

## CLAIMS

What is claimed is:

1. A method for determining the presence of soluble, misfolded  $\alpha$ -synuclein ( $\alpha$ -syn) protein in a biological sample, comprising:

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

- (1) a monomeric  $\alpha$ -syn protein;
- (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:

(1) two or more times on the incubation mixture effective to form an amplified portion of misfolded  $\alpha$ -syn protein from the monomeric  $\alpha$ -syn protein, each incubation cycle comprising:

(i) incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric  $\alpha$ -syn protein in the presence of the soluble, misfolded  $\alpha$ -syn protein; and

(ii) physically disrupting the incubation mixture;

(2) in the presence of a  $\text{Si}_3\text{N}_4$  bead comprising a coating of bovine serum albumin (BSA); and

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

2. The method of claim 1, wherein the  $\text{Si}_3\text{N}_4$  bead has a diameter of from about 1 mm to about 5 mm.

3. The method of claim 1, wherein the  $\text{Si}_3\text{N}_4$  bead has a diameter of about 2.38 mm.

4. The method of claim 1, wherein the biological sample comprises human cerebrospinal fluid (CSF).
5. The method of claim 1, wherein the monomeric  $\alpha$ -syn protein is present in a concentration of from about 10  $\mu$ M to about 30  $\mu$ M.
6. The method of claim 1, wherein the monomeric  $\alpha$ -syn protein comprises SEQ ID NO. 2.
7. The method of claim 6, wherein the monomeric  $\alpha$ -syn protein is present in a concentration of about 19.6  $\mu$ M.
8. The method of claim 1, wherein the buffer composition has a pH of between about 6.2 to about 6.5.
9. The method of claim 1, wherein the buffer composition comprises PIPES.
10. The method of claim 1, wherein the salt comprises NaCl.
11. The method of claim 1, wherein the salt comprises NaCl in a concentration between about 500 mM to about 700 mM.
12. The method of claim 1, wherein the indicator comprises thioflavin T (ThT).
13. The method of claim 12, wherein the detection comprises measuring ThT fluorescence.
14. The method of claim 1, wherein the physically disrupting comprises shaking.
15. A method for determining the presence of soluble, misfolded  $\alpha$ -syn protein in human CSF, comprising:
  - (A) contacting the human CSF with a pre-incubation mixture, the pre-incubation mixture comprising:

- (1) a monomeric  $\alpha$ -syn protein in a concentration of from about 10  $\mu$ M to about 30  $\mu$ M;
- (2) a buffer composition having a pH between about 6.2 to about 6.5;
- (3) NaCl in a concentration between about 500 mM to about 700 mM; and
- (4) ThT,

to form an incubation mixture;

(B) conducting an incubation cycle on the incubation mixture, the incubation cycle being conducted:

- (1) two or more times on the incubation mixture effective to form an amplified portion of misfolded  $\alpha$ -syn protein from the monomeric  $\alpha$ -syn protein, each incubation cycle comprising:

- (i) incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric  $\alpha$ -syn protein in the presence of the soluble, misfolded  $\alpha$ -syn protein; and

- (ii) shaking the incubation mixture;

- (2) in the presence of a  $\text{Si}_3\text{N}_4$  bead comprising a coating of BVA and having a diameter greater than 2.3 mm; and

(C) determining if a detectable amount of misfolded  $\alpha$ -syn aggregate is present in the biological sample, wherein detection of misfolded  $\alpha$ -syn aggregate indicates the presence of soluble, misfolded  $\alpha$ -syn protein in the biological sample, and wherein the detection comprises measuring ThT fluorescence.

16. The method of claim 15, wherein the monomeric  $\alpha$ -syn protein comprises SEQ ID NO. 2.

17. The method of claim 16, wherein the monomeric  $\alpha$ -syn protein is present in a concentration of about 19.6  $\mu$ M.

18. A method for determining the presence of soluble, misfolded  $\alpha$ -syn protein in a biological sample, comprising:

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

- (1) a monomeric  $\alpha$ -syn protein comprising SEQ ID NO. 2;
- (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:

(1) two or more times on the incubation mixture effective to form an amplified portion of misfolded  $\alpha$ -syn protein from the monomeric  $\alpha$ -syn protein, each incubation cycle comprising:

(i) incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric  $\alpha$ -syn protein in the presence of the soluble, misfolded  $\alpha$ -syn protein; and

(ii) physically disrupting the incubation mixture;

(2) in the presence of a borosilicate glass bead having a diameter greater than 2.3 mm; and

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

19. The method of claim 18, wherein the biological sample is human CSF.

20. The method of claim 18, wherein:

(i) the monomeric  $\alpha$ -S protein is present in a concentration of from about 10  $\mu$ M to about 30  $\mu$ M;

(ii) the buffer composition comprises PIPES and has a pH of from about 6.2 to about 6.5;

(iii) the salt comprises NaCl in a concentration of from about 500 mM to about 700 mM;

- (iv) the borosilicate glass bead has a diameter of 2.45 mm;
- (v) the indicator comprises ThT in a concentration of from about 5  $\mu\text{M}$  to about 10  $\mu\text{M}$ ; and
- (vi) the detection comprises measuring ThT fluorescence.