopes. This information was then subjected to software analysis against a database of known T-cell epitopes to identify optimal amino acid substitutions within those potential epitopes. These procedures are described in detail in the Examples.

[0037] In one aspect of these embodiments, the 1-9 additional amino acid substitutions (in addition to any substitutions that have eliminated the glycosylation signal) are selected from those set forth in Table 1. In a more specific aspect of the embodiment set forth above, the polypeptide comprises a variant of amino acids 1-217 SEQ ID NO:1 or a variant of amino acids 1-217 of SEQ ID NO:2 having only a specific single amino acid substitution, wherein the substitution is selected from one of the substitutions set forth in Table 3:

Table 3. Specific De-Immunizing Single Amino Acid Substitutions in Amino Acids 1-217 of SEQ ID NO:1, or Amino Acids 1-217 of SEQ ID NO:2

G48H	G48K	G48R	G48S
G48T	T51G	T51H	T51K
T51P	T51R	T51Q	T51N
Y54G	Y54H	Y54K	Y54P
Y54R	T56G	T56H	T56K
T56P	T56R	M135A	M135D
M135G	M135H	M135K	M135N
M135R	M135T	R140A	R140D
R140E	R140G	R140H	R140Q
F141D	F141E	N143A	N143G
S173G	S173P	M176G	M176H
M176K	M176N	D178G	D178N
D178Q	D178S	W181G	W181H
W181K	W181R	S173K	K174R
M176R	D178T		

[0038] In an even more specific aspect of these embodiments, the specific single amino acid substitution is not in epitope 2 (amino acids 135-143 of SEQ ID NO:1).

[0039] In some embodiments, the polypeptide comprises a variant of amino acids 1-217 SEQ ID NO:1 or a variant of amino acids 1-217 of SEQ ID NO:2 having 2-9 amino acid substitutions (in addition to any substitutions that have eliminated the glycosylation signal), wherein the substitutions are in at least two of epitopes 1, 2 and 3, and wherein the substitutions are selected from those set forth in Tables 1 and 2. In a more specific aspect, at least two substitutions in the variant of amino acids 1-217 SEQ ID NO:1 or the variant of amino acids 1-217 of SEQ ID NO:2 are selected from those set forth in Table 1.

In an even more specific aspect the polypeptide comprises a variant of SEQ ID NO:1 or SEQ ID NO:2 that has only two amino acid substitutions, wherein the substitutions are selected from any of the specific two amino acid substitutions set forth in Table 4:

Table 4. Specific De-Immunizing Two Amino Acid Substitutions in Amino Acids 1-217 of SEQ ID NO:1, or Amino Acids 1-217 of SEQ ID NO:2:

Y54K and M135K	Y54K and M135T	Y54K and R140Q	Y54R and M135K
Y54R and M135T	Y54R and R140Q	T56H and M135K	T56H and M135T
T56H and R140Q	T56K and M135K	T56K and M135T	T56K and R140Q
Y54K and D178N	Y54K and W181H	Y54K and W181R	Y54K and K174R
Y54R and D178N	Y54R and W181H	Y54R and W181R	Y54R and K174R
T56H and D178N	T56H and W181H	T56H and W181R	T56H and K174R
T56K and D178N	T56K and W181H	T56K and W181R	T56K and K174R
M135K and D178N	M135K and W181H	M135K and W181R	M135K and K174R
M135T and D178N	M135T and W181H	M135T and W181R	M135T and K174R
R140Q and D178N	R140Q and W181H	R140Q and W181R	R140Q and K174R

[0040] In a more specific aspect of these embodiments, neither of the two amino acid substitutions are in epitope 2 (amino acids 135-143 of SEQ ID NO:1). In an even more specific aspect of these embodiments, the two amino acid substitutions are T56H and K174R.

[0041] In another embodiment, the polypeptide comprises a variant of amino acids 1-217 SEQ ID NO:1 or a variant of amino acids 1-217 of SEQ ID NO:2, having 3-9 amino acid substitutions (in addition to any substitutions that have eliminated the glycosylation signal), wherein at least one amino acid substitution is in each of epitopes 1, 2 and 3, and wherein the substitutions are selected from substitutions set forth in Table 1 and Table 2. In a more specific aspect, at least three amino acids substitution in the variant of amino acids 1-217 SEQ ID NO:1 or the variant of amino acids 1-217 of SEQ ID NO:2 are selected from substitutions set forth in Table 2. In an even more specific aspect, the

polypeptide comprising the variant of amino acids 1-217 SEQ ID NO:1 or the variant of amino acids 1-217 of SEQ ID NO:2 has only three amino acid substitutions, wherein the substitutions are selected from any of the specific three amino acid substitutions set forth in Table 5.

Table 5. Specific De-Immunizing Three Amino Acid Substitutions in Amino Acids 1-215 of SEQ ID NO:1, Amino Acids 1-217 of SEQ ID NO:2:

Y54K, M135K and D178N	Y54K, M135T and D178N	Y54K, R140Q and D178N	Y54R, M135K and D178N
Y54R, M135T and D178N	Y54R, R140Q and D178N	T56H, M135K and D178N	T56H, M135T and D178N
T56H, R140Q and D178N	T56K, M135K and D178N	T56K, M135T and D178N	T56K, R140Q and D178N
Y54K, M135K and W181H	Y54K, M135T and W181H	Y54K, R140Q and W181H	Y54R, M135K and W181H
Y54R, M135T and W181H	Y54R, R140Q and W181H	T56H, M135K and W181H	T56H, M135T and W181H
T56H, R140Q and W181H	T56K, M135K and W181H	T56K, M135T and W181H	T56K, R140Q and W181H
Y54K, M135K and W181R	Y54K, M135T and W181R	Y54K, R140Q and W181R	Y54R, M135K and W181R
Y54R, M135T and W181R	Y54R, R140Q and W181R	T56H, M135K and W181R	T56H, M135T and W181R
T56H, R140Q and W181R	T56K, M135K and W181R	T56K, M135T and W181R	T56K, R140Q and W181R
Y54K, M135K and K174R	Y54K, M135T and K174R	Y54K, R140Q and K174R	Y54R, M135K and K174R
Y54R, M135T and K174R	Y54R, R140Q and K174R	T56H, M135K and K174R	T56H, M135T and K174R
T56H, R140Q and K174R	T56K, M135K and K174R	T56K, M135T and K174R	T56K, R140Q and K174R

[0042] In another embodiment, the invention provides a polypeptide comprising a g3p variant wherein one of the 1 to 9 substitution is a substitution in epitope 4 selected from V215A, V215S, V215G or V215T, V215C, V215D, V215E, V215F, V215H, V215K, V215N, V215P, V215Q, or V215R. In still another embodiment, the invention provides a polypeptide comprising a variant of amino acids 1-217 of SEQ ID NO:1, wherein one of the 1 to 9 substitutions is a substitution in epitope 4 selected from V215A, V215S, V215G, V215T, V215C, V215D, V215E, V215F, V215H, V215K, V215N, V215P, V215Q, or V215R. Through testing of overlapping potential T-cell epitope peptide portions of the N1-N2 sequence, applicants have determined that V215 in SEQ ID NO:1 is part of a potential T-cell epitope (epitope 4 in FIG. 1) spanning amino acids 215-223 of SEQ ID (the end of N2 through a portion of the glycine-rich linker). In a more specific aspect of this embodiments, epitope 4 has a V215A and G220E substitution (as in SEQ ID NO:2). In addition, a single V215G substitution in epitope 4 as compared to SEQ ID NO:1 did not affect the ability of the polypeptide to bind to or disaggregate amyloid. Each of the other substitutions for V215 set forth above are similarly predicted by software and database analysis to eliminate the T-cell epitope, while having little or no effect on amyloid binding.

[0043] In a more specific aspect, the polypeptide comprising a variant of amino acids 1-217 SEQ ID NO:1 has a modification that removes the putative glycosylation site at amino acids 39-41; any one of the V215 substitutions set forth above; as well as 1-8 of the amino acid substitutions set forth in Table 1 or Table 2. In an even more specific embodiment, the 1-8 amino acid substitutions are selected from those set forth in Table 1. In an even more specific aspect, the polypeptide has a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A; a V215 substitution selected from V215A, V215S, V215G or V215T, V215C, V215D, V215E, V215F, V215H, V215K, V215N, V215P, V215Q, and V215R; and one additional single amino acid substitution selected from those set forth in Table 3, wherein the single amino acid substitution is not in epitope 2. In a more specific aspect, the polypeptide comprising a variant of amino acids 1-217 SEQ ID NO:1 has a modification that removes the putative glycosylation site at amino acids 39-41; any one of the V215 substitutions set forth above; and 2-8 additional amino acid substitutions, wherein the additional substitutions are in at least two of epitopes 1, 2 and 3, and wherein the substitutions are selected from those set forth in Table 1 or Table 2. In an even more specific embodiment, the at least one substitution in at least two of epitopes 1, 2 and 3, is selected from the substitutions set forth in Table 1. In a still more specific embodiment, the polypeptide has a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A; a V215 substitution selected from V215A, V215S, V215G or V215T, V215C, V215D, V215E, V215F, V215H, V215K, V215N, V215P, V215Q, and V215R; and one of the specific two amino acid substitutions set forth in Table 4, wherein the neither of the amino acid substitutions are in epitope 2.

[0044] In a more specific aspect, the polypeptide comprising a variant of amino acids 1-217 of SEQ ID NO:1 has a modification that removes the putative glycosylation site at amino acids 39-41; any one the V215 substitutions set forth above; and 3-8 additional amino acid substitutions selected from those set forth in Table 1 or Table 2, wherein each of epitopes 1, 2 and 3, comprise one of the additional substitutions. In an even more specific embodiment, the substitution in each of epitopes 1, 2 and 3, is selected from those set forth in Table 1. In a still more specific embodiment, the polypeptide has a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A; a V215 substitution selected from V215A, V215S, V215G or V215T, V215C, V215D, V215E, V215F, V215H, V215K, V215N, V215P, V215Q, and V215R; an optional G220E substitution; and one of the specific three amino acid substitutions set forth in Table 5.

[0045] In an even more specific embodiment, the polypeptide comprises a variant of amino acids 1-217 of SEQ ID NO:2 having a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A; and a pair of substitutions selected from those set forth in Table 4, wherein one of the substitutions is in epitope 1 and the other is in epitope 3. In one aspect of this embodiment, the T41 substitution is T41G. In an alternate aspect of this embodiment, the pair of substitutions, wherein one of the substitutions is in epitope 1 and the other is in

epitope 3 is T56H and K174R. In an even more specific aspect of this embodiment, the polypeptide comprises amino acids 1-215 of SEQ ID NO:5. In another embodiment, the polypeptide of the invention is a fusion protein consisting essentially of a human or humanized immunoglobulin Fc polypeptide sequence fused via a peptide linker or directly to the C-terminus of the variant g3p amino acid sequence. The term "peptide linker" as used herein refers to a series of consecutive amino acids that will not interfere with the function of the polypeptide. As set forth above, in SEQ ID NOs: 1 and 3, amino acids 218-256 represent the glycine-rich linker that is normally present in the M13 g3p protein. That linker may be used or a different linker may be substituted therefor in the polypeptides of the invention. Alternatively, the Fc polypeptide sequence may be linked directly to the last amino acid encoding N2 (e.g., amino acid 217 of SEQ ID NOs 1 and 3). The choice of linker sequence and/or its absence may be made by those of skill in the art taking into account vectors available for the recombinant expression of the polypeptide of the invention, and any secondary or tertiary structure such a linker may impart to the polypeptide. In one aspect of this embodiment, the Fc polypeptide is the Fc portion of a human IgG. In a more specific aspect the polypeptide is a variant of SEQ ID NO:1 or SEQ ID NO:3 having a modification that removes the putative glycosylation site at amino acids 39-41. In an even more specific aspect, the polypeptide is a variant of SEQ ID NO:1 or SEQ ID NO:2 having a modification that removes the putative glycosylation site at amino acids 39-41; and 1 to 9 additional amino acid residue substitutions therein selected from the group of amino acid substitutions set forth in Table 1, Table 2, or Table 6, or Table 7, below:

Table 6. Deimmunizing Amino Acid Substitutions to Amino Acids 215-223 of SEQ ID NO:1.

Epitope	Amino Acid #	Amino Acid present in Amino Acids 1-215 of SEQ ID NO: 1	Substitution
4	215	V*	A*, S, G, T
4	218	G	C, E, N, P, Q, S, T
4	220	G*	E*, D, F, W
4	221	S	D, E, G
4	223	G	D, P

Table 7. Alternate and Additional Deimmunizing Amino Acid Substitutions to Amino Acids 215-223 of SEQ ID NO:1.

Epitope	Amino Acid #	Amino Acid present in Amino Acids 1-215 of SEQ ID NOs 1-3	Substitution
4	215	V*	C, D, E, F, H, K, N, P, Q, R
4	218	G	A, H, W
4	220	G*	M, Y
4	223	G	E, K, N, R, T

*V215A and G220E are already substituted in SEQ ID NO:2 so that a variant of SEQ ID NO:2 would not contain a further substitution at these amino acid residues.

[0046] In one embodiment, the polypeptide is a variant of SEQ ID NO:1 having a modification that removes the putative glycosylation site at amino acids 39-41; and 2-9 additional amino acid substitutions, wherein one of the additional substitutions is a substitution set forth in Table 6 and Table 7; and at least one other of the substitutions is a substitution set forth in Table 1 and Table 2. In a more specific aspect of this embodiment, one of the additional substitutions is a substitution set forth in Table 6; and at least one other of the substitutions is a substitution set forth in Table 1. In an even more specific aspect of this embodiment, the polypeptide does not have a substitution in epitope 2. In another even more specific aspect of this embodiment, the polypeptide has a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A. In a still more specific aspect of this embodiment, the polypeptide has a T41G substitution.

[0047] In another embodiment, the polypeptide is a variant of SEQ ID NO:1 having a modification that removes the putative glycosylation site at amino acids 39-41; and has 3-9 additional amino acid substitutions, wherein at least one of the additional substitutions is selected from substitutions set forth in Table 6 and Table 7, and wherein at least two of epitopes 1, 2, and 3 contain at least one substitution selected from the substitutions set forth in Table 1 and Table 2. In a more specific aspect, the polypeptide has at least one of the substitutions set forth in Table 6 and at least one substitution in at least two of epitopes 1, 2 and 3 selected from the substitutions set forth in Table 1. In an even more specific aspect of this embodiment, the polypeptide does not have a substitution in epitope 2. In another even more specific aspect of this embodiment, the polypeptide has a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A. In a still more specific aspect of this embodiment, the polypeptide has a T41G substitution. In an alternate aspect of this embodiment, the polypeptide has only two additional substitutions, wherein one is in epitope 1 and the other is in epitope 3. In an even more specific aspect of this embodiment, the polypeptide has only two additional substitutions, wherein one is T56H and the other is K174R.

[0048] In another embodiment, the polypeptide is a variant of SEQ ID NO:1 having a modification that removes the putative glycosylation site at amino acids 39-41; and has 4-9 amino acid substitutions; at least one of substitutions set forth in Table 6 and Table 7; and at least one substitution in each of

epitopes 1, 2 and 3 selected from the substitutions set forth in Table 1 and Table 2. In a specific aspect of this embodiment, the polypeptide has at least one of the substitutions set forth in Table 6 and at least one substitution in each of epitopes 1, 2 and 3 selected from those set forth in Table 1. In another more specific aspect, the polypeptide has at least one of substitutions set forth in Table 6; and at least one of the specific substitutions one, two or three amino acid substitutions set forth in Table 3, Table 4 or Table 5, respectively. In still another more specific aspect of this embodiment, the polypeptide is a variant of SEQ ID NO:1 and has only one of the amino acid substitutions set forth in Table 6 and only one, two or three additional amino acid substitutions selected from one of the specific one, two or three amino acid substitutions set forth in Table 3, Table 4 or Table 5, respectively. In another even more specific aspect of this embodiment, the polypeptide has a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A. In a still more specific aspect of this embodiment, the polypeptide has a T41G substitution.

[0049] In an alternate embodiment, the polypeptide is a variant of SEQ ID NO:2 having a modification that removes the putative glycosylation site at amino acids 39-41; and 1 to 9 additional amino acid residue substitutions selected from the group of amino acid substitutions set forth in Table 1, and Table 2. In a more specific aspect, at least one additional substitution is set forth in Table 1. In an even more specific aspect of this embodiment, the polypeptide does not have a substitution in epitope 2. In another even more specific aspect of this embodiment, the polypeptide has a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A. In a still more specific aspect of this embodiment, the polypeptide has a T41G substitution.

[0050] In another embodiment, the polypeptide is a variant of SEQ ID NO:2 having a modification that removes the putative glycosylation site at amino acids 39-41; and 2-9 additional amino acid substitutions and at least one substitution in at least two of epitopes 1, 2 and 3 selected from any of the substitutions set forth in Table 1 and 2. In a more specific aspect, at least one substitution in at least two of epitopes 1, 2 and 3 is selected from those set forth in Table 1. In an even more specific aspect of this embodiment, the polypeptide does not have a substitution in epitope 2. In another even more specific aspect of this embodiment, the polypeptide has a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A. In a still more specific aspect of this embodiment, the polypeptide has a T41G substitution. In an alternate aspect of this embodiment, the polypeptide comprises at least one amino acid substitution in epitope 1 and at least one amino acid substitution in epitope 3. In an even more specific aspect of this embodiment, the polypeptide comprises a T56H and a K174R substitution.

[0051] In another more specific aspect, the polypeptide is a variant of SEQ ID NO:2 or SEQ ID NO:5 and has 3-9 amino acid substitutions, wherein at least one substitution is in each of epitopes 1, 2 and 3 and is selected from any of the substitutions set forth in Table 1 and 2. In a more specific aspect, the at least one substitution in each of epitopes 1, 2 and 3 is selected from those set forth in Table 1. In an even more specific embodiment, the polypeptide is a variant of SEQ ID NO:2 or SEQ ID NO:5 and has

only one, two or three amino acid substitutions selected from one of the specific one, two or three amino acid substitutions set forth in Table 3, Table 4 or Table 5, respectively. In another even more specific aspect of this embodiment, the polypeptide has a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A. In a still more specific aspect of this embodiment, the polypeptide has a T41G substitution.

[0052] In another embodiment, the polypeptide of the invention is a variant of SEQ ID NO:2 having a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A; and only 2 additional amino acid substitutions selected from any of the specific two amino acid substitutions set forth in Table 4. In a specific aspect of this embodiment, the polypeptide has a T41G substitution. In a more specific aspect of this embodiment, the polypeptide has a T41G substitution. In another more specific aspect of this embodiment, one additional amino acid substitution is in epitope 1 and the other additional amino acid substitution is in epitope 3. In a still more specific aspect of this embodiment, one additional amino acid substitution is T56H and the other additional amino acid substitution is K174R. In an even more specific aspect of this embodiment, the polypeptide has the amino acid sequence set forth in SEQ ID NO:5

[0053] In another embodiment, the polypeptide of the invention is a variant of SEQ ID NO:2 having a T41 substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A; and only 3 additional amino acid substitutions selected from any of the specific three amino acid substitutions set forth in Table 5. In a specific aspect of this embodiment, the polypeptide has a T41G substitution.

Nucleic Acid Molecules, Sequences, Vectors and Host Cells

[0054] In other embodiments, the invention provides an isolated nucleic acid molecule that comprises a nucleic acid sequence coding for any of the polypeptides or fusion proteins comprising a g3p variant described above. In one aspect of this embodiment, the isolated nucleic acid molecule comprises a variant of nucleotides 64-714 of SEQ ID NO:3 or nucleotides 64-708 of SEQ ID NO:5, that is modified by a codon substitution, an in-frame codon insertion or an in-frame codon deletion that destroys the putative glycosylation site encoded by nucleotides 181-189 of SEQ ID NOS:3 or 4 (corresponding to the amino acids NAT at amino acids 39-41 of SEQ ID NOS:1 or 3). In a more specific aspect of these embodiments, the variant of nucleotides 64-714 of SEQ ID NOS:3 or 4 is modified by a codon substitution that destroys the putative glycosylation site encoded by nucleotides 181-189 of SEQ ID NOS:3 or 4. In an even more specific aspect of these embodiments, the variant of nucleotides 64-714 of SEQ ID NOS:3 or 4 is modified by a codon substitution at nucleotides 187-189 (which encodes T41 of SEQ ID NOS:1 and 2) that encodes an amino acid substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A. In an even more specific aspect of these embodiments, the substituted codon substitution is selected from gga, tgg, cat, ggt, att, ctt, agg, aaa, tat, ttc, gac, gag, cag, aat, and gct. In an even more specific aspect of these

embodiments, the variant of nucleotides 64-714 of SEQ ID NOS:3 or 4 is modified by a codon substitution at nucleotides 187-189 that encodes the amino acid substitution T41G. In an even more specific aspect of these embodiments, the substituted codon substitution is gga.

[0055] In another embodiment, in addition to the modification that destroys the putative glycosylation site encoded by nucleotides 181-189 of SEQ ID NOS:3 or 4, the variant of nucleotides 64-714 of SEQ ID NOS:3 or 4 further consists of 1-9 codon substitutions, wherein each codon substitution corresponds to an amino acid substitution selected from the substitutions set forth in Table 1, and Table 2, and any one of the following V215 amino acid substitutions: V215A, V215S, V215G or V215T, V215C, V215D, V215E, V215F, V215H, V215K, V215N, V215P, V215Q, and V215R. In an even more specific aspect of these embodiments the variant nucleic acid sequence is modified by one codon substitution selected to code for any one of the V215 amino acid substitutions set forth above; and from 1-8 additional codon substitutions, wherein each of the additional codon substitutions is selected to code for an amino acid substitution set forth in Table 1. In a still more specific aspect of these embodiments the variant nucleic acid sequence is modified by one codon substitution selected to code for any one of the V215 amino acid substitutions set forth above; and from 2-8 additional codon substitutions, wherein each additional codon substitutions encodes an amino acid substitution set forth in Table 1, and a codon substitution is present in each of at least two of epitopes 1, 2 and 3. In a still more specific embodiment, the variant nucleic acid sequence is modified by one codon substitution selected to code for any one of the V215 amino acid substitutions set forth above; and from 3-8 additional codon substitutions, wherein each additional codon substitution encodes an amino acid substitution set forth in Table 1, and a codon substitution is present in each of epitopes 1, 2 and 3. In a still more specific embodiment, the variant nucleic acid sequence is modified by one codon substitution selected to code for a V215A amino acid substitution; and one additional codon substitution selected to code for one of the single amino acid substitutions set forth in Table 3. In a more specific aspect of this embodiment, the one additional codon substitution selected to code for one of the single amino acid substitutions set forth in Table 3 does not code for an amino acid substitution in epitope 2. In another specific embodiment, the variant nucleic acid sequence is modified by one codon substitution selected to code for a V215A amino acid substitution set forth above; and two additional codon substitutions selected to code for one of the specific two amino acid substitutions set forth in Table 4. In a more specific aspect of this embodiment, the two additional codon substitutions selected to code for one of the specific two amino acid substitutions set forth in Table 4 does not code for an amino acid substitution in epitope 2. In a still more specific embodiment, the variant nucleic acid sequence is modified by one codon substitution selected to code for a V215 amino acid substitution set forth above; and three additional codon substitutions selected to code for one of the specific three amino acid substitutions set forth in Table 5.

[0056] In still other embodiments, the isolated nucleic acid molecule comprises a variant of nucleotides 64-1530 of SEQ ID NO:3 or nucleotides 64-1524 of SEQ ID NO:6, wherein the sequence is modified by a codon substitution, an in-frame codon insertion or an in-frame codon deletion that destroys the

putative glycosylation site encoded by nucleotides 181-189 of SEQ ID NOS:3 or 4 (corresponding to the amino acids NAT at amino acids 39-41 of SEQ ID NOS:1 or 3). In a more specific aspect of these embodiments, the variant of nucleotides 64-1530 of SEQ ID NO:3 or nucleotides 64-1524 of SEQ ID NO:4 is modified by a codon substitution that destroys the putative glycosylation site encoded by nucleotides 181-189 of SEQ ID NOS:3 or 4. In an even more specific aspect of these embodiments, the variant of nucleotides 64-1530 of SEQ ID NO:3 or nucleotides 64-1524 of SEQ ID NO:4 is modified by a codon substitution at nucleotides 187-189 (aca, which encodes T41 of SEQ ID NOS:1 and 2) that encodes an amino acid substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A. In an even more specific aspect of these embodiments, the substituted codon substitution is selected from gga, tgg, cat, gtt, att, ett, agg, aaa, tat, ttc, gac, gag, cag, aat, and gct. In an even more specific aspect of these embodiments, the variant of nucleotides 64-714 of SEQ ID NOS:3 or 4 is modified by a codon substitution at nucleotides 187-189 that encodes the amino acid substitution T41G. In an even more specific aspect of these embodiments, the substituted codon substitution is gga

[0057] In another embodiment, in addition to the modification that destroys the putative glycosylation site encoded by nucleotides 181-189 of SEQ ID NOS:3 or 4, the variant of nucleotides 64-1530 of SEQ ID NO:3 or nucleotides 64-1524 of SEQ ID NO:4 further consists of 1-9 codon substitutions, wherein each codon substitution corresponds to an amino acid substitution selected from the substitutions set forth in Table 1, Table 2, and any one of the following V215 amino acid substitutions: V215S, V215G or V215T, V215C, V215D, V215E, V215F, V215H, V215K, V215N, V215P, V215Q, and V215R. In a more specific aspect of this embodiment, each codon substitution corresponds to an amino acid substitution selected from the substitutions set forth in Table 1, and any one of the V215 substitutions set forth above. In an even more specific embodiment, the variant nucleic acid sequence is modified by one codon substitution selected to code for any one of the V215 amino acid substitutions set forth above and from 1-8 additional codon substitutions, wherein each of the additional codon substitutions corresponds to an amino acid substitution selected from the substitutions set forth in Table 1. In a more specific aspect, the variant has one additional codon substitution corresponding to one of the specific one amino acid substitutions set forth in Table 3. In a more specific aspect of this embodiment, the one additional codon substitution selected to code for one of the single amino acid substitutions set forth in Table 3 does not code for an amino acid substitution in epitope 2.

[0058] In another embodiment, in addition to the modification that destroys the putative glycosylation site encoded by nucleotides 181-189 of SEQ ID NOS:3 or 4, the variant of nucleotides 64-1530 of SEQ ID NO:3, or nucleotides 64-1524 of SEQ ID NO:6 has a modification that consists of one codon substitution selected to code for any one of the V215 amino acid substitution set forth above; and from 2-8 additional codon substitutions, wherein each additional codon substitution corresponds to an amino acid substitution set forth in Table 1, and a codon substitution is present in each of at least two of epitopes 1, 2 and 3. In a more specific aspect, the variant has two additional codon substitutions

corresponding to one of the specific two amino acid substitutions set forth in Table 4. In a more specific aspect of this embodiment, the two additional codon substitutions selected to code for one of the specific two amino acid substitutions set forth in Table 4 does not code for an amino acid substitution in epitope 2. In an even more specific aspect of this embodiment, the specific two amino acid substitutions from Table 4 is T56H and K174R. In an even more specific aspect of this embodiment, the variant nucleotide sequence is SEQ ID NO:8.

[0059] In another embodiment, in addition to the modification that destroys the putative glycosylation site encoded by nucleotides 181-189 of SEQ ID NOS:3 or 4, the variant of any one of nucleotides 64-1530 of SEQ ID NO:3, or nucleotides 64-1524 of SEQ ID NO:6, has a modification that consists of one codon substitution selected to code for any one of the V215 amino acid substitution set forth above; and from 3-8 additional codon substitutions, wherein each additional codon substitution corresponds to an amino acid substitution set forth in Table 1, and a codon substitution is present in each of epitopes 1, 2 and 3. In a more specific aspect, the variant has three additional codon substitutions corresponding to one of the specific three amino acid substitutions set forth in Table 5.

[0060] In still other embodiments of the nucleic acid molecules of the invention, the nucleic acid molecule further comprises nucleic acid sequences encoding a signal sequence fused in phase and directly to the 5' end of the nucleic acid sequence encoding the variant g3p. In one aspect of these embodiments, the nucleic acid sequence encoding the signal sequence is nucleotides 1-63 of SEQ ID NO:3.

[0061] The nucleic acid molecules of the invention encompass nucleic acid sequences that are degenerative to, but encode the same amino acid sequence as encoded by any of the nucleic acid nucleic acid molecules described above.

[0062] For recombinant production, any of the nucleic acid molecules of the invention may be inserted into an appropriate expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence, or in the case of an RNA viral vector, the necessary elements for replication and translation. The encoding nucleic acid is inserted into the vector in proper reading frame. Accordingly, the invention provides vectors comprising nucleic acid molecule and sequences of the invention. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, etc. The choice of appropriate vector in which to clone the nucleic acid molecules and sequences of the invention may be made by those of skill in the art using well-known knowledge of the compatibility of the vector with the chosen host cell in which to carry out expression. This may be done in any of mammalian cells, plant cells, insect cells, bacterial cells, yeast cells, etc. Appropriate vectors for each of these cell types are well-known in the art and are generally commercially available.

[0063] In another embodiment, the invention provides a host cell harboring the vector containing a nucleic acid molecule or nucleic acid sequence of the invention. Methods of transfecting or transforming or otherwise getting a vector of the invention into a host cell are known in the art. A cell

harboring the vector, when cultured under appropriate conditions, will produce the polypeptides of the invention. Specific examples of vectors and cells used for the recombinant production of the polypeptides of the invention are set forth in the example section below.

Pharmaceutical Compositions

[0064] In some embodiments, the invention provides a pharmaceutical composition comprising any polypeptide or fusion protein comprising a variant g3p, optionally together with a pharmaceutically acceptable carrier, diluent or excipient. A "pharmaceutical composition" refers to a therapeutically effective amount of a composition as described herein with a physiologically suitable carrier and/or excipient. A pharmaceutical composition does not cause significant irritation to an organism. The phrases "physiologically suitable carrier" and "pharmaceutically acceptable carrier" which may be used interchangeably refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered composition. The term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, include, for example, saline, calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols, and surfactants, including, for example, polysorbate 20.

[0065] Pharmaceutical compositions for use in accordance with the present invention may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into compositions which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen and upon the nature of the composition delivered (e.g., size and solubility of the polypeptide). In one aspect of these embodiments, the pharmaceutical composition is formulated for injection or infusion into the bloodstream of a patient. In another aspect of these embodiments, the pharmaceutical composition is formulated for direct administration to the brain or central nervous system of the patient, for example, by direct intramedullary, intrathecal, or intraventricular injection.

[0066] The compositions described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Pharmaceutical compositions for parenteral administration include aqueous solutions of the composition in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents (e.g., surfactants such as polysorbate (Tween 20)) which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions. A protein based agent such as, for example, albumin may

be used to prevent adsorption of polypeptide of the invention to the delivery surface (i.e., IV bag, catheter, needle, etc.).

[0067] For oral administration, the compositions can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art.

[0068] Formulations may be presented in unit dosage form, e.g., in vials, ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Single dosage forms may be in a liquid or a solid form. Single dosage forms may be administered directly to a patient without modification or may be diluted or reconstituted prior to administration. In certain embodiments, a single dosage form may be administered in bolus form, e.g., single injection, single oral dose, including an oral dose that comprises multiple tablets, capsule, pills, etc. In alternate embodiments, a single dosage form may be administered over a period of time, such as by infusion, or via an implanted pump, such as an ICV pump. In the latter embodiment, the single dosage form may be an infusion bag or pump reservoir pre-filled with the appropriate amount of a polypeptide or fusion protein comprising a variant g3p. Alternatively, the infusion bag or pump reservoir may be prepared just prior to administration to a patient by mixing an appropriate dose of the variant g3p with the infusion bag or pump reservoir solution.

[0069] Another aspect of the invention includes methods for preparing a pharmaceutical composition of the invention. Techniques for formulation of drugs may be found, for example, in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition, which is incorporated herein by reference in its entirety.

[0070] Pharmaceutical compositions suitable for use in the context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose.

[0071] Determination of a therapeutically or diagnostically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0072] Dosage amount and interval may be adjusted individually to provide brain levels of the phage display vehicle which are sufficient to treat or diagnose a particular brain disease, disorder, or condition (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics.

[0073] Dosage intervals can also be determined using the MEC value. Preparations should be administered using a regimen, which maintains brain levels above the MEC for 10-90% of the time, preferable between 30-90% and most preferably 50-90%.

[0074] Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

[0075] The amount of a composition to be administered will, of course, be dependent on the subject being treated or diagnosed, the severity of the affliction, the judgment of the prescribing physician, etc. In certain embodiments, the amount of polypeptide to be administered is selected from 0.1-100 mg/kg subject body weight; 0.5-50 mg/kg; 1-30 mg/kg; 1-10 mg/kg; 3-30 mg/kg; 1-3 mg/kg; 3-10 mg/kg; and 10-30 mg/kg. In some embodiments, the peptide is administered to the subject once a week, once every two weeks, once every three weeks, once every four weeks, or once a month.

[0076] Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as if further detailed above.

[0077] It is to be understood that both the foregoing and following description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

Therapeutic Uses

[0078] Another aspect of the invention relates to the use of any of the polypeptides, nucleic acid molecules, or compositions of the invention, in the treatment of protein misfolding diseases, including, but not limited to, those diseases involving any of: $\text{fA}\beta 42$, *f*asyn or *f*tau.

[0079] In the context of treatments, the terms "patient", "subject" and "recipient" are used interchangeably and include humans as well as other mammals. In some embodiments, a patient is a human who is positive for a biomarker associated with a protein misfolding disease. In one embodiment, the patient exhibits β -amyloid deposits as detected by PET imaging with florbetapir.

[0080] The term "treating" and its cognates are intended to mean reducing, slowing, or reversing the progression of a disease in a patient exhibiting one or more clinical symptoms of a disease. "Treating" is also intended to mean reducing, slowing, or reversing the symptoms of a disease in a patient exhibiting one more clinical symptoms of a disease. In one embodiment, the patient exhibits β -amyloid deposits as detected by PET imaging with florbetapir and the number of β -amyloid deposits is reduced by the treatment. In one embodiment, the patient exhibits β -amyloid deposits as detected by the polypeptide or polypeptide compositions of the present invention and the number of β -amyloid deposits are reduced or maintained by the treatment. In another embodiment, the patient exhibits any type of

amyloid deposits as detected by PET imaging and the cognitive function of the patient is improved by the treatment. Improvement in cognitive function may be assayed by the methods and tests of McKhann et al., *Alzheimer's & Dementia* 7(3):263-9(2011).

[0081] "Prophylaxis" is distinct from treating and refers to administration of a composition to an individual before the onset of any clinical symptoms. Prophylaxis using any of the polypeptides or compositions thereof of the present invention is encompassed. Prophylaxis may be implicated in individuals who are known to be at increased risk for a disease, or whom are certain to develop a disease, solely on the basis of one or more genetic markers. Many genetic markers have been identified for the various protein misfolding diseases. For examples, individuals with one or more of the Swedish mutation, the Indiana mutation, or the London mutation in human amyloid precursor protein (hAPP) are at increased risk for developing early-onset Alzheimer's Disease and so are candidates for prophylaxis. Likewise, individuals with the trinucleotide CAG repeats in the huntingtin gene, particularly those with 36 or more repeats, will eventually develop Huntington's Disease and so are candidates for prophylaxis.

[0082] The term "protein misfolding" refers to diseases characterized by formation of amyloid protein by an aggregating protein (amyloid forming peptide), such as, but not limited to, β -amyloid, serum amyloid A, cystatin C, IgG kappa light chain, or a prion protein. Diseases known to be associated with misfolded and/or aggregated amyloid protein include Alzheimer's disease, which includes early onset Alzheimer's disease, late onset Alzheimer's disease, and presymptomatic Alzheimer's disease, Parkinson's disease, SAA amyloidosis, cystatin C, hereditary Icelandic syndrome, senility, multiple myeloma, prion diseases including but not limited to kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker disease (GSS), fatal familial insomnia (FFI), scrapie, and bovine spongiform encephalitis (BSE); amyotrophic lateral sclerosis (ALS), spinocerebellar ataxia (SCA1), (SCA3), (SCA6), (SCA7), Huntington disease, entorubral-pallidolusian atrophy, spinal and bulbar muscular atrophy, hereditary cerebral amyloid angiopathy, familial amyloidosis, frontotemporal lobe dementia, British/Danish dementia, Progressive Supranuclear Palsy (PSP), and familial encephalopathy. The polypeptides and compositions of the invention may be used to treat "protein misfolding" diseases.

[0083] Many of these misfolded and/or aggregated amyloid protein diseases occur in the central nervous system (CNS). Some examples of diseases occurring in the CNS are Parkinson's Disease; Alzheimer's Disease; frontotemporal dementia (FTD) including those patients having the following clinical syndromes: behavioral variant FTD (bvFTD), progressive non-fluent aphasia (PNFA) and semantic dementia (SD); frontotemporal lobar degenerations (FTLDs); and Huntington's Disease. The polypeptides and compositions of the invention may be used to treat diseases characterized by misfolded and/or aggregated amyloid protein that occur in the central nervous system (CNS).

[0084] Misfolding and/or aggregation of proteins may also occur outside the CNS. Amyloidosis A (AA) (for which the precursor protein is serum acute phase apolipoprotein, SAA) and multiple myeloma (precursor proteins immunoglobulin light and/or heavy chain) are two widely known protein misfolding and/or aggregated protein diseases that occur outside the CNS. Other examples include disease

involving amyloid formed by $\alpha 2$ -microglobulin, transthyretin (Familial Amyloidotic Polyneuropathy [FAP], Familial Amyloidotic Cardiomyopathy [FAC], and Senile Systemic Amyloidosis [SSA]), (apo)serum AA, apolipoproteins AI, AII, and AIV, gelsolin (Finnish form of Familial Amyloidotic Polyneuropathy), lysozyme, fibrinogen, cystatin C (Cerebral Amyloid Angiopathy, Hereditary Cerebral Hemorrhage with Amyloidosis, Icelandic Type), (pro)calcitonin, islet amyloid polypeptide (IAPP amyloidosis), atrial natriuretic factor, prolactin, insulin, lactahedrin, kerato-epithelin, lactoferrin, odontogenic ameloblast-associated protein, and semenogelin I. The polypeptides and compositions of the invention may be used to treat diseases involving misfolding and/or aggregation of proteins that occur outside the CNS.

[0085] Neurodegenerative diseases may also involve tau lesions. Reviewed in Lee et al., *Annu. Rev. Neurosci.* 24:1121-159 (2001). Tau proteins are microtubule-associated proteins expressed in axons of both central and peripheral nervous system neurons. Neurodegenerative tauopathies (sometimes referred to as tauopathies) are encompassed. Examples of tauopathies include Alzheimer's Disease, Amyotrophic lateral sclerosis/parkinsonism-dementia complex, Argyrophilic grain dementia, Corticobasal degeneration, Creutzfeldt-Jakob disease, Dementia pugilistica, diffuse neurofibrillary tangles with calcification, Down's syndrome, Frontotemporal dementias including frontotemporal dementia with parkinsonism linked to chromosome 17, Gerstmann-Sträussler-Scheinker disease, Hallervorden-Spatz disease, Myotonic dystrophy, Niemann-Pick disease type C, Non-Guamanian motor neuron disease with neurofibrillary tangles, Pick's disease, Postencephalitic parkinsonism, Prion protein cerebral amyloid angiopathy, Progressive subcortical gliosis, Progressive supranuclear palsy, Subacute sclerosing panencephalitis, and Tangle only dementia. Some of these diseases may also include deposits of fibrillar amyloid β peptides. For example, Alzheimer's disease exhibits both amyloid β deposits and tau lesions. Similarly, prion-mediated diseases such as Creutzfeldt-Jakob disease, prion protein cerebral amyloid angiopathy, and Gerstmann-Sträussler-Scheinker syndrome may have also have tau lesions. Thus an indication that a disease is a "tauopathy" should not be interpreted as excluding the disease from other neurodegenerative disease classifications or groupings, which are provided merely as a convenience. The polypeptides and compositions of the invention may be used to treat neurodegenerative diseases as well as diseases involving tau lesions.

[0086] In one embodiment, a pharmaceutical composition or formulation is for use in a method of reducing amyloid in a patient exhibiting symptoms related to the presence of amyloid or that is positive for a biomarker associated with a protein misfolding disease, such as florbetapir (AV-45, Eli Lilly), comprising administering to the patient an effective amount of a pharmaceutical composition or formulation as described herein. In one embodiment, the route of administration is selected from intrathecal injection or infusion, direct intraventricular injection or infusion, intraparenchymal injection or infusion, or intravenous injection or infusion.

[0087] In one embodiment, a pharmaceutical composition or formulation is for use in a method of maintaining the level of amyloid in a patient exhibiting symptoms related to the presence of amyloid or

that is positive for a biomarker associated with a protein misfolding disease, such as florbetapir (AV-45, Eli Lilly), comprising administering to the patient an effective amount of a pharmaceutical composition or formulation as described herein. In one embodiment, the route of administration is selected from intrathecal injection or infusion, direct intraventricular injection or infusion, intraparenchymal injection or infusion, or intravenous injection or infusion.

[0088] In one embodiment, a pharmaceutical composition or formulation is for use in a method of disaggregating amyloid in a patient comprising administering to a patient having amyloid an effective amount of a pharmaceutical composition or formulation as described herein. In one embodiment, the route of administration is selected from intrathecal injection or infusion, direct intraventricular injection or infusion, intraparenchymal injection or infusion, or intravenous injection or infusion.

[0089] In one embodiment, a pharmaceutical composition or formulation of the invention is for use in a method of causing the disaggregation of β -amyloid deposits in the brain, comprising injecting directly into the brain of a patient in need thereof an effective amount of pharmaceutical composition as described herein, thereby causing a reduction in β -amyloid deposits in the brain. In an alternate embodiment, a pharmaceutical composition or formulation of the invention is for use in a method of causing the disaggregation of β -amyloid deposits in the brain, comprising injecting intravenous delivery into a patient in need thereof an effective amount of pharmaceutical composition as described herein, thereby causing a reduction in β -amyloid deposits in the brain.

[0090] In one embodiment, a pharmaceutical composition or formulation is for use in a method of reducing amyloid formation in the brain. Reducing amyloid formation in the brain may prevent, treat or reduce the symptoms or severity of a protein-misfolding or neurodegenerative disease. In one embodiment, the route of administration is selected from intrathecal injection or infusion, direct intraventricular injection or infusion, intraparenchymal injection or infusion, or intravenous injection or infusion.

[0091] In one embodiment, a pharmaceutical composition or formulation of the invention is for use in a method for promoting amyloid clearance in the brain. Promoting amyloid clearance may prevent, treat or reduce the symptoms or severity of a protein-misfolding or neurodegenerative disease. In one embodiment, the route of administration is selected from intrathecal injection or infusion, direct intraventricular injection or infusion, intraparenchymal injection or infusion, or intravenous injection or infusion.

[0092] In one embodiment, a pharmaceutical composition or formulation of the invention is for use in a method for inhibiting amyloid aggregation in the brain. Inhibiting amyloid aggregation in the brain may prevent, treat or reduce the symptoms or severity of a protein-misfolding or neurodegenerative disease. In one embodiment, the route of administration is selected from intrathecal injection or infusion, direct intraventricular injection or infusion, intraparenchymal injection or infusion, or intravenous injection or infusion.

[0093] In one embodiment, a pharmaceutical composition or formulation of the invention is for use in a method for clearing toxic amyloid oligomers in the brain. Clearing toxic amyloid oligomers in the brain may prevent, treat or reduce the symptoms or severity of a protein-misfolding or neurodegenerative disease. In one embodiment, the route of administration is selected from intrathecal injection or infusion, direct intraventricular injection or infusion, intraparenchymal injection or infusion, or intravenous injection or infusion.

[0094] In one embodiment, a pharmaceutical composition or formulation of the invention is for use in a method for preventing the formation of toxic amyloid oligomers in the brain. Preventing the formation of toxic oligomers in the brain may prevent, treat or reduce the symptoms or severity of a protein-misfolding or neurodegenerative disease. In one embodiment, the route of administration is selected from intrathecal injection or infusion, direct intraventricular injection or infusion, intraparenchymal injection or infusion, or intravenous injection or infusion.

[0095] In one embodiment, a pharmaceutical composition or formulation of the invention is for use in a method for protecting neurons from amyloid damage. Protecting neurons from amyloid damage may prevent, treat or reduce the symptoms or severity of a protein-misfolding or neurodegenerative disease. In one embodiment, the route of administration is selected from intrathecal injection or infusion, direct intraventricular injection or infusion, intraparenchymal injection or infusion, or intravenous injection or infusion. In one embodiment, a pharmaceutical composition or formulation of the invention for use in protecting neurons from amyloid damage is given prophylactically.

[0096] In some embodiments, the patient is positive for a biomarker associated with a protein misfolding and/or aggregation disease. In one embodiment, the biomarker is florbetapir (AV45, Eli Lilly).

[0097] In some embodiments, the patient is exhibiting symptoms of a neurodegenerative disease that is associated with the presence of amyloid. In various embodiments, the amyloid is any of $\text{fA}\beta 42$, $\text{f}\alpha\text{syn}$ or $\text{f}\tau\text{au}$.

[0098] In certain embodiments, the neurodegenerative disease is Parkinson's disease, Alzheimer's disease, or Huntington's disease. In one embodiment, the neurodegenerative disease is Alzheimer's disease. In one embodiment, the neurodegenerative disease is Alzheimer's disease and the patient exhibits β -amyloid as detected by the imaging agent florbetapir (AV-45, Eli Lilly).

[0099] In some embodiments, the patient is exhibiting symptoms of a prion-mediated disease.

[0100] In certain embodiments, the prion-mediated disease is chosen from Creutzfeldt-Jakob disease, kuru, fatal familial insomnia, or Gerstmann-Sträussler-Scheinker syndrome.

[0101] In some embodiments, the patient is exhibiting symptoms of a neurodegenerative tauopathy other than Alzheimer's disease. In certain embodiments, the disease to be treated is selected from Argyrophilic grain dementia, Corticobasal degeneration, Dementia pugilistica, diffuse neurofibrillary tangles with calcification, Down's syndrome, Frontotemporal dementias including frontotemporal dementia with parkinsonism linked to chromosome 17, Hallervorden-Spatz disease, Myotonic

dystrophy, Niemann-Pick disease type C, Non-Guamanian motor neuron disease with neurofibrillary tangles, Pick's disease, Postencephalitic parkinsonism, Progressive subcortical gliosis, Progressive supranuclear palsy, Subacute sclerosing panencephalitis, and Tangle only dementia.

[0102] In another embodiment, any of the disease conditions described above may be treated by administration of a nucleic acid molecule of the invention (i.e., one that encodes a variant g3p that exhibits reduced immunogenicity and possessing the ability to bind to amyloid, disaggregate amyloid plaques, and/or prevent aggregation of amyloid) alone or associated with a suitable carrier, such as, e.g., a lipid nanoparticle, a polymeric carrier, or a vector, such as a viral vector directly to a patient by any suitable route, such as, e.g., inhalation and intravenous infusion. The nucleic acid molecule encoding the variant g3p of the invention suitable for this treatment may be DNA or RNA.

Diagnostics

[0103] In another aspect of the invention, the polypeptides and compositions described herein, are used in diagnostic applications associated with the various diseases described herein. For example, binding of a composition of the invention when used as an imaging agent either *in vivo* or *in vitro* may be part of a diagnosis of one of the protein misfolding diseases described. When used as diagnostic agents, the polypeptides of the invention may further comprise a detectable label, or may be otherwise detected *in vivo*. Various labels can be attached to the amyloid binding component of the diagnostic composition using standard techniques for labeling proteins. Examples of labels include fluorescent labels and radiolabels. There are a wide variety of radiolabels that can be used, but in general the label is often selected from radiolabels including, but not limited to, ^{18}F , ^{11}C , and ^{123}I . These and other radioisotopes can be attached to the protein using well known chemistry. In one embodiment, the label is detected using positron emission tomography (PET). However, any other suitable technique for detection of radioisotopes may also be used to detect the radiotracer.

[0104] The polypeptides and compositions of the invention may be used as diagnostic imaging agents in combination with an imaging agent that is specific for β -amyloid such as, for example, F18-AV-45, Eli Lilly. Since there are currently no known imaging agents for non- β -amyloid aggregates, the use of a diagnostic composition of the invention together with a β -amyloid-specific imaging agent will result in the detection of non- β -amyloid aggregates based on differential detection. Thus, in one embodiment, a diagnostic composition of the invention is used as an imaging agent in combination with a β -amyloid imaging agent to detect non- β -amyloid aggregates.

[0105] In another embodiment, the polypeptides or compositions of the invention is used as a diagnostic imaging agent to detect β -amyloid in the CNS, including the brain.

[0106] Diagnostic compositions of the invention may be administered using the same routes described for therapeutic compositions. In one embodiment, the route of administration is selected from intrathecal injection or infusion, direct intraventricular injection or infusion, intraparenchymal injection or infusion, or intravenous injection or infusion.

Examples

Example 1: Mapping of CD4+ T Cell Epitopes in g3p

[0107] 87 overlapping peptides spanning the sequence of amino acids 1-240 of SEQ ID NO:1 (15 amino acids long with 12 amino acid overlaps) were synthesized and tested in a T cell epitope mapping assay for responses from human CD4+ T cells. Individual peptides were tested in sextuplicate PBMC cultures and T cell responses were assessed in order to identify the location of epitopes as well as their relative potency.

[0108] PBMC (peripheral blood mononuclear cells) were isolated from healthy community donor buffy coats (from blood drawn within 24 hours) obtained from the UK National Blood Transfusion Service (Addenbrooke's Hospital, Cambridge, UK) and according to approval granted by Addenbrooke's Hospital Local Research Ethics Committee by Lymphoprep (Axis-shield, Dundee, UK) density centrifugation. CD8⁺ T cells were depleted using CD8⁺ RosetteSep™ (StemCell Technologies Inc, London, UK). Donors were characterized by identifying HLA-DR haplotypes using an HLA SSP-PCR based tissue-typing kit (Biotest, Solihull, UK). T cell responses to a control neoantigen protein (KLH protein (Pierce (Perbio), Cramlington, UK) and peptides derived from IFV and EBV) were also determined. PBMC were then frozen and stored in liquid nitrogen until required.

[0109] A cohort of 55 donors was selected for the assay to best represent the number and frequency of HLA-DR allotypes expressed in the world population. Analysis of the allotypes expressed in the cohort against those expressed in the world population revealed that coverage of >80% was achieved and that all major HLA-DR alleles (individual allotypes with a frequency >5% expressed in the world population) were well represented. Details of individual donor haplotypes and a comparison of the frequency of MHC class II haplotypes expressed in the world population and the sample population are shown in Table 8 and FIG. 3, respectively.

Table 8. Donor details and haplotypes

Donor No.	Haplotype
1	DRB1*04:01,DRB1*16:01;DRB4*01:03;DQB1*03:02;DQB1*05:02
2	DRB1*01:01,DRB1*13:02;DRB3*03:01;DQB1*05:01;DQB1*06:04
3	DRB1*03:01,DRB1*07:01;DRB3*01:01;DRB4*01:03;DQB1*02:01;DQB1*03:03
4	DRB1*09:01,DRB1*13:01;DRB3*02:02;DRB4*01:03;DQB1*03:03;DQB1*06:03
5	DRB1*13:01,DRB1*13:02;DRB3*01:01;DRB3*03:01;DQB1*06:03;DQB1*06:04
6	DRB1*04:01,DRB1*04:07;DRB4*01:03;DQB1*03:01
7	DRB1*13:01;DRB3*01:01;DQB1*06:03
8	DRB1*13:01,DRB1*15:01;DRB3*02:02;DRB5*01:01;DQB1*06:02;DQB1*06:03
9	DRB1*04:01,DRB1*11:01;DRB3*02:02;DRB4*01:03;DQB1*03:01;DQB1*03:02
10	DRB1*04:04,DRB1*12:01;DRB3*02:02;DRB4*01:03;DQB1*03:01;DQB1*03:02
11	DRB1*13:02,DRB1*15:01;DRB3*01:01;DRB5*01:01;DQB1*06:02;DQB1*06:04
12	DRB1*04:01,DRB1*15:01;DRB4*01:03;DRB5*01:01;DQB1*03:02;DQB1*06:02
13	DRB1*04:02,DRB1*07:01;DRB4*01:01;DRB4*01:03;DQB1*02:01
14	DRB1*03:01,DRB1*16:01;DRB3*01:01;DRB5*02:02;DQB1*02:01;DQB1*05:02
15	DRB1*03:01,DRB1*13:01;DRB3*02:02;DQB1*02:01;DQB1*06:03
16	DRB1*01:01,DRB1*15:01;DRB5*01:01;DQB1*05:01;DQB1*06:02

Donor No.	Haplotype
17	DRB1*01:01,DRB1*07:01,DRB4*01:03,DQB1*03:03,DQB1*05:01
18	DRB1*01:01,DRB1*09:01,DRB4*01:03,DQB1*03:03,DQB1*05:01
19	DRB1*03:01,DRB1*11:02,DRB3*01:01,DRB3*02:02,DQB1*02:01,DQB1*03:01
20	DRB1*13:01,DRB3*01:01,DRB3*02:02,DQB1*06:03
21	DRB1*01:01,DRB1*13:02,DRB3*03:01,DQB1*05:01,DQB1*06:04
22	DRB1*04:01,DRB1*04:03,DRB4*01:03,DQB1*03:02
23	DRB1*08:01,DRB1*13:01,DRB3*01:01,DQB1*04:02,DQB1*06:03
24	DRB1*03:01,DRB1*15:01,DRB3*01:01,DRB5*01:01,DQB1*02:01,DQB1*06:02
25	DRB1*03:01,DRB4*01:01,DRB3*01:01,DRB4*01:03,DQB1*02:01,DQB1*03:01
26	DRB1*01:01,DRB1*15:01,DRB5*01:01,DQB1*05:01,DQB1*06:02
27	DRB1*04:04,DRB1*07:01,DRB4*01:01,DRB4*01:03,DQB1*02:02,DQB1*03:02
28	DRB1*11:01,DRB1*15:01,DRB3*02:01,DRB5*01:01,DQB1*03:01,DQB1*06:01
29	DRB1*08:01,DRB1*15:01,DRB5*01:01,DQB1*04:02,DQB1*06:02
30	DRB1*13:02,DRB1*15:01,DRB3*03:01,DRB5*01:01,DQB1*06:02,DQB1*06:09
31	DRB1*04:01,DRB1*16:01,DRB4*01:03,DRB5*02:02,DQB1*03:02,DQB1*06:03
32	DRB1*13:02,DRB1*15:01,DRB3*03:01,DRB5*01:01,DQB1*06:02,DQB1*06:04
33	DRB1*07:01,DRB1*11:04,DRB3*02:02,DRB4*01:01,DQB1*02:02,DQB1*03:01
34	DRB1*01:03,DRB1*15:01,DRB5*01:01,DQB1*03:01,DQB1*06:02
35	DRB1*03:01,DRB1*14:01,DRB3*01:01,DRB3*02:02,DQB1*02:01,DQB1*05:03
36	DRB1*03:01,DRB1*08:01,DRB3*01:01,DQB1*02:01,DQB1*04:02
37	DRB1*03:01,DRB1*11:01,DRB3*01:01,DRB3*02:02,DQB1*02:01,DQB1*03:01
38	DRB1*07:01,DRB1*15:01,DRB4*01:03,DRB5*01:01,DQB1*02:02,DQB1*06:02
39	DRB1*03:01,DRB1*13:02,DRB3*02:02,DRB3*03:01,DQB1*02:01,DQB1*06:09
40	DRB1*01:01,DRB1*13:02,DRB3*01:01,DQB1*05:01,DQB1*06:04
41	DRB1*04:07,DRB1*15:01,DRB4*01:03,DRB5*01:01,DQB1*03:01,DQB1*06:02
42	DRB1*07:01,DRB4*01:03,DQB1*02:02,DQB1*03:03
43	DRB1*03:01,DRB1*15:01,DRB3*01:05,DRB5*01:01,DQB1*02:01,DQB1*06:02
44	DRB1*07:01,DRB1*11:04,DRB3*02:02,DRB4*01:01,DQB1*02:02,DQB1*03:01
45	DRB1*03:01,DRB1*04:04,DRB3*01:01,DRB4*01:03,DQB1*02:01,DQB1*03:02
46	DRB1*04:04,DRB1*13:01,DRB3*02:02,DRB4*01:03,DQB1*03:02,DQB1*06:03
47	DRB1*04:01,DRB1*11:01,DRB3*02:02,DRB4*01:03,DQB1*03:01
48	DRB1*03:01,DRB1*04:01,DRB3*01:06,DRB4*01:03,DQB1*02:01,DQB1*03:02
49	DRB1*01:02,DRB1*13:03,DRB3*01:01,DQB1*03:01,DQB1*05:01
50	DRB1*04:07,DRB1*15:01,DRB4*01:03,DRB5*01:01,DQB1*03:01,DQB1*06:02
51	DRB1*04:07,DRB1*13:02,DRB3*03:01,DRB4*01:03,DQB1*03:01,DQB1*06:04
52	DRB1*03:01,DRB3*01:05,DQB1*02:01
53	DRB1*03:01,DRB1*07:01,DRB3*01:01,DRB4*01:01,DQB1*02:01,DQB1*02:02
54	DRB1*04:04,DRB1*15:01,DRB4*01:03,DQB1*03:02,DQB1*06:02
55	DRB1*03:01,DRB1*04:01,DRB3*01:01,DRB4*01:03,DQB1*02:01,DQB1*03:01

[0110] PBMC from each donor were thawed, counted and viability was assessed. Cells were revived in room temperature AIM-V® culture medium (Invitrogen, Paisley, UK) before adjusting the cell density to $2-3 \times 10^6$ PBMC/ml (proliferation cell stock). The 15 amino acid long peptides were synthesized on a 1-3 mg scale with free N-terminal amine and C-terminal carboxylic acid. Peptides were dissolved in DMSO to a concentration of 10 mM and peptide culture stocks prepared by diluting into AIM-V® culture medium to a final concentration of 5 μ M in the well. For each peptide and each donor, sextuplicate cultures were established in a flat bottomed 96 well plate. Both positive and negative control cultures were also tested in sextuplicate. For each donor, three controls (KLH protein

and peptides derived from IFV and EBV) were also included. For a positive control, PHA (Sigma, Dorset, UK) was used at a final concentration of 2.5 µg/ml.

[0111] Cultures were incubated for a total of 6 days before adding 0.75 µCi ³[H]-thymidine (Perkin Elmer®, Beaconsfield, UK) to each well. Cultures were incubated for a further 18 hours before harvesting onto filter mats using a TomTec Mach III cell harvester. Cpm for each well were determined by Meltilex™ (Perkin Elmer®, Beaconsfield, UK) scintillation counting on a Microplate Beta Counter (Perkin Elmer®, Beaconsfield, UK) in paralux, low background counting mode.

[0112] For analysis of the data, a threshold of a stimulation index (SI) equal to or greater SI ≥ 2.00 was used (with consideration of borderline SI ≥ 1.90-1.99 responses). Positive responses were defined by the following statistical and empirical thresholds:

1. Significance ($p < 0.05$) of the response by comparing cpm of test wells against medium control wells using unpaired two sample Student's t-test;
2. Stimulation index greater than 2.00 (SI ≥ 2.00), where SI = mean cpm of test wells / mean cpm medium control wells. Data presented in this way is indicated as SI ≥ 2.00, $p < 0.05$.

[0113] In addition, intra-assay variation was assessed by calculating the CV and SD of the raw data from replicate cultures. Proliferation assays were set up in sextuplicate cultures ("non-adjusted data"). To ensure that intra-assay variability was low, the data were also analysed after removing the maximum and minimum cpm values ("adjusted data") and the SI of donor responses was compared using both data sets. T cell epitopes were identified by calculating the average frequency of positive responses (defined above) to all peptides in the study plus SD to give a background response rate. Any peptide that induced proliferative responses above the background response rate in both the adjusted and non-adjusted data was considered to contain a T cell epitope. When two overlapping peptides induced a proliferative response rate the T-cell epitope was considered to be in the overlap region. Based upon this the following T-cell epitopes were identified in the tested polypeptide:

Epitope 1: C T G D E T Q C Y G T W (amino acids 46-57 of SEQ ID NO:1)

Epitope 2: T F M F Q N N R F R N R (amino acids 133-144 of SEQ ID NO:1)

Epitope 3: S S K A M Y D A Y W N G (amino acids of 172-183 of SEQ ID NO:1)

Epitope 4: P V N A G G G S G G G S (amino acids 214-225 of SEQ ID NO:1)

Epitope 5: S G S G A M V R S D K T H T C (amino acids 253-267 of SEQ ID NO:1)

Example 2: Design of Substitutions in T Cell Epitopes 4 and 5 by *In Silico* Analysis

[0114] The sequences of peptides that were positive in the T cell assay were analysed using overlapping 9-mers from the epitope region using iTope™ and TCED™ *in silico* technologies. [Perry et al., *Drugs R D* 9(6):385-96 (2008).] Each 9-mer was tested against a database of MHC class II alleles (34 in total) and scored based on the fit and interactions with the MHC class II molecules. In addition, each 9-mer was BLAST searched against a database of known CD4+ T cell epitopes in order to identify any high sequence homology between that of the 9-mer and of database peptides from

unrelated proteins that stimulated T cell responses in previous T cell assays. On the basis of information from the *in silico* analysis, substitutions were identified for potential removal of CD4+ T cell epitope activity from the identified epitopes.

[0115] Epitope 5 spans the C-terminus of the native N2-CT Gly-rich linker, the amino acids coded for by the multiple cloning site ("MCS") of the pFUSE vector used to produce the N1-N2-human Ig Fc fusion protein of SEQ ID NO:1, and the N-terminus of the human Ig Fc region. *In silico* analysis implicated M258 and V259 of SEQ ID NO:1 as the P1 anchors responsible T-cell activity. Based on their location outside of the N1-N2 coding region, removal of these two amino acids was not expected to cause a loss of function. These two amino acids were encoded by the MCS. Therefore, a double-stranded DNA molecule that modified the MCS and eliminated the nucleotides encoding M258 and V259 of SEQ ID NO:1 was produced by site-directed mutagenesis using appropriate oligonucleotide primers. This was followed by recloning the resulting mutagenized DNA sequence back into the pFUSE vector using the using *EcoRI* and *BglII* restriction sites in the MCS. The resulting mature (lacking the signal sequence) fusion protein omitted M258 and V259. That fusion protein retained the same ability to bind Abeta in the assay described below as the SEQ ID NO:1 fusion protein.

[0116] Epitope 4 overlaps the N2 domain and the native Gly-rich linker. Crystal structure of the g3p protein (not shown) suggested that Epitope 4 is located away from amyloid binding region and therefore would be tolerant to amino acid substitutions without affecting activity. V215 (SEQ ID NO:1), which was identified as a P1 anchor, is surface exposed with slight orientation of side chain towards the protein core. From structural analysis, any of the substitutions for V215 set forth in Tables 6 and 7 should remove the epitope. In addition any of the substitutions of other amino acids within this epitope as set forth in Tables 6 and 7 should also be accommodated. A nucleic acid sequence encoding an N1-N2-Ig Fc comprising a V215A substitution (SEQ ID NO:4) and omitting M258 and V259 was derived from the above-described nucleotide sequence by site-directed mutagenesis using appropriate oligonucleotide primers. The resulting mature fusion protein (SEQ ID NO:2) demonstrated increased binding to Abeta in the binding assay as compared to a fusion protein having the amino acid sequence of either SEQ ID NO:1 or the mature fusion protein lacking M258 and V239, described above. The nucleic acid sequence of SEQ ID NO:4 was used as the parent sequence to create genes incorporating all modifications in epitopes 1, 2 and 3.

Example 3: Design of Substitutions in T Cell Epitopes 1, 2 and 3 by *In Silico* Analysis

[0117] Epitope 1 lies just C-terminal to a putative Abeta binding portion of N1-N2. *In silico* analysis of Epitope 1 highlighted amino acids 48-56 of SEQ ID NO:1 as an area for amino acid substitution and removal of the T-cell epitope. Amino acids within this 9-mer were targeted for substitution based upon the nature of the existing amino acid, surface exposure, and interaction with the amyloid binding region of g3p, as interpreted from the X-ray crystal structure of g3p. In particular, G48, T51, Y54 and T56

were targeted for substitution with the changes indicated in Table 1. Other potential amino acid substitutions in this region are set forth in Table 2.

[0118] iTope™ analysis of Epitope 2 pointed to amino acids 135-143 of SEQ ID NO:1 as a target for reducing or eliminating that epitope. Based on the X-ray crystal structure, amino acids 136-139 of SEQ ID NO:1 form a loop region that forms bonds with the hinge region of N1-N2 and thus may be important for amyloid binding activity. Changes to these amino acids are less preferred and are only presented in Table 2. The more preferred changes are to M135, R140, F141 and N143 and are set forth in Table 1. Other potential changes to this nine amino acid region are set forth in Table 2.

[0119] Amino acids 173-182 of SEQ ID NO:1 were identified within Epitope 3 as targets for substitution by *in silico* analysis. Epitope 3 is located in an alpha helical portion of the N2 domain, thus the strategy was to avoid introduction of hydrophobic residues and small polar uncharged residues. In addition, we wanted to avoid introducing polar residues acidic residues towards the C-terminus of this epitope. Based on X-ray crystallographic data, we targeted S173, D174, M176, D178 and W182 for substitution with the changes indicated in Table 1. Other potential amino acid substitutions in this region are set forth in Table 2.

Example 4: Generation of N1-N2-Human IgG Fc Polypeptides Having Reduced T-Cell Eptiopes

[0120] Fifty-eight different nucleic acid molecules, each encoding N1-N2-human IgG Fc fusion proteins containing a different single amino acid substitution set forth in Table 3 were prepared. This was achieved by site-directed mutagenesis of SEQ ID NO:4 using appropriate oligonucleotide primers to introduce the desired substitution, followed by recloning of the PCR-amplified mutagenized sequence into the pFUSE-hIgG1-Fc2 vector (Invivogen®, Toulouse, France, Catalogue No. pfuse-hg1fc2).

[0121] Genes encoding these “deimmunized” Fc fusion polypeptides were transiently expressed in individual pFUSE-hIgG1-Fc2 vectors in FreeStyle 293-F cells (Invitrogen, Paisley, Scotland, Catalogue # R790-07). On the day of transfection, cells were diluted to 1×10^6 /mL in FreeStyle 293 Media (Invitrogen, Catalogue # 12338) ensuring a viability of >90%. Plasmid DNA and polyethylenimine (PEI) were diluted separately in Optimem (Invitrogen, Catalogue # 31985) and incubated for 5 minutes following which the PEI was added slowly to the DNA, and the DNA/PEI mixtures were incubated for 5 minutes at room temperature. After incubation, the DNA/PEI mixtures were added dropwise to the 293-F cells whilst swirling the flask. Transfected cultures were incubated at 37°C, 8% CO₂ on an orbital shaker platform rotating at 135 rpm for 6–7 days, following which they were harvested.

[0122] Culture medium containing the polypeptide was harvested by centrifugation and pH adjusted using 10x PBS. Proteins were bound to Protein A Sepharose beads (Sigma, Dorset, UK) by rotating overnight at 4°C. The beads were washed twice with 1x PBS and transferred to SigmaPrep spin columns (Sigma). Samples were eluted by centrifugation using 0.1M Glycine pH3.0 and neutralized in the collection tube using 1/10th volume 1M Tris-HCl pH8.0. Eluates were buffer exchanged into 1x PBS

using 2ml ZebaSpin columns (Pierce, Cramlington, UK, Catalogue #89890). Samples were filter-sterilized and the absorbance at 280nm was measured for each sample.

Example 5: ABeta Binding Analysis of Deimmunized Polypeptides

[0123] *A. ABeta (A β) Fiber Preparation.* A β 42 (1mg, rPeptide A-1002-2) was dissolved in hexafluoroisopropanol (HFIP, 1mL), vortexed thoroughly and incubated at room temperature for 2-18 hours until a clear solution appears. Aliquots (100 μ l, 100 μ g) were placed in 1.5mL Eppendorf tubes and dry under vacuum (speed Vac, Eppendorf, Concentrator 5301) for 2-3hr. The resulting monomers were resuspended in 20 μ L DMSO, pipetted and vortexed thoroughly until completely dissolved. The solution was diluted with 260 μ L of 10mM HCl solution (final A β 42 concentration is 80 μ M) and vortexed for 20 seconds. The clear solution is incubated (without shaking) for 3 days at 37°C to allow for aggregation.

[0124] For use in the assay A β 42 fibers from the resulting stock solution were diluted 50-fold to 1.6 μ M final concentration in PBS.

[0125] *B. ELISA Plate Preparation.* To each well of a 96-well plate (F96 MAXISORP NUNC-IMMUNO PLATE; Catalog number: 442404, Lot 125436 and 128158; Denmark) was added 200 μ L of a 1% BSA solution. The plates were sealed and incubated at 7°C for 3 hr. Plates were then washed with PBS (250 μ L/well) x3. We added 50 μ L of the diluted A β 42 fiber solution (1.6 μ M) to each well and incubated uncovered at 37°C overnight to complete dryness. PBS (50 μ L/well) is added to control wells (without A β 42 fibers). Plates were then washed 2X with water and 1X with PBS (250 μ L/well for each washing).

[0126] *C. ELISA Assay.* Varying concentrations of each polypeptide (as well as the polypeptide of SEQ ID NO:2) in 50 μ L were added to each well, as well as to non-A β 42 fiber coated wells and incubated for 1h at 37°C. Plates were then washed 3X with PBS-T (0.05% Tween 20 in PBS) and 3X with PBS (250 μ L/well for each washing). We then added 50 μ L of HRP-conjugated Goat anti-Human anti Fcy (Jackson Labs, Catalog number, 109-035-008, Lot number: 106617) diluted 1:2500 (0.32 μ g/mL final) in PBS-T + 1% Milk (Difco™ Skim Milk, Becton, Dickinson and Company, USA, Catalog number: 232100, Lot number: 7320448) to each well and incubated for 40 min at 37°C. Plates were then washed 6X with PBS-T and 2X with PBS (250 μ L/well for each washing). We then added 50 μ L/well OPD solution (15 mg/7.5 ml 0.05 M Citrate buffer pH-5.5/3 μ L H₂O₂) and let color to develop for 3-6 min. We next added 25 μ L/well of 4N HCl solution to stop reaction. Plates were read for absorbance at 492 nm and 405 nm. The 405 nm absorbance was subtracted from the 492 nm absorbance and the results plotted as a function of polypeptide concentrations. An IC₅₀ for binding for each deimmunized polypeptide was then calculated and compared to the IC₅₀ calculated for the polypeptide of SEQ ID NO:2. The results are shown in Table 9, below.

Table 9. Relative Change in ABeta Binding IC₅₀ for Polypeptides with a Single Additional Amino Acid Substitution in Epitope 1, 2 or 3 as Compared to Polypeptide of SEQ ID NO:2

Amino Acid Substitution	IC ₅₀ Relative to SEQ ID NO:2*
Epitope 1 G48H	1.8
Epitope 1 G48K	1.1
Epitope 1 G48R	1.9
Epitope 1 G48S	1.2
Epitope 1 G48T	1.0
Epitope 1 T51G	0.8
Epitope 1 T51H	1.5
Epitope 1 T51K	2.5
Epitope 1 T51P	0.2
Epitope 1 T51R	2.0
Epitope 1 T51Q	0.8
Epitope 1 T51N	0.5
Epitope 1 Y54G	0.02 / 0.2
Epitope 1 Y54H	0.3
Epitope 1 Y54K	0.13 / 0.32
Epitope 1 Y54P	0.07
Epitope 1 Y54R	0.15 / 0.25
Epitope 1 T56G	0.1
Epitope 1 T56H	0.47 / 0.77
Epitope 1 T56K	0.5 / 0.66

Amino Acid Substitution	IC ₅₀ Relative to SEQ ID NO:2*
Epitope 1 T56P	0.1 / 0.09
Epitope 1 T56R	0.8
Epitope 2 M135A	0.4
Epitope 2 M135D	0.5
Epitope 2 M135G	0.2
Epitope 2 M135H	0.1
Epitope 2 M135K	0.4 / 0.2
Epitope 2 M135N	0.3
Epitope 2 M135R	0.1
Epitope 2 M135T	0.14 / 0.3
Epitope 2 R140A	0.2
Epitope 2 R140D	0.3
Epitope 2 R140E	0.3
Epitope 2 R140G	0.2
Epitope 2 R140H	0.2
Epitope 2 R140Q	0.28 / 0.22
Epitope 2 F141D	0.2
Epitope 2 F141E	0.2
Epitope 2 N143A	1.9 / 1.1

Amino Acid Substitution	IC ₅₀ Relative to SEQ ID NO:2*
Epitope 2 N143G	0.19 / 0.08
Epitope 3 S173G	0.2
Epitope 3 S173P	0.4
Epitope 3 M176G	0.3
Epitope 3 M176H	0.4
Epitope 3 M176K	0.2
Epitope 3 M176N	0.5
Epitope 3 D178G	0.2
Epitope 3 D178N	0.5 / 0.4
Epitope 3 D178Q	0.6
Epitope 3 D178S	0.3
Epitope 3 W181G	0.5
Epitope 3 W181H	0.47 / 0.87
Epitope 3 W181K	0.3
Epitope 3 W181R	0.5/0.8
Epitope 3 S173K	0.17 / 0.07
Epitope 3 K174R	1.2 / 1.0
Epitope 3 M176R	0.2
Epitope 3 D178T	0.4

*Numbers reflect IC₅₀ (substituted polypeptide)/IC₅₀ (polypeptide of SEQ ID NO:2). Multiple values reflect duplicate testing in the binding assay.

Example 6: Analysis of Whole Protein CD4+ T cell Responses

[0127] In order to analyze CD4+ T cell responses from any of the polypeptides of the invention in comparison to SEQ ID NO:1, a whole protein T cell assay was performed. PBMCs were isolated from 20 healthy human donor buffy coats prepared as in Example 1. PBMCs were revived from frozen in AIM-V® culture medium and CD14⁺ cells were isolated using Miltenyi CD14 Microbeads and LS columns (Miltenyi Biotech, Oxford, UK). Monocytes were resuspended in AIM-V® supplemented with 1000U/ml IL-4 and 1000U/ml GM-CSF ("DC culture medium") to 4-6x10⁶ PBMC/ml and then distributed in 24 well plates (2ml final culture volume). Cells were fed on day 2 by replacement of a half volume DC culture medium. By day 3, monocytes had differentiated to semi-mature dendritic cells (DC) which were pre-incubated with antigens comprising either 40ug/ml of test polypeptide or 40ug/ml of the polypeptide of SEQ ID NO:1 and 100μg/ml KLH or medium only. Semi-mature DC were incubated with antigen for 24 hours after which excess antigen was removed by washing the cells twice and resuspending in DC culture medium supplemented with 50ng/ml TNF-α (Peprotech, London, UK). DC were fed on

day 7 by replacement of a half volume DC culture medium supplemented with 50ng/ml TNF α and mature DC were harvested on day 8. The harvested mature DC were counted and viability assessed using trypan blue dye exclusion. The DC were then γ -irradiated (4000 rads) and resuspended at 2×10^5 cells per ml in AIM-V medium before use analysis in T cell proliferation and ELISpot assays as below. Additionally, on day 8, fresh CD4 $^+$ T cells were also prepared. To purify CD4 $^+$ T cells, PBMCs were revived in AIM-V $^{\circledR}$ culture medium and CD4 $^+$ cells isolated using Miltenyi CD4 Microbeads and LS columns (Miltenyi Biotech, Oxford, UK) and resuspended in AIM-V $^{\circledR}$ medium at 2×10^6 cells/ml.

[0128] On day 8, T cell proliferation assays were established whereby 1×10^5 autologous CD4 $^+$ T cells were added to 1×10^4 antigen-loaded DC (ratio of 10:1) in 96 well U-bottomed plates, with AIM-V $^{\circledR}$ medium added to a final volume 200ul/well. On day 14, assay plates were pulsed with 1uCi [3 H] (Perkin Elmer, Beaconsfield, UK) per well in 25ul AIM-V $^{\circledR}$ for 6 hours before harvesting onto filter mats (Perkin Elmer) using a TomTec Mach III (Hamden CT, USA) cell harvester. All polypeptides were tested in sextuplet cultures. Counts per minute (cpm) for each well were determined by Meltilex $^{\text{TM}}$ (Perkin Elmer) scintillation counting on a 1450 Microbeta Wallac Trilux Liquid Scintillation Counter (Perkin Elmer) in paralux, low background counting. Counts per minute for each antigen were normalised to the AIM-V $^{\circledR}$ medium only control.

[0129] For ELISpot assays, ELISpot plates (Millipore, Watford, UK) were coated with 100ul/well IL-2 capture antibody (R&D Systems, Abingdon, UK) in PBS. Plates were then washed twice in PBS, incubated overnight in block buffer (1% BSA (Sigma) in PBS) and washed in AIM-V $^{\circledR}$ medium. On day 8, 1×10^5 autologous CD4 $^+$ T cells were added to 1×10^4 antigen loaded DC (ratio of 10:1) in 96 well ELISpot plates. All polypeptide preparations were tested in sextuplet cultures. For each donor PBMC, a negative control (AIM-V $^{\circledR}$ medium alone), no cells control and a PHA (10ug/ml) positive control were also included.

[0130] After a further 7 day incubation period, ELISpot plates were developed by three sequential washes in dH $_2$ O and PBS prior to the addition of 100ul filtered biotinylated detection antibody (R&D Systems, Abingdon, UK) in PBS/1% BSA. Following incubation at 37°C for 1.5 hour, plates were further washed three times in PBS and 100ul filtered streptavidin-AP (R&D Systems) in PBS/1% BSA was added for 1 hour (incubation at room temperature). Streptavidin-AP was discarded and plates were washed four times in PBS. BCIP/NBT (R&D Systems) was added to each well and incubated for 30 minutes at room temperature. Spot development was stopped by washing the wells and the backs of the wells three times with dH $_2$ O. Dried plates were scanned on an Immunoscan $^{\text{TM}}$ Analyser and spots per well (spw) were determined using Immunoscan $^{\text{TM}}$ Version 4 software.

[0131] For both proliferation and IL-2 ELISpot assays, results were expressed as a Stimulation Index (SI) defined as the ratio of cpm (proliferation assay) or spots (ELISpot assay) for the test polypeptide against a medium-only control using a threshold of SI equal to or greater than 2 ($SI \geq 2.0$) for positive T cell responses.

Example 7: Design of Double and Triple Substitutions in Two or More of T Cell Epitopes 1, 2 and 3.

[0132] Based on the results of the binding assay, the following substitutions were chosen at epitopes 1, 2 and 3 to be present in polypeptides that contain two amino acid substitutions as compared to SEQ ID NO:2, each substitution in a different epitope.

Table 10. Amino Acid Substitutions for Variants Comprising Two Epitope and Three Epitope Modifications.

Epitope	Amino Acid	Original Amino Acid in SEQ ID NO:2	Substitution Amino Acids
1	54	Y	K, R
1	56	T	H, K
2	135	M	K, T
2	140	R	Q
3	174	K	R
3	178	D	N
3	181	W	H, R

[0133] DNA encoding N1-N2-Human IG Fc fusion proteins having two of the amino acid substitutions set forth in Table 10, each in a different epitope, were prepared by using site-directed mutagenesis of the appropriate starting DNA (typically the DNA encoding for one of the two substitutions prepared as set forth in Example 3. The resulting DNA encoding these fusion proteins were used to transform cells and were expressed and purified as set forth in Example 4, and tested for binding as set forth in Example 5. Polypeptides having one substitution in each of epitopes 1, 2 and 3 were then designed based on the results of the binding assay on the two amino acid substituted polypeptides. Polypeptides having one substitution in each of epitopes 1, 2 and 3 are assayed for both ABeta binding, as well as T-cell response as set forth in Example 6. In particular, the following double and triple epitope variants were made by substituting certain amino acids in SEQ ID NO:2 as indicated in Table 11, below.

Table 11. Double and Triple Epitope Variant Polypeptides of the Invention.

Polypeptide No.	Starting Sequence	Epitope 1 Substitution	Epitope 2 Substitution	Epitope 3 Substitution
63	SEQ ID NO:2	Y54K	M135K	
64	SEQ ID NO:2	Y54K	M135T	

Polypeptide No.	Starting Sequence	Epitope 1 Substitution	Epitope 2 Substitution	Epitope 3 Substitution
65	SEQ ID NO:2	Y54K	R140Q	
66	SEQ ID NO:2	Y54R	M135K	
67	SEQ ID NO:2	Y54R	M135T	
68	SEQ ID NO:2	Y54R	R140Q	
69	SEQ ID NO:2	T56H	M135K	
70	SEQ ID NO:2	T56H	M135T	
71	SEQ ID NO:2	T56H	R140Q	
72	SEQ ID NO:2	T56K	M135K	
73	SEQ ID NO:2	T56K	M135T	
74	SEQ ID NO:2	T56K	R140Q	
75	SEQ ID NO:2	Y54K		D178N
76	SEQ ID NO:2	Y54K		W181H
77	SEQ ID NO:2	Y54K		W181R
78	SEQ ID NO:2	Y54K		K174R
79	SEQ ID NO:2	Y54R		D178N
80	SEQ ID NO:2	Y54R		W181H
81	SEQ ID NO:2	Y54R		W181R
82	SEQ ID NO:2	Y54R		K174R
83	SEQ ID NO:2	T56H	D178N	
84	SEQ ID NO:2	T56H		W181H
85	SEQ ID NO:2	T56H		W181R
86	SEQ ID NO:2	T56H		K174R
87	SEQ ID NO:2	T56K		D178N
88	SEQ ID NO:2	T56K		W181H
89	SEQ ID NO:2	T56K		W181R
90	SEQ ID NO:2	T56K		K174R
91	SEQ ID NO:2		M135K	D178N
92	SEQ ID NO:2		M135K	W181H
93	SEQ ID NO:2		M135K	W181R
94	SEQ ID NO:2		M135K	K174R
95	SEQ ID NO:2		M135T	D178N
96	SEQ ID NO:2		M135T	W181H
97	SEQ ID NO:2		M135T	W181R
98	SEQ ID NO:2		M135T	K174R
99	SEQ ID NO:2		R140Q	D178N
100	SEQ ID NO:2		R140Q	W181H
101	SEQ ID NO:2		R140Q	W181R
102	SEQ ID NO:2		R140Q	K174R

[0134] The above-indicated polypeptides were assayed for binding to beta-amyloid using the ELISA assay set forth in Example 5. The results are set forth in Tables 12 and 13. Relative binding values reflect IC_{50} (polypeptide of SEQ ID NO:2)/ IC_{50} (tested polypeptide) (e.g., the lower the value the greater the binding of the polypeptide as compared to a polypeptide of SEQ ID NO:2). Multiple values reflect duplicate testing in the binding assay.

Table 12. Relative Binding Values of a Polypeptide of SEQ ID NO:2 Versus Exemplary Polypeptides of the Invention.

Polypeptide No.	Relative Binding Value
63	0.12
64	0.14
65	0.18
66	0.08
67	0.1
68	0.19
69	0.29, 0.37
70	0.43, 0.45
71	0.42
72	0.40, 0.27
73	0.25, 0.39
74	0.26
75	0.11

Polypeptide No.	Relative Binding Value
76	0.13
77	0.18, 0.16
78	0.24, 0.20
79	0.08
80	0.16
81	0.14
82	0.2
83	0.18, 0.36
84	0.26, 0.48
85	0.24, 0.79
86	0.51, 1.08
87	0.51, 0.83
88	0.65, 1.30
89	0.71, 1.05

Polypeptide No.	Relative Binding Value
90	1.43, 1.64
91	0.14
92	0.24
93	0.34
94	0.53, 0.48
95	0.07
96	0.15
97	0.14
98	0.21, 0.61
99	0.11
100	0.36
101	0.2
102	0.23

Example 8: Cellulose acetate filter retardation assay.

[0135] This assay was used to monitor the destabilization (disaggregation) or remodeling of amyloid fibers into non-amyloidogenic or soluble aggregates. The assay was primarily adapted from Chang, E. and Kuret, J., *Anal Biochem* 373, 330-6, (2008) and Wanker, E. E. et al., *Methods Enzymol* 309, 375-86, (1999). Specifically, 2.5 μ M preparations of fA β amyloid fibers were pre-incubated with different concentrations of the variant fusion polypeptides of the invention (1 nM to 2 μ M) at 37°C for 3 days. After incubation, fibers with and without fusion polypeptide were diluted and spotted on cellulose acetate membranes on vacuum blots. The membranes were extensively washed with PBS and probed with an antibody specific for the N-terminal of A β for 1 hr. HRP-conjugated secondary Ab was used to quantitate the fibrillar aggregates retained on the membrane. Spot color was analyzed and digitized using a

densitometric scanner. An EC₅₀ (half maximal effective concentration) was calculated based upon the intensities of the signal of each spot versus the concentration of fusion polypeptide added to each spot.

[0136] As can be seen from the above Examples, the variant polypeptides of the invention all exhibited binding to A β as determined by the ELISA assay. Most of the variant polypeptides tested also exhibited disaggregation of A β , as determined by the dot blot assay.

Example 9: Construction and Analysis of Polypeptides with a Modified Glycosylation

Signal

[0137] We constructed polypeptides lacking a glycosylation signal at amino acids 39-41 of SEQ ID NO:1 or SEQ ID NO:2 using the nucleotide sequence of either SEQ ID NO:3 or a modified version of nucleotide sequence SEQ ID NO:4 that encoded Polypeptide No. 86 as starting material for site-direct mutagenesis.

[0138] A plasmid vector derived from pFUSE-hIgG1-Fc2 vector (InVivogen) and encoding Polypeptide 86 fused to a mammalian signal sequence, was mutagenized using the QuickChange Site-Directed Mutagenesis Kit (Agilent) and the following primers:

Forward primer: GCTGTCTGTGGAATGCTGGAGGCGTTGTAGTTTG (SEQ ID NO:8)

Reverse primer: CAAACTACAACGCCTCCAGCATTCACAGACAGC (SEQ ID NO:9)

following manufacturer's directions to create a T41G substitution. The resulting vector (SEQ ID NO:7) was used to transform NEB 5-alpha competent E. coli cells in order to isolate and sequence the desired plasmid using standard techniques.

[0139] The purified vector was then used to transform Expi293 cells using the commercially available Expi293TM Expression System (Life Technologies). One day before transfection, Expi293 cells were seeded at a density of 2×10^6 viable cells/ml. On the day of transfection, 500 μ g of the filter-sterilized plasmid was diluted into Opti-MEM I to a total volume of 25 ml. In a separate tube, 1.333 ml ExpiFectamineTM 293 Reagent was diluted in 25 ml Opti-MEM I and mixed by inverting. After five minutes incubation at room temperature the diluted DNA was added to the diluted ExpiFectamineTM 293 Reagent and incubated for an additional 20-30 minutes. The DNA-ExpiFectamineTM 293 Reagent complex was slowly added to 500 ml cells ($>3 \times 10^6$ cells/ml) while gently swirling the flask. ExpiFectamineTM 293 Transfection Enhancers I and II, 2.5ml and 25ml respectively, were added to the transfected cells after approximately 18 hours and cells are incubated for another 5 days at 37°C, 8% CO₂, 135 rpm on an orbital shaker. The expressed fusion protein (termed "Polypeptide 86-T41G")-containing media was harvested by

centrifugation at 10,000 rpm at 4°C for 20 minutes. The supernatant was purified on a 5ml HiTrap rProtein A FF column (GE Healthcare), with all steps being performed at 4°C. The column was regenerated with 5 volumes of elution buffer (0.1M glycine, pH 3), and washed in 5-10 volumes 20 mM sodium phosphate buffer before applying the cell media using a flow rate of 5ml/min. The column was washed with 5-10 volumes 20 mM sodium phosphate buffer before eluting off bound Polypeptide 86-T41G with 0.1M glycine pH 3. One to three ml fractions were collected in tubes with 1M Tris-HCl pH 9 to adjust pH. Yield was determined by absorbance at 280 nm on a Nanodrop 2000C. Five µl of each protein-containing fraction was separated on a SDS-PAGE TGX gel (BioRad) and Coomassie stained for 2 hours. Fractions containing Polypeptide 86-T41G were pooled and dialyzed in D-PBS overnight at 4°C. The final Polypeptide 86-T41G sample was sterilized on Ultrafree spin filters and the concentration was measured on the Nanodrop 2000C.

[0140] Purified Polypeptide 86-T41G (SEQ ID NO:6) was analyzed by SDS-PAGE and migrated as a single band with slightly lower molecular weight (apparent ~500 dalton less) than Polypeptide 86 (Figure 9). We believe this lower molecular weight is due to both the T to G change at amino acid 41, as well as the loss of glycosylation on N39.

[0141] Purified Polypeptide 86-T41G was also analyzed by size exclusion chromatography on a Superdex200 increase 10/300 column. The column was washed and equilibrated with 100 ml of phosphate buffered saline ("PBS"). One hundred micrograms (100 µg) of Polypeptide 86-T41G was diluted in PBS to a final volume of 200 µL and loaded onto the column. The column was then eluted with 1.5 column volumes of PBS at a rate of 0.75 mL/minute. Protein in fractions was monitored by spectrophotometrically at 214 nm and 280 nm and demonstrated a sharp peak indicating homogeneity (data not shown).

[0142] Purified Polypeptide 86-T41G was analyzed for Abeta binding using the ELISA described in Example 5. The EC₅₀ for Abeta binding in this assay was calculated to be 13.15 nM, compared to 20.6-27.01 nM for the polypeptide of SEQ ID NO:1 and 34.5 nM for Polypeptide 86.

[0143] Purified Polypeptide 86-T41G was also compared to the polypeptide of SEQ ID NO:1 and Polypeptide 86 for Abeta binding using the cellulose acetate filter retardation assay described in Example 8. The results of this assay are shown in Figure 10.

[0144] Purified Polypeptide 86-T41G was then compared to Polypeptide 86 and the polypeptide of SEQ ID NO:1 (as well as humanized A33 antibody and keyhole limpet hemocyanin as positive controls) in the whole protein CD4+ T cell Response assay using 50 different PBMC donors

representing 95% of the human HLA haplotypes; and in the ELISpot cytokine (IL-2) assays described in Example 6. The results are shown in Tables 13 and 14, below.

Table 13. PBMC T-cell Proliferative Response Assay Results.

Sample	Mean SI	SD	% Response
SEQ ID NO:1	2.21	0.32	12
Polypeptide 86	2.66	1.02	4
Polypeptide 86 T41G	2.11	0.15	4
Humanized A33	3.29	1.83	12
KLH	4.74	3.28	84

Table 14. ELISpot IL-2 Assay Results.

Sample	Mean SI	SD	% Response
SEQ ID NO:1	2.51	0.64	14
Polypeptide 86	2.83	1.03	4
Polypeptide 86 T41G	2.33	0.22	4
Humanized A33	2.46	0.33	20
KLH	4.57	4.32	86

[0145] As can be seen from the above Tables the polypeptide of SEQ ID NO:1 (no amino acid changes in either the putative glycosylation site at amino acids 39-41 or any putative T-cell epitopes) elicited proliferative responses ("SI") >2 times background for 12% of the donors (6/50). Polypeptide 86 and Polypeptide 86 T41G elicited proliferative responses from significantly fewer donor PBMCs (4%; 2/50) with responders having proliferative response also slightly higher than 2 times background. This indicates lower projected immunogenicity of Polypeptide 86 T41G for human subjects. The IL-2 assay confirms the T-cell response assay results.

[0146] Polypeptide 86 T41G was also compared to the polypeptide of SEQ ID NO:1 for binding to Abeta42 fibers, NAC fibers and tau-mtbr fibers.

[0147] *Fiber and ELISA Plate Preparation.* A β 42 peptide (rPeptide A-1002-2) was dissolved in hexafluoroisopropanol by vortexing and incubation at room temperature for 18 hours. Aliquots were dried under vacuum and stored at -20°C. 100 ug of A β 42 monomers were dissolved in 20 μ l DMSO, dissolved by vortex and diluted to 80 μ M in 10 mM HCl solution. The A β 42 peptide solution was incubated for 3 days at 37°C and fiber formation verified with ThT fluorescence assay.

[0148] The non-amyloid beta component (NAC) of senile plaque is an aggregated fragment of alpha-synuclein, the aggregate that is the hallmark of Parkinson's disease. NAC peptide ((Bachem H2598) was dissolved in 20mM NaHCO₃ at 600uM and centrifuged for 1 hour, 100,000xg at 4°C. Supernatant was neutralized with 2N HCl and mixed 1:1 with 10mM HCl. The peptide was incubated for 4 days at 37°C and fiber formation confirmed by ThT fluorescence assay.

[0149] Fibers comprising the microtubule binding portion of Tau (Tau-mtbr fibers) were made according to Frost et al. J Biol Chem. 2009 May 8;284(19):12845-52. Briefly, 40uM of tau-mtbr protein was incubated with 40uM low-molecular weight heparin (Fisher Scientific, BP2524) and 2mM DTT for 3 days at 37°C. Fibril formation was confirmed by ThT fluorescence assay.

[0150] Fibers were diluted to 1uM in PBSA-0.02% and dry-coated on Maxisorp Nunc Immunoplate ELISA plates (ThermoFisher Cat no.442404) by incubation over night at 37°C. Wells were blocked, 200ul/well, in Superblock (ThermoFisher Cat no. 37515) for 1 hour at room temperature and washed in PBST-0.05%.

[0151] *Binding Assay and Results.* The polypeptide of SEQ ID NO:1 and Polypeptide 86 T41G were separately added to the fiber ELISA at 50 and 200nM and incubated for 1 hour at 37°C. Wells were washed in PBST-0.05% 6x200ul before incubation with goat anti-human IgG Fc fragment specific-HRP (Jackson labs Cat no. 109-035-008), 1:2500 in TBST-0.05%; 1% milk block (LabScientific Cat no. 732-291-1940), for 45 minutes in room temperature. Plates were washed in 4x200 ul TBST-0.05%, 2x200 ul PBS before adding 50 ul TMB solution (Sigma T0440) per well. The reaction was left to develop for 8 minutes and stopped by adding 50 ul 2N HCl per well. The absorbance at 450 nm was recorded in a Tecan plate reader (Infinite M1000Pro).

[0152] Data points were taken from the average of triplicate wells with standard deviation calculated with GraphPad Prism. The values were corrected for background by subtracting the mean absorbance in wells incubated without either polypeptide for each substrate.

[0153] As shown in Figure 11, Polypeptide 86 T41G bind Aβ42m NAC and tau-mtbr fibers with the same or higher affinity compared to the polypeptide of SEQ ID NO:1.

[0154] We also constructed by similar protocols the following variants of SEQ ID NO:1 modified only to eliminate the putative glycosylation site (substitution indicated in parentheses):

Polypeptide 200 (N39A)	Polypeptide 202 (T41M)	Polypeptide 204 (T41H)
Polypeptide 201 (N39Q)	Polypeptide 203 (T41W)	Polypeptide 205 (T41V)

Polypeptide 206 (T41I)	Polypeptide 211 (T41F)	Polypeptide 216 (T41A)
Polypeptide 207 (T41L)	Polypeptide 212 (T41D)	Polypeptide 217 (T41G).
Polypeptide 208 (T41R)	Polypeptide 213 (T41E)	
Polypeptide 209 (T41K)	Polypeptide 214 (T41Q)	
Polypeptide 210 (T41Y)	Polypeptide 215 (T41N)	

Example 10: Pharmacokinetic (PK) Studies of Polypeptide 217 and SEQ ID NO:1.

[0155] *Animal treatment and sample collection.* C57Bl6 mice (8 - 12 wks; Hilltop Lab Animals) were administered a single 20 mg/kg intraperitoneal dose of the polypeptide of SEQ ID NO:1 (n=22) or Polypeptide 217 (n=22) used. The polypeptide of SEQ ID NO:1 was administered once (20 mg/kg, i.p.) to 22 mice. Polypeptide 217 was administered once (20 mg/kg, i.p.) to a separate set of 22 mice. Blood was collected once each animal at different times (0h, 6h, 9h, 12h, 1d, 3d, 7d and 14d post-dosing). Plasma was isolated from the blood samples, stored in 100 μ L aliquots, and used for all subsequent analyses. After collection of the blood, mice were euthanized, transcardially perfused with PBS and their brains harvested. The brains were hemisected and each hemisphere further sectioned into a rostral, caudal, hippocampus and cerebellum portion. Plasma was shipped to Intertek (San Diego, CA) for pharmacokinetic analysis, while left frontal cortex was shipped to Cambridge Biomedical (Boston, MA) for PK analysis.

[0156] *Plasma ELISA Analysis.* All standards and samples that were analyzed were exposed to 217 mM acetic acid for 30 min at room temperature ("RT"), and then neutralized in (1:1.5 v/v 1M Tris pH 9.5:sample). The acid dissociation step solubilized polypeptide present in an insoluble fraction.

[0157] A sandwich ELISA assay was used to measure polypeptide levels in plasma. MaxiSorp™ plates were coated with rabbit anti-M13 (Abcam: ab6188) at 1:1,000 dilution from stock (3.7 μ g/mL, 0.37 μ g/well) overnight in carbonate buffer (pH 9.6) at 4 °C. Plates were washed three times with PBS containing 0.1% Tween-20 ("PBST") and blocked with 1% milk in PBS for 2h at 37 °C followed by 1h at RT. Plates were again washed three times with PBST and then samples or standards were added to wells and incubated for 1h at 37 °C. Wells were then washed 3X with PBST, and incubated with HRP-labeled goat anti-Human IgG (heavy & light chains, Bethel: A80-219P; 1:10,000) for 30 min at RT. Wells were washed 3x with PBST, and the plates were

then developed at RT with TMB substrate. Reactions were stopped after the A_{450} of the highest standards was between 0.6 – 0.8. Levels of polypeptide were quantified from the absorbance read at 450 nm, minus the reference absorbance at 650 nm. Plasma was analyzed at dilutions of 1:20, 1:300 and 1:3,000; no matrix interference was observed at these dilutions. The results are shown below in Table 15.

Table 15. Plasma Pharmacokinetic Parameters.

Parameter	SEQ ID NO:1	Polypeptide 217
C_{max}	140 $\mu\text{g/mL}$	179 $\mu\text{g/mL}$
T_{max}	6 h	6 h
Beta-phase $\frac{1}{2}$ life	5 days	10 days
Clearance	24.5 mL/day/kg	8.3 mL/day/kg
AUC	816.33 day* $\mu\text{g/mL}$	2396.1 day* $\mu\text{g/mL}$

[0158] *Brain ELISA Analysis.* Brain tissue (left frontal cortex) was homogenized in cold PBS using trip Pure M-Bio Grade beaded tubes and a Precellys024 Lysis Homogenizer (5,000 RPM twice for 20 sec, with a 5 sec interval between homogenization cycles). Homogenate was centrifuged at 14,000 rpm for 5 min at 4 °C. Supernatant was removed to a new tube and used for all subsequent analyses. Protein content of brain lysate was determined using a Pierce BCA protein assay kit. Lysate was used at a 1:2 dilution.

[0159] A sandwich ELISA assay was used to measure polypeptide levels in brain. MaxiSorp™ plates were coated with rabbit anti-M13 (Abcam: ab6188) at 1:1,000 (3.7 $\mu\text{g/mL}$, 0.37 $\mu\text{g/well}$) overnight in carbonate buffer at 4 °C. Plates were washed 3x with PBST and blocked with 1% milk in PBS for 2h at 37 °C, followed by 1h at RT. Plates were then washed 3x with PBST, and samples or standards were added to wells and incubated for 1h at 37 °C. Plates were again washed 3x with PBST, and then wells were incubated with HRP-labeled donkey anti-Human IgG (heavy & light chains, Jackson ImmunoResearch: 709-035-149; 1:10,000) for 30 min at RT. After 3x washes with PBST, plates were developed for 15 min at RT with TMB substrate. Reactions

were stopped and absorbance read at 450 nm. Levels of polypeptide in brain were expressed relative to protein content of lysates. The results are shown below in Table 16.

Table 16. Brain Pharmacokinetic Parameters

Parameter	SEQ ID NO:1	Polypeptide 217
C _{max}	2.5 ng/mg	2.7 ng/mg
T _{max}	3 d	3 d
Beta-phase ½ life	3 days	7 days
AUC	14.44 day*ng/mg	32.90 day*ng/mg

Claims:

1. A polypeptide comprising a variant of a starting amino acid sequence, wherein the starting amino acid sequence is selected from: amino acids 1-217 of SEQ ID NO:1 or amino acids 1-217 of SEQ ID NO:2 and mutants of any of the foregoing having one or more of the following modifications: substitution of VVV at amino acids 43-45 with AAA; substitution C53W; deletion of amino acids 96-103; substitution of QPP at amino acids 212-214 with AGA; substitutions W181A, F190A and F194A; deletion of amino acid 1; deletion of amino acids 1 and 2; and addition of a N-terminal methionine residue, wherein:

- (a) the starting amino acid sequence is modified to remove the putative glycosylation signal at amino acids 39-41; and
- (b) the polypeptide binds to and/or disaggregates amyloid.

2. The polypeptide according to claim 1, wherein the modification at amino acids 39-41 is selected from substitution of one or more of N39, A40 and/or T41; deletion of one or more of N39, A40 and/or T41; insertion of one or more amino acids between N39 and A40; and insertion of one or more amino acids between A40 and T41.

3. The polypeptide of claim 1 or 2, wherein the starting amino acid sequence is selected from: amino acids 1-217 of SEQ ID NO:1, and amino acids 1-217 of SEQ ID NO:2 .

4. The polypeptide of any one of claims 1-3, wherein the modification at amino acids 39-41 of SEQ ID NO:1 or SEQ ID NO:2 is selected from amino acid substitution of one or more of N39 and/or T41.

5. The polypeptide of claim 4, wherein the modification that removes the putative glycosylation signal at amino acids 39-41 of SEQ ID NO:1 or SEQ ID NO:2 is an amino acid substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A.

6. The polypeptide of claim 5, wherein the modification that removes the putative glycosylation signal at amino acids 39-41 of SEQ ID NO:1 or SEQ ID NO:2 is a T41G substitution.

7. The polypeptide of any one of claims 3-6, wherein:

- (c) the polypeptide has reduced immunogenicity as compared to a corresponding polypeptide comprising the starting amino acid sequence; and

(d) the variant has from 1 to 9 amino acid substitutions in addition to any amino acid substitution at amino acids 39-41 of SEQ ID NO:1 or SEQ ID NO:2 or mutants thereof, wherein each amino acid substitution is selected from the group of amino acid substitutions set forth below:

Amino Acid #	Amino Acid present in the Starting Amino Acid Sequence	Amino Acid Substitutions
48	G	H, K, R, S, T, D, P
50	E	G, H, K, P, R
51	T	G, H, K, R, P, Q, N, W
53	C	F, H, K, N, Q, R, W, Y
54	Y	G, H, K, R, P
56	T	G, H, K, R, P
135	M	A, D, G, K, N, T, H, R, C, E, P, Q, S
137	Q	D, E
138	N	D, E, G, H, P, Q, S, T
140	R	D, E, H, Q, A, G, M, N, P, S, Y
141	F	D, E
143	N	A, G
173	S	G, P, K, D, H, R, T
174	K	R
175	A	G, H, K, P, R
176	M	G, H, K, N, R, P, Q, W
178	D	G, N, Q, S, T, F, H, K, R, W, Y
179	A	H, K, P, R
181	W	G, H, K, R, P

and

(e) when the starting amino acid sequence is amino acids 1-217 of SEQ ID NO:1 any of the 1 to 9 amino acid substitutions is optionally additionally selected from the group of amino acid substitutions set forth below:

Amino Acid #	Amino Acid present in the Starting Amino Acid Sequence	Amino Acid Substitutions
215	V	S, T, C, D, E, F, H, K, N, P, Q, R
218	G	C, E, N, P, Q, S, T, A, H, W
220	G	E, D, F, W, M, Y
221	S	D, E, G
223	G	D, P, E, K, N, R, T

8. The polypeptide of claim 7, wherein each of the 1 to 9 amino acid substitutions is selected from the group of amino acid substitutions set forth below:

Amino Acid #	Amino Acid present in the Starting Amino Acid Sequence	Amino Acid Substitutions
48	G	H, K, R, S, T
51	T	G, H, K, R, P, Q, N
54	Y	G, H, K, R, P
56	T	G, H, K, R, P
135	M	A, D, G, K, N, T, H, R
140	R	D, E, H, Q, A, G
141	F	D, E
143	N	A, G
173	S	G, P, K
174	K	R
176	M	G, H, K, N, R
178	D	G, N, Q, S, T
181	W	G, H, K, R

9. The polypeptide of any one of claims 3-8, wherein the starting amino acid sequence is selected from amino acids 1-217 of SEQ ID NO:1, and amino acids 1-217 of SEQ ID NO:2.

10. The polypeptide of any one of claims 3-9, wherein the variant amino acid sequence has 2 to 9 amino acid substitutions in addition to any amino acid substitution at amino acids 39-41 of SEQ ID NO:1 or SEQ ID NO:2 or mutants thereof, wherein at least one substitution is present in epitope 1, comprising amino acids 48-56 of SEQ ID NO:1 or SEQ ID NO:2; and wherein at least one substitution is present in epitope 3, comprising amino acids 173-181 of SEQ ID NO:1, or SEQ ID NO:2.

11. The polypeptide of claim 10, wherein the variant amino acid sequence has only two amino acid substitutions, in addition to any amino acid substitution at amino acids 39-41 of SEQ ID NO:1

or SEQ ID NO:2 or mutants thereof; and wherein the substitutions are selected from the group of two amino acid substitutions set forth below:

Y54K and D178N	Y54K and W181H	Y54K and W181R	Y54K and K174R
Y54R and D178N	Y54R and W181H	Y54R and W181R	Y54R and K174R
T56H and D178N	T56H and W181H	T56H and W181R	T56H and K174R
T56K and D178N	T56K and W181H	T56K and W181R	T56K and K174R

12. The polypeptide of any one of claims 1-11, consisting essentially of a human or humanized immunoglobulin Fc polypeptide sequence fused either via a peptide linker or directly to the C-terminus of the variant amino acid sequence.

13. The polypeptide of claim 12, wherein the immunoglobulin Fc polypeptide sequence is the Fc portion of a human IgG.

14. The polypeptide of claim 13, wherein the amino acid sequence of the peptide linker and Fc portion of human IgG is selected from amino acids 218-488 of SEQ ID NO:1, and amino acids 218-486 of SEQ ID NO:2.

15. A polypeptide consisting of a variant of a starting amino acid sequence, wherein the starting amino acid sequence is selected from SEQ ID NO:1 or SEQ ID NO:2 and the starting amino acid sequence is modified to remove the putative glycosylation signal at amino acids 39-41 of SEQ ID NO:1 or SEQ ID NO:2, wherein the modification is selected from amino acid substitution of one or more of N39, A40 and/or T41; deletion of one or more of N39, A40 and/or T41; insertion of one or more amino acids between N39 and A40; and insertion of one or more amino acids between A40 and T41.

16. The polypeptide of claim 15, wherein the modification that removes the putative glycosylation signal at amino acids 39-41 of SEQ ID NO:1 or SEQ ID NO:2 is selected from amino acid substitution of one or more of N39 and/or T41.

17. The polypeptide of claim 16, wherein the modification that removes the putative glycosylation signal at amino acids 39-41 of SEQ ID NO:1 or SEQ ID NO:2 is an amino acid substitution selected from T41G, T41W, T41H, T41V, T41I, T41L, T41R, T41K, T41Y, T41F, T41D, T41E, T41Q, T41N, and T41A.

18. The polypeptide of claim 17, wherein the modification that removes the putative glycosylation signal at amino acids 39-41 of SEQ ID NO:1 or SEQ ID NO:2 is a T41G substitution.

19. The polypeptide of any one of claims 15-18, wherein the starting amino acid sequence is SEQ ID NO:2, and the polypeptide has two amino acid substitutions in addition to any amino acid substitution at amino acids 39-41 of SEQ ID NO:2 selected from any of the following:

Polypeptide No.	Epitope 1 Substitution	Epitope 2 Substitution	Epitope 3 Substitution
75	Y54K	None	D178N
76	Y54K	None	W181H
77	Y54K	None	W181R
78	Y54K	None	K174R
79	Y54R	None	D178N
80	Y54R	None	W181H
81	Y54R	None	W181R
82	Y54R	None	K174R
83	T56H	None	D178N
84	T56H	None	W181H
85	T56H	None	W181R
86	T56H	None	K174R
87	T56K	None	D178N
88	T56K	None	W181H
89	T56K	None	W181R
90	T56K	None	K174R

20. The polypeptide of claim 19, wherein the polypeptide has the amino acid sequence of SEQ ID NO:5.

21. A pharmaceutical composition comprising the polypeptide of any one of claims 1-20 and a pharmaceutically acceptable carrier.

22. The pharmaceutical composition of claim 21, wherein the composition is formulated for injection or infusion into the bloodstream of a patient.

23. The pharmaceutical composition of claim 21, wherein the composition is formulated for direct administration to the brain or CNS.

24. A method of reducing amyloid or tau protein aggregates in a patient in need thereof, comprising administering to the patient an effective amount of the polypeptide of any one of claims 1-18 or the pharmaceutical composition of any one of claims 21-24.

25. The method of claim 24, wherein the patient is exhibiting symptoms of a neurodegenerative disease that is associated with the presence of amyloid or tau protein aggregates.

26. The method of claim 24 or claim 25, wherein the patient is positive for the biomarker florbetapir when that biomarker is used as an imaging agent in positron emission tomography.

27. The method of any one of claims 24-26, wherein the patient is suffering from a neurodegenerative disease selected from Alzheimer's disease, which includes early onset Alzheimer's disease, late onset Alzheimer's disease, and presymptomatic Alzheimer's disease, Parkinson's disease, SAA amyloidosis, cystatin C, hereditary Icelandic syndrome, senility, multiple myeloma, prion diseases including but not limited to kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker disease (GSS), fatal familial insomnia (FFI), scrapie, and bovine spongiform encephalitis (BSE); amyotrophic lateral sclerosis (ALS), spinocerebellar ataxia (SCA1, SCA3, SCA6, or SCA7), Huntington disease, entorubral-pallidolusian atrophy, spinal and bulbar muscular atrophy, hereditary cerebral amyloid angiopathy, familial amyloidosis, British/Danish dementia, familial encephalopathy, Amyotrophic lateral sclerosis/parkinsonism-dementia complex, Argyrophilic grain dementia, Corticobasal degeneration, Dementia pugilistica, diffuse neurofibrillary tangles with calcification, Down's syndrome, Gerstmann-Sträussler-Scheinker disease, Hallervorden-Spatz disease, Myotonic dystrophy, Niemann-Pick disease type C, Non-Guamanian motor neuron disease with neurofibrillary tangles, Pick's disease, Postencephalitic parkinsonism, Prion protein cerebral amyloid angiopathy, Progressive subcortical gliosis, Progressive supranuclear palsy, Subacute sclerosing panencephalitis, Tangle only dementia, frontotemporal lobar degenerations (FTLDs), and frontotemporal lobe dementia (FTD) including a patient having one or more of the following clinical syndromes: behavioral variant FTD (bvFTD), progressive non-fluent aphasia (PNFA), frontotemporal dementia with parkinsonism linked to chromosome 17, Progressive Supranuclear Palsy (PSP), and semantic dementia (SD).

28. The method of claim 27, wherein the neurodegenerative disease is Parkinson's disease, Alzheimer's disease, or Huntington's disease.

29. The method of claim 28, wherein the neurodegenerative disease is Alzheimer's disease.

30. The method of claim 27, wherein the patient is suffering from prion-mediated disease selected from Creutzfeldt-Jakob disease, kuru, fatal familial insomnia, or Gerstmann-Sträussler-Scheinker syndrome.

31. A nucleic acid sequence encoding any one of the polypeptides of claims 1-20.

32. The nucleic acid sequence of claim 31 further encoding a mammalian signal sequence fused to and in frame with the polypeptide encoding sequence.

33. The nucleic acid sequence of claim 32, wherein the nucleic acid sequence in SEQ ID NO:6.

34. A vector comprising a nucleic acid sequence of any one of claims 31-33, wherein the nucleic acid sequence is operatively linked to an expression control sequence in the vector.

35. A host cell containing the vector of claim 34.

36. A method of making a polypeptide of any one of claims 1-20 comprising the steps of expressing the protein encoded by the nucleic acid sequence of any one of claims 31-33; and isolating the expressed polypeptide.

37. A method of making a polypeptide of any one of claims 1-20 comprising the steps of culturing the host cell of claim 35 under conditions sufficient to allow expression of the polypeptide; and isolating the expressed polypeptide.

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FIG. 1 (SEQ ID NO:1)

1	A E T V E S C L A K P H T E N S F T N V W K	22
23	D D K T L D R Y A N Y E G C L W <u>N A T</u> G V V	44
45	V C T <u>G D E T Q C Y G T</u> W V P I G L A I P E	66
67	N <u>E G G G S E G G G S E G G G S E G G G</u> T K	88
89	P P E Y G D T P I P G Y T Y I N P L D G T Y	110
111	P P G T E Q N P A N P N P S L E E S Q P L N	132
133	T F <u>M F Q N N R F R N</u> R Q G A L T V Y T G T	154
155	F T Q G T D P V K T Y Y Q Y T P V S <u>S K A M</u>	176
177	<u>Y D A Y W</u> N G K F R D C A F H S G F N E D P	198
199	F V C E Y Q G Q S S D L P Q P P <u>V N A G G G</u>	220
221	<u>S G G</u> <u>G S Q G G S E G G G S E G G G S E S G</u>	242
243	<u>G S E G G G S E G G G S G S G</u> A <u>M V R S D K T</u>	264
265	<u>H T</u> C P P C P A P E L L G G P S V F L F P P	286
287	K P K D T L M I S R T P E V T C V V V D V S	308
309	H E D P E V K F N W Y V D G V E V H N A K T	330
331	K P R E E Q Y N S T Y R V V S V L T V L H Q	352
353	D W L N G K E Y K C K V S N K A L P A P I E	374
375	K T I S K A K G Q P R E P Q V Y T L P P S R	396
397	E E M T K N Q V S L T C L V K G F Y P S D I	418
419	A V E W E S N G Q P E N N Y K T T P P V L D	440
441	S D G S F F L Y S K L T V D K S R W Q Q G N	462
463	V F S C S V M H E A L H N H Y T Q K S L S L	484
485	S P G K	

FIG. 2 (SEQ ID NO:2)

1	A E T V E S C L A K P H T E N S F T N V W K	22
23	D D K T L D R Y A N Y E G C L W <u>N A T</u> G V V	44
45	V C T <u>G D E T Q C Y G T</u> W V P I G L A I P E	66
67	N E G G G S E G G G S E G G G S E G G G T K	88
89	P P E Y G D T P I P G Y T Y I N P L D G T Y	110
111	P P G T E Q N P A N P N P S L E E S Q P L N	132
133	T F <u>M F Q N N R F R N</u> R Q G A L T V Y T G T	154
155	F T Q G T D P V K T Y Y Q Y T P V S <u>S K A M</u>	176
177	<u>Y D A Y W</u> N G K F R D C A F H S G F N E D P	198
199	F V C E Y Q G Q S S D L P Q P P A N A G G E	220
221	S G G G S G G G S E G G G S E G G G S E G G	242
243	G S E G G G S G G G S G S G A R S D K T	262
263	H T C P P C P A P E L L G G P S V F L F P P	284
285	K P K D T L M I S R T P E V T C V V V D V S	306
307	H E D P E V K F N W Y V D G V E V H N A K T	328
329	K P R E E Q Y N S T Y R V V S V L T V L H Q	350
351	D W L N G K E Y K C K V S N K A L F A P I E	372
373	K T I S K A K G Q P R E P Q V Y T L P P S R	394
395	E E M T K N Q V S L T C L V K G F Y P S D I	416
417	A V E W E S N G Q P E N N Y K T T P P V L D	438
439	S D G S F F L Y S K L T V D K S R W Q Q G N	460
461	V F S C S V M H E A L H N H Y T Q K S L S L	482
483	S P G K	

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SEQ ID NO:3

ATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCAAGAAATTCGATGGCTGAAACTGTTGAAAG
 TTGTTTAGCAAAACCCCATACAGAAATTCATTACTAACGCTGGAAGACGACAAAACTTTAGATCGTTACGCTAACT
 ATGAGGGCTGTCTGTGGAATGCTACAGCGCTTGTAGTTGTACTGGTGACGAAACTCAGTGTACGGTACATGGGTTCCCT
 ATTGGGCTTGCTATCCCTGAATAATGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGTTCTGAGGGTGGCGG
 TACTAAACCTCCTGAGTACGGTGATACACCTATTCGGGCTATACCTTATCAACCCCTCTCGACGGCACTTATCCGCCTG
 GTACTGAGCAAAACCCCGCTAATCCTAATCCTTCTCTTGAGGAGTCTCAGCCTCTTAATACTTTCATGTTCAGAAATAAT
 AGGTTCCGAAATAGCGAGGGGCATTAACTGTTTATACGGGCACTTTTACTCAAGGCACCTGACCCCGTTAAACCTTATTA
 CCAGTACACTCCTGTATCATCAAAAGCCATGTATGACGCTTACTGGAACGGTAAATTCAGAGACTGCGCTTCCATTCTG
 GCTTTAATGAGGATCCATTCTGTTGTGAATATCAAGGCCAATCGTCTGACCTGACCTCAACCTCTGTCAATGTGCGCGC
 GGCTCTGGTGGTCTGTTGGCGGCTCTGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGG
 AGGCGGTTCCGGTGGTGGCTCTGGTTCGGTGCCATGGTTAGATCTGACAAAACTCACACATGCCCACTGCCAGCAC
 CTGAACTCCTGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAG
 GTCACATGCGTGGTGGACGTGAGCCACGAACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
 TAATGCCAAGACAAAGCCGCGGAGGAGCAACACAGCACGTACCGTGTGGTCAAGCTCCTCAGCTCCTGCACCCAGG
 ACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAGCCCTCCAGCCCTCATCGAGAAACCATCTCCAAA
 GCCAAAGGCAGCCCCGAGAACCCACAGGTGTACACCTGCCCCCATCCCGGAGGAGATGACCAAGAACCCAGTCAAGCCT
 GACCTGCCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAAGCCGGAGAACACTACA
 AGACCAAGCCTCCCGTGGACTCCGACGGCTCCTTCTCTACAGCAAGCTCACCGTGGACAAAGAGCAGGTGGCAG
 CAGGGAAACGCTCTTCTCATGTCTCCGTGATGCACGAGGCTCTGCAACAACCACTACACGCAAGAGCCTCTCCCTGTCTCC
 GGTAAATGA

FIG. 3

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SEQ ID NO:4

ATGTACAGGATGCAACTCCTGTCTTGCATTGCACCTAAGTCTTGCACTTGTCAAGAAATTCGATGGCTGAAACTGTTGAAAG
 TTGTTTAGCAAAACCCCATACAGAAATTCATTACTAACGCTGGAAGACGACAAAACTTTAGATCGTTACGCTAACT
 ATGAGGGCTGTCTGTGGAATGCTACAGCGCTTGTAGTTGTACTGGTGACGAAACTCAGTGTACGGTACATGGGTTCCCT
 ATTGGGCTTGCTATCCCTGAATAATGAGGGTGGTGGCTCTGAGGGTGGCGGTCTCTGAGGGTGGCGGTCTCTGAGGGTGGCGG
 TACTAAACCTCCTGAGTACGGTGATACACCTATTCCGGGCTATACCTATCAACCCCTCTCGACGGCACTTATCCGCCTG
 GTACTGAGCAAAACCCCGCTAATCCTAATCCTCTCTTGAGGAGTCTCAGCCTCTTAATACTTTTCATGTTCAGAAATAAT
 AGGTTCCGAAATAGGCAGGGGCATTAACTGTTTATACGGGCCTTTTACTCAAGGCACCTGACCCCGTTAAACTTATTA
 CCAGTACACTCCTGTATCATCAAAAGCCATGTATGACGCTTACTGGAACGGTAAATTCAGAGACTGCGCTTTCATTTCTG
 GCTTTAATGAGGATCCATTCTGTTGTGAATATCAAGCCCAATCGTCTGACCTGCTCAACCTCTGCAATGTGCGCGG
 GAgTCTGTGGTGGTCTGTGGTGGGCTCTGAGGGTGGTGGCTCTGAGGGTGGCGGTCTCTGAGGGTGGCGGTCTCTGAGGG
 AGGCGGTTCCGGTGGTGGCTCTGTGTTCCGGTGGCAGATCTGACAAACTCAACATGCCCCACCGTGCCCCAGCACCTGAAC
 TCCTGGGGGACCGTCAGTCTTCCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCACA
 TGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGAGGTGCATAATGC
 CAAGACAAAGCCGGGAGGAGCAGTACAAACAGCACGTACCGTGTGGTCAAGGTCTCACCGTCTGCAACGAGACTGGC
 TGAATGGCAAGGAGTACAAGTCAAGTCTCCAACAAGCCCTCCAGCCCCCATCGAGAAACCATCTCCAAGCCAAA
 GGCAGCCCCGAGAACCCACAGGTGTACACCCCTGCCCCCATCCCGGAGGAGATGACCAAGAACCCAGTCAAGCTGACCTG
 CCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAAGCCGGAGAACCACTACAAGACCA
 CGCCTCCCGTGGACTCCGACGGCTCCTTCTTCCCTCTACAGCAAGCTCACCGTGGACAGAGCAGGTGGCAGCAGGGG
 AACGTCTTCTCATGTCCGTGATGCACGAGGCTCTGACAAACCACTACACGCAAGAGCCCTCTCCCTGTCTCCGGGTAA
 ATGA

FIG. 4

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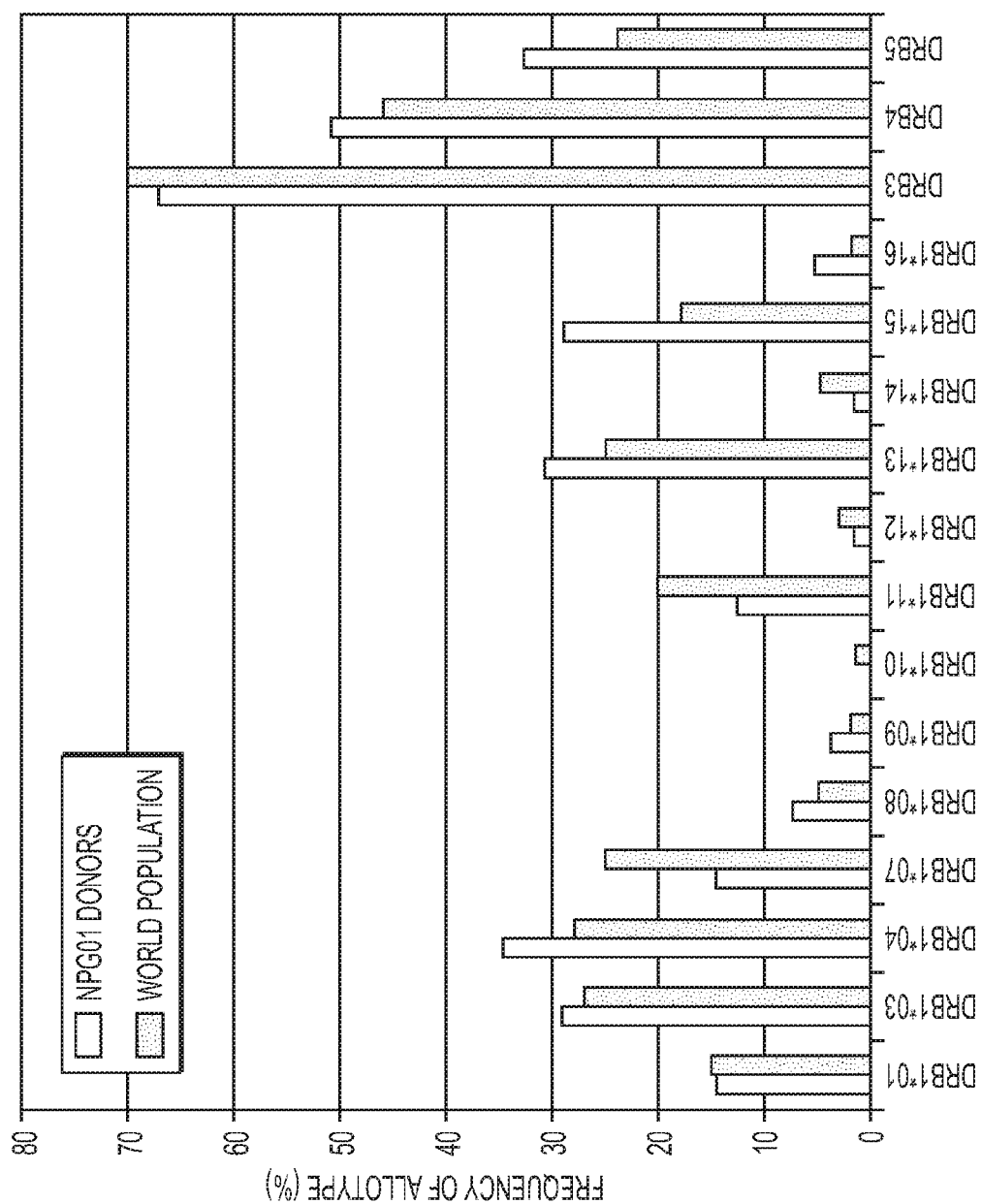


FIG. 5

FIG. 6 (SEQ ID NO:5)

1	A E T V E S C L A K P H T E N S F T N V W K	22
23	D D K T L D R Y A N Y E G C L W N A G G V V	44
45	V C T G D E T Q C Y G X W V P I G L A I P E	66
67	N E G G G S E G G G S E G G G S E G G G T K	88
89	P P E Y G D T P I P G Y T Y I N P L D G T Y	110
111	P P G T E Q N P A N P N P S L E E S Q P L N	132
133	T F M F Q N N R F R N R Q G A L T V Y T G T	154
155	F T Q G T D P V K T Y Y Q Y T P V S S X A M	176
177	Y D A Y W N G K F R D C A F H S G F N E D P	198
199	F V C E Y Q G Q S S D L P Q P P A N A G G E	220
221	S G G G S G G G S E G G G S E G G G S E G G	242
243	G S E G G G S G G G S G S G A R S D K T	262
263	H T C P P C P A P E L L G G P S V F L F P P	284
285	K P K D T L M I S R T P E V T C V V V D V S	306
307	H E D P E V K F N W Y V D G V E V H N A K T	328
329	K P R E E Q Y N S T Y R V V S V L T V L H Q	350
351	D W L N G K E Y K C K V S N K A L P A P I E	372
373	K T I S K A K G Q P R E P Q V Y T L P P S R	394
395	E E M T K N Q V S L T C L V K G F Y P S D I	416
417	A V E W E S N G Q P E N N Y K T T P P V L D	438
439	S D G S F F L Y S K L T V D K S R W Q Q G N	460
461	V F S C S V M H E A L H N H Y T Q K S L S L	482
483	S P G K	

SEQ ID NO: 6

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GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTGCGCAATTGAACGGGT
 GCCTAGAGAACGTTGGCGCGGGGTAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCAGGGTGGGGGAGAACCGTATATAAGTGC
 AGTAGTCGCGGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCC
 GCCCTACCTGAGGCGCCCATCCACGCGGTTGAGTCGCGTTCTGCCGCTCCGCGCTGTGGTGCCCTGAACTCGCTCGCGCTCTAGGTAAGT
 TTAAGCTCAGGTCGAGACCGGGCTTTGTCCGGCGCTCCCTGGAGCCTACCTAGACTCAGCGGGCTCTCCACGCTTTGCTGACCTGCTTGC
 TCAACTCTACGTCCTTTGTTCTGTTCTGCGCGCTTACAGATCCAAGCTGTGACCGCGCGCTACCTGAGATCACCGGCGAAGGAGGGCCA
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 CCCCATACAGAAAATTCATTACTAAGCTCTGGAAGACGACAAAATTTAGATCGTTACGTAACCTATGAGGGCTGTCTGTGGAATGCTACAGG
 CGTTGTAGTTTGTACTGGTGACGAACTCAGTGTACGGTCATTGGGTTCTTATTGGGCTTGCTATCCCTGAAAATGAGGGTGGTGGCTCTGAGG
 GTGGCGGTTCTGAGGGTGGCGGTTCTGAGGGTGGCGGTACTAAACCTCCTGAGTACGGTGATACACCTATTCCGGGTATACCTTATATCAACCT
 CTCGACGGCACTTATCCGCTGGTACTGAGCAAAACCCGCTAATCCTAATCCTTCTCTTGGAGAGTCTCAGCCTCTTAATACTTTTCATGTTTCA
 GAATAATAGTTTCCGAAATAGGAGGGGGCATTAACTGTTTATACGGGCACTTTTACTCAAGGCACTGACCCGTTAAAATTTATTACAGTACA
 CTCCTGTATCATCAAGAGCATGTATGACGCTTACTGGAACGGTAAATTCACAGACTGCGCTTTCCATTCTGGCTTTAATGAGGATCCATTGCTT
 TGTGAATATCAAGGCAATCGTCTGACCTGCTCAACCTCCTGCCAATGCTGGCGCGAGTCTGGTGGTGGTCTGGTGGCGCTCTGAGGGTGG
 TGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGGGGCTTCCGGTGGTCTGGTTCGGGTGCCAGATCTGACAAAACCTCACA
 CATGCCACCTGCCAGCAGCTGAACTCCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGGACC
 CCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGTGCATAATGCCAA
 GACAAAGCCCGGGAGGAGCAGTACAACAGCAGTACCGTGTGGTCAGCGTCTCACCCTCTGCACCGAGTGGTGAATGGCAAGGAGTACA
 AGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACACAGGTGTACACCTG
 CCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGGTCAAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAG
 CAATGGGCAGCCGGAGAACAATAACAAGACCAGCCTCCCGTGTGGACTCCGACGGCTCCTTCTCTCTACAGCAAGCTCACCCTGGACAAGA
 GCAGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAGAGGCTCTGCACAACCACTACACGCAGAGAGCCTCTCCCTGTCTCCGGGT
 AAATGAGTGTAGCTGGCCAGACATGATAACATACATTGATGAGTTTGGACAAACCACAACAGTGAATGCAGTGAATAAATGCTTTATTTGTGAA
 ATTTGTGATGCTATTGCTTTATTTGTAACATTATAAGCTGCAATAAACAAGTTAACAACAACAAATGCAATTCATTTTATGTTTCAGGTTACGGG
 GGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAATTTAAATACAGCATAGCAAACTTTAACCTCCAA
 ATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTGCGCAATGTGCATTAGCTGTTTGCAGCCTCACT
 TCTTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAAGTACTCTTCAATTTCTTTATGTTTAAATGCAGTACCTCCACATTTCCC
 TTTTATAGTAAATATTCAGAAATAATTTAATAACATCATTGCAATGAAATAAATGTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTTCA
 TAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGT
 CCTGCTCCTCTGCCACAAAGTGACGCAAGTTGCGCGCCGGGTGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTCATGGCC
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 GTCCGGCACCACTGCTGACCGCGCTGATGAACAGGTCACGTCGTCCCGACCAACCGCGGAAGTGTCTCCACGAAGTCCCGGAGAG
 ACCCGAGCGGCTCGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGCTCAACTTGGCCATGATGGCTCCT
 CctgtcaggagaggaagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAA
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 TCACAGGAGGAGAGAGGAGAGGCTTGAGACAGACCCGCGGACCGCCAACTGCGAGGGGACGTGCTAGGGCGGCTTCTTTTATGGTGCGCCG
 GCCCTCGGAGGAGGCGCTCGGGAGGCTTAGCGGCCAATCTGCGGTGGCAGGAGGCGGGCCGAGGGCCGTGCTGACCAATCCGGAGCACAT
 AGGAGTCTCAGCCCCCGCCCCAAAGCAAGGGGAAGTACCGCGCTGTAGCGCCAGCGTGTGTGAATGGGGCTTGGGGGGTGGGGCCCTG
 ACTAGTCAAAACAACTCCATTGACGTCAATGGGTGGAGACTTGAATCCCGTGAGTCAAAACCGCTATCCACGCCATTGATGTACTGCCA
 AAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCAATGTACTGGGCATTAATGCCAGG
 CGGGCCATTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGTATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCC
 ACCCATGACGTCAATGGAAGTCCCTATTGGCGTTACTATGGGAACATACTGATTAATTGACGTCAATGGCGGGGGTCTTGGCGGTGACGCC
 AGGCGGGCCATTACCGTAAGTTATGTAACGCTGCAGGTTAATTAAGAATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGC
 CGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTAT
 AAAGATACCAGCGTTTTCCCTGGAAGCTCCCTCGTGGCTCTCCTGTTCCGACCTGCGGCTTACCGGATACCTGTCCGCTTCTCCTCTCG
 GGAAGCGTGGCGTTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCCGTTAGGTGCTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGCT
 TCAGCCGACCGCTGCGCTTATCCGGTAACCTATCGTCTTGTAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACA
 GGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGC
 GCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTCATCCGGCAACAAACACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAA
 GCAGCAGATTACCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGAAAACTCAGCTTAAG
 GGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGCGCAATAAAATATCTTTATTTTATTACATCTGTGTGTGGTTTTTTTGTGTG
 AATCGTAACATAACGCTCTCCATCAAAACAAACGAAACAAACAACTAGCAAAATAGGCTGTCCCGAGTGAAGTGCAGGTGCCAGAAC
 TTCTCTATCGAA

FIG. 7

SEQ ID NO:7

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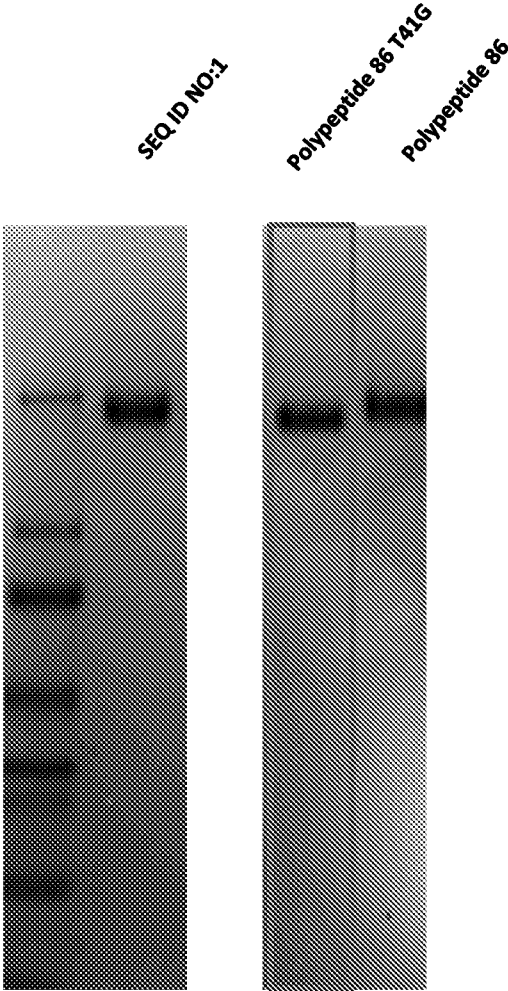
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GCCTAGAGAAGGTGGCGCGGGTAAACTGGGAAGTGATGTCGTGACTGGCTCCGCCCTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGC
AGTAGTCGCGGTGAACGTTCTTTTTCGCAACGGGTTTGGCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCC
GCCCTACCTGAGGCGCCCATCCACGCGGGTTGACTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCCTGAACGCGTCCGCGCTCTAGTAAGT
TTAAAGCTCAGGTCGAGACCGGGCTTTGTCCGCGCTCCCTTGGAGCCTACCTAGACTCAGCGGCTCTCCACGCTTTGCTGACCTGCTTGC
TCAACTCTACGCTTTTGTTCGTTTCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCGCTACCTGAGATCACCGGCGAAGGAGGGCCA
CCATGTACAGGATGCAACTCCTGCTTTCGATTGCACTAAGTCTTGCACCTTGTACGAATTGATGGCTGAAACTGTTGAAAGTTGTTTAGCAAAA
CCCCATACAGAAAATTCATTTACTAACGCTCTGGAAAGACGACAAAACCTTAGATCGTTACGCTAACTATGAGGGCTGTCTGTGGAATGCTGGAGG
CGTTGTAGTTTGTACTGCTGACGAAACTCAGTGTACGGTCACTGGGTTCTTATGGGCTTGCTATCCCTGAAAATGAGGGTGGTGGCTCTGAGG
GTGGCGGTTCTGAGGGTGGCGGTTCTGAGGGTGGCGGTACTAAACCTCCTGAGTACGGTGATACACCTATTCCGGGCTATACTTATATCAACCT
CTCGACGGCACTTATCCGCTGGTACTGAGCAAAACCCGCTAATCCTAATCCTTCTCTTGGAGTCTCAGCCTCTTAATACTTTCATGTTTCA
GAATAATAGGTTCCGAAATAGGCAGGGGGCATTAACTGTTTATACGGGCACTTTTACTCAAGGCACTGACCCCGTTAAAACTTATTACCAGTACA
CTCCTGTATCATCAAGAGCCATGTATGACGCTTACTGGAACGGTAAATTCAGAGACTCGGCTTTCCATTCTGGCTTAAATGAGGATCCATTGCTT
TGTGAATATCAAGGCCAATCGTCTGACCTGCCCTCAACCTCCTGCCAATGCTGGCGGCGAGTCTGCTGGTGGTCTGCTGGTGGCGGCTCTGAGGGTGG
TGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGGCGGTTCCGCTGGTGGCTCTGGTTCCGGTGCCAGATCTGACAAAACCTCACA
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GACAAAGCCCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAAGCTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAGCCCTCCAGCCCCATCGAGAAAACATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCCTG
CCCCATCCCGGGAGGAGATGACCAAGAACAGGTGAGCCTGACCTGCTGGTCAAAGGCTTCTATCCAGCGACATCGCGGTGAGTGGGAGAG
CAATGGGACGCGGAGAACAACTACAAGACCACGCTCCCGTGGTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGA
GCAGGTGGCAGGAGGGAACGCTTCTCATGCTCCGTGATGCACGAGGCTCTGCACAACCACTACACGCAGAAGACCTCTCCCTGTCTCCGGGT
AAATGAGTCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAA
ATTGTGATGCTATTGCTTTATTTGTAAACCATATAAGCTGCAATAAACAAGTTAACAACAACATTCGATTCAATTTATGTTTCAGGTTTCAGGG
GGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTAATTTCTAAATACAGCATAGCAAACTTTAACTCCAA
ATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTGCAATGTGCATTAGCTGTTTGCAGCCTCACT
TCTTTCATGGAGTTTAAAGATAGTGTATTTTCCCAAGGTTTGAACCTAGCTCTTCATTTCTTTATGTTTAAATGCAGTACCTCCACATTTCC
TTTTTAGTAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTTTTTTATAGGCAGAAATCCAGATGCTCAAGGCCTTCA
TAATATCCCCAGTTTAGTAGTTGGAATTAGGGAACAAAGGAACCTTTAATAGAAATTTGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGT
CCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCGGGGTGCGCGAGGGCGAACTCCCGCCCCACGGCTGCTGCGCATCTCGGTATGGCC
GGCCCGGAGGCGTCCCGGAAGTTCTGTGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGCGCGCGCACCCACACCCAGGCCAGGGTGT
GTCCGGCACCACTGGTCTGGACCGCGCTGATGAACAGGCTCAGCTCGTCCCGGACCAACCGCGGAAGTCTGCTCCACGAAGTCCCGGAGA
ACCCGAGCCGCTGGTCCAGAACTCGACCGCTCCGGGACGTCGCGCGGGTGAGCACCGGAACGGCACTGGTCAACTTGCCATGATGGCTCCT
CctgtcaggagaggaaagagaagaaggttagtacaattgCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAA
ACTAGGGCTGCAgggttcagatgtgcaacttttctgcactgccccatctctgcccaccccttccagcagatagacagtcagtgacttaeCAAAC
TCACAGGAGGGAGAAGGCAGAACCTTGAGACAGACCCGCGGGACCGCGAAGTCCGAGGGGACGTGGCTAGGGCGGCTCTCTTTTATGGTGGCGCG
GCCCTCGGAGGCGAGGCGCTCGGGGAGGCTAGCGGCAATCTGCGGTGGCAGGAGGCGGGGCGAAGGCCGTGCTGACCAATCCGAGCACAT
AGGAGTCTCAGCCCCCGCCCCAAAGCAAGGGGAAGTCAAGCGCTGTAGCGCAGCGTGTGTGAAATGGGGGCTTGGGGGGGTGGGGCCCTG
ACTAGTCAAAACAACTCCCATTTGACGTCAATGGGGTGGAGACTTGAATAACCCGCTGAGTCAAAACCGCTATCCACGCCCATTTGATGTACTGCCA
AAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAGGTCATGTACTGGGCATAATGCCAGG
CGGGCCATTTACCGTCATTGACGTCAATAGGGGGGCTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCC
ACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGGCGGGGTGCTTGGCGGTGACGC
AGGCGGGCCATTTACCGTAAGTTATGTAACGCCCTGCAGGTAAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGC
CGCGTGTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTAT
AAAGATACCAGGCGTTTCCCTGGAAGCTCCCTCGTGGCTCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCG
GGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGT
TCAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGTAGTCCAACCCGTAAGACACGACTATCGCCACTGGCAGCAGCCACTGGTAACA
GGAATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGC
GCTCTGCTGAAGCCAGTTACCTTCGAAAAAGACTTGTAGCTCTTGATCCGGCAACAAACCCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAA
GCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTACGGGGTCTGACGCTCAGTGAACGAAAACTCACGTTAAG
GGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGCGCGCAATAAATATCTTTATTTTTCATACATCTGTGTGTGGTTTTTGTGTG
AATCGTAACATAACGCTCTCCATCAAAACAAACGAAACAAACAACTAGCAAAATAGGCTGTCCCGAGTCAAGTGCAGGTGCCAGAACA
TTTCTCTATCGAA

FIG. 8

SUBSTITUTE SHEET (RULE 26)

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FIG. 9



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FIG. 10.

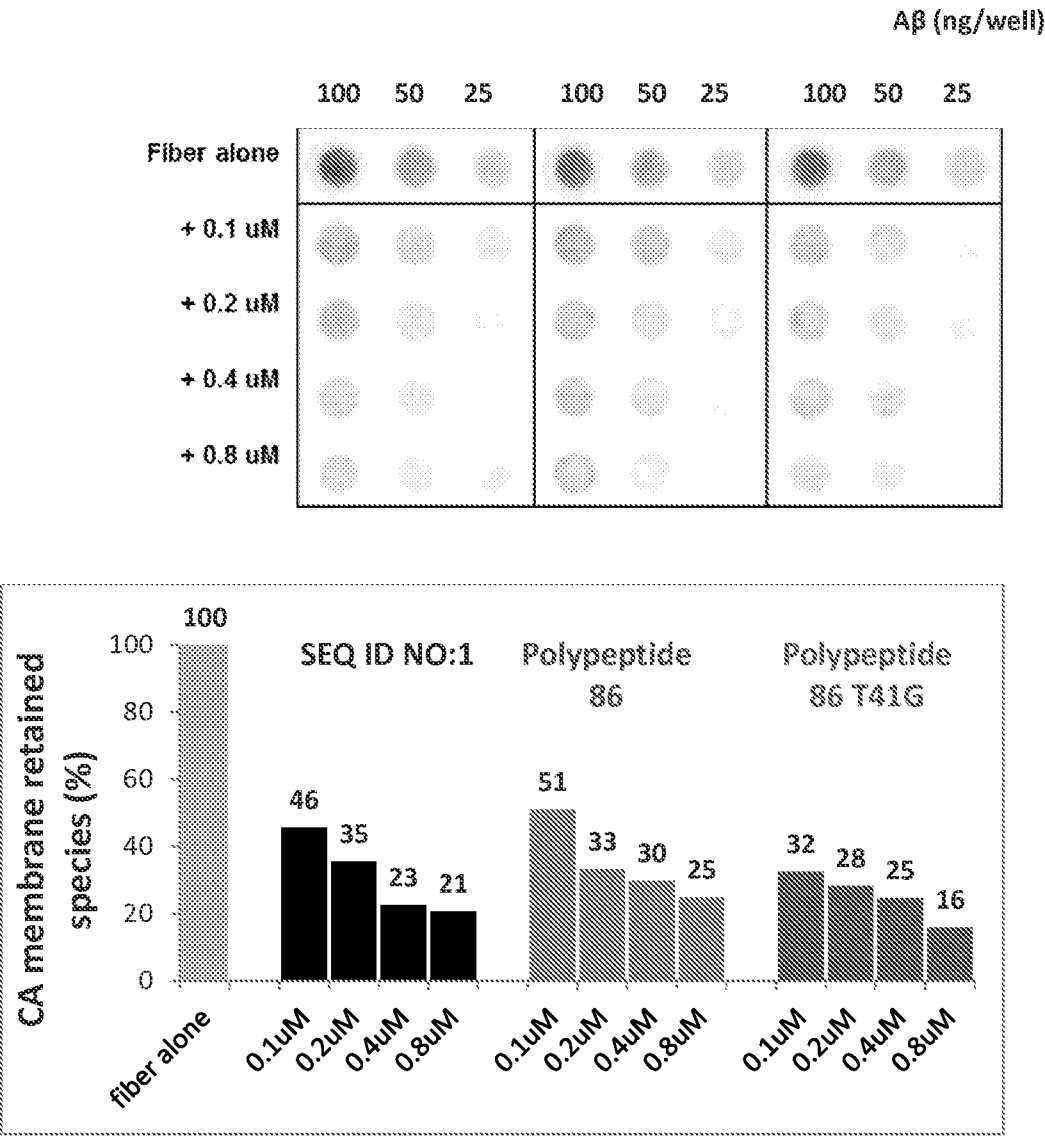


FIG. 11.

