

CLAIMS

1. An in vitro method for ~~determining~~detecting the presence of ~~soluble, misfolded~~ α -synuclein (α -syn) ~~protein aggregate~~ in a biological sample, the method comprising:

(A) providing a biological sample;

~~(B) ——— contacting the biological sample with~~providing a pre-incubation mixture, the pre-incubation mixture comprising:

(1) a monomeric α -syn protein;

(2) a buffer composition;

(3) a salt; ~~and~~

(4) a fluorescent protein aggregation~~an~~ indicator; and

(5) one or more Si₃N₄ beads having a diameter of 1 mm to 5 mm;

(C) combining the biological sample and the pre-incubation mixture 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 α -syn protein from the monomeric α -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 α -syn protein in the presence of the soluble, misfolded α -syn protein; and~~

~~(ii) ——— physically disrupting the incubation mixture;~~

~~(2) — in the presence of a Si₃N₄ bead comprising a coating of bovine serum albumin (BSA)~~

~~(D) incubating the incubation mixture with intermittent agitation cycles to form an incubated mixture;~~

~~(E) illuminating the incubated mixture with a wavelength of light that excites the fluorescent protein aggregation indicator~~

; and

~~(C) — (F) determining if a detectable amount of misfolded α -syn aggregate is present in the biological sample, a level of fluorescence during incubation,~~

wherein ~~an increase in the level of fluorescence during incubation~~ ~~detection of misfolded α -syn aggregate~~ indicates the presence of ~~soluble, misfolded α -syn protein aggregate~~ in the biological sample.

2. The method of claim 1, wherein the Si₃N₄ bead has a diameter of ~~from about 12.3~~ mm to ~~about 5~~ mm.

3. The method of claim 1, wherein the Si₃N₄ bead ~~has a diameter of about 2.38 mm~~ comprises a coating of bovine serum albumin (BSA).

4. ~~The method of claim 1, wherein the biological sample comprises human cerebrospinal fluid (CSF).~~

~~5. —~~ The method of claim 1, wherein the monomeric α -syn protein is present in a concentration of from ~~about 10 μ M $\pm$ 10%~~ to ~~about 30 μ M $\pm$ 10%~~.

~~6.~~ The method of claim 1, wherein the monomeric α -syn protein comprises SEQ ID NO. 2.

~~76. The method of claim 6, wherein the monomeric α -syn protein is present in a concentration of about 19.6 μ M.~~

~~8. The method of claim 1, wherein the buffer composition has a pH of between about 6.2 $\pm$ 10% to about 6.5 $\pm$ 10%.~~

~~97. The method of claim 1, wherein the buffer composition comprises PIPES.~~

~~108. The method of claim 1, wherein the salt comprises NaCl.~~

~~119. The method of claim 1, wherein the salt comprises NaCl in a concentration between about 500 $\pm$ 10% mM to about 700 $\pm$ 10% mM.~~

~~1210. The method of claim 1, wherein the fluorescent protein aggregation 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 α -syn protein in human CSF, comprising:~~

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

~~(1) — a monomeric α -syn protein in a concentration of from about 10 μ M to about 30 μ 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 α -syn protein from the monomeric α -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 α -syn protein in the presence of the soluble, misfolded α -syn protein; and~~

~~(ii) — shaking the incubation mixture;~~

~~(2) — in the presence of a Si_3N_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 α -syn aggregate is present in the biological sample, wherein detection of misfolded α -syn aggregate indicates the presence of soluble, misfolded α -syn protein in the biological sample, and wherein the detection comprises measuring ThT fluorescence.~~

~~16. — The method of claim 15, wherein the monomeric α -syn protein comprises SEQ ID NO. 2.~~

~~17. — The method of claim 16, wherein the monomeric α -syn protein is present in a concentration of about 19.6 μM .~~

181. An in vitro method for determining-detecting the presence of α -synuclein (soluble, misfolded α -syn) protein in a biological sample, the method comprising:

(A) providing a biological sample~~contacting the biological sample with~~

(B) -providing a pre-incubation mixture, the pre-incubation mixture comprising:

(1) a monomeric α -syn protein comprising SEQ ID NO. 2;

(2) a buffer composition;

(3) a salt; ~~and~~

(4) ~~an~~ a fluorescent protein aggregation indicator; and

(5) a borosilicate glass bead having a diameter greater than 2.3 mm;

(C) combining the biological sample and the pre-incubation mixture;

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 α -syn protein from the monomeric α -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 α -syn protein in the presence of the soluble, misfolded α -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~~(D) incubating the incubation mixture with intermittent agitation cycles to form an incubated mixture;

(E) illuminating the incubated mixture with a wavelength of light that excites the protein aggregation indicator; and

(F) (C) determining if a detectable amount of misfolded α -syn aggregate is present in the biological samplea level of fluorescence during incubation, wherein an increase in the level of fluorescence during incubation~~detection of misfolded α -syn aggregate~~indicates the presence of soluble, misfolded α -syn protein aggregate in the biological sample.

~~19~~12. The method of claim 18, wherein the biological sample is human CSF.

~~20.~~—The method of claim 18~~11~~, wherein:

- (i) the monomeric α -~~S~~-syn protein is present in a concentration of from ~~about~~10 μM ~~$\pm 10\%$~~ to ~~about~~30 μM ~~$\pm 10\%$~~ ;
- (ii) the buffer composition comprises PIPES and has a pH of from ~~about~~6.2 ~~$\pm 10\%$~~ to ~~about~~6.5 ~~$\pm 10\%$~~ ;
- (iii) the salt comprises NaCl in a concentration of from ~~about~~500 ~~$\pm 10\%$~~ mM to ~~about~~700 mM ~~$\pm 10\%$~~ ;
- (iv) the borosilicate glass bead has a diameter of 2.45 mm ~~$\pm 10\%$~~ ;
- (v) the fluorescent protein aggregation indicator comprises thioflavin T (ThT) in a concentration of from ~~about~~5 μM to ~~about~~10 μM ; and
- (vi) the detection comprises measuring ThT fluorescence.

13. The method of claim 1, wherein the pre-incubation mixture further comprises sarkosyl.

14. The method of claim 12, wherein the pre-incubation mixture further comprises sarkosyl.

15. The method of claim 1, wherein the pre-incubation mixture further comprises sarkosyl in a concentration of 0.1% w/v in IX PBS.