

CLAIMS

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

contacting the sample with a monomeric, folded α S protein corresponding to the soluble, misfolded α S protein to form an incubation mixture, the incubation mixture comprising the monomeric, folded α S protein in a concentration range of 100 nM to 20 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 effective to form an amplified portion of misfolded α S 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 α S protein in the presence of the soluble, misfolded α S 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 α S protein in the sample by detecting at least a portion of the soluble, misfolded α S protein,

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

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

2. The method of claim 1:

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

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

3. The method of claim 2, the indicator of the soluble, misfolded α S protein comprising one or more of: Thioflavin T, Congo Red, m-I-Stilbene, Chrysamine G, luminescent conjugated polythiophenes, or a fluorescent protein.

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

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

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

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

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

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

determining an amount of the soluble, misfolded α S 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 α S 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 α S protein in labeled form, the detecting the soluble, misfolded α S protein comprising detecting the monomeric, folded α S protein in labeled form as incorporated into the amplified portion of misfolded α S protein.
8. The method of claim 1, the biological fluid comprising one or more of: blood; cerebrospinal fluid; plasma; saliva; and urine.
9. The method of claim 1, further comprising selectively concentrating the soluble, misfolded α S protein in one or more of the sample and the incubation mixture.
10. The method of claim 9, the selectively concentrating the soluble, misfolded α S 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 α S protein specific antibodies to the soluble, misfolded α S protein to form a captured soluble, misfolded protein, the one or more soluble, misfolded α S protein specific antibodies comprising one or more of: an antibody specific for an amino acid sequence of the soluble, misfolded α S protein and an antibody specific for a conformation of the soluble, misfolded α S protein.

11. The method of claim 1, comprising preparing the incubation mixture comprising the monomeric, folded protein at a concentration of one of: between 1 μ M and 10 μ M; 7 μ M; and 2 μ M.
12. The method of claim 1, comprising preparing the incubation mixture comprising a salt composition at a total concentration of between 1 μ M and 500 mM, the salt composition comprising one or more of: NaCl and KCl.
13. The method of claim 1, provided the sample excludes α S fibrils and tau fibrils.
14. The method of claim 1, the monomeric, folded α S protein and/or the soluble, misfolded α S protein comprising one or more of: one or more peptides formed via proteolytic cleavage of α S-140 protein; α S-140; α S-126; and α S-112.
15. The method of claim 1, the incubation mixture comprising the buffer composition at a total concentration of between 1 μ M and 1M.
16. 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.
17. 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.
18. 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.
19. 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.

20. 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.

21. A method for determining the presence of a soluble, misfolded alpha synuclein (α S) protein in a sample comprising a biological fluid, comprising:

contacting the sample with a monomeric, folded α S protein corresponding to the soluble, misfolded α S protein to form an incubation mixture, the incubation mixture comprising the monomeric, folded α S protein in a concentration range of 1 μ M to 100 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 effective to form an amplified portion of misfolded α S 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 α S protein in the presence of the soluble, misfolded α S 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 α S protein in the sample by detecting at least a portion of the soluble, misfolded α S protein,

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

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

22. The method of claim 21, comprising the monomeric, folded α S protein in a concentration range of 6.54 μ M to 65.4 μ M