

CLAIMS

1. An in-vitro method for detecting the presence of soluble, misfolded α -synuclein (α -S) aggregates in a biological fluid, the method comprising:

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

- (1) monomeric, folded protein comprising α -S ~~protein or an isoform or fragment thereof~~ in a concentration range of 100 nM to 20 μ M;
- (2) a buffer composition;
- (3) a salt; and
- (4) an indicator comprising a fluorophore,

(B) combining the biological fluid and the pre-incubation mixture to form an incubation mixture;

(C) ~~incubating the incubation mixture with intermittent physical disruption~~ conducting two or more incubation cycles, each incubation cycle comprising:

- (1) incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric, folded protein comprising α -S in the presence of any soluble, misfolded α -S aggregate present in the biological fluid; and
- (2) physically disrupting the incubation mixture effective to break up at least a portion of any protein aggregate present ~~to form an incubated mixture;~~

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

- (E) determining a level of fluorescence during incubation, wherein an increase in the level of fluorescence indicates the presence of soluble, misfolded α -S aggregates in the biological fluid.
2. The method of claim 1, wherein the biological fluid 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 increase in the level of fluorescence is an increase in the level of fluorescence of the incubated mixture at maximum fluorescence of at least two times the standard deviation of the fluorescence of the incubated mixture at maximum fluorescence compared to the level of fluorescence of the incubated mixture during a lag phase.

10. The method of claim 1, wherein the ~~physical disruption~~ physically disrupting comprises shaking.

11. The method of claim 1, wherein the biological fluid comprises one or more of: amniotic fluid; bile; blood; blood plasma; 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.

12. The method of claim ~~11~~ 1, wherein the monomeric, folded protein comprising α -S ~~protein or an isoform or fragment thereof are~~ is present in a concentration of about 19.6 μ M.

13. An in-vitro method for detecting the presence of soluble, misfolded α -S aggregates in human CSF, comprising:

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

- (1) monomeric, folded protein comprising α -S ~~protein or an isoform or fragment thereof~~ in a concentration range of 100 nM to 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,

(B) combining the human CSF and the pre-incubation mixture to form an incubation mixture

(C) ~~incubating the incubation mixture with intermittent physical disruption~~ conducting two or more incubation cycles, each incubation cycle comprising:

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

(2) physically disrupting the incubation mixture effective to break up at least a portion of any protein aggregate present ~~to form an incubated mixture;~~

(D) illuminating the incubated mixture with a wavelength of light that excites the ThT; and

(E) determining a level of fluorescence during incubation, wherein an increase in the level of fluorescence indicates the presence of soluble, misfolded α -S aggregate in the human CSF.

14. The method of claim 13, wherein the increase in the level of fluorescence is an increase in the level of fluorescence of the incubated mixture at maximum fluorescence of at least two times the standard deviation of the fluorescence of the incubated mixture at maximum fluorescence compared to the level of fluorescence of the incubated mixture during a lag phase.

15. The method of claim 13, wherein the monomeric, folded protein comprising α -S ~~protein or an isoform or fragment thereof~~ is present in a concentration of about 19.6 μ M.

16. An in-vitro method for detecting the presence of soluble, misfolded α -S aggregates in a biological fluid, the method comprising:

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

- (1) monomeric, folded protein comprising α -S ~~protein or an isoform or fragment thereof~~ in a concentration range of 1 μ M to 100 μ M;
- (2) a buffer composition having a pH between 6 and 7;
- (3) a salt composition at a total concentration between 450 mM and 550 mM;
- and
- (4) an indicator comprising a fluorophore,

(B) combining the biological fluid and the pre-incubation mixture to form an incubation mixture

(C) ~~incubating the incubation mixture with intermittent physical disruption~~ conducting two or more incubation cycles, each incubation cycle comprising:

- (1) incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric, folded protein comprising α -S in the presence of any soluble, misfolded α -S aggregate present in the biological fluid; and
- (2) physically disrupting the incubation mixture effective to break up at least a portion of any protein aggregate present ~~to form an incubated mixture;~~

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

- (E) determining a level of fluorescence during incubation, wherein an increase in the level of fluorescence indicates the presence of soluble, misfolded α -S aggregates in the biological fluid.
17. The method of claim 16, wherein the biological fluid 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 increase in the level of fluorescence is an increase in the level of fluorescence of the incubated mixture at maximum fluorescence of at least two times the standard deviation of the fluorescence of the incubated mixture at maximum fluorescence compared to the level of fluorescence of the incubated mixture during a lag phase.

26. The method of claim 16, wherein the ~~physical disruption~~ physically disrupting comprises shaking.

27. The method of claim 16, wherein the biological fluid comprises one or more of: amniotic fluid; bile; blood; blood plasma; 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.

28. The method of claim ~~27~~ 16, wherein the monomeric, folded protein comprising α -S ~~protein or an isoform or fragment thereof are~~ is present in a concentration of about 65.4 μ M.

29. An in-vitro method for detecting the presence of soluble, misfolded α -S aggregates in human CSF, comprising:

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

- (1) monomeric, folded protein comprising α -S ~~protein or an isoform or fragment thereof~~ in a concentration range of 1 μ M to 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,
- (B) combining the human CSF and the pre-incubation mixture to form an incubation mixture
- (C) ~~incubating the incubation mixture with intermittent physical disruption~~ conducting two or more incubation cycles, each incubation cycle comprising:
 - (1) incubating the incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the monomeric, folded protein comprising α -S in the presence of any soluble, misfolded α -S aggregate present in the biological fluid; and
 - (2) physically disrupting the incubation mixture effective to break up at least a portion of any protein aggregate present ~~to form an incubated mixture;~~
- (D) illuminating the incubated mixture with a wavelength of light that excites the ThT; and
- (E) determining a level of fluorescence during incubation, wherein an increase in the level of fluorescence indicates the presence of soluble, misfolded α -S aggregate in the human CSF.

30. The method of claim 29, wherein the increase in the level of fluorescence is an increase in the level of fluorescence of the incubated mixture at maximum fluorescence of at least two times the standard deviation of the fluorescence of the incubated mixture at maximum fluorescence compared to the level of fluorescence of the incubated mixture during a lag phase.

31. The method of claim ~~30~~ 29, wherein the monomeric, folded protein comprising α -S protein ~~or an isoform or fragment thereof are~~ is present in a concentration of about 65.4 μ M.

32. ~~— A kit to detect the presence of α -synuclein (α -S) aggregates in a biological fluid, the kit comprising: a pre-incubation mixture comprising:~~

~~(1) monomeric, folded α -S proteins or an isoform or fragment thereof;~~

~~(2) a buffer composition having a pH between 6 and 7;~~

~~(3) a salt composition at a total concentration of between 450 mM and 550 mM; and~~

~~(4) thioflavin T (ThT);~~

~~and optionally comprising instructions for the use thereof.~~