CLAIMS

1. A method for determining a presence of a soluble, misfolded alpha synuclein (αS) protein in a sample comprising a biological fluid, comprising:

contacting the sample with a monomeric, folded αS protein corresponding to the soluble, misfolded αS protein to form an incubation mixture, the incubation mixture comprising the monomeric, folded αS protein in a concentration range of 100 nM to 20 μM;

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 effective to form an amplified portion of misfolded αS 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 αS protein in the presence of the soluble, misfolded αS 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 αS protein in the sample by detecting at least a portion of the soluble, misfolded αS protein,

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

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

2. The method of claim 1:

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

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

3. The method of claim 2, the indicator of the soluble, misfolded αS 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 αS protein in the sample comprising one or more of:

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

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

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

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

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

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

5. The method of claim 1, the incubating comprising incubating the incubation mixture between 12° C and 45° C.

6. The method of claim 1, the detecting the soluble, misfolded αS 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 αS protein in labeled form, the detecting the soluble, misfolded αS protein comprising detecting the monomeric, folded αS protein in labeled form as incorporated into the amplified portion of misfolded αS protein.

8. The method of claim 1,

the biological fluid 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.

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

10. The method of claim 9, the selectively concentrating the soluble, misfolded αS 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 αS protein specific antibodies to the soluble, misfolded αS protein to form a captured soluble, misfolded protein, the one or more soluble, misfolded αS protein specific antibodies comprising one or more of: an antibody

specific for an amino acid sequence of the soluble, misfolded αS protein and an antibody specific for a conformation of the soluble, misfolded αS protein.

11. A method for determining a presence of soluble, misfolded protein in a sample comprising a biological fluid, 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.

12. The method of claim 11, the incubating comprising incubating the incubation mixture at one or more of: between 4 °C and 60 °C.

13. The method of claim 11, 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.

14. The method of claim 11, 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.

15. The method of claim 14, the solid phase comprising one or more of a magnetic bead and a multiwell plate.

16. The method of claim 1, comprising preparing the incubation mixture comprising the monomeric, folded protein at a concentration of one of: between 1 μM and 10 μM; 7 μM; and 2 μM.

17. The method of claim 1, comprising preparing the incubation mixture comprising a salt composition at a total concentration of between 1 μm and 500 mM, the salt composition comprising one or more of: NaCl and KCl.

18. The method of claim 1, provided the sample excludes αS fibrils and tau fibrils.

19. The method of claim 1, the monomeric, folded αS protein and/or the soluble, misfolded αS protein comprising one or more of: one or more peptides formed via proteolytic cleavage of αS-140 protein; αS-140; αS-126; and αS-112.

20. The method of claim 1, the incubation mixture comprising the buffer composition at a total concentration of between 1 μm and 1M.

21. The method of claim 1, comprising preparing or maintaining the incubation mixture with a pH of one or more of: between 5 and 9; between 6 and 8.5; between 7 and 8; and 7.4.

22. The method of claim 1, the determining the presence of the soluble, misfolded protein in the sample comprising determining an amount of the soluble, misfolded protein in the sample compared to a control sample.

23. The method of claim 1, 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.

24. The method of claim 1, the physically disrupting the incubation mixture comprising cyclic agitation, the cyclic agitation being conducted for one or more of: between 50 rotations per minute (RPM) and 10,000 RPM, between 200 RPM and 2000 RPM, and at 500 RPM.

25. The method of claim 1, each incubation cycle comprising independently incubating and physically disrupting the incubation mixture for one or more of: incubating between 5 minutes and 5 hours and physically disrupting between 5 seconds and 10 minutes; incubating between 10 minutes and 2 hours and physically disrupting between 30 sec and 1 minute; incubating between 15 minutes and 1 hour and physically disrupting between 45 sec and 1 minute; incubating between 25 minutes and 45 minutes and physically disrupting between 45 sec and 1 minute; and incubating 1 minute and physically disrupting 1 minute.

26. The method of claim 11:

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.

27. The method of claim 11, 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, derivatives thereof.

28. The method of claim 11, the determining the presence of the soluble, misfolded protein in the sample comprising one of:

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

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

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

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

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

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

29. The method of claim 11, comprising preparing the incubation mixture comprising the monomeric, folded protein at a concentration of one or more of: between 1 nM and 2 mM; between 10 nM and 200 μM; between 100 nM and 20 μM; between 1 μM and 10 μM; and 2 μM.

30. The method of claim 11, comprising preparing the incubation mixture comprising a buffer composition including one or more of: Tris-HCL, PBS, MES, PIPES, MOPS, BES, TES, and HEPES, the incubation mixture comprising the buffer composition at a total concentration of between 1 μm and 1M.

31. The method of claim 11, comprising preparing the incubation mixture comprising a salt composition at a total concentration of between 1 μm and 500 mM, the salt composition comprising one or more of: NaCl and KCl.

32. The method of claim 11, the detecting the soluble, misfolded protein comprising one or more of: a Western 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.

33. The method of claim 11, further comprising providing the monomeric, folded protein in labeled form, the monomeric, folded protein in labeled form comprising one or more of: a covalently incorporated radioactive amino acid, a covalently incorporated, isotopically labeled amino acid, and a covalently incorporated fluorophore, the detecting the soluble, misfolded protein comprising detecting the monomeric, folded protein in labeled form as incorporated into the amplified portion of misfolded protein.

34. The method of claim 11, the biological fluid comprising one or more of: amniotic fluid; bile; blood; cerebrospinal fluid; cerumen; skin; exudate; feces; gastric fluid; lymph; milk; mucus; mucosal membrane; peritoneal fluid; plasma; pleural fluid; pus; saliva; sebum; semen; sweat; synovial fluid; tears; and urine.

35. The method of claim 11, comprising contacting the sample with a molar excess of the monomeric, folded protein to form the incubation mixture, the molar excess being greater than a total molar amount of protein monomer included in the soluble, misfolded protein in the sample.

36. The method of claim 11, provided the sample excludes αS fibrils and tau fibrils.

37. The method of claim 11, comprising preparing or maintaining the incubation mixture with a pH of one or more of: between 5 and 9; between 6 and 8.5; between 7 and 8; and 7.4.

38. The method of claim 11, the determining the presence of the soluble, misfolded protein in the sample comprising determining an amount of the soluble, misfolded protein in the sample compared to a control sample.

39. The method of claim 11, 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.

40. The method of claim 11, the physically disrupting the incubation mixture comprising cyclic agitation, the cyclic agitation being conducted for one or more of: between 50 rotations per minute (RPM) and 10,000 RPM, between 200 RPM and 2000 RPM, and at 500 RPM.

41. The method of claim 11, each incubation cycle comprising independently incubating and physically disrupting the incubation mixture for one or more of: incubating between 5 minutes and 5 hours and physically disrupting between 5 seconds and 10 minutes; incubating between 10 minutes and 2 hours and physically disrupting between 30 sec and 1 minute; incubating between 15 minutes and 1 hour and physically disrupting between 45 sec and 1 minute; incubating between 25 minutes and 45 minutes and physically disrupting between 45 sec and 1 minute; and incubating 1 minute and physically disrupting 1 minute.

42. The method of claim 11, the conducting the incubation cycle being repeated for one or more of: between 2 times and 1000 times, between 5 times and 500 times, between 50 times and 500 times, and between 150 times and 250 times.