

CLAIMS

What is claimed is:

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

(A) contacting the biological sample with a pre-incubation mixture, the pre-incubation mixture comprising:

(1) a monomeric α -S protein in a concentration range of about 100 nM to about 20 μ M;

(2) a buffer composition;

(3) a salt; and

(4) an indicator,

to form an incubation mixture;

(B) conducting an incubation cycle on the incubation mixture, the incubation cycle being conducted two or more times on the incubation mixture effective to form an amplified portion of misfolded α -S protein from the monomeric α -S protein, each incubation cycle comprising:

(1) incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric α -S protein in the presence of the soluble, misfolded α -S protein; and

(2) physically disrupting the incubation mixture, and

(C) determining if a detectable amount of misfolded α -S aggregate is present in the biological sample, wherein detection of misfolded α -S aggregate indicates the presence of soluble, misfolded α -S protein in the biological sample.

2. The method of claim 1, wherein the biological sample comprises human CSF.
3. The method of claim 1, wherein the buffer composition has a pH of about 6.5.
4. The method of claim 1, wherein the buffer composition comprises PIPES.
5. The method of claim 1, wherein the buffer composition comprises about 100 mM PIPES.
6. The method of claim 1, wherein the salt comprises NaCl.
7. The method of claim 1, wherein the salt comprises NaCl in a concentration of about 500 mM.
8. The method of claim 1, wherein the indicator comprises thioflavin T (ThT).
9. The method of claim 8, wherein the detection comprises measuring ThT fluorescence at about 485 nm after excitation at about 435 nm.
10. The method of claim 1, wherein the physically disrupting comprises shaking.
11. The method of claim 1, wherein the monomeric α -S protein comprises a recombinant C-terminal His Tag monomeric α -S protein.

12. The method of claim 11, wherein the monomeric α -S protein is present in a concentration of about 19.6 μ M.

13. A method for determining the presence of soluble, misfolded α -S protein in human CSF, comprising:

(A) contacting the human CSF with a pre-incubation mixture, the pre-incubation mixture comprising:

- (1) a monomeric α -S protein in a concentration range of about 100 nM to about 20 μ M;
- (2) a buffer composition comprising PIPES and having a pH of about 6.5;
- (3) NaCl in a concentration of about 500 mM; and
- (4) ThT,

to form an incubation mixture;

(B) conducting an incubation cycle on the incubation mixture, the incubation cycle being conducted two or more times on the incubation mixture effective to form an amplified portion of misfolded α -S protein from the monomeric α -S protein, each incubation cycle comprising:

- (1) incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric α -S protein in the presence of the soluble, misfolded α -S protein; and
- (2) shaking the incubation mixture, and

(C) determining if a detectable amount of misfolded α -S aggregate is present in the biological sample, wherein detection of misfolded α -S aggregate indicates the presence of soluble, misfolded α -S protein in the biological sample, and wherein the detection comprises measuring ThT fluorescence at about 485 nm after excitation at about 435 nm.

14. The method of claim 13, wherein the monomeric α -S protein comprises a recombinant C-terminal His Tag monomeric α -S protein.

15. The method of claim 14, wherein the monomeric α -S protein is present in a concentration of about 19.6 μ M.

16. A method for determining the presence of soluble, misfolded α -S in a biological sample, comprising:

(A) contacting the biological sample with a pre-incubation mixture, the pre-incubation mixture comprising:

(1) a monomeric α -S protein in a concentration range of about 1 μ M to about 100 μ M;

(2) a buffer composition;

(3) a salt; and

(4) an indicator,

to form an incubation mixture;

(B) conducting an incubation cycle on the incubation mixture, the incubation cycle being conducted two or more times on the incubation mixture effective to form an

amplified portion of misfolded α -S protein from the monomeric α -S protein, each incubation cycle comprising:

- (1) incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric α -S protein in the presence of the soluble, misfolded α -S protein; and
 - (2) physically disrupting the incubation mixture, and
- (C) determining if a detectable amount of misfolded α -S aggregate is present in the biological sample, wherein detection of misfolded α -S aggregate indicates the presence of soluble, misfolded α -S protein in the biological sample.

17. The method of claim 16, wherein the biological sample comprises human CSF.
18. The method of claim 16, wherein the buffer composition has a pH of about 6.5.
19. The method of claim 16, wherein the buffer composition comprises PIPES.
20. The method of claim 16, wherein the buffer composition comprises about 100 mM PIPES.
21. The method of claim 16, wherein the salt comprises NaCl.
22. The method of claim 16, wherein the salt comprises NaCl in a concentration of about 500 mM.

23. The method of claim 16, wherein the indicator comprises ThT.
24. The method of claim 23, wherein the detection comprises measuring ThT fluorescence at about 485 nm after excitation at about 435 nm.
25. The method of claim 16, wherein the indicator comprises ThT in a concentration of about 5 μ M.
26. The method of claim 16, wherein the physically disrupting comprises shaking.
27. The method of claim 16, wherein the monomeric α -S protein comprises a recombinant C-terminal His Tag monomeric α -S protein.
28. The method of claim 27, wherein the monomeric α -S protein is present in a concentration of about 65.4 μ M.
29. A method for determining the presence of soluble, misfolded α -S protein in human CSF, comprising:
- (A) contacting the human CSF with a pre-incubation mixture, the pre-incubation mixture comprising:
- (1) a monomeric α -S protein in a concentration range of about 1 μ M to about 100 μ M;
- (2) a buffer composition comprising PIPES and having a pH of about 6.5;

(3) NaCl in a concentration of about 500 mM; and

(4) ThT,

to form an incubation mixture;

(B) conducting an incubation cycle on the incubation mixture, the incubation cycle being conducted two or more times on the incubation mixture effective to form an amplified portion of misfolded α -S protein from the monomeric α -S protein, each incubation cycle comprising:

(1) incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric α -S protein in the presence of the soluble, misfolded α -S protein; and

(2) shaking the incubation mixture, and

(C) determining if a detectable amount of misfolded α -S aggregate is present in the biological sample, wherein detection of misfolded α -S aggregate indicates the presence of soluble, misfolded α -S in the biological sample, and wherein the detection comprises measuring ThT fluorescence at about 485 nm after excitation at about 435 nm.

30. The method of claim 33, wherein the monomeric α -S protein comprises a recombinant C-terminal His Tag monomeric α -S protein.

31. The method of claim 34, wherein the monomeric α -S protein is present in a concentration of about 65.4 μ M.