

CLAIMS

1. A method for determining a presence of a soluble, misfolded protein in a sample, comprising:

contacting the sample with a monomeric, folded protein corresponding to the soluble, misfolded protein to form an incubation mixture;

conducting an incubation cycle two or more times effective to form an amplified portion of misfolded protein, each incubation cycle comprising:

incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric, folded protein in the presence of the soluble, misfolded protein;

physically disrupting the incubation mixture effective to break up at least a portion of any protein aggregate present; and

determining the presence of the soluble, misfolded protein in the sample by detecting at least a portion of the soluble, misfolded protein,

the soluble, misfolded protein comprising one or more of: a soluble, misfolded monomer and a soluble, misfolded aggregate; and

the amplified portion of misfolded protein comprising one or more of: an amplified portion of the soluble, misfolded monomer, an amplified portion of the soluble, misfolded aggregate, and an insoluble, misfolded aggregate,

provided that the monomeric, folded protein and the soluble, misfolded protein exclude prion protein (PrP) and isoforms thereof.

2. The method of claim 1:

further comprising contacting an indicator of the soluble, misfolded protein to the incubation mixture, the indicator of the soluble, misfolded protein being characterized by an indicating state in the presence of the soluble, misfolded protein and a non-indicating state in the absence of the soluble, misfolded protein; and

wherein the determining the presence of the soluble, misfolded protein in the sample comprises detecting the indicating state of the indicator of the soluble, misfolded protein.

3. The method of claim 2, the indicator of the soluble, misfolded protein comprising one or more of: Thioflavin T, Congo Red, m-I-Stilbene, Chrysamine G, PIB, BF-227, X-34, TZDM, FDDNP, MeO-X-04, IMPY, NIAD-4, luminescent conjugated polythiophenes, a fluorescent protein, and derivatives thereof.

4. The method of claim 1, the determining the presence of the soluble, misfolded protein in the sample comprising one or more of:

determining an amount of the soluble, misfolded protein in the sample;

detecting the amount of the soluble, misfolded protein in the sample at a sensitivity of at least about 80%;

detecting the amount of the soluble, misfolded protein in the sample at less than about 100 nmol;

detecting the amount of the soluble, misfolded protein in the sample in a molar ratio to monomeric, folded protein comprised by the sample, the molar ratio being less than about 1:100;

detecting the soluble, misfolded protein in the sample with a specificity of at least about 80%; and

determining an amount of the soluble, misfolded protein in the sample compared to a control sample.

5. The method of claim 1, the incubating comprising incubating the incubation mixture at one or more of: between about 4 °C and about 35 °C.
6. The method of claim 1, the detecting the soluble, misfolded protein comprising one or more of: a Western Blot assay, a dot blot assay, an enzyme-linked immunosorbent assay (ELISA), a thioflavin T binding assay, a Congo Red binding assay, a sedimentation assay, electron microscopy, atomic force microscopy, surface plasmon resonance, and spectroscopy.
7. The method of claim 1, further comprising providing the monomeric, folded protein in labeled form, the detecting the soluble, misfolded protein comprising detecting the monomeric, folded protein in labeled form as incorporated into the amplified portion of misfolded protein.
8. The method of claim 1, the sample being taken from a subject, further comprising determining or diagnosing the presence of a protein misfolding disorder (PMD) in the subject according to the presence of the soluble, misfolded protein in the sample, the PMD comprising at least one of: an amyloidosis; a synucleinopathy; a triplet repeat disorder; amyotrophic lateral sclerosis; Alzheimer's disease; systemic amyloidosis; Parkinson's disease; Lewy body dementia; multiple system atrophy; synuclein-related neuroaxonal dystrophy; and Huntington's disease.
9. The method of claim 1, the sample being taken from a subject, further comprising determining or diagnosing the presence of a PMD in the subject according to one or more of:
 - the presence of the soluble, misfolded protein in the sample compared to a control sample taken from a control subject;
 - an amount of the soluble, misfolded protein in the sample compared to a predetermined threshold amount, the detecting comprising detecting the amount of the soluble, misfolded protein in the sample;

the presence of the soluble, misfolded protein in the sample, the subject exhibiting no clinical signs of dementia according to cognitive testing;

as a contributing factor to one or more clinical signs of dementia in the subject according to the presence of the soluble, misfolded protein in the sample, the subject exhibiting the one or more clinical signs of dementia according to cognitive testing;

the presence of the soluble, misfolded protein in the sample, the subject exhibiting no clinical signs of dementia according to cognitive testing; and

a progression or homeostasis of a PMD in the subject by comparing the amount of the soluble, misfolded protein in the sample to an amount of the soluble, misfolded protein in a comparison sample taken from the subject at a different time compared to the sample.

10. The method of claim 1, further comprising obtaining the sample from a subject,

the sample comprising one or more of: amniotic fluid; bile; blood; cerebrospinal fluid; cerumen; skin; exudate; feces; gastric fluid; lymph; milk; mucus; mucosal membrane; nasal secretions; peritoneal fluid; plasma; pleural fluid; pus; saliva; sebum; semen; sweat; synovial fluid; tears; and urine; and

the subject being one of a: human, mouse, rat, dog, cat, cattle, horse, deer, elk, sheep, goat, pig, and non-human primate.

11. The method of claim 1, the subject being treated with a PMD modulating therapy, further comprising:

comparing the amount of the soluble, misfolded protein in the sample to an amount of the soluble, misfolded protein in a comparison sample, the sample and the comparison sample being

taken from the subject at different times over a period of time under the PMD modulating therapy;
and

determining the subject is one of: responsive to the PMD modulating therapy according to a change in the soluble, misfolded protein over the period of time, or non-responsive to the PMD modulating therapy according to homeostasis of the soluble, misfolded protein over the period of time.

12. The method of claim 1, further comprising selectively concentrating the soluble, misfolded protein in one or more of the sample and the incubation mixture.

13. The method of claim 12, the selectively concentrating the soluble, misfolded protein comprising one or more of:

pre-treating the sample prior to forming the incubation mixture;

pre-treating the incubation mixture prior to incubating the incubation mixture; and

contacting one or more soluble, misfolded protein specific antibodies to the soluble, misfolded protein to form a captured soluble, misfolded protein, the one or more soluble, misfolded protein specific antibodies comprising one or more of: an antibody specific for an amino acid sequence of the soluble, misfolded protein and an antibody specific for a conformation of the soluble, misfolded protein.

14. A method for determining a presence of soluble, misfolded protein in a sample, comprising:

capturing soluble, misfolded protein from the sample to form a captured soluble, misfolded protein;

contacting the captured soluble, misfolded protein with a molar excess of a monomeric, folded protein corresponding to the soluble, misfolded protein to form an incubation mixture, the molar excess being greater than an amount of protein monomer included in the captured soluble, misfolded protein;

conducting an incubation cycle two or more times effective to form an amplified portion of misfolded protein, each incubation cycle comprising:

incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric, folded protein in the presence of the captured soluble, misfolded protein;

physically disrupting the incubation mixture effective to break up at least a portion of any protein aggregate present; and

determining the presence of the soluble, misfolded protein in the sample by detecting at least a portion of the soluble, misfolded protein,

the soluble, misfolded protein comprising one or more of: a soluble, misfolded monomer and a soluble, misfolded aggregate;

the captured, soluble, misfolded protein comprising one or more of: a captured, soluble, misfolded monomer and a captured, soluble, misfolded aggregate; and

the amplified portion of misfolded protein comprising one or more of: an amplified portion of the soluble, misfolded monomer, an amplified portion of the soluble, misfolded aggregate, and an insoluble, misfolded aggregate,

provided that the monomeric, folded protein and the soluble, misfolded protein exclude prion protein (PrP) and isoforms thereof.

15. The method of claim 14, the incubating comprising incubating the incubation mixture at one or more of: between about 4 °C and about 60 °C.

16. The method of claim 14, the sample being taken from a subject, further comprising determining or diagnosing the presence of a PMD in the subject according to one or more of:

the presence of the soluble, misfolded protein in the sample compared to a control sample taken from a control subject;

an amount of the soluble, misfolded protein in the sample compared to a predetermined threshold amount, the detecting comprising detecting the amount of the soluble, misfolded protein in the sample;

the presence of the soluble, misfolded protein in the sample, the subject exhibiting no clinical signs of dementia according to cognitive testing;

as a contributing factor to one or more clinical signs of dementia in the subject according to the presence of the soluble, misfolded protein in the sample, the subject exhibiting the one or more clinical signs of dementia according to cognitive testing;

a progression or homeostasis of a PMD in the subject by comparing the amount of the soluble, misfolded protein in the sample to an amount of the soluble, misfolded protein in a comparison sample taken from the subject at a different time compared to the sample.

17. The method of claim 14, the subject being treated with a PMD modulating therapy, further comprising:

comparing the amount of the soluble, misfolded protein in the sample to an amount of the soluble, misfolded protein in a comparison sample, the sample and the comparison sample being taken from the subject at different times over a period of time under the PMD modulating therapy; and

determining the subject is one of: responsive to the PMD modulating therapy according to a change in the soluble, misfolded protein over the period of time, or non-responsive to the PMD modulating therapy according to homeostasis of the soluble, misfolded protein over the period of time.

18. The method of claim 14, the capturing the soluble, misfolded protein from the sample to form a captured soluble, misfolded protein being conducted using one or more soluble, misfolded protein-specific antibodies, the one or more soluble, misfolded protein specific antibodies comprising one or more of: an antibody specific for an amino acid sequence of the soluble, misfolded protein and an antibody specific for a conformation of the soluble, misfolded protein.

19. The method of claim 14, the capturing the soluble, misfolded protein from the sample to form a captured soluble, misfolded protein being conducted using one or more soluble, misfolded protein-specific antibodies, the one or more soluble, misfolded protein specific antibodies being coupled to a solid phase.

20. The method of claim 18, the solid phase comprising one or more of a magnetic bead and a multiwell plate.

21. A method for determining a presence of a soluble, misfolded A β protein in a sample, comprising:

contacting the sample with a monomeric, folded A β protein in the presence of a biological fluid to form an incubation mixture, the incubation mixture comprising the monomeric, folded A β protein in a concentration range of about 1 μ M to about 10 μ M;

providing a buffer in the incubation mixture, the buffer comprising one of: Tris-HCL, MES, PIPES, MOPS, BES, TES, and HEPES;

conducting an incubation cycle two or more times on the incubation mixture effective to form an amplified portion of misfolded A β protein from the monomeric, folded A β protein, each incubation cycle comprising:

incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric, folded A β protein in the presence of the soluble, misfolded A β protein, the incubating being conducted at a temperature between about 12 °C and about 30 °C;

physically disrupting the incubation mixture effective to at least partly de-aggregate at least a portion of a misfolded A β aggregate present; and

determining the presence of the soluble, misfolded A β protein in the sample by detecting at least a portion of the amplified portion of misfolded A β protein,

the soluble, misfolded A β protein comprising one or more of: a soluble, misfolded A β monomer and a soluble, misfolded A β aggregate; and

the amplified portion of misfolded A β protein comprising one or more of: an amplified portion of the soluble, misfolded A β monomer, an amplified portion of the soluble, misfolded A β aggregate, and an insoluble, misfolded A β aggregate.

22. The method of claim 21:

further comprising contacting an indicator of misfolded A β protein to the incubation mixture, the indicator of misfolded A β protein being characterized by an indicating state in the presence of misfolded A β protein and a non-indicating state in the absence of misfolded A β protein; and

wherein the determining the presence of the soluble, misfolded A β protein in the sample comprises detecting the indicating state of the indicator of misfolded A β protein in the incubation mixture.

23. The method of claim 22, the indicator of misfolded A β protein comprising one or more of: Thioflavin T, Congo Red, m-I-Stilbene, Chrysamine G, PIB, BF-227, X-34, TZDM, FDDNP, MeO-X-04, IMPY, NIAD-4, luminescent conjugated polythiophenes, a fluorescent protein, and derivatives thereof.

24. The method of claim 21, the determining the presence of the soluble, misfolded A β protein in the sample comprising one or more of:

determining an amount of the soluble, misfolded A β protein in the sample;

determining the amount of the soluble, misfolded A β protein in the sample at a sensitivity of at least about 80%;

determining the amount of the soluble, misfolded A β protein in the sample at less than about 100 nmol;

determining the amount of the soluble, misfolded A β protein in the sample in a molar ratio to monomeric, folded A β protein comprised by the sample, the molar ratio being less than about 1:100;

determining the soluble, misfolded A β protein in the sample with a specificity of at least about 80%; and

determining an amount of the soluble, misfolded A β protein in the sample compared to a control sample.

25. The method of claim 21, the incubating comprising incubating the incubation mixture at between about 18 °C and about 26 °C.

26. The method of claim 21, the detecting at least the portion of the amplified portion of misfolded A β protein comprising one or more of: a Western Blot assay, a dot blot assay, an enzyme-linked immunosorbent assay (ELISA), a thioflavin T binding assay, a Congo Red

binding assay, a sedimentation assay, electron microscopy, atomic force microscopy, surface plasmon resonance, and spectroscopy.

27. The method of claim 21, further comprising providing the monomeric, folded A β protein in labeled form, the detecting at least the portion of the amplified portion of misfolded A β protein comprising detecting the monomeric, folded A β protein in labeled form as incorporated into the amplified portion of misfolded A β protein.

28. The method of claim 21, the sample being taken from a subject, further comprising determining or diagnosing one or more of:

the presence of AD (Alzheimer's disease) in the subject according to the presence of the soluble, misfolded A β protein in the sample;

the presence of AD in the subject according to the presence of the soluble, misfolded A β protein in the sample compared to a control sample taken from a control subject;

the presence of AD in the subject by comparing an amount of the soluble, misfolded A β protein in the sample to a predetermined threshold amount, the detecting at least the portion of the amplified portion of misfolded A β protein comprising detecting an amount of the soluble, misfolded A β protein in the sample;

the presence of AD in the subject according to the presence of the soluble, misfolded A β protein in the sample, the subject exhibiting no clinical signs of dementia according to cognitive testing;

the presence of AD in the subject according to the presence of the soluble, misfolded A β protein in the sample, the subject exhibiting no cortex plaques or tangles according to amyloid beta contrast imaging;

the presence of AD as a contributing factor to one or more clinical signs of dementia in the subject according to the presence of the soluble, misfolded A β protein in the sample, the subject exhibiting the one or more clinical signs of dementia according to cognitive testing; and

a progression or homeostasis of AD in the subject by comparing the amount of the soluble, misfolded A β protein in the sample to an amount of the soluble, misfolded A β protein in a comparison sample taken from the subject at a different time compared to the sample.

29. The method of claim 21, further comprising obtaining the sample from a subject,

the sample comprising one or more of: amniotic fluid; bile; blood; cerebrospinal fluid; cerumen; skin; exudate; feces; gastric fluid; lymph; milk; mucus; mucosal membrane; nasal secretions; peritoneal fluid; plasma; pleural fluid; pus; saliva; sebum; semen; sweat; synovial fluid; tears; and urine; and

the subject being one of a: human, mouse, rat, dog, cat, cattle, horse, deer, elk, sheep, goat, pig, and non-human primate.

30. The method of claim 21, further comprising:

comparing the amount of the soluble, misfolded A β protein in the sample to an amount of the soluble, misfolded A β protein in a comparison sample, the sample and the comparison sample being taken from a subject being treated with an A β modulating therapy, the sample and the comparison sample being taken from the subject at different times over a period of time under the A β modulating therapy; and

determining the subject is one of: responsive to the A β modulating therapy according to a change in the soluble, misfolded A β protein over the period of time, or non-responsive to the A β

modulating therapy according to homeostasis of the soluble, misfolded A β protein over the period of time.

31. The method of claim 21, the sample being taken from a subject, the subject being treated with an A β modulating therapy, the A β modulating therapy comprising administration of one or more of: an inhibitor of BACE1 (beta-secretase 1); an inhibitor of γ -secretase; a modulator of A β homeostasis; E2609; MK-8931; LY2886721; AZD3293; semagacestat (LY-450139); avagacestat (BMS-708163); solanezumab; (SEQ ID NO: 11 (VH))(SEQ ID NO: 12 (VL)); crenezumab (SEQ ID NO: 13 (VH))(SEQ ID NO: 14 (VL)); bapineuzumab (SEQ ID NO: 15 (VH))(SEQ ID NO: 16 (VL)); BIIB037 (SEQ ID NO: 17 (VH))(SEQ ID NO: 18 (VL)); CAD106; antibodies raised against A β globulomers; ACC-001; V950; and Affitrope AD02.

32. The method of claim 21, the physically disrupting the incubation mixture comprising one or more of: sonication, stirring, shaking, freezing/thawing, laser irradiation, autoclave incubation, high pressure, homogenization, and cyclic agitation.

33. The method of claim 21, the monomeric, folded A β protein and/or the soluble, misfolded A β protein comprising peptides formed via β - or γ -secretase cleavage of one or more of: amyloid precursor protein (SEQ ID NO: 1); Abeta40 (SEQ ID NO: 2); and Abeta42 (SEQ ID NO: 3).

34. The method of claim 21, comprising:

contacting the sample with Thioflavin T and the monomeric, folded A β protein to form the incubation mixture;

conducting the incubation cycle two or more times on the incubation mixture effective to form the amplified portion of misfolded A β protein from the monomeric, folded A β protein, each incubation cycle comprising:

incubating the incubation mixture effective to cause misfolding and/or aggregation of at least the portion of the monomeric, folded A β protein in the presence of the soluble, misfolded A β protein;

shaking the incubation mixture effective to at least partly de-aggregate at least the portion of a misfolded A β aggregate present; and

determining the presence of the soluble, misfolded A β protein in the sample by detecting a fluorescence of the Thioflavin T corresponding to at least the portion of the amplified portion of misfolded A β protein.

35. A method for determining a presence of a soluble, misfolded A β protein in a sample, comprising:

capturing a soluble, misfolded A β protein from the sample to form a captured soluble, misfolded A β protein;

contacting the captured, soluble misfolded A β protein with a molar excess of monomeric, folded A β protein to form an incubation mixture, the molar excess being greater than an amount of A β protein monomer included in the captured soluble, misfolded A β protein;

conducting an incubation cycle two or more times on the incubation mixture effective to form an amplified portion of misfolded A β protein from the monomeric, folded A β protein, each incubation cycle comprising:

incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric, folded A β protein in the presence of the captured soluble, misfolded A β protein;

physically disrupting the incubation mixture effective to at least partly de-aggregate at least a portion of a misfolded A β aggregate present; and

determining the presence of the soluble, misfolded A β protein in the sample by detecting at least a portion of the amplified portion of misfolded A β protein,

the soluble, misfolded A β protein comprising one or more of: a soluble, misfolded A β monomer and a soluble, misfolded A β aggregate; and

the captured, soluble, misfolded A β protein comprising one or more of: a captured, soluble, misfolded A β monomer and a captured, soluble, misfolded A β aggregate; and

the amplified portion of misfolded A β protein comprising one or more of: an amplified portion of the soluble, misfolded A β monomer, an amplified portion of the soluble, misfolded A β aggregate, and an insoluble, misfolded A β aggregate.

36. The method of claim 35, the capturing comprising selectively concentrating the soluble, misfolded A β protein in one or more of the sample and the incubation mixture.

37. The method of claim 36, the selectively concentrating the soluble, misfolded A β protein comprising one or more of: pre-treating the sample prior to forming the incubation mixture and pre-treating the incubation mixture prior to incubating the incubation mixture.

38. The method of claim 36, the selectively concentrating the soluble, misfolded A β protein comprising contacting one or more A β specific antibodies to the soluble, misfolded A β protein to form a captured soluble, misfolded A β protein, the one or more A β specific antibodies comprising one or more of: 6E10, 4G8, 82E1, A11, X-40/42, 16ADV, an antibody specific for an amino acid sequence of A β , and an antibody specific for a conformation of the soluble, misfolded A β protein.

39. The method of claim 38, the one or more A β specific antibodies being coupled to a solid phase.

40. The method of claim 39, the solid phase comprising one or more of a magnetic bead and a multiwell plate.

41. The method of claim 35, further comprising:

comparing the amount of the soluble, misfolded A β protein in the sample to an amount of the soluble, misfolded A β protein in a comparison sample, the sample and the comparison sample being taken from a subject being treated with an A β modulating therapy, the sample and the comparison sample being taken from the subject at different times over a period of time under the A β modulating therapy; and

determining the subject is one of: responsive to the A β modulating therapy according to a change in the soluble, misfolded A β protein over the period of time, or non-responsive to the A β modulating therapy according to homeostasis of the soluble, misfolded A β protein over the period of time.

42. The method of claim 35, the sample being taken from a subject, further comprising determining or diagnosing one or more of:

the presence of AD in the subject according to the presence of the soluble, misfolded A β protein in the sample;

the presence of AD in the subject according to the presence of the soluble, misfolded A β protein in the sample compared to a control sample taken from a control subject;

the presence of AD in the subject by comparing an amount of the soluble, misfolded A β protein in the sample to a predetermined threshold amount, the detecting at least the portion of the amplified portion of misfolded A β protein comprising detecting an amount of the soluble, misfolded A β protein in the sample;

the presence of AD in the subject according to the presence of the soluble, misfolded A β protein in the sample, the subject exhibiting no clinical signs of dementia according to cognitive testing;

the presence of AD in the subject according to the presence of the soluble, misfolded A β protein in the sample, the subject exhibiting no cortex plaques or tangles according to amyloid beta contrast imaging;

the presence of AD as a contributing factor to one or more clinical signs of dementia in the subject according to the presence of the soluble, misfolded A β protein in the sample, the subject exhibiting the one or more clinical signs of dementia according to cognitive testing; and

a progression or homeostasis of AD in the subject by comparing the amount of the soluble, misfolded A β protein in the sample to an amount of the soluble, misfolded A β protein in a comparison sample taken from the subject at a different time compared to the sample.