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 (Aβ), alpha synuclein, and 3R tau.

~~5. The method of claim 4, the second substrate protein comprising one of: amyloid-beta (Aβ), alpha synuclein, and 3R tau.~~

~~6.~~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.

~~7. The method of claim 3, comprising determining the presence in the sample of the first misfolded protein aggregate and the second misfolded protein aggregate.~~

~~8. The method of claim 7, further comprising determining the presence or absence in the sample of at least a third misfolded protein aggregate.~~

~~9. The method of claim 8, further comprising performing at least a third PMCA procedure to determine the presence or absence in the sample of at least the third misfolded protein aggregate, comprising:~~

~~forming a third incubation mixture by contacting a third portion of the sample with a third substrate protein, the third substrate protein being subject to pathological misfolding and/or aggregation in vivo to form the third misfolded protein aggregate;~~

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

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

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

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

the third misfolded protein aggregate comprising the third substrate protein and the third amplified, misfolded protein aggregate comprising the third substrate protein.

10. The method of claim 9, the first substrate protein comprising 4R tau, the second substrate protein comprising Aβ, and the third substrate protein comprising alpha synuclein.

11. The method of claim 8, comprising determining the presence in the sample of the first misfolded protein aggregate, the second misfolded protein aggregate, and the third misfolded protein aggregate.

12. The method of claim 8, further comprising determining the presence or absence in the sample of at least a third misfolded protein aggregate.

13. The method of claim 12, further comprising performing at least a fourth PMCA procedure to determine the presence or absence in the sample of at least the fourth misfolded protein aggregate, comprising:

forming a fourth incubation mixture by contacting a fourth portion of the sample with a fourth substrate protein, the fourth substrate protein being subject to pathological misfolding and/or aggregation in vivo to form the fourth misfolded protein aggregate;

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

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

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

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

~~the fourth misfolded protein aggregate comprising the fourth substrate protein and the fourth amplified, misfolded protein aggregate comprising the fourth substrate protein.~~

~~14. The method of claim 13, the first substrate protein comprising 4R tau, the second substrate protein comprising Aβ, the third substrate protein comprising alpha synuclein, and the fourth substrate protein comprising 3R tau.~~

~~15.~~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.

~~16. The method of claim 15, the method further comprising:~~

~~performing at least a second PMCA procedure to determine the presence or absence in the sample of a misfolded protein aggregate comprising a second substrate protein, comprising:~~

~~forming a second incubation mixture by contacting a second portion of the 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 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.~~

~~17. The method of claim 16, the subject comprising the tauopathy, further comprising characterizing an identity of the tauopathy in the subject according to: the presence in the~~

~~sample of the first misfolded protein aggregate; and the presence or absence in the sample of the second misfolded protein aggregate.~~

~~18. The method of claim 16, the second substrate protein comprising one of: amyloid-beta (Aβ), alpha synuclein, and 3R tau.~~

~~19. The method of claim 16, the method further comprising:~~

~~performing at least a third PMCA procedure to determine the presence or absence in the sample of a misfolded protein aggregate comprising a third substrate protein, comprising:~~

~~forming a third incubation mixture by contacting a third portion of the sample with a third substrate protein, the third substrate protein being subject to pathological misfolding and/or aggregation in vivo to form the third misfolded protein aggregate;~~

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

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

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

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

~~the third misfolded protein aggregate comprising the third substrate protein and the third amplified, misfolded protein aggregate comprising the third substrate protein.~~

~~20. The method of claim 19, the method further comprising:~~

~~performing at least a fourth PMCA procedure to determine the presence or absence in the sample of a misfolded protein aggregate comprising a fourth substrate protein, comprising:~~

~~forming a fourth incubation mixture by contacting a fourth portion of the sample with a fourth substrate protein, the fourth substrate protein being subject to pathological misfolding and/or aggregation in vivo to form the fourth misfolded protein aggregate;~~

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

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

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

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

~~the fourth misfolded protein aggregate comprising the fourth substrate protein and the fourth amplified, misfolded protein aggregate comprising the fourth substrate protein.~~

~~21. The method of claim 15, the tauopathy comprising a primary tauopathy or a secondary tauopathy.~~

~~22. The method of claim 15, the tauopathy characterized at least in part by misfolding and/or aggregation of 4R tau protein.~~

~~23. The method of claim 16, the second substrate protein comprising 3R tau, the method further comprising characterizing the tauopathy by a ratio between the first misfolded protein aggregate and the second misfolded protein aggregate, and determining the tauopathy corresponds to a known tauopathy according to a known ratio of misfolding and/or aggregation of 4R tau protein to 3R tau protein that is characteristic of the known tauopathy.~~

~~24.~~7. The method of claim ~~15~~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).

~~25. The method of claim 24, characterizing the identity of the tauopathy comprising:~~

~~determining the one or more corresponding PMCA kinetic parameters, comprising one or more of: lag phase, T_{50}, amplification rate, and amplification extent;~~

~~comparing the one or more corresponding PMCA kinetic parameters to one or more corresponding predetermined corresponding PMCA 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.~~

~~26.~~8. The method of claim ~~24~~7, characterizing the identity of the tauopathy comprising using one or more of: an antibody ~~selective for a conformational epitope of a tauopathy-specific misfolded tau protein aggregate~~; 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.

~~27. The method of claim 15, provided that the tauopathy excludes Pick's disease.~~

~~28. The method of claim 15, determining or diagnosing the presence or absence of a tauopathy in the subject comprising comparing the presence or absence of the first misfolded protein aggregate in the sample to a control sample taken from a control subject.~~

~~29. The method of claim 1, the detecting comprising detecting an amount of the first misfolded protein aggregate in the sample, the sample being taken from a subject, further comprising determining or diagnosing the presence or absence of a tauopathy in the subject by comparing the amount of the first misfolded protein aggregate in the sample to a predetermined threshold amount.~~

~~30. The method of claim 1, the sample being taken from a subject exhibiting no 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.~~

~~31. The method of claim 1, the sample being taken from a subject exhibiting no cortex plaques or tangles according to contrast imaging, 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.~~

~~32. The method of claim 1, the sample being taken from a subject exhibiting clinical signs of dementia according to cognitive testing, further comprising determining or diagnosing the presence or absence of a tauopathy as a contributing factor to the clinical signs of dementia in the subject according to the presence or absence of the first misfolded protein aggregate in the sample.~~

~~33~~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 ~~and the subject exhibiting a predisposition to dementia according to genetic 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.

~~34~~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.

~~35~~11. The method of claim 1, comprising preparing or maintaining the first incubation mixture characterized by one or more of:

~~the first incubation mixture comprising~~ the first substrate protein at a concentration between about 0.001 µM and about 2000 µM~~.~~;

~~36. The method of claim 1, comprising preparing the first incubation mixture characterized by~~ heparin at a concentration between about 0.001 µM and about 75 µM~~.~~;

~~37. The method of claim 1,~~ comprising ~~preparing the first incubation mixture comprising~~ a buffer composition of one or more of: Tris-HCL, PBS, MES, PIPES, MOPS, BES, TES, and HEPES, .

~~38. The method of claim 37, comprising preparing the first incubation mixture comprising~~ the buffer composition at a total concentration of between about 1 µM and about 1 M~~.~~;

~~39. The method of claim 1, comprising preparing the first incubation mixture~~ comprising a salt composition at a total concentration of between about 1 µM and about 1 M~~.~~;

~~40. The method of claim 39, the salt composition comprising one or more of: NaCl and KCl.~~

~~41. The method of claim 1, comprising preparing or maintaining the first incubation mixture at~~ a pH of between about 5 and about 9~~.~~; and

~~42. The method of claim 1, comprising preparing the first incubation mixture comprising~~ an indicator at a total concentration of between about 1 nM and about 1 mM.

~~43. The method of claim 1, the incubating comprising heating or maintaining the first incubation mixture at a temperature between about 5˚C and about 60˚C.~~

~~44~~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.

~~45. The method of claim 44, the indicating state of the indicator and the non-indicating state of the indicator being characterized by a difference in fluorescence, the determining the presence of the first misfolded protein aggregate in the sample comprising detecting the difference in fluorescence.~~

~~46. The method of claim 44, comprising contacting a molar excess of the indicator of the first misfolded protein aggregate to the first incubation mixture, the molar excess being greater than a total molar amount of protein monomer included in the first substrate protein and the first misfolded protein aggregate in the first incubation mixture.~~

~~47. The method of claim 44, the indicator of the first misfolded protein aggregate comprising one or more of: a thioflavin, Congo Red, m-I-Stilbene, Chrysamine G, PIB, BF-227, X-34, TZDM, FDDNP, MeO-XO4, IMPY, NIAD-4, luminescent conjugated polythiophenes, a fluorescent protein, and derivatives thereof.~~

~~48. The method of claim 1, the determining the presence of the first misfolded protein aggregate in the sample comprising determining an amount of the first misfolded protein aggregate in the sample.~~

~~49. The method of claim 47, comprising detecting the amount of the first misfolded protein aggregate in the sample at a sensitivity of at least about 70%.~~

~~50. The method of claim 47, comprising detecting the amount of the first misfolded protein aggregate in the sample at less than about 100 nmol.~~

~~51. The method of claim 47, comprising detecting the amount of the first misfolded protein aggregate in the sample in a molar ratio to the first substrate protein comprised by the sample, the molar ratio being less than about 1:100.~~

~~52. The method of claim 47, comprising determining the amount of the first misfolded protein aggregate in the sample compared to a control sample.~~

~~53. The method of claim 1, comprising detecting the first misfolded protein aggregate in the sample with a specificity of at least about 70%.~~

~~54~~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, ~~and~~ spectroscopy;~~.~~

~~55. The method of claim 54, the ELISA comprising a two-sided sandwich ELISA.~~

~~56. The method of claim 54, the spectroscopy comprising one or more of: quasi-light scattering spectroscopy, multispectral ultraviolet spectroscopy, confocal dual-color fluorescence correlation spectroscopy, Fourier-transform infrared spectroscopy, capillary electrophoresis with spectroscopic detection, electron spin resonance spectroscopy, nuclear magnetic resonance spectroscopy, and Fluorescence Resonance Energy Transfer (FRET) spectroscopy.~~

~~57. The method of claim 1, the detecting the first misfolded protein aggregate comprising:~~

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.

~~58. The method of claim 1, further comprising providing the first substrate protein in labeled form.~~

~~59. The method of claim 58, the first substrate protein in labeled form comprising one or more of: a covalently incorporated radioactive amino acid, a covalently incorporated, isotopically labeled amino acid, and a covalently incorporated fluorophore.~~

~~60. The method of claim 59, the detecting the first misfolded protein aggregate comprising detecting the first substrate protein in labeled form as incorporated into the first amplified, misfolded protein aggregate.~~

~~61~~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~~.~~;

~~62. The method of claim 1, the sample~~ 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

~~63. The method of claim 1,~~ each portion of the biological sample characterized by a volume from about 1 µL to about 1000 µL.

~~64. The method of claim 1, each portion of the sample comprising CSF characterized by a volume from about 10 µL to about 80 µL.~~

~~65. The method of claim 1, each portion of the sample comprising one of:~~

~~plasma characterized by a volume from about 250 µL to about 750 µL; and~~

~~blood characterized by a volume from about 200 µL to about 1000 µL.~~

~~66~~15. The method of claim 1, further comprising obtaining the biological sample from a subject~~.~~.

~~67. The method of claim 66, the subject being one of a: human, mouse, rat, dog, cat, cattle, horse, deer, elk, sheep, goat, pig, and non-human primate.~~

~~68. The method of claim 66,~~ the subject being one or more of: at risk of a tauopathy, having the tauopathy, and under treatment for the tauopathy.

~~69. The method of claim 1, the sample being taken from a subject, further comprising determining a progression or homeostasis of a tauopathy in the subject by comparing the amount of the first misfolded protein aggregate in the sample to an amount of the first misfolded protein aggregate in a comparison sample taken from the subject at a different time compared to the sample.~~

~~70~~16. The method of claim 1~~5~~, the subject being treated ~~with~~ for ~~a~~ tauopathy ~~modulating therapy,~~ 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 ~~tauopathy modulating therapy~~treatment; and

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

~~71. The method of claim 70, further comprising treating the subject determined to be responsive to the tauopathy modulating therapy with the tauopathy modulating therapy.~~

~~72. The method of claim 1, the sample being taken from a subject, the subject being treated with a tauopathy modulating therapy to inhibit production of the first substrate protein or to inhibit aggregation of the first misfolded protein aggregate.~~

~~73~~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.

~~74. The method of claim 73, the selectively concentrating the first misfolded protein aggregate comprising pre-treating the sample prior to forming the first incubation mixture.~~

~~75. The method of claim 70, the selectively concentrating the first misfolded protein aggregate comprising pre-treating the first incubation mixture prior to incubating the first incubation mixture.~~

~~76. The method of claim 73, the selectively concentrating the first misfolded protein aggregate comprising contacting one or more antibodies capable of binding the first misfolded protein aggregate to form a captured first misfolded protein aggregate.~~

~~77. The method of claim 76, the one or more antibodies capable of binding the first misfolded protein aggregate comprising one or more of: an antibody specific for an amino acid epitope sequence of the first misfolded protein aggregate, and an antibody specific for a conformation of the first misfolded protein aggregate.~~

~~78. The method of claim 77, the antibody specific for a conformation of the first misfolded protein aggregate being selective for a conformational epitope of a tauopathy-specific misfolded tau aggregate.~~

~~79. The method of claim 77, the one or more one or more antibodies capable of binding the first misfolded protein aggregate being coupled to a solid phase.~~

~~80. The method of claim 79, the solid phase comprising one or more of a magnetic bead and a multiwell plate.~~

~~81. The method of claim 76, the contacting the sample with the first substrate protein to form the first incubation mixture comprising contacting a molar excess of the first substrate protein to the sample comprising the captured first misfolded protein aggregate, the molar excess of the first substrate protein being greater than a total molar amount of protein monomer included in the captured first misfolded protein aggregate.~~

~~82. The method of claim 81, the incubating the first incubation mixture being effective to cause misfolding and/or aggregation of at least a portion of the first substrate protein in the presence of the captured first misfolded protein aggregate to form the first amplified, misfolded protein aggregate.~~

~~83~~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.

~~84. The method of claim 1, the disrupting the first incubation mixture comprising cyclic agitation.~~

~~85. The method of claim 84, the cyclic agitation being conducted for one or more of: between about 50 rotations per minute (RPM) and 10,000 RPM, between about 200 RPM and about 2000 RPM, and at about 500 RPM.~~

~~86. The method of claim 1, the disrupting the first incubation mixture being conducted in each incubation cycle for one or more of: between about 5 seconds and about 10 minutes, between about 30 sec and about 1 minute, between about 45 sec and about 1 minute, and about 1 minute.~~

~~87. The method of claim 1, the incubating the first incubation mixture being independently conducted, in each incubation cycle for one or more of: between about 1 minute and about 5 hours, between about 5 minutes and about 5 hours, between about 10 minutes and about 2 hours, between about 15 minutes and about 1 hour, and between about 25 minutes and about 45 minutes.~~

~~88. The method of claim 1, each incubation cycle comprising independently incubating and disrupting the first incubation mixture for one or more of: incubating between about 1 minute and about 5 hours and disrupting between about 5 seconds and about 10 minutes; incubating between about 5 minutes and about 5 hours and disrupting between about 5 seconds and about 10 minutes; incubating between about 10 minutes and about 2 hours and disrupting between about 30 sec and about 1 minute; incubating between about 15 minutes and about 1 hour and disrupting between about 45 sec and about 1 minute; incubating between about 25 minutes and about 45 minutes and disrupting between about 45 sec and about 90 seconds; incubating for about 29 minutes and for about 1 minute; and incubating about 1 minute and disrupting about 1 minute.~~

~~89. The method of claim 1, the conducting the incubation cycle being repeated for one or more of: between about 2 times and about 1000 times, between about 5 times and about 500 times, between about 50 times and about 500 times, and between about 150 times and about 250 times.~~

~~90. The method of claim 1, the contacting the sample with the first substrate protein to form the first incubation mixture being conducted under physiological conditions.~~

~~91. The method of claim 1, comprising contacting the sample with a molar excess of the first substrate protein to form the first incubation mixture, the molar excess being greater than a total molar amount of protein monomer included in the first misfolded protein aggregate in the sample.~~

~~92~~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.

~~93. The method of claim 1, the first substrate protein being produced by one of: chemical synthesis, recombinant production, and extraction from non-recombinant biological samples.~~

~~94. The method of claim 1,~~

~~the first misfolded protein aggregate comprising one or more of a soluble first misfolded protein aggregate and an insoluble first misfolded protein aggregate; and~~

~~the first amplified, misfolded protein aggregate comprising one or more of: a soluble portion and an insoluble portion,~~

~~95. The method of claim 94, the first misfolded protein aggregate substantially being the soluble first misfolded protein aggregate.~~

~~96~~20. The method of claim 1, provided the sample excludes tau fibrils.

~~97. A method for determining a presence or absence in a subject of a tauopathy corresponding to a first misfolded protein aggregate, comprising:~~

~~providing a sample from the subject;~~

performing at least 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 sample with a first substrate protein, the first substrate protein comprising at a tau isoform, the first substrate protein being subject to pathological misfolding and/or aggregation in vivo to form the first misfolded protein aggregate, the first substrate protein comprising 4R tau protein;

conducting an incubation cycle two or more times under conditions 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 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 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; and

determining the presence or absence of the tauopathy in the subject according the presence or absence of the first misfolded protein aggregate in the sample,

the first misfolded protein aggregate comprising the first substrate protein and the first amplified, misfolded protein aggregate comprising the first substrate protein,

provided that the tauopathy excludes Pick's disease when the first substrate protein consists of monomeric 3R tau.

98. The method of claim 97, the method further comprising:

performing at least a second PMCA procedure to determine the presence or absence in the sample of at least a second misfolded protein aggregate, comprising:

forming a second incubation mixture by contacting a second portion of the 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 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 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.

99. The method of claim 98, the second substrate protein being distinct from the first substrate protein and the second substrate protein comprising one of: amyloid-beta (Aβ), alpha synuclein, 3R tau, and 4R tau.

100. (Canceled)

101. The method of claim 98, the method further comprising:

performing at least a third PMCA procedure to determine the presence or absence in the sample of at least a third misfolded protein aggregate, comprising:

forming a third incubation mixture by contacting a third portion of the sample with a third substrate protein, the third substrate protein being subject to pathological misfolding and/or aggregation in vivo to form the third misfolded protein aggregate;

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

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

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

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

the third misfolded protein aggregate comprising the third substrate protein and the third amplified, misfolded protein aggregate comprising the third substrate protein.

102. The method of claim 101, the tauopathy being present in the subject, further comprising characterizing an identity of the tauopathy in the subject according to: the presence in the

sample of the first misfolded protein aggregate; and the presence or absence in the sample of the second misfolded protein aggregate.

103. The method of claim 97, provided that the tauopathy is not primarily characterized by misfolding and/or aggregation of 3R tau protein.

104. The method of claim 97, comprising:

contacting the 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 protein monomer included in the first misfolded protein aggregate in the 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 at least the portion 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 or absence of the first misfolded protein aggregate in the sample by detecting a fluorescence of the thioflavin corresponding to the first misfolded protein aggregate.

105. A method for determining a presence or absence in a sample of a first misfolded protein aggregate using capturing, comprising:

capturing the first misfolded protein aggregate from the sample to form a captured first misfolded protein aggregate;

performing at least a first protein misfolding cyclic amplification (PMCA) procedure, the first PMCA procedure comprising:

forming a first incubation mixture by contacting the captured first misfolded protein aggregate with a molar excess of a first substrate protein, the first substrate protein being subject to pathological misfolding and/or aggregation in vivo to form the first misfolded protein aggregate, and the first substrate protein comprising 4R tau protein;

the molar excess being greater than an amount of protein monomer included in the captured first misfolded protein aggregate;

conducting an 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 at least a portion of the first substrate protein in the presence of the captured first misfolded protein aggregate;

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

determining the presence or absence of the first misfolded protein aggregate in the sample by detecting at least a portion 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.

106. The method of claim 105, the capturing the first misfolded protein aggregate from the sample to form a captured first misfolded protein aggregate being conducted using one or more antibodies specific for the first misfolded protein aggregate, comprising one or more of: an antibody specific for an amino acid epitope sequence of the first misfolded protein aggregate and an antibody specific for a conformation of the first misfolded protein aggregate.

107. The method of claim 106, the antibody specific for a conformation of the first misfolded protein aggregate being selective for a conformational epitope of a tauopathy-specific first misfolded protein aggregate.

108. The method of claim 106, the antibody specific for the conformation of the first misfolded protein aggregate corresponding to 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).

109. The method of claim 105, the one or more antibodies specific for the first misfolded protein aggregate being coupled to a solid phase.

110. The method of claim 109, the solid phase comprising one or more of a magnetic bead and a multiwell plate.

111. The method of claim 109, the contacting the sample with the first substrate protein to form the first incubation mixture comprising contacting a molar excess of the first substrate

protein to the sample, the molar excess of the first substrate protein being greater than a total molar amount of protein monomer included in the captured first misfolded protein aggregate.

112. The method of claim 109, the incubating the first incubation mixture being effective to cause misfolding and/or aggregation of at least a portion of the first substrate protein in the presence of the captured first misfolded protein aggregate to form the first amplified, misfolded protein aggregate.

113. (Canceled)

114. A method for determining a presence or absence of a tauopathy in a subject, the tauopathy comprising Alzheimer's disease (AD), the method comprising:

providing the subject;

obtaining a sample from the subject, the sample comprising one or more of: a bio-fluid, a biomaterial, a homogenized tissue, and a cell lysate;

performing at least a first protein misfolding cyclic amplification (PMCA) procedure, the first PMCA procedure comprising:

forming a first incubation mixture by contacting the sample with a first substrate protein, the first substrate protein comprising 4R tau;

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

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

determining the presence or absence in the sample of the first misfolded protein aggregate by detecting in the first incubation mixture the presence or absence of the first amplified, misfolded protein aggregate; and

determining the presence or absence in the subject of AD according to the presence or absence of the first misfolded protein aggregate in the sample.

115. The method of claim 114, determining the presence or absence in the subject of AD further comprising distinguishing AD from one or more additional tauopathies by determining a signature of AD tau protein aggregate comprising one or more of: one or more AD-specific corresponding PMCA kinetic parameters of: lag phase, T_{50}, amplification rate, and amplification

extent; an assay using an antibody selective for a conformational epitope of AD tau protein aggregate; an indicator selective for AD tau protein aggregate; and a spectrum characteristic of AD tau protein aggregate.

116. A method for determining a presence or absence of a tauopathy in a subject, the tauopathy comprising Parkinson's Disease (PD), the method comprising:

providing the subject;

obtaining a sample from the subject, the sample comprising one or more of: a bio-fluid, a biomaterial, a homogenized tissue, and a cell lysate;

performing at least a first PMCA procedure comprising:

forming a first incubation mixture by contacting the sample with a first substrate protein, the first substrate protein comprising 4R tau;

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

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

determining the presence or absence in the sample of the first misfolded protein aggregate by detecting in the first incubation mixture the presence or absence of the first amplified, misfolded protein aggregate; and

determining the presence or absence in the subject of PD according to the presence or absence of the first misfolded protein aggregate in the sample.

117. The method of claim 116, determining the presence or absence in the subject of PD further comprising distinguishing PD from one or more additional tauopathies by determining a signature of PD tau protein aggregate comprising one or more of: one or more PD-specific corresponding PMCA kinetic parameters of: lag phase, T_{50}, amplification rate, and amplification extent; an assay using an antibody selective for a conformational epitope of PD tau protein aggregate; an indicator selective for PD tau protein aggregate; and a spectrum characteristic of PD tau protein aggregate.

118. A method for determining a presence or absence of a tauopathy in a subject, the tauopathy comprising Progressive Supranuclear Palsy (PSP), the method comprising:

providing the subject;

obtaining a sample from the subject, the sample comprising one or more of: a bio-fluid, a biomaterial, a homogenized tissue, and a cell lysate;

performing at least a first PMCA procedure comprising:

forming a first incubation mixture by contacting the sample with a first substrate protein, the first substrate protein comprising 4R tau;

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

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

determining the presence or absence in the sample of the first misfolded protein aggregate by detecting in the first incubation mixture the presence or absence of the first amplified, misfolded protein aggregate; and

determining the presence or absence in the subject of PSP according to the presence or absence of the first misfolded protein aggregate in the sample.

119. The method of claim 118, determining the presence or absence in the subject of PSP further comprising distinguishing PSP from one or more additional tauopathies by determining a signature of PSP tau protein aggregate comprising one or more of: one or more PSP-specific corresponding PMCA kinetic parameters of: lag phase, T_{50}, amplification rate, and amplification extent; an assay using an antibody selective for a conformational epitope of PSP tau protein aggregate; an indicator selective for PSP tau protein aggregate; and a spectrum characteristic of PSP tau protein aggregate.

120. A method for determining a presence or absence of a tauopathy in a subject, the tauopathy comprising FrontoTemporal Dementia (FTD), the method comprising:

providing the subject;

obtaining a sample from the subject, the sample comprising one or more of: a bio-fluid, a biomaterial, a homogenized tissue, and a cell lysate;

performing at least a first PMCA procedure comprising:

forming a first incubation mixture by contacting the sample with a first substrate protein, the first substrate protein comprising 4R tau;

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

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

determining the presence or absence in the sample of the first misfolded protein aggregate by detecting in the first incubation mixture the presence or absence of the first amplified, misfolded protein aggregate; and

determining the presence or absence in the subject of FTD according to the presence or absence of the first misfolded protein aggregate in the sample.

121. The method of claim 120, determining the presence or absence in the subject of FTD further comprising distinguishing FTD from one or more additional tauopathies by determining a signature of FTD tau protein aggregate comprising one or more of: one or more FTD-specific corresponding PMCA kinetic parameters of: lag phase, T_{50}, amplification rate, and amplification extent; an assay using an antibody selective for a conformational epitope of FTD tau protein aggregate; an indicator selective for FTD tau protein aggregate; and a spectrum characteristic of FTD tau protein aggregate.

122. A method for determining a presence or absence of a tauopathy in a subject, the tauopathy comprising Corticobasal degeneration (CBD), the method comprising:

providing the subject;

obtaining a sample from the subject, the sample comprising one or more of: a bio-fluid, a biomaterial, a homogenized tissue, and a cell lysate;

performing at least a first protein misfolding cyclic amplification (PMCA) procedure, the first PMCA procedure comprising:

forming a first incubation mixture by contacting the sample with a first substrate protein, the first substrate protein comprising 4R tau;

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

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

determining the presence or absence in the sample of the first misfolded protein aggregate by detecting in the first incubation mixture the presence or absence of the first amplified, misfolded protein aggregate; and

determining the presence or absence in the subject of CBD according to the presence or absence of the first misfolded protein aggregate in the sample.

123. The method of claim 122, determining the presence or absence in the subject of CBD further comprising distinguishing CBD from one or more additional tauopathies by determining a signature of CBD tau protein aggregate comprising one or more of: one or more CBD-specific corresponding PMCA kinetic parameters of: lag phase, T_{50}, amplification rate, and amplification extent; an assay using an antibody selective for a conformational epitope of CBD tau protein aggregate; an indicator selective for CBD tau protein aggregate; and a spectrum characteristic of CBD tau protein aggregate.

124. The method of any one of claims 114, 116, 118, 120, and 120, further comprising:

performing at least a second PMCA procedure to determine the presence or absence in the sample of at least a second misfolded protein aggregate, comprising:

forming a second incubation mixture by contacting a second portion of the sample with a second substrate protein, the second substrate protein being subject to pathological misfolding and/or aggregation;

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 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; and

the tauopathy being present in the subject, further comprising characterizing an identity of the tauopathy in the subject according to: the presence in the sample of the first misfolded

protein aggregate; and the presence or absence in the sample of the second misfolded protein aggregate.

the second misfolded protein aggregate comprising the second substrate protein,

the second amplified, misfolded protein aggregate comprising the second substrate protein, and

the second substrate protein being distinct from the first substrate protein and the second substrate protein comprising one of: amyloid-beta (Aβ), alpha-synuclein, and 3R tau.

125. The method of claim 124, determining the presence or absence in the subject of each tauopathy further comprising distinguishing each tauopathy from one or more additional tauopathies by analyzing for at least one signature of one or more misfolded aggregates each corresponding to one of Aβ, alpha-synuclein, and 3R tau, each signature corresponding to one or more of: an assay using an antibody selective for a conformational epitope of any of the one or more misfolded aggregates; an assay using an antibody selective for a conformational epitope of any of the one or more misfolded aggregates; one or more PMCA kinetic parameters of the one or more misfolded aggregates, comprising one or more of: lag phase, T_{50}, amplification rate, and amplification extent; an indicator selective for any of the one or more misfolded aggregates; and a spectrum characteristic of any of the one or more misfolded aggregates.

126. A kit for determining a presence or absence in a sample of a first misfolded protein aggregate, comprising:

a first substrate protein comprising 4R tau;

an indicator of the first misfolded protein aggregate, the first misfolded protein aggregate comprising the first substrate protein and the first misfolded protein aggregate corresponding to a tauopathy;

a buffer;

heparin; and

a salt;

instructions, the instructions directing a user to:

obtain the sample;

perform at least a first PMCA procedure, comprising:

forming a first incubation mixture by contacting a first portion of the sample with the first substrate protein, the indicator of the first misfolded protein aggregate, the buffer, the heparin, and the salt, the first incubation mixture being

formed with a concentration of one or more of: the first substrate protein of less than about 20 μM; the heparin of less than about 75 μM; the salt as NaCl of less than about 190 mM; and the indicator of the first misfolded protein aggregate as Thioflavin T of less than about 9.5 μM;

conducting an incubation cycle two or more times effective to form a 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;

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

determine the presence or absence in the 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 according to the indicator of the first misfolded protein aggregate.

127. The kit of claim 126, the instructions directing the user to obtain the sample from a subject, the sample comprising one or more of: a bio-fluid, a biomaterial, a homogenized tissue, and a cell lysate, and the instructions directing the user to determine or diagnose a tauopathy in the subject according to the presence or absence in the sample of the first misfolded protein aggregate.

128. The kit of claim 126:

further comprising a second substrate protein and an indicator of a second misfolded protein aggregate, the second misfolded protein aggregate comprising the second substrate protein, the second substrate protein being distinct from the first substrate protein and the second substrate protein comprising one of: amyloid-beta (Aβ), alpha synuclein, 3R tau, and 4R tau;

the instructions further directing the user to:

perform at least a second PMCA procedure, comprising:

forming a second incubation mixture by contacting a second portion of the sample with the second substrate protein and the indicator of the second misfolded protein aggregate;

conducting an incubation cycle two or more times 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 the second substrate protein in the presence of the second misfolded protein aggregate;

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

determine the presence or absence in the 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,

characterize the sample for an identity of a tauopathy according to: the presence in the sample of the first misfolded protein aggregate; and the presence or absence in the sample of the second misfolded protein aggregate.

129. The kit of claim 126, further comprising a PMCA apparatus, the PMCA apparatus comprising one or more of: a multiwall microtitre plate; a microfluidic plate; a shaking apparatus; a spectrometer; and an incubator.

130. The kit of claim 126, the antibody specific for the conformation of the first misfolded protein aggregate corresponding to 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), the instructions comprising determining, according to a binding assay using the antibody specific for the conformation of the first misfolded protein aggregate, the presence or absence in the subject of one of AD, PD, PSP, FTD, CBD, MCI, AgD, TBI, CTE, and DP.