

CLAIMS

1. A method for determining a presence or absence in a biological sample of a first misfolded protein aggregate, the method comprising:
 - performing a first protein misfolding cyclic amplification (PMCA) procedure, the first PMCA procedure comprising:
 - forming a first incubation mixture by contacting a first portion of the biological sample with a first substrate protein, the first substrate protein comprising monomeric seed-free 4R tau;
 - conducting an incubation cycle two or more times under conditions effective to form a first amplified, misfolded protein aggregate, each incubation cycle comprising:
 - incubating the first incubation mixture at a temperature between about 15° C. and about 40° C effective to cause misfolding and/or aggregation of at least a portion of the first substrate protein in the presence of the first misfolded protein aggregate;
 - disrupting the first incubation mixture effective to form the first amplified, misfolded protein aggregate; and
 - determining the presence or absence in the biological sample of the first misfolded protein aggregate by analyzing the first incubation mixture for the presence or absence of the first amplified, misfolded protein aggregate,
 - the first misfolded protein aggregate comprising the first substrate protein and the first amplified, misfolded protein aggregate comprising the first substrate protein.
2. The method of claim 1, comprising determining the presence in the biological sample of the first misfolded protein aggregate, the first misfolded protein aggregate being misfolded 4R tau aggregate.
3. The method of claim 1, further comprising determining the presence or absence in the biological sample of at least a second misfolded protein aggregate.
4. The method of claim 3, further comprising performing at least a second PMCA procedure to determine the presence or absence in the biological sample of at least the second misfolded protein aggregate, comprising:
 - forming a second incubation mixture by contacting a second portion of the biological sample with a second substrate protein, the second substrate protein being subject to pathological misfolding and/or aggregation in vivo to form the second misfolded protein aggregate;

conducting an incubation cycle two or more times under conditions effective to form a second amplified, misfolded protein aggregate, each incubation cycle comprising:

incubating the second incubation mixture effective to cause misfolding and/or aggregation of at least a portion of the second substrate protein in the presence of the second misfolded protein aggregate;

disrupting the second incubation mixture effective to form the second amplified, misfolded protein aggregate; and

determining the presence or absence in the biological sample of the second misfolded protein aggregate by analyzing the second incubation mixture for the presence or absence of the second amplified, misfolded protein aggregate,

the second misfolded protein aggregate comprising the second substrate protein and the second amplified, misfolded protein aggregate comprising the second substrate protein, the second substrate protein comprising one of: amyloid-beta (A β), alpha synuclein, and 3R tau.

5. The method of claim 4, the second substrate protein comprising 3R tau, the method further comprising determining a ratio of the 4R tau and the 3R tau in the biological sample of between about 1:99 and about 99:1.

6. The method of claim 1, the biological sample being taken from a subject, further comprising determining or diagnosing the presence or absence of a tauopathy in the subject according to the presence or absence of the first misfolded protein aggregate in the biological sample.

7. The method of claim 6, further comprising characterizing an identity of the tauopathy by analyzing the first amplified, misfolded protein aggregate or one or more corresponding PMCA 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).

8. The method of claim 7, characterizing the identity of the tauopathy comprising 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.

9. The method of claim 1, the biological sample being taken from a subject characterized by one of:

exhibiting no clinical signs of dementia according to cognitive testing

exhibiting no cortex plaques or tangles according to contrast imaging; and

exhibiting clinical signs of dementia according to cognitive testing,

further comprising determining or diagnosing the presence or absence of a tauopathy in the subject according to the presence or absence of the first misfolded protein aggregate in the sample.

10. The method of claim 1, comprising preparing the first incubation mixture characterized by at least one concentration of:

the first substrate protein of less than about 20 μM ;

heparin of less than about 75 μM ;

NaCl of less than about 190 mM; and

Thioflavin T of less than about 9.5 μM .

11. The method of claim 1, comprising preparing or maintaining the first incubation mixture characterized by one or more of:

the first substrate protein at a concentration between about 0.001 μM and about 2000 μM ;

heparin at a concentration between about 0.001 μM and about 75 μM ;

comprising a buffer composition of one or more of: Tris-HCL, PBS, MES, PIPES, MOPS, BES, TES, and HEPES, .

the buffer composition at a total concentration of between about 1 μM and about 1 M;

comprising a salt composition at a total concentration of between about 1 μM and about 1 M;

a pH of between about 5 and about 9; and

an indicator at a total concentration of between about 1 nM and about 1 mM.

12. The method of claim 1:

further comprising contacting an indicator of the first misfolded protein aggregate to the first incubation mixture, the indicator of the first misfolded protein aggregate being characterized by an indicating state in the presence of the first misfolded protein aggregate and a non-indicating state in the absence of the first misfolded protein aggregate; and

wherein the determining the presence of the first misfolded protein aggregate in the biological sample comprises detecting the indicating state of the indicator of the first misfolded protein aggregate.

13. The method of claim 1, the detecting the first misfolded protein aggregate 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 Congo Red binding assay, a sedimentation assay, electron microscopy, atomic force microscopy, surface plasmon resonance, spectroscopy;

contacting the first incubation mixture with a protease; and

detecting the first misfolded protein aggregate 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.

14. The method of claim 1, the biological sample characterized by one or more of: comprising one or more of: amniotic fluid; bile; blood; 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;

derived from cells or tissue of one or more of: skin, brain, heart, liver, pancreas, lung, kidney, gastro-intestine, nerve, mucous membrane, blood cell, gland, and muscle; and

each portion of the biological sample characterized by a volume from about 1 μ L to about 1000 μ L.

15. The method of claim 1, further comprising obtaining the biological sample from a subject,

the subject being one or more of: at risk of a tauopathy, having the tauopathy, and under treatment for the tauopathy.

16. The method of claim 15, the subject being treated for tauopathy, further comprising:

comparing the amount of the first misfolded protein aggregate in the biological sample to an amount of the first misfolded protein aggregate in a comparison sample, the biological sample and the comparison sample being taken from the subject at different times over a period of time under the treatment; and

determining the subject is one of: responsive to the treatment according to a change in the first misfolded protein aggregate over the period of time, or non-responsive to the treatment according to homeostasis of the first misfolded protein aggregate over the period of time.

17. The method of claim 1, further comprising selectively concentrating the first misfolded protein aggregate in one or more of the biological sample and the first incubation mixture.

18. The method of claim 1, the disrupting the first incubation mixture comprising one or more of: sonication, stirring, shaking, freezing/thawing, laser irradiation, autoclave incubation, high pressure, and homogenization.

19. The method of claim 1, comprising:

contacting the biological sample with a thioflavin and a molar excess of the first substrate protein to form the first incubation mixture, the molar excess being greater than an amount of the first substrate protein included in the first misfolded protein aggregate in the biological sample;

conducting the incubation cycle two or more times effective to form the first amplified, misfolded protein aggregate, each incubation cycle comprising:

incubating the first incubation mixture effective to cause misfolding and/or aggregation of the first substrate protein in the presence of the first misfolded protein aggregate;

shaking the first incubation mixture effective to form the first amplified, misfolded protein aggregate; and

determining the presence of the first misfolded protein aggregate in the biological sample by detecting a fluorescence of the thioflavin corresponding to the first misfolded protein aggregate.

20. The method of claim 1, provided the sample excludes tau fibrils.