CLAIMS

1. An in vitro method for detecting the presence of misfolded 4R tau protein aggregates in a first cerebrospinal fluid (CSF) sample, the method comprising:

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

(1) a monomeric 4R tau protein having a concentration of 12.5 μM ±10%, the monomeric 4R tau protein without the catalytic activity for misfolding and aggregation associated with misfolded seeds;

(2) heparin having a concentration of 1.25 μM ±10%; and

(3) Thioflavin T (ThT) in a concentration of 5 μM ±10%,

(B) combining the first CSF sample and the first pre-incubation mixture in a first well of a reaction vessel to form a first incubation mixture;

(C) incubating the first incubation mixture with intermittent cyclic agitation cycles to form a first incubated mixture;

(D) illuminating the first incubated mixture; and

(E) determining a level of fluorescence during incubation, wherein an increase in the level of fluorescence of the first incubated mixture at maximum fluorescence compared to the level of fluorescence of the first incubated mixture at any time point up to 50 hours of incubation with intermittent agitation cycles indicates the presence of misfolded 4R tau protein aggregates in the first CSF sample.

2. The method of claim 1, further comprising

(A) providing a second pre-incubation mixture that is free of tau seeds, the second pre-incubation mixture comprising:

(1) a monomeric 4R tau protein having a concentration of 12.5 μM ±10%, the monomeric 4R tau protein without the catalytic activity for misfolding and aggregation associated with misfolded seeds;

(2) heparin having a concentration of 1.25 μM ±10%; and

(3) Thioflavin T (ThT) in a concentration of 5 μM ±10%,

(B) combining a second CSF sample that is free of tau seeds as a negative control sample and the second pre-incubation mixture in a second well of the reaction vessel to form a second incubation mixture;

(C) incubating the second incubation mixture with intermittent cyclic agitation cycles to form a second incubated mixture;

(D) illuminating the second incubated mixture; and

(E) determining a level of fluorescence during incubation of the second incubated mixture and comparing the level of maximum fluorescence of the first incubated mixture with the level of maximum fluorescence of the second incubated mixture, wherein an increase in the level of fluorescence of the first incubated mixture at maximum fluorescence of at least two times the standard deviation of the fluorescence of the first incubated mixture at maximum fluorescence compared to the level of fluorescence of the second incubated mixture after at least 200 hours of incubation with intermittent agitation cycles indicates the presence of misfolded 4R tau protein aggregates in the first CSF sample.

3. The method of claim 1, wherein the presence of the misfolded 4R tau protein aggregates is indicative of a tauopathy, the method further comprising characterizing an identity of the tauopathy by analyzing the fluorescence kinetic parameters thereof for a signature of at least one of: Alzheimer's disease (AD), Parkinson's Disease (PD), Progressive Supranuclear Palsy (PSP), FrontoTemporal Dementia (FTD), Corticobasal degeneration (CBD), Mild cognitive impairment (MCI), Argyrophilic grain disease (AgD) Traumatic Brain Injury (TBI), Chronic Traumatic Encephalopathy (CTE), and Dementia Pugilistica (DP).

4. The method of claim 1, wherein the presence of the misfolded 4R tau protein aggregates is indicative of a tauopathy, the method further comprising characterizing the identity of the tauopathy using one or more of: an antibody; an indicator selective for the tauopathy-specific misfolded tau protein aggregate; a spectrum characteristic of the tauopathy-specific misfolded tau protein aggregate; a proteolytic resistance of the tauopathy-specific misfolded tau protein aggregate; and a stability to denaturation of the tauopathy-specific misfolded tau protein aggregate.

5. The method of claim 1, wherein the incubating comprises incubating the first incubation mixture at a temperature of 37 °C ±10%.

6. The method of claim 1, wherein the intermittent cyclic agitation cycles comprise 1 ±10% minute of shaking at 500 ±10% revolutions per minute followed by 29 ±10% minutes without shaking.

7. The method of claim 1, wherein the illuminating comprises illuminating the first incubated mixture with an excitation wavelength of 435 ±10% nanometers and an emission wavelength of 485 ±10% nanometers.

8. The method of claim 1, wherein the detecting further comprising one or more of: a Western Blot assay, a dot blot assay, an enzyme-linked immunosorbent assay (ELISA), a fluorescent protein/peptide binding assay, a thioflavin binding assay, a sedimentation assay, electron microscopy, atomic force microscopy, surface plasmon resonance, spectroscopy;

contacting the first incubated mixture with a protease; and

detecting the misfolded 4R tau protein aggregates using anti-misfolded protein antibodies or antibodies specific for a misfolded tau aggregate in one or more of: a Western Blot assay, a dot blot assay, and an ELISA.

9. The method of claim 3, wherein the analyzing comprising comparing one or more of: lag phase, T50, amplification rate, and amplification extent to one or more corresponding predetermined corresponding fluorescence kinetic parameters that are characteristic of the identity of the tauopathy to determine a similarity or difference effective to characterize the identity of the tauopathy.

10. A method for the preparation of misfolded 4R tau protein aggregates, the method comprising:

(A) providing a cerebrospinal fluid (CSF) sample;

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

(1) a monomeric 4R tau protein having a concentration of 12.5 μM ±10%, the monomeric 4R tau protein without the catalytic activity for misfolding and aggregation associated with misfolded seeds; and

(2) heparin having a concentration of 1.25 μM ±10%;

(C) combining the CSF sample and the pre-incubation mixture to form an incubation mixture; and

(D) incubating the incubation mixture with intermittent cyclic agitation cycles.

11. The method of claim 10, further comprising detecting the misfolded 4R tau protein aggregates, the detecting comprising:

(A) illuminating the incubated mixture; and

(B) determining a level of fluorescence during incubation, wherein an increase in the level of fluorescence of the incubated mixture at maximum fluorescence compared to the level of fluorescence of the incubation mixture at any time point up to 50 hours of incubation with intermittent agitation cycles indicates the presence of misfolded 4R tau protein aggregates in the CSF sample.

12. The method of claim 10, further comprising characterizing the misfolded 4R tau protein aggregates via contacting the misfolded 4R tau protein aggregates with a proteinase selected from the group consisting of proteinase K, trypsin, and chymotrypsin.

13. The method of claim 10, wherein the incubating comprises incubating the first incubation mixture at a temperature of 37 ℃ ±10%.

14. The method of claim 10, wherein the intermittent cyclic agitation cycles comprise 1 ±10% minute of shaking at 500 ±10% revolutions per minute followed by 29 ±10% minutes without shaking.

15. The method of claim 11, wherein the illuminating comprising illuminating the first incubated mixture with an excitation wavelength of 435 ±10% nanometers and an emission wavelength of 485 ±10% nanometers.