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(71) Applicant (for all designated States except US): **UNIVERSITY OF DELAWARE** [US/US]; 1 Innovation Way, Delaware Technology Park, Building 1, Suite 500, Newark, DE 19711 (US).

(72) Inventors; and

(71) Applicants (for US only): **COLBY, David, Wesley** [US/US]; 227 W 14th St, Wilmington, DE 19801 (US). **MOROZOVA, Olga, Andreevna** [US/US]; 356 East Main Street, Apt 201, Newark, DE 19711 (US).

(74) Agents: **DONNELLY, Rex, A.** et al.; RatnerPrestia, P.O. Box 980, Valley Forge, PA 19482 (US).

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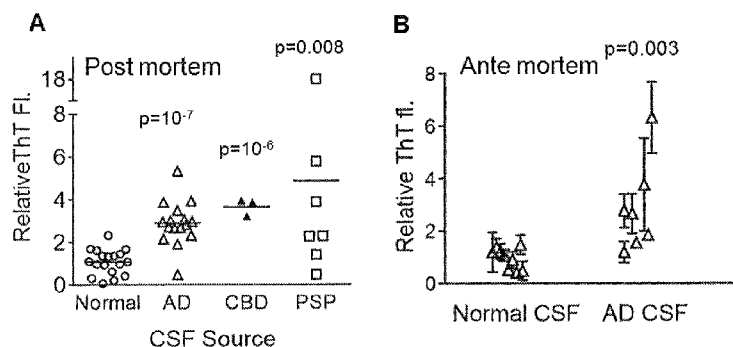
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(54) Title: DETECTION OF DISEASE-ASSOCIATED TAU PROTEIN CONFORMATIONS AND APPLICATIONS THEREOF

FIG. 4



(57) Abstract: The present invention provides a method for detecting an amyloidogenic tau protein in a bodily fluid (e.g., CSF) sample from a subject. The method comprises incubating the sample or a purified fraction thereof *in vitro* with a monomeric tau protein to form tau amyloid, and detecting the presence of the tau amyloid. The method may further comprise determining a conformational feature of the tau amyloid and comparing it with a reference conformational feature of tau amyloid formed *in vitro* in the presence of an amyloidogenic tau protein from a patient who suffers from a neurological disease or disorder. The reference conformational feature may be absent from tau amyloid formed *in vitro* in the presence of an amyloidogenic tau protein from a healthy control. Also provided are kits useful for carrying out the detection method.

DETECTION OF DISEASE-ASSOCIATED TAU PROTEIN CONFORMATIONS AND APPLICATIONS THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 62/108,818, filed January 28, 2015 and U.S. Provisional Application No. 62/195,915, filed July 23, 2015, the contents of which are incorporated herein by reference in their entireties for all purposes.

REFERENCE TO U.S. GOVERNMENT SUPPORT

This work is supported by a grant from the U.S. National Institutes of Health (NIH) (NIH Grant # 064143). The United States has certain rights in the invention.

FIELD OF THE INVENTION

The invention relates generally to detection of disease-associated tau protein conformations and applications thereof.

BACKGROUND OF THE INVENTION

Tau is a microtubule-associated protein that stabilizes the microtubule network within neurons. Tau is a highly soluble, unfolded, protein under normal physiological conditions, but aggregates under pathological conditions. Intracellular deposits of tau protein in brain tissues are associated with Alzheimer's and other neurological diseases or disorders, such as Parkinson's disease, Huntington's disease, Chronic Traumatic Encephalopathy, progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). Increased concentrations of soluble tau in the cerebrospinal fluid (CSF) of patients with AD and other neurological diseases or disorders have been observed, but suitable methods for analysis of the conformational state of tau in bodily fluid samples such as a CSF sample from a patient, especially a living patient, has not been reported.

By established criteria, clinical AD diagnosis is <90% accurate, with lower accuracy at early stages of AD. For other less common neurological diseases or disorders, highly accurate clinical tests or biochemical tests are not available. Additional diagnostic biomarkers or methods are desirable.

Misfolded protein amplification assays are useful tools for detection of abnormally folded proteins. These assays rely on the seeded conversion of a monomeric protein substrate into an amyloidogenic protein, also called a seed protein, to increase the amount of misfolded protein in a sample. The misfolded protein is then detected using standard methods, such as the Thioflavin T (ThT) fluorescence dye for amyloid detection, protease resistance, filter retardation, turbidity, circular dichroism, etc. Faithful amplification of tau fibrils in brain tissues of

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AD patients with recombinant tau protein has been reported for characterizing conformational features of the tau fibrils. Compartmentalization or separation of a sample comprising aggregatory seeds into a plurality of microdroplets to increase the local concentration of the aggregatory seeds has been suggested for detecting
5 tau protein in a human sample such as a CSF sample. No detection of disease-associated tau conformations in bodily fluid samples from patients with different neurological diseases or disorders has been reported.

Therefore, there remains a need for sensitive methods for detection of disease-associated conformational features of amyloidogenic tau protein in bodily
10 fluid samples, especially from living patients.

SUMMARY OF THE INVENTION

The present invention relates to detection of disease-associate conformational features of amyloidogenic tau protein in bodily fluid samples and kits useful for the detection thereof.

15 A method for detecting an amyloidogenic tau protein in a bodily fluid sample from a subject is provided. The method comprises incubating the sample or a purified fraction thereof *in vitro* with a monomeric tau protein to form tau amyloid, and detecting the presence of the tau amyloid. The detecting step may comprise determining the amount of the tau amyloid and comparing the amount of the tau
20 amyloid with one or more controls.

The method may further comprise determining a conformational feature of the tau amyloid. The method may further comprise comparing the conformational feature of the tau amyloid with a reference conformational feature of tau amyloid formed *in vitro* in the presence of an amyloidogenic tau protein from a patient
25 suffering from a neurological disease or condition, wherein the reference conformation feature is absent from tau amyloid formed *in vitro* in the presence of an amyloidogenic tau protein from a healthy control.

The subject may be a human being. For example, the subject may be a patient who suffers from the neurological disease or condition, or predisposed to the
30 neurological disease or condition.

The neurological disease or condition may be selected from the group consisting of Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, Chronic Traumatic Encephalopathy (CTE), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Lewy body dementia, frontotemporal dementia,
35 traumatic brain injury, and multiple sclerosis (MS). Preferably, the neurological disease or condition is selected from the group consisting of Alzheimer's disease (AD), Cortical Basal Degeneration (CBD) and Progressive Supranuclear Palsy (PSP).

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The bodily fluid may be selected from the group consisting of blood, cerebrospinal fluid (CSF), lymphatic fluid, saliva, tear and urine. Preferably, the bodily fluid is cerebrospinal fluid (CSF).

The monomeric tau protein may be a polypeptide having an amino acid sequence at least 90% identical to a sequence selected from SEQ ID NOs: 1-47, or a functional fragment thereof. The amyloidogenic tau protein may comprise a polypeptide having an amino acid sequence at least 90% identical to a sequence selected from SEQ ID NOs: 1-47, or a functional fragment thereof. The amyloidogenic tau protein may comprise an amino acid sequence at least 90% identical to that of the monomeric tau protein or a functional fragment thereof.

In the incubating step, the concentration of the monomeric tau protein may be greater than 0.08 mg/ml, and the amount of the amyloidogenic tau protein may be less than 1 pg.

The method may further comprise purifying the amyloidogenic tau protein from the sample before the incubating step.

The method may further comprise determining the concentration of the amyloidogenic tau protein in the sample.

The conformational feature may be selected from the group consisting of fibril width, twist periodicity, and a combination thereof. The reference conformational feature may be predetermined.

Where the conformational feature of the tau amyloid is substantially similar to the reference conformational feature, the method may further comprise administering to the subject a therapy effective for treating the disease. The disease may be selected from the group consisting of Alzheimer's disease (AD), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD).

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates detection of brain-derived fibrils. A dose-dependent response was observed upon addition of varying titers of both (A) recombinant and (B) AD brain-derived tau fibers. (C) The limit of detection was determined to be ~10 pg of recombinant tau fibers by serial dilution of the seed titer. (D) A seeding effect was observed upon addition of as little as ~100 pg tau fibers isolated from AD brain compared to normal brain controls.

Fig. 2 shows the detection of pathogenic tau conformations in CSF, which is specific to tau protein relative to Amyloid Beta (A β) and prions. (A) Characteristic kinetic traces of recombinant tau amyloid formation in the presence (squares) and absence (circles) of preformed recombinant tau amyloid seed, monitored by ThT fluorescence. (B) Kinetic traces of amyloid formation in the presence of misfolded

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tau isolated from the brains of individuals affected with AD (squares), and a mock isolate from normal brain (triangles). (C) Kinetic traces of amyloid formation in the presence of AD CSF (squares) and Normal CSF (triangles). (D) Thioflavin T fluorescence measured at 30 h for recombinant tau amyloid formation reactions containing misfolded proteins, including partially purified prions (PrP^{Sc}), and synthetic A β oligomers. Reactions containing mock seeds from three control brains and three AD brains are also shown. Measured ThT fluorescence values for specificity controls were indistinguishable from normal brain control samples ($p=0.97$ for PrP^{Sc}, and $p=0.64$ for A β ; $p=0.003$ for AD). Anti-tau 5A6 antibody bound to magnetic beads was used to immunopurify tau from CSF. (E) CSF before and after tau immunopurification shows total tau levels decrease by western blots. The same amount of CSF was added to the assay reaction before and after tau depletion, and the reaction kinetics were monitored by ThT fluorescence increase. (F) The depletion of tau from CSF significantly ($p=0.05$) removed the seeding activity.

Fig. 3 shows no significant correlation for either MCI or clinical AD CSF based on the comparison of relative ThT values normalized to clinically normal CSF samples with standard AD biomarker concentrations (total tau/ A β 42). False positives and false negatives in the biomarker measurements can be identified in the clinically diagnosed AD CSF samples with the comparison to the relative ThT data.

Fig. 4 shows detection of amyloidogenic tau conformers in cerebrospinal fluid (CSF) of AD and other tauopathies. (A) The relative ThT fluorescence observed for postmortem AD CSF samples (triangles) at a sensitive time point in the fibrillization kinetics was significantly higher than that observed for normal control samples (circles) run simultaneously. The relative ThT fluorescence for CSF from CBD (black triangles), and PSP (open squares) samples was significantly higher than that observed for the normal control samples. (B) The relative ThT fluorescence for pre-mortem AD CSF was significantly higher at CDR1 and CDR 2 compared to normal control samples.

Fig. 5 shows that analysis of assay products allows one to identify which tauopathy the patient has. Tau protein was immunopurified from CSF with magnetic beads bound to 5A6 antibody. The beads bound to tau were incubated in a reaction with recombinant tau, ThT, and 5mM BME in PBS at 37 °C. The fibrillization reaction was monitored with ThT fluorescence increase. (A) Representative electron microscopy images of CSF-seeded recombinant tau structures from AD ($n=5$), PSP ($n=2$), CBD ($n=2$) and spontaneous fibrillization in the Normal controls ($n=3$). (B) Quantified width and periodicity measurements for all structures. The width of the AD CSF-seeded, PSP CSF-seeded and CBD CSF-seeded recombinant tau fibrils are

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significantly different from the spontaneous tau fibrils from the Normal CSF. AD CSF-seeded recombinant tau fibrils do not have a significantly different width from PSP CSF-seeded structures, but are significantly different from CBD CSF-seeded tau fibrils. The average periodicity is significantly different across the structures seeded with all three tauopathies.

Fig. 6 shows that alternative buffer conditions and sample preparation techniques improve the sensitivity of amyloidogenic tau detection. (A) The limit of detection of synthetic recombinant tau with the improved detection assay buffer conditions and sample preparation is less than 1pg, which is a 1000x improvement in the limit of detection. (B) Relative AD CSF ThT values at a sensitive time point normalized to normal controls show that improved detection assay buffer conditions increase the signal to noise ratio by 5 times.

Fig. 7 shows methods used for analysis of amyloid formation data. Quantitative metrics derived either from simple analysis of ThT measurements (A) or parameters from a fit to the entire data set by the Sigmoidal Equation (B). (A) ThT measurements were obtained and analyzed by direct comparisons of ThT values of AD and Control, determined by the plateau, the lag phase and the optimal ratio between the two data sets. Preferably, an empirical fit to the entire data set was made with the Sigmoidal equation (B), with the representative parameters of the fit juxtaposed by the representative kinetic curve.

Fig. 8 shows methods used for analysis of tau fibrillization data. Spearman-Kärber analysis of the data obtained by quantifying significant seeding in a titration of tau amyloid isolated from AD brains provides a quantitative measure of amyloidogenic tau. (A) The lag phase ThT ratio at a sensitive time point of the endpoint titration of the disease tau conformations (filled markers) and the mock control sample (empty markers) was analyzed by measuring significant ThT increase over background. (B) Endpoint titration analysis of the sigmoidal function fit lag phase and slope values of the tau fibrillization reactions seeded with a titration of disease tau conformations (filled markers) and normal controls (empty parkers). Different analysis of the data may alter the quantification of the seeding sensitivity. Fitting the tau fibrillization data to an empirical function significantly increases the sensitivity of the seeding analysis.

Fig. 9 shows quantitative relationship between the parameters and initial seed concentration for a predictive power related to a standard titration. (A) The optimized relationship between Thioflavin T data analysis and the amyloidogenic load in a sample shows a significant power correlation. (B) The optimized relationship between the 3 parameters of the sigmoidal equation that fully captures the kinetic

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trace of the amyloid growth data and the amyloidogenic load in a sample shows a significantly positive power correlation. A comparison to such a standard curve in an experiment can be used as a comparison to calculate the amyloid concentration in a sample.

5 Fig. 10 shows the detection of amyloidogenic tau conformations in the Mild Cognitive Impairment (CDR=0.5) stage of Alzheimer's disease compared to normal controls (CDR=0). The relative ThT fluorescence ratio normalized to the normal controls (CDR 0) at a sensitive time point shows an increase for 2 CSF patients with Mild Cognitive Impairment.

10 Fig. 11 shows the detection of amyloidogenic tau in Parkinson's Disease (PD), multiple sclerosis (MS) and Huntington's Disease (HD). Addition of HD, MS and PD CSF to the reaction of tau protein in the presence of heparin accelerates amyloid formation and ThT fluorescence increase compared to Normal CSF controls.

 Fig. 12 illustrates a device/kit/method of detecting altered tau protein
15 conformation in a sample according to the methods described in the invention. The kit includes recombinant tau protein (rec tau) which has been validated for function in the assay, anti-tau magnetic beads for purification of tau from 96 samples, a detection buffer, a 96-well plate for fluorescence measurements, positive and negative controls for quality assurance, and a data analysis protocol for a
20 standardized output along with guidelines for interpretation of the results. Briefly, he sample is incubated with the anti-tau magnetic beads, which are subsequently washed. Rec tau protein is diluted in the detection butter before the addition of the washed magnetic beads for each sample, and the solution is added to a multiwall plate for fluorescence detection over time.

25 Fig. 13 shows the amino acid sequences of human tau isoforms having 381 aa (Genbank Accession No. NP_001190180; SEQ ID NO: 1), 412 aa (Genbank Accession No. NP_001116539; SEQ ID NO: 2), 352 aa (Genbank Accession No. NP_058525; SEQ ID NO: 3), 383 aa (Genbank Accession No. NP_058518; SEQ ID NO: 4), 441 aa (Genbank Accession No. NP_005901 NP_776088; SEQ ID NO: 5) or
30 410 aa (Genbank Accession No. NP_001190181; SEQ ID NO: 6).

 Fig. 14 shows the amino acid sequences of rat tau isoforms having 343 aa (SEQ ID NO: 7), 374aa (SEQ ID NO: 8), 403 aa (Genbank Accession No. XP_008766500.1; predicted; SEQ ID NO: 9), 432 aa (GenBank Accession No. XP_008766499; predicted; SEQ ID NO: 10) or 469 aa (Genbank Accession No.
35 XP_008766497.1; predicted; SEQ ID NO: 11), 498 aa (Genbank Accession No. XP_008766496.1; predicted; SEQ ID NO: 12).

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Fig. 15 shows the amino acid sequences of mouse tau isoforms having 372 aa (Genbank Accession No. NP_034968.3; SEQ ID NO: 13), 341 aa (Genbank Accession No. NP_001272385.1; SEQ ID NO: 14), 430 aa (Genbank Accession No. NP_001033698.1; SEQ ID NO: 15), 350 aa (Genbank Accession No. NP_001272384.1; SEQ ID NO: 16) or 364 aa (Genbank Accession No. NP_001272383.1; SEQ ID NO: 17).

Fig. 16 shows the amino acid sequences of cattle tau isoforms having 448 aa (Genbank Accession No. NP_776531; SEQ ID NO: 18) or 370 aa (Genbank Accession No. AAI09942; SEQ ID NO: 19).

Fig. 17 shows the amino acid sequences of tree frog tau isoforms having 403 aa (GenBank Accession No. NP_001072465.1; SEQ ID NO: 20), 421 aa (GenBank Accession No. XP_012808026; predicted; SEQ ID NO: 21), 465 aa (GenBank Accession No. XP_012808025.1; predicted; SEQ ID NO: 22), 482 aa (GenBank Accession No. XP_012808024; predicted; SEQ ID NO: 23), 483 aa (GenBank Accession No. XP_012808023; predicted; SEQ ID NO: 24) or 324 aa (GenBank Accession No. XP_011544655.1; predicted; SEQ ID NO: 25).

Fig. 18 shows the amino acid sequences of Japanese medaka tau isoforms having 483 aa (GenBank Accession No. XP_011486741.1; predicted; SEQ ID NO: 26); 486 aa (GenBank Accession No. XP_011486740.1; predicted; SEQ ID NO: 27), 523 aa (GenBank Accession No. XP_011486739.1; predicted; SEQ ID NO: 28) or 524 aa (GenBank Accession No. XP_011486737.1; predicted; SEQ ID NO: 29).

Fig. 19 shows the amino acid sequences of black rockcod tau isoforms having 483 aa (GenBank Accession No. XP_010782995.1; predicted; SEQ ID NO: 30), 529 aa (GenBank Accession No. XP_010782994.1; predicted; SEQ ID NO: 31) or 530 aa (GenBank Accession No. XP_010782992.1; predicted; SEQ ID NO: 32).

Fig. 20 shows the amino acid sequences of chicken tau isoforms having 432 aa (GenBank Accession No. NP_001186122.1; SEQ ID NO: 33), 345 aa (GenBank Accession No. ABY86222; SEQ ID NO: 34), 348 aa (GenBank Accession No. ABY86221; SEQ ID NO: 35), 400 aa (GenBank Accession No. XP_015154842; predicted; SEQ ID NO: 36), 410 aa (GenBank Accession No. XP_015154838; predicted; SEQ ID NO: 37), 429 aa (GenBank Accession No. XP_015154837.1; predicted; SEQ ID NO: 38), 463 aa (GenBank Accession No. XP_015154833.1; predicted; SEQ ID NO: 39), 467 aa (GenBank Accession No. XP_015154832; predicted; SEQ ID NO: 40) or 520 aa (GenBank Accession No. XP_015154831.1; predicted; SEQ ID NO: 41).

Fig. 21 shows alignment of homologous portions R1 (SEQ ID NO: 42), R2 (SEQ ID NO: 43), R3 (SEQ ID NO: 44) and R4 (SEQ ID NO: 45) of the human tau

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protein region which form the core of tau amyloid fibers. The sequence contains four copies a shorter sequence with >86% homology to one another; a suitable substrate for the assay will contain a polypeptide with ~86% homology to one or more of these four sequences.

5 DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel and highly sensitive method for detecting and characterizing conformational features of tau amyloid formed *in vitro* in the presence of an amyloidogenic tau protein in biological samples, including bodily fluid samples. Such conformational features may be compared with reference
10 conformational features of tau amyloid associated with diseases, including neurological diseases or disorders such as Alzheimer's disease (AD), Cortical Basal Degeneration (CBD), and Progressive Supranuclear Palsy (PSP), for identifying suitable therapeutic treatments for patients having certain tau pathologies. Kits useful for the detection method are also provided.

15 The term "protein" used herein refers to a biological molecule comprising amino acid residues. A protein may comprise one or more polypeptides. Each polypeptide may be a subunit of a protein. The protein may be in a native or modified form, and may exhibit a biological function when its polypeptide or polypeptides are properly folded or assembled.

20 The term "polypeptide" used herein refers to a polymer of amino acid residues with no limitation with respect to the minimum length of the polymer. Preferably, the polypeptide has at least 4 amino acids. A polypeptide may be a full-length protein, or a fragment or variant thereof.

The term "fragment" of a protein used herein refers to a polypeptide having
25 an amino acid sequence that is the same as a part, but not all, of the amino acid sequence of the protein. Preferably, a fragment is a functional fragment of a protein that retains the same function as the protein.

The term "variant" of a protein used herein refers to a polypeptide having an amino acid sequence that is the same as that of the protein except having at least
30 one amino acid modified, for example, deleted, inserted, or replaced, respectively. The amino acid replacement may be a conservative amino acid substitution, preferably at a non-essential amino acid residue in the protein. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues
35 having similar side chains are known in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine,

glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). A variant of a protein may
5 have an amino acid sequence at least about 80%, 90%, 95%, or 99%, preferably at least about 90%, more preferably at least about 95%, identical to the amino acid sequence of the protein. Preferably, a variant is a functional variant of a protein that retains the same function as the protein.

The term "derived from" used herein refers to an origin or source, and may
10 include naturally occurring, recombinant, unpurified or purified molecules. A protein or polypeptide derived from an original protein or polypeptide may comprise the original protein or polypeptide, in part or in whole, and may be a fragment or variant of the original protein or polypeptide.

The term "native" used herein refers to a molecule (e.g., a protein or
15 polypeptide) that is naturally occurring. The term "artificial" used herein refers to a molecule (e.g., a protein or polypeptide) that is not naturally occurring, but synthesized artificially, for example, recombinantly or chemically.

The present invention provides a method for detecting an amyloidogenic tau protein in a biological sample from a subject. The method comprises incubating the
20 biological sample, or a purified fraction thereof, *in vitro* with a monomeric tau protein to form tau amyloid, and detecting the presence of the tau amyloid. In some embodiments, the detection method does not involve microdroplets. For example, the detection method does not include incubating a plurality of (e.g., at least about 1,000) microdroplets (e.g., each having a volume of less than about 1 μ l)
25 comprising the amyloidogenic tau protein and the monomeric tau protein.

The subject may be any mammal. Examples of the subject may include human being, cat, chicken, cow, deer, dog, horse, monkeys, mouse, rat, rabbit, guinea pig, pig and sheep. The subject may also be a frog or fish. Preferably, the subject is a human being. The subject may suffer from or be predisposed to a
30 disease, for example a neurological disease or disorder.

The neurological disease or disorder may be a neurodegenerative disease. Examples of a neurodegenerative disease may include Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, Chronic Traumatic Encephalopathy, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Lewy body
35 dementia, frontotemporal dementia, multiple sclerosis (MS) and traumatic brain injury. Preferably, the neurological disease or disorder is Alzheimer's disease (AD), Cortical Basal Degeneration (CBD) or Progressive Supranuclear Palsy (PSP).

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A biological sample may be any sample obtained from the subject. The biological sample may be a bodily fluid sample. Exemplary bodily fluid samples may include blood (e.g., serum or plasma), cerebrospinal fluid (CSF), lymphatic fluid, saliva, tear and urine samples. Preferably, the biological sample is a CSF sample.

5 A biological sample may be prepared by using conventional techniques. For example, CSF may be obtained from patients by standard protocols by licensed physicians. CSF samples may be stored at -80 °C in one milliliter aliquots until ready for use, and then thawed at room temperature for 30 minutes.

In some embodiments of the invention, the biological sample may be purified.
10 The purification may be accomplished by methods known in the art, for example, immunoprecipitation. Upon purification, the concentration of the amyloidogenic tau protein in a purified fraction of the biological sample may be increased by at least about 10, 100 or 1,000 folds. This may enhance the formation of the tau amyloid when a purified fraction of the biological sample is used in the incubation step.

15 A tau protein consists of a single polypeptide, and may have one or more isoforms produced by alternative splicing of the tau gene in a species. The amino acid sequences of tau isoforms in different species are shown in Figs. 13-20.

A functional fragment of the tau protein retains the same function as the tau protein, for example, as used as a substrate for amyloidogenic tau protein detection
20 in this invention. A tau functional fragment may comprise an amino acid sequence required for retaining a biological function, for example, to form amyloid more rapidly in the presence of amyloidogenic tau. Homologous portions R1 (SEQ ID NO: 42), R2 (SEQ ID NO: 43), R3 (SEQ ID NO: 44) and R4 (SEQ ID NO: 45) of the human tau protein region form the core of tau amyloid fibers. Peptides VQIINKK
25 (SEQ ID NO: 46) and VQIVYK (SEQ ID NO: 47), derived from the human tau protein sequence, form amyloid capable of seeding tau fiber formation and may be useful as a substrate in the assay in place of tau protein monomers. The tau protein contains a microtubule binding domain that has been found important for promoting and stabilizing tau fibrillization. A tau functional fragment may comprise an amino acid
30 sequence at least about 80%, 90%, 95%, 99% or 100%, preferably at least about 90%, more preferably at least about 100%, identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 42-47, or the amino acid sequence of a microtubule binding domain. The actual or predicted location of the microtubule binding domain in exemplary tau isoforms is provided in Table 1.

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Table 1. Microtubule Binding Domain Location

Tau Protein	Microtubule Binding Domain Location
Human tau 381 aa (SEQ ID NO: 1)	Residues 174-307
Human tau 412 aa (SEQ ID NO: 2)	Residues 223-338
Human tau 352 aa (SEQ ID NO: 3)	Residues 194-278
Human tau 383 aa (SEQ ID NO: 4)	Residues 194-309
Human tau 441 aa (SEQ ID NO: 5)	Residues 252-367
Human tau 410 aa (SEQ ID NO: 6)	Residues 252-336
Rat tau 343 aa (SEQ ID NO: 7)	Residues 185-269
Rat tau 374 aa (SEQ ID NO: 8)	Residues 185-268
Rat Tau Protein 403 aa (SEQ ID NO: 9)	Residues 214-329
Rat Tau Protein 432 aa (SEQ ID NO: 10)	Residues 243-358
Rat Tau Protein 469 aa (SEQ ID NO: 11)	Residues 280-395
Rat Tau Protein 498 aa (SEQ ID NO: 12)	Residues 309-424
Mouse tau 372 aa (SEQ ID NO: 13)	Residues 185-298
Mouse tau 341 aa (SEQ ID NO: 14)	Residues 183-267
Mouse tau 430 aa (SEQ ID NO: 15)	Residues 241-356
Mouse tau 350 aa (SEQ ID NO: 16)	Residues 161-276
Mouse tau 364 aa (SEQ ID NO: 17)	Residues 183-267
Cattle tau 448 aa (SEQ ID NO: 18)	Residues 259-374
Cattle Tau Protein 370 aa (SEQ ID NO: 19)	Residues 212-296
Tree frog tau 403 aa (SEQ ID NO: 20)	Residues 215-329
Tree frog tau 421 aa (SEQ ID NO: 21)	Residues 233-347
Tree frog tau 465 aa (SEQ ID NO: 22)	Residues 277-391
Tree frog tau 482 aa (SEQ ID NO: 23)	Residues 294-408
Tree frog tau 483 aa (SEQ ID NO: 24)	Residues 295-409
Tree frog tau 324 aa (SEQ ID NO: 25)	Residues 135-250
Japanese medaka tau 483 aa (SEQ ID NO: 26)	Residues 209-415
Japanese medaka tau 486 aa (SEQ ID NO: 27)	Residues 212-418
Japanese medaka tau 523 aa (SEQ ID NO: 28)	Residues 249-455
Japanese medaka tau 524 aa (SEQ ID NO: 29)	Residues 250-456
Black rockcod tau 483 aa (SEQ ID NO: 30)	Residues 302-415
Black rockcod tau 529 aa (SEQ ID NO: 31)	Residues 255-461
Black rockcod tau 530 aa (SEQ ID NO: 32)	Residues 256-462
Chicken tau 432 aa (SEQ ID NO: 33)	Residues 243-358
Chicken tau 345 aa (SEQ ID NO: 34)	Residues 156-271
Chicken tau 348 aa (SEQ ID NO: 35)	Residues 190-273
Chicken tau 400 aa (SEQ ID NO: 36)	Residues 181-326
Chicken tau 410 aa (SEQ ID NO: 37)	Residues 190-336
Chicken tau 429 aa (SEQ ID NO: 38)	Residues 209-355
Chicken tau 463 aa (SEQ ID NO: 39)	Residues 243-289
Chicken tau 467 aa (SEQ ID NO: 40)	Residues 247-393
Chicken tau 520 aa (SEQ ID NO: 41)	Residues 300-414

The term "amyloidogenic tau protein" used herein refers to a tau protein that is capable of stimulating conversion of monomeric tau protein into an amyloid conformation (i.e., an aggregate of tau proteins with a fiber-like morphology). The amyloidogenic tau protein may comprise a single or a plurality of a polypeptide, for

example, at least about 10, 100, 1,000 or 10,000 copies of the polypeptide. This polypeptide may be the endogenous tau protein in the subject, or a functional fragment or variant thereof. The polypeptide may comprise the microtubule binding domain of the endogenous tau protein. The amyloidogenic tau protein may have an amino acid sequence at least about 80%, 90%, 95%, 99% or 100%, preferably at least about 90%, more preferably at least about 100%, identical to the amino acid sequence of the full length endogenous tau protein in the subject (e.g., SEQ ID NOs: 1-41) or a tau functional fragment (e.g., SEQ ID NOs: 42-47, or microtubule binding domain).

The detection method according to the present invention is highly sensitive. It may detect a very small amount of the amyloidogenic tau protein in a biological sample, for example, at less than about 50 ng or 1 pg. Where an antemortem CSF sample is used, this method may detect as little as 0.01-0.1 pg of the amyloidogenic tau protein.

The monomeric tau protein may be a native tau protein or an artificial polypeptide derived from a native tau protein. The native tau protein is preferably in the same species as the polypeptide made up of the amyloidogenic tau protein, which is to be detected. Preferably, the monomeric tau protein comprises an amino acid sequence at least about 80%, 90%, 95%, 99% or 100%, preferably at least about 90%, more preferably at least about 100%, identical to the amino acid sequence of the polypeptide made up of the amyloidogenic tau protein or a functional fragment thereof. The native monomeric tau protein may be prepared from a biological sample obtained from the subject while the artificial monomeric tau protein may be prepared by a recombinant method. Preferably, the monomeric tau protein is a recombinant protein.

The monomeric tau protein may comprise an amino acid sequence at least about 80%, 90%, 95%, 99% or 100%, preferably at least about 90%, more preferably at least about 100%, identical to the amino acid sequence of a full length tau protein (e.g., SEQ ID NOs: 1-41) or a tau functional fragment (e.g., SEQ ID NOs: 42-47, or microtubule binding domain).

The concentration of the monomeric tau protein used in the incubation step for formation of tau amyloid may vary depending on several factors, including the quality of the monomeric tau protein, the nature of the amyloidogenic tau protein and the source of the biological sample. The monomeric tau protein may be present at a concentration greater than the limit of solubility for the monomeric tau protein in the presence of the specific amyloidogenic tau protein to be detected, under the buffer conditions present, which we have determined to be 0.08 mg/ml for AD, PiD,

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and CBD disease conformations under buffer conditions of 5 mM BME in pH 7.4 phosphate buffered saline, but preferably at about 0.1-0.3 mg/mL, more preferably at about 0.15-0.3 mg/mL.

In one embodiment, recombinant tau protein is produced according to the method described by Levy et al (*The Journal of biological chemistry* 280, 13520-13528, doi:10.1074/jbc.M413490200 (2005)), with slight modification. Briefly, tau protein is purified from BL21 *E. coli* (New England BioLabs) transformed with an IPTG-inducible pET 11a vector encoding the 0N4R human tau isoform (SEQ ID NO: 4) under the T7 promoter. Bacteria are grown at 37°C in 5 ml LB media, 100 mg/ml ampicillin for 12 hours before inoculation into 2L culture. When the culture reaches an OD_{600 nm} of 0.6-0.8, protein expression is induced for 3 hours with 0.5 μM IPTG before harvesting cells. Cells are then lysed by sonication in BRB80 buffer (80 mM PIPES, 1 mM EGTA, 1 mM MgCl, pH 6.8). The cell lysate is boiled for 20 minutes to denature cellular proteins and isolate heat-stable tau protein. The recombinant tau protein is then separated by ion-exchange chromatography on phosphocellulose resin and eluted in 0.3 M NaCl in BRB80 buffer. The purity and concentration of the purified recombinant tau protein are characterized by SDS-PAGE and UV spectroscopy. Purified tau fractions are pooled, concentrated, and stored at a concentration of 1 mg/ml in 0.3 M NaCl in BRB80 at -80°C until use.

To increase the sensitivity of detection of the amyloidogenic tau, the incubation conditions (e.g., ionic strength and pH) and the reagents (e.g., the concentration of the monomeric tau protein and the amount of the amyloidogenic tau protein) may be adjusted. The formation of the tau amyloid may be detected by monitoring and quantifying using various methods such as aggregation-specific dyes, turbidity, light scattering, filter retardation assays, circular dichroism, protein stability, protein protease resistance, and detection by conformationally-specific antibodies, through comparison to positive and negative controls. For example, the detection of the amyloidogenic tau may comprise determining the amount of tau amyloid formed and comparing the amount of the tau amyloid formed with one or more controls, which may include a positive control and a negative control.

In one embodiment for monitoring the kinetics of *in vitro* amyloid formation, recombinant tau is boiled for 5 minutes in the presence of β-mercaptoethanol and heparin and diluted in PBS and ThT to a final concentration of 125 μg/ml recombinant protein, 5 mM β-mercaptoethanol, 15 μg/ml heparin, and 40 μM ThT in PBS (pH 7.4). To this substrate solution, 25 μl of CSF is added. Reactions are incubated at a volume of 190 μl in 96-well plates. Plates are sealed with sealing tape and incubated at 37°C in a SpectraMax M2 plate reader (Molecular Devices).

Fluorescence readings are taken every 5 minutes with excitation and emission filters set to 444 nm and 485 nm, respectively. The plates are shaken for 5 seconds once per 5 minutes. The experiment is stopped when positive controls have reached saturating ThT values.

5 The ThT signal may be normalized to normal healthy negative controls. To quantitatively evaluate whether or not a sample reacted positively in the assay reaction, the average ThT fluorescence value of the seeded reactions (reactions with disease CSF) may be compared with their appropriate controls (reactions with normal CSF), at a sensitive time point p , values may be calculated by Student's two-
10 sample unpaired t test; a sample may be deemed positive if (Mean ThT of sample at time = p) > (Mean ThT of negative control samples at time = p).

 The detection method may further comprise determining the concentration of the amyloidogenic tau protein in the biological sample. Quantification of the amyloidogenic tau protein may be calculated based on the amount of the tau
15 amyloid formed during the incubation step as compared with a standard curve generated by the amounts of tau amyloid formed using an amyloidogenic tau protein in a series of known amounts under the same incubation conditions.

 In additional embodiments, combinations of approaches from these embodiments may be combined for optimal detection limits. The method may
20 further comprise determining a conformational feature of the tau amyloid. The term "conformational feature" used herein refers to the three-dimensional (3D) structural features such as width, twist periodicity, length, specific exposed protein sequences, and affinity to conformational antibodies of tau fibrils in a biological sample or tau amyloid formed *in vitro*. The conformational feature may be associated with a
25 disease, for example, a neurological disease or disorder, as well as the bodily fluid or tissue from which the tau protein is isolated. Tau amyloid may have a width of about 20-30 nm and a twist periodicity of about 100-170 nm in a postmortem CSF sample from an AD patient; a width of about 20-30 nm and a twist periodicity of about 230-280 nm in a postmortem CSF sample from a PSP patient; and a width of about 15-
30 20 nm and a twist periodicity of about 140-180 nm in a postmortem CSF sample from a CBD patient. Tau fibrils in a postmortem biological sample are expected to share substantial similarity in conformational features such as width and twist periodicity with those in a corresponding antemortem biological sample, i.e., of the same type such as bodily fluid (e.g., blood, CSF, lymphatic fluid, saliva, tear or urine)
35 from the same subject. For a single AD CSF sample tested, this was found to be true. Tau amyloid formed *in vitro* during the incubation step according to the present invention exhibit a conformational feature that is substantially similar to that of the

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tau fibrils in the biological sample, when that biological sample is composed of brain tissue. We hypothesize the same is true of other bodily fluids and tissues. Yet, the conformational feature may vary for antemortem bodily fluids compared to postmortem bodily fluids, as the composition of such fluids is known to change during the postmortem interval between death and isolation of the bodily fluid. However, we hypothesize that the conformational feature of amyloid derived from living subjects will be characteristic of the neurological disease or condition which the subject has. Thus, the exact measurements of the conformational feature for antemortem CSF may vary from what is reported here but is still expected to be distinct for each disease or disorder, as may be determined by one skilled in the art using the methods described here.

Where a conformational feature can be characterized by a numerical value, the term "substantial similarity" or "substantially similar" used herein refers to similarity of at least about 80%, 90%, 95%, 99% or 100%, preferably about 90%, more preferably about 95%, most preferably about 100%.

A conformational feature of tau amyloid may be used for clinical diagnosis. The conformational feature of tau amyloid formed *in vitro* in the presence of an amyloidogenic tau protein in a test biological sample obtained from a subject may be compared with a reference conformational feature of tau amyloid formed *in vitro* in the presence of amyloidogenic tau protein in a reference biological sample obtained from a patient known to have had a disease such as a neurological disease or disorder (or disease-associated conformational feature). The reference conformational feature may be absent from tau amyloid formed *in vitro* in the presence of an amyloidogenic tau protein in a control sample obtained from a healthy control. The test biological sample, the reference sample and the control sample are preferably of the same type, for example, bodily fluid such as CSF, from the same species. Substantial similarity of a conformational feature of the tau amyloid to the reference conformational feature indicates that the subject suffers from the disease, and a therapy effective for treating the disease may be administered to the subject.

The disease-associated conformational feature of tau fibrils or tau amyloid may be predetermined based on the characterization of the tau fibrils in samples obtained from patients suffering from the disease or tau amyloid formed *in vitro* in the presence of an amyloidogenic tau protein in a reference biological sample obtained from a patient suffering from the disease. For example, the disease-associated conformation of tau fibrils may be predetermined based on the conformation of tau fibrils in a bodily fluid sample from a subject who suffers from the neurological disease or disorder and the conformation of tau fibrils in a bodily

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fluid sample from a subject who does not suffer from the neurological disease or disorder. The disease may be a neurological disease or disorder such as Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, Chronic Traumatic Encephalopathy, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Lewy body dementia, frontotemporal dementia, multiple sclerosis (MS) and traumatic brain injury, preferably AD, PSP and CBD. The predetermined AD-associated conformational features of tau fibrils or tau amyloid may include a width of 20-30 nm and a twist periodicity of 100-170 nm. The predetermined PSP-associated conformational features of tau fibrils or tau amyloid may include a width of 20-30 nm and a twist periodicity of 230-280 nm. The predetermined CBD-associated conformational features of tau fibrils or tau amyloid may include a width of 15-20 nm and a twist periodicity of 140-180 nm.

In one embodiment, the tau amyloid formed *in vitro* using an amyloidogenic tau protein in a biological sample from a subject may have a conformational feature (e.g., width or twist periodicity) substantially similar to that of tau fibrils from an AD patient. For example, the tau amyloid may have a width of 20-30 nm and a twist periodicity of 100-170 nm. The substantial similarity indicates that the subject suffers from AD. The subject may be treated with a therapy effective for treating AD.

In another embodiment, the tau amyloid formed *in vitro* using an amyloidogenic tau protein in a biological sample from a subject may have a conformational feature (e.g., width or twist periodicity) substantially similar to that of tau fibrils from a PSP patient. For example, the tau amyloid may have a width of 20-30 nm and a twist periodicity of 230-280 nm. The substantial similarity indicates that the subject suffers from PSP. The subject may be treated with a therapy effective for treating PSP.

In yet another embodiment, the tau amyloid formed *in vitro* using an amyloidogenic tau protein in a biological sample from a subject may have a conformational feature (e.g., width or twist periodicity) substantially similar to that of tau fibrils from a CBD patient. For example, the tau amyloid may have a width of 15-20 nm and a twist periodicity of 140-180 nm. The substantial similarity indicates that the subject suffers from CBD. The subject may be treated with a therapy effective for treating CBD.

In a preferred embodiment, recombinant tau monomers are incubated with an unknown biological sample (e.g., brain, CSF, saliva, blood, etc.), which may or may not contain disease-relevant conformational features of the tau protein, from which tau protein has been partially purified by immunoprecipitation or other means. Samples which contain the disease-relevant conformational features can be

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distinguished from those that do not by measuring the amount of the tau amyloid formed in the incubation solution using the fluorescent dye Thioflavin T over two or more points in time, compared to positive and negative controls. The analysis of the amount of the tau amyloid as a function of time includes a rigorous statistical
5 comparison based on a sigmoidal fit to the growth kinetics of the aggregated tau in the reaction with the unknown biological sample compared to the control reactions. A standard curve of aggregated tau added to the reaction can be a point of comparison to calculate the concentration of the disease-relevant tau conformation in the biological sample. The reaction products may be analyzed for biochemical
10 properties (e.g., electron microscopy) for distinct morphological properties associated with different neurological diseases or disorders.

According to the present invention, a sample may be incubated with a synthetic or recombinant polypeptide derived from a tau protein and monitored for formation of aggregated protein. Unknown samples may be compared to controls
15 including (a) a positive control, for example, a sample containing a disease-associated conformation of tau and (b) a negative control, for example, a sample of an equivalent composition which lacks disease-associated tau conformations. The assay may have sub-picogram sensitivity when performed under ideal conditions. The reaction product may be analyzed, if desired, for protein aggregates that form
20 during the reaction. Samples comprising a tau protein from individuals with different neurological diseases or disorders produce such aggregates with distinct structures, permitting the identification of the disease manifested in the individual from which the sample was obtained.

The present invention also provides kits useful for carry out the detection
25 method. The kit comprises a monomeric tau protein and a detection buffer. The monomeric tau protein may be recombinant tau protein at a concentration of 1 mg/ml in BRB80 buffer (80 mM PIPES, 1 mM EGTA, 1 mM MgCl, pH 6.8). The detection buffer may be a phosphate buffer at pH range 5.5-8.5 and ionic strength range 20 mM – 600 mM with 0-100 mM 2-Mercaptoethanol and 20-80 μ M Thioflavin
30 T. The kit may also comprise a positive and negative controls for quality assurance. The positive control may be a titration of synthetic recombinant tau fibrils produced in the same reaction buffer. The negative control may be a reaction buffer or a mock isolation of tau from a non-diseased biological sample. The kit may further comprise a data analysis protocol for a standardized output.

35 The term "about" as used herein when referring to a measurable value such as an amount, a percentage, and the like, is meant to encompass variations of $\pm 20\%$

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or $\pm 10\%$, more preferably $\pm 5\%$, even more preferably $\pm 1\%$, and still more preferably $\pm 0.1\%$ from the specified value, as such variations are appropriate.

Assay reaction volumes and vessels. An ideal vessel has minimal volume with a geometry which permits the detection of amyloid and determination of a conformational feature of the amyloid, allowing many replicate reactions to be performed with a low confidence interval in measurement of the SD50 by spearman-karber analysis, while minimizing the cost of reagents (especially recombinant tau protein); multiwell plates are well suited when combined with the use of one or more fluorescent dyes that detect amyloid and which have spectral properties dependent upon amyloid conformation. The useful assay reaction volumes and vessels may include nanocapillary arrays with submicroliter volumes, nanowells with submicroliter volumes, multiwell plates of 24, 96, 384, or more wells with 1 to 3000 microliter volumes, eppendorf tubes, test tubes of any size.

Temperature. We demonstrate detection of amyloidogenic tau at 37 °C, but the assay may function at any temperature permitting the solution to remain a liquid, ranging from under 0 °C to over 100 °C (the melting and boiling temperatures of water adjusted by the effect of salts). Amyloids have been shown to form at temperatures as low as 4 °C and as high as 100 °C.

Additives. Polyanionic additives (RNA, DNA, polyglutamate, anionic detergents, sulfonated glycans, arachidonic acid, and thiazine red, heparin) have been shown to stimulate spontaneous tau amyloid formation. These and other polyanionic modifiers of tau amyloid formation may be used in conjunction with the assay described here, or to alter conditions used to detect amyloidogenic tau in a biological sample, or be used in place of standards or controls.

Protein modifications. Modification of tau protein by phosphorylation, acetylation, methylation, nitration, glycation, and glycosylation has also been shown to induce or inhibit spontaneous tau amyloid formation *in vitro*. These and other protein modifications of the tau monomer may be used in conjunction with the assay described here, or to alter conditions used to detect amyloidogenic tau in a biological sample.

Sample pre-treatment. Sonication of tau amyloids has been shown to increase its seeding potency. Altering the amount of energy, number of pulses, timing, or intensity of sonication may improve the detection limit reported here. Addition or removal of the protein modifications listed above, which have varying effects on the ability of tau protein to form amyloid, may be used in conjunction with the assay to improve the limit of detection. Means of tau purification other than immunopurification mediated by magnetic beads (e.g.

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sucrose fractionation, size exclusion, other antibody based purification schemes) are well known and may be used in conjunction or in place of immunopurification to detect amyloidogenic tau. Other antibodies recognizing disease-associated tau proteins may be used to purify the amyloidogenic tau.

- 5 Agitation of reaction contents. During the incubation period, the reaction may be agitated constantly, every fraction of a second, once per second, one to sixty times per minute, one to sixty times per hour, one to twenty-four hours per day, or not at all.

10 Preparation of tau monomer. Alternate methods of producing tau monomer may produce recombinant protein that is effective in the assay; generally, the protein produced must be >90% pure and free of polyanions to be functional in the assay. Alternately, polyanions may be deactivated by reaction with cations or polycations.

Example 1. Detection of Tau Fibrillization

- 15 A. Magnetic bead assisted depletion of tau from CSF

20 Twenty microliters of magnetic beads (e.g. from New England Bio Labs, binding capacity 2 µg) conjugated to anti-mouse IgG secondary antibodies were washed with PBS buffer and incubated with a solution of 2 µg of primary antibody (e.g. 5A6, Iowa Hybridoma Bank) in 200 µL of 0.1% BSA in PBS buffer at room temperature with continuous shaking. After 2 h of incubation, magnetic beads were removed and washed 3 times with PBST buffer. The beads were re-suspended in 1000 µL of PBS.

25 Ten microliters of the magnetic beads were added to 0.1 to 100 µL of CSF and incubated at room temperature, shaking for 2 hrs. Magnetic beads were removed, and the remaining CSF solution was used in the amyloid seeding assay in the same concentration as non-depleted CSF. The recovered magnetic beads were used to seed structural propagation of misfolded tau in CSF *in vitro*. Beads bound to tau protein from CSF were sonicated for 30 sec (10 sec pulses) before the addition to the reaction with monomeric tau protein.

- 30 B. Tau Monomer Preparation

Fresh BRB80 buffer and PBS (pH 7.4) buffer were prepared.

35 BRB80 buffer contains 80 mM PIPES buffer (piperazine-N,N'-bis(2-ethanesulfonic acid)), 1 mM EGTA, and 1 mM magnesium chloride, with pH adjusted to 6.8 with KOH. Milky white color from PIPES should change to clear with sufficient addition of KOH.

Recombinant monomeric tau protein was diluted to 1 mg/ml in BRB80 buffer (with 0.3 M NaCl) and boiled at 100 °C for 5 min with 25 mM β-mercaptoethanol.

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Boiling times could vary from 0-15 min. Boiling could be replaced with salting out the purified protein sample with 60% ammonium sulfate and removing polyanions. β -mercaptoethanol concentrations could vary from 0 mM to 500 mM.

5 The monomeric protein solution was rapidly diluted 1:5 and cooled to 20 °C in PBS, pH 7.4, to a final concentration of 0.2 mg/ml of recombinant tau and 5 mM β -mercaptoethanol. The resulting tau protein concentration could range from 0.15 mg/mL to 0.3 mg/mL. The buffer may have a pH at 5.5-8.5. The ionic strength of the PBS buffer could be 10-600 mM.

10 The diluted recombinant tau solution was supplemented with 40 μ M Thioflavin T (ThT) and filtered through a sterile 0.2 μ m filter. ThT could be replaced with any method for monitoring protein aggregation or amyloid formation.

To obtain optimal results, the following adjustments may be made:

- a. The critical concentration of recombinant tau to seed is 0.08 mg/ml and may vary based on the origin of the seed.
- 15 b. The average extent of reaction for brain-derived seeding is ~10%, as assessed by sedimentation at 20,000xg for 1 hr. The extent of reaction may be increased by the addition of monomeric tau after the fibrillization kinetics plateau.
- c. Seeds may have significantly decreased seeding after storage in -80 °C for up to 45 months.
- 20 d. The working range for seeding is from less than one picogram to up to 50 ng tau in the sample. Impurities may inhibit the seeding reaction.
- e. pH can vary over a broad range encompassing at least 5.5 to 8.5.

C. Monitoring Tau Fibrillization Kinetics

25 An opaque 96-well plate was prepared with a 3 mm glass bead added to each well to increase agitation. The recombinant tau and seed solution mixture was added to the plate in 200 μ l reaction volumes (total). It is recommended for each reaction to have at least 3 technical replicates, prepared simultaneously. The plate was sealed with sealing tape to prevent evaporation and incubated in the plate reader at 37 °C. The plate reader was set to shake for 5 sec before each 5 min fluorescence
30 time reading.

Thioflavin T fluorescence was monitored over time with excitation and emission filters set to 444 nm and 485 nm, respectively. Fluorescence readings were taken every 5 minutes, with agitation for 3 seconds before each reading. The lag phase of seeding varies from 10-100 hrs depending on the protein prep and seed
35 purity.

D. Quantification of disease-associated tau protein in sample: Data Analysis and Statistical Analysis

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We used simple logistic regression to model tau polymerization *in vitro*, which is well-suited to model a variety of autocatalytic processes such as template-driven amyloid fibrillization, given by:

$$C_{\tau}^* = \frac{M}{1 + e^{\frac{4S(L-t)+2}{M}}} \quad (1)$$

5 where C_{τ}^* concentration of misfolded tau in the reaction, M is the maximum ThT fluorescence signal, S is the maximum rate of ThT fluorescence increase, and L is the lag phase.

Spearman-Kärber analysis (Hamilton et al., *Environmental Science and Technology* 11, 714-719 (1977)) is used to determine the seeding dose of CSF
10 material at which 50% of reactions were positive based on a t-test of the above parameters to the negative controls. For this analysis, a dilution series of CSF subjected to immunopurification was prepared such that at least one titer of positive control and CSF caused 100%-positive reactions to increase ThT fluorescence before unseeded reaction and at least one dilution caused 0%-positive reactions. The
15 positive correlation was determined with Spearman's correlation coefficient to determine the "SD50," at which 50% of reactions appeared to contain seeds.

E. Conformational Analysis of reaction products identifies the disease manifest in the patient from whom the sample was obtained

Electron microscopy may be used to quantify the width and periodicity of
20 fibrillar aggregates found in the reaction products. Reactions seeded with postmortem CSF samples from 2-5 patients with three different tauopathies have been analyzed and have distinct appearances. The width of AD CSF-seeded, PSP CSF-seeded and CBD CSF-seeded recombinant tau fibrils are significantly different from the spontaneous tau fibrils from the Normal CSF and from one another. AD and
25 PSP CSF-seeded recombinant tau fibrils have widths of 20-30 nm. CBD seeded fibrils have widths of 15-20 nm. The periodicity of AD CSF seeded reactions is 100-170 nm, while that of CBD CSF seeded reactions is 140-180 nm, and that of PSP CSF is 230-280 nm.

We predict that many of the >20 different diseases involving tau will produce
30 structures which are distinct for the disease of the patient.

Such structural analysis could be accomplished by many different biochemical assays, such as shifts in the spectra of protein fiber binding dyes, 1-

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Anilidonaphthalene-8-Sulfonic Acid (ANS), Circular Dichroism (CD), stability analysis by thermal or chemical denaturation, etc.

F. Tau Fibrillization Results

In the presence of disease-associated aggregated tau conformations,
5 recombinant monomeric tau is sequestered by the aggregates and the disease-specific conformation is propagated in the reaction.

Thioflavin T (ThT) binds to the disease-relevant tau conformation in solution and undergoes enhanced and shifted fluorescence. This fluorescence change is detected over time in a 96 well plate as the recombinant tau monomers are
10 converted to the aggregated conformation in the presence of tau aggregates from a biological sample.

Following incubation of the tau monomer with a disease-relevant tau conformation, the disease-relevant conformation propagates in a reaction with the tau monomer. This conformation is specific to the disease the original sample is
15 taken from. The morphological differences are analyzed by electron microscopy.

The resulting tau aggregation kinetics monitored by ThT have a sigmoidal behavior, and are analyzed by fitting to a sigmoidal curve and statistically comparing the parameters from the curve of the normal and the disease structures. The lag phase of the kinetic reaction correlates with the amount of aggregates tau added to
20 the reaction. A standard curve can be prepared by adding a titration of aggregated tau of known concentrations to the reaction with monomeric tau, and the kinetic analysis of aggregation growth can be used as a comparison to calculate the amount of tau aggregates present at the start of the reaction.

G. Additional Methods

25 *Production and purification of recombinant tau proteins*

Recombinant tau protein was purified from BL21 *E. coli* (New England BioLabs) that was transformed with an IPTG-inducible pET-11a vector encoding the human 0N4R tau isoform under the T7 promoter. A fresh plate was made by streaking an LB and 100 mg/L ampicillin plate with an *E. coli* glycerol stock. The
30 plate was incubated at 37 °C overnight. A single colony was picked from the plate and grown at 37 °C in 5 mL of LB, 100 mg/mL ampicillin culture for 12 hours before the addition to a 200 mL LP and ampicillin (100 mg/L) culture. Cells were grown overnight and then used as an inoculum for a 20 L of TB and ampicillin (100 mg/L) fermentation in a New Brunswick BioFlo 4500 fermenter with 30% dissolved oxygen,
35 200 rpm agitation at 37 °C. Cells were grown until the culture reached an OD600 of 0.4-0.6 and were then induced with 0.5 mM IPTG. The cells were grown for 3 hours

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before harvesting by centrifugation at 5000 x g for 15 min at 4 °C. Cell pellets were re-suspended in purification buffer BRB80 (80mM PIPES buffer, 1mM EGTA, 1mM MgCl, pH 6.8) with added protease inhibitors (1 mM PMSF and 1mM protease inhibitor cocktail, Sigma) and sonicated with a Fisher Scientific Model 120 sonicator for 10 total minutes at 80% amplitude (10 sec on/10 sec off) on ice. The cell lysate was centrifuged at 5000xg for 15 min at 4 °C and the supernatant was boiled for 15 min. Tau protein has a high thermal resistivity at 100°C like most other proteins. Incubating the lysate in a boiling water bath therefore keeps tau intact and denatures a large portion of other proteins present. The boiled lysate was centrifuged at 5000xg for 15 min at 4 °C and the supernatant was used for further purification of tau by ion exchange. Ion exchange chromatography was done with activated phosphocellulose resin (C2258 SIGMA) with a 60 mL gravity column. The lysate was loaded onto the column (5 g of resin per 1 L of culture), washed with 1 lysate volume equivalent of BRB80 buffer and then washed with 1 lysate volume equivalent of 0.045 M NaCl in BRB80 buffer. Pure recombinant tau was eluted in 0.1 M NaCl in BRB80 buffer. The resulting pure recombinant tau concentration was measured by UV A280 spectroscopy and purity was measured with Coomassie densitometry. The purified protein was frozen at -80 °C until immediately before use. Monomeric recombinant tau protein showed characteristics of random coil structure by Circular Dichroism with no signs of misfolding by Electron Microscopy.

Preparation of brain homogenates

To prepare 5% (w/v) brain homogenates, nine volumes of ice-cold homogenization buffer (10 mM Tris, 1 mM EDTA, 0.8 M NaCl, 10% sucrose, and 0.1% Triton X-100) supplemented with protease inhibitors (1 mM PMSF; protease inhibitor cocktail P8340, Sigma) were added to brain tissue in a 50-ml tube. Brain tissue was homogenized on ice by extrusion through progressively smaller needles (10 passages each through 16, 18, and 21 gauge needles). The sample was centrifuged at 1000xg for 5 min at 4°C. The supernatant (S1) was collected, and the pellet was rehomogenized in 9 volumes homogenization buffer and protease inhibitors by 10 passages through a 21-gauge needle. This suspension was then centrifuged at 1000xg for 5 min at 4°C, and the resulting supernatant was combined with S1, yielding a 5% brain homogenate.

Partial Purification of Misfolded Tau from Brain Homogenates

We used a previously published protocol to partially purify misfolded tau from human brain samples. Briefly, 4 ml of 5% brain homogenate in 1% (w/v) Sarkosyl and 0.2% dithiothreitol was incubated at room temperature for 2.5 h with stirring. Multimeric tau species were pelleted by ultracentrifugation at 300,000xg for

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1.5 h at 4°C. The pellet was washed with PBS, ultracentrifuged at 300,000×g for 1.5 h at 4°C, and resuspended in 4 ml PBS by stirring overnight at room temperature and passing 5 times through a 27-gauge needle to break up any residual large aggregates. A portion was used immediately in the assay reaction and the remainder
5 was aliquoted and stored at -80°C. A decrease in seeding was seen after long storage of purified seeds.

Purification of mouse prions

We used a previously published protocol to purify prions from mouse brain. Briefly, 10% brain homogenate in PBS was prepared by needle extrusion from
10 the brain of an FVB mouse infected with the Chandler/RML prion strain. A 10% solution of phosphotungstic acid (pH 7.4) was added to 500 µl brain homogenate to a final concentration of 0.5%. Samples were incubated with shaking for 1 h at 37°C, followed by centrifugation at 14,000×g for 30 min at room temperature. We washed the pellet with 2% Sarkosyl and re-centrifuged the pellet at 14,000×g for 30 min at
15 room temperature. The pellet was re-suspended in 150 µl water and stored at -80°C.

Preparation of synthetic Aβ oligomers

Synthetic Aβ₁₋₄₂ peptide (Abcam) was dissolved at 1 mg/ml in 100% HFIP for one hour at room temperature with occasional vortexing, followed by 10 min sonication in a water bath sonicator. HFIP was evaporated under a gentle Ar
20 stream, and the peptide was re-suspended in DMSO at 5mM. For oligomer assembly, the peptide solution was diluted to 100µM in F-12 cell culture medium (Sigma) and incubated for 24 h at 4°C, as described previously.

Electron microscopy CSF seeded tau fibrils and Aβ oligomers

Oligomers were adsorbed onto 300 mesh carbon-coated copper grids
25 (Electron Microscopy Sciences) for 1 min before extra solution was syphoned off with filter paper, and the protein was allowed to dry on the grid for 5 min. After two successive 1 min washes with deionized water, each sample was stained with 1% phosphotungstic acid for 20 sec. The grids were then observed on a Tecnai G2 12 Twin transmission electron microscope.

30 Image analysis was done with ImageJ. The width of each fibril was measured every 20 nm with the scaled line selection drawing tool. The periodicity measurement was taken with the same technique in ImageJ by measuring the distance between each complete twist that was available in every imaged fibril. The available number (n) of measurements for each fibril characteristic represents all of
35 the measurements taken across biological and technical replicates. All fibril images were chosen from a random area on the EM grid to eliminate observer bias.

Immunogold-Electron Microscopy

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Tau protein species found in the CSF were characterized in following a previously described method. 5 µl of CSF was deposited to a freshly glow-discharged 300-mesh carbon coated EM grid (Electron Microscopy Sciences) and incubated at room temperature for 1 min. Excess solution was removed with filter paper and the grid was allowed to air-dry for 5 min. The grids were then blocked with blocking solution (0.1% BSA in PBS) for 15 min. the grids were then transferred to a solution of 5A6 primary antibody diluted 1:1000 in blocking buffer and were incubated for 1 h. The grids were then washed 6 times in a drop of blocking buffer supplemented with 0.1% Tween-20 (2min/drop) and 2 times in water. The grids were then transferred to an anti-mouse secondary antibody conjugated with 10nm colloidal gold particles (Electron Microscopy Sciences) diluted 1:200 in blocking solution for 1 h. The grids were washed as before and air-dried for 10 min. Staining was performed by adding 3ul of 1% phosphotungstic acid (PTA) for 20s. Excess stain was removed with filter paper and the grids were allowed to air-dry. Images were taken with a transmission electron microscopy (Tecnai 12 Biotwin) at 120 V.

Quantification of labeling was performed on areas selected by systematic random sampling. A cluster greater than 1 was defined as a group of particles that had a significantly closer average distance than a given distance from a typical particle.

Quantification of Partially Purified Misfolded Tau

Partially purified brain homogenate (10 µl) was diluted 1:1 with SDS loading buffer (4% SDS, 20% glycerol, 125 mM Tris, 0.1% β-mercaptoethanol, 0.02% bromophenol blue), boiled for 5 min, and subjected to SDS-PAGE analysis on 4-20% polyacrylamide gels (Pierce) in Tris-HEPES-SDS buffer (Pierce) at 150V for 30 min. Proteins were blotted to a nitrocellulose membrane in transfer buffer (50 mM Tris, 390 mM glycine, 0.05% SDS, 10% ethanol) at 25V for 1 h. Blots were blocked with 1% nonfat dry milk in PBS overnight, probed with the N-terminal tau antibody 5A6 (diluted 1:1000; Iowa Hybridoma Bank), and a mouse secondary HRP antibody (Invitrogen). Protein content was detected with chemiluminescence using SuperSignal West Dura substrate (Pierce).

Example 2. Detection of distinct tau strains in brain and CSF of neurodegenerative disease patients

To find the sensitivity of amyloid seeding for tau, insoluble tau protein was purified (Greenberg and Davies, *Proceedings of the National Academy of Sciences of the United States of America* 87, 5827-5831 (1990)) from frontal cortex samples of human AD brain. Addition of recombinant (Fig. 1A) and AD brain-derived tau fibers (Fig. 1B) to solutions of recombinant tau protein resulted in accelerated amyloid

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formation. A dose-dependent response was observed upon addition of varying titers of both recombinant and AD brain-derived tau fibers. The limit of detection was determined to be ~1 ng of recombinant tau fibers or ~100 pg tau fibers isolated from AD brain by serial dilution (Figs 1C, 1D).

5 We added CSF to the assay reaction to determine whether we could detect aberrant conformations of tau in the postmortem CSF. The addition of 25 µl of neuropathologically-confirmed AD CSF seeded the fibrillization reaction with a reduction in the lag phase compared to the addition of 25 µl normal CSF control (Fig. 2).

10 To verify the specificity of the fibrillization reaction to the detection of misfolded tau and not other misfolded proteins, we added synthetic Aβ oligomers and prions purified from the brains of infected mice to the assay reaction. Aβ oligomers were made from synthetic Aβ₁₋₄₂ peptide (Stine et al., *The Journal of biological chemistry* 278, 11612-11622, doi:10.1074/jbc.M210207200 (2003)),
15 while mouse-adapted scrapie prions (PrP^{Sc}) were purified by phosphotungstic acid precipitation (Safar et al., *Nature Medicine* 4, 1157-1165 (1998)) from the brain of an FVB mouse infected with the Chandler/RML prion strain. In all cases activity in the assay reaction, as measured by ThT fluorescence, was comparable to normal control samples, indicating the assay specifically detected tau fibers but not Aβ
20 oligomers or prions (Fig. 2). As an additional test for specificity, tau was depleted from the AD CSF samples with magnetic bead-assisted immunopurification. After the depletion, most of the tau was removed from the samples, and seeding was significantly ($p < 0.001$) halted (Fig. 2).

A total of 17 human CSF samples with neuropathologically-confirmed AD and
25 15 CSF samples neuropathologically normal human controls were added the assay reaction. We observed significant ($p < 0.001$) three-fold average increase in ThT fluorescence at a sensitive time point for AD CSF samples compared to the normal CSF samples (Fig. 4A). To determine whether we can detect misfolded tau in different diseases, we added 25 µl of human CSF from patients with other
30 neuropathologically-confirmed tauopathies (PSP and CBD) to the fibrillization reaction and observed the fibrillization kinetics with ThT. Significant ($p < 0.01$) ThT increase at a sensitive time point was observed in all three tauopathies compared to normal CSF controls (Fig. 4A).

To determine whether we could detect misfolded tau in ante-mortem CSF, we
35 added to 25 µl of CSF obtained from 7 patients with clinical AD and 7 clinically healthy individuals to the assay reaction. A significant ($p < 0.01$; Fig. 4B) ThT fluorescence increase was observed in AD CSF samples with clinical dementia rating

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of 1 and 2, compared to healthy normal CSF samples. We also compared the ThT fluorescence increase from the addition of CSF from 2 patients with clinical mild cognitive impairment (MCI) to normal healthy controls. Only 1 out of 2 CSF samples had significantly ($p < 0.01$) higher ThT fluorescence compared to healthy control CSF (Fig. 10). A significant ($p < 0.001$) positive correlation was observed between ThT fluorescence increase and CDR, with the highest ThT fluorescence difference in postmortem samples. This is consistent with the trend observed between neurofibrillary tangle density and CDR in postmortem brain tissue.

There was no significant correlation between the relative ThT values of the antemortem AD samples and the individual sample measurements for standard biomarkers. This comparison to the standard biomarker measurements also revealed possible false positive and false negatives (Fig. 3).

We used immunogold-EM to characterize any presence of higher-order misfolded tau species in the CSF. CSF samples were deposited on carbon-coated EM grids, and tau protein was detected with a 5A6 antibody coupled to a 10nm gold nanoparticle. Disease CSF contained clusters of tau protein. The gold particle-conjugated antibodies are significantly ($p < 0.01$) more clustered in the disease CSF than in normal healthy CSF. These clusters may be fibrillization nuclei that seed the fibrillization reaction.

The addition of tau fibrils to a solution of recombinant tau sequesters monomeric recombinant tau into the amyloid and amplifies the original seed structure. We characterized the structural propagation of the seeds found in the CSF, and observed that the structures were specific for the different diseases (Fig. 5). Tau protein was immunopurified from 100 ul of CSF from different tauopathies (AD, PSP, CBD) with magnetic beads bound to 5A6 antibody. The beads bound to CSF tau were washed and incubated in a reaction with recombinant tau, ThT, and 5mM BME in PBS at 37 °C. The fibrillization reaction was monitored with ThT fluorescence increase. The resulting tau fibrils were deposited on carbon-coated grids and stained with 1%PTA. Structures from different biological replicates from each tauopathy were homogeneous between biological replicates and distinct.

AD CSF tau fibrils had a width of 28 nm and a periodicity of 120 nm. PSP CSF tau fibrils had a width of 25 nm and a periodicity of 250, which was significantly ($p < 0.001$) longer than the AD CSF tau fibrils. The CBD CSF tau fibrils were narrow, with a width of 17 nm and a periodicity of 180, which were significantly ($p < 0.001$) different from the measurements for the AD CSF and PSP CSF samples. The healthy control CSF tau induced fibrillization to make narrow 18 nm in with fibrils with no detectable twisting.

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In AD, intracellular tau fibril formation precedes Abeta plaque formation, decades before the increase of tau in the CSF. The conformation-specific tau biomarkers can provide an additional metric that may be used in combination with tau concentration and phosphorylation to diagnose and monitor disease progression.

5 There is no significant correlation between the assay measured ThT increase in the clinically diagnosed AD CSF and other biomarkers. This suggests that the detection of misfolded tau in CSF may be a good candidate for a complimentary biomarker to help identify false positives and false negatives with previously established methods.

10 An emerging notion in neurodegenerative disease research is that many misfolded proteins share characteristics with prions. Prions propagate through the brain by protein-protein interactions in the interstitial space. Mounting evidence suggests that intracellular misfolded tau may escape host cells and 'infect' other cells in a similar fashion, potentially contributing to the pathogenesis of AD.

15 We have demonstrated that aberrant conformations of tau protein derived from human AD brain samples sensitively seed recombinant tau amyloid formation. We used the assay based on this observation to selectively detect misfolded tau in the CSF of humans with AD, PSP, and CBD, which have been previously described to have distinct tau misfolding pathology. Our findings establish that amyloidogenic
20 conformers of the intracellular tau protein are carried through the extracellular space in the CSF.

 In tauopathies, the severity of NFT pathology correlates with cognitive decline, supporting the link between tau pathology, functional impairment and neurodegeneration. Therefore, the detection of the disease-associated conformation
25 of tau may be an important biomarker for not only AD, but also other neurodegenerative diseases.

 Biochemical markers for AD and other tauopathies detect increased tau protein in the CSF by ELISA. The availability of epitopes on tau molecules targeted by antibodies used in these ELISAs may be differentially exposed in the amyloid
30 state compared to the soluble state, which may impact these readings. ELISAs to selectively detect hyperphosphorylated tau have demonstrated improved sensitivity over those that detect total tau. However, recent evidence suggests that phosphorylation may be secondary to tau misfolding, and phosphorylation sites evolve as the disease progresses. Other tauopathies have a wide spectrum of clinical
35 presentation and the development of imaging and fluid biomarkers is still in the early stages. Therefore, detection of misfolded tau in AD and other tau-related

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neurodegenerative diseases CSF may be a good candidate biomarker for AD to complement ELISA methods at earlier stages of the disease.

Profound neuronal loss occurs in even mild AD. This may result in the release misfolded tau conformers into the extracellular space. In cell culture, tau protein aggregates added to the culture medium enter the cytoplasm and recruit endogenous tau protein, suggesting that misfolded protein which has entered the interstitial space may enter nearby cells and propagate through a prion-like mechanism. Our results indicate that misfolded tau is present in the human AD CSF, which may reflect a clearance mechanism in which the CSF washes away extracellular tau aggregates.

High concentrations of A β oligomers have been shown to stimulate tau amyloid formation and it is known that A β oligomers are present in CSF, raising the possibility that our observed results arise from cross seeding of tau with A β . However, cross seeding has only been observed at A β concentrations several orders of magnitude above those observed in CSF, and our addition of A β oligomers to the reaction assay at physiologically relevant concentrations did not produce a seeding effect (Fig. 2).

Here, we show that we can amplify distinct tau fibril structures from different tauopathy CSF to study the tau fibril morphology found in the CSF. Tau fibrils from brains of different tauopathies have been shown to have disease-specific morphology, which is also reflected in the distinct structures that are propagated from different tauopathy CSF. This adds a higher level of specificity to the detection of misfolded tau as a biomarker.

By detecting tau molecules in a conformation with an increased propensity to form amyloid fibers, the seeding assay mechanistically links tau in CSF to a main pathological event in AD and other neurodegenerative diseases.

All documents, books, manuals, papers, patents, published patent applications, guides, abstracts, and/or other references cited herein are incorporated by reference in their entirety. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

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What is Claimed:

1. A method for detecting an amyloidogenic tau protein in a bodily fluid sample from a subject, comprising:
 - (a) incubating the sample or a purified fraction thereof *in vitro* with a monomeric tau protein to form tau amyloid, and
 - (b) detecting the presence of the tau amyloid.
2. The method of claim 1, wherein step (b) comprises determining the amount of the tau amyloid and comparing the amount of the tau amyloid with one or more controls.
3. The method of claim 1, further comprising determining a conformational feature of the tau amyloid.
4. The method of claim 3, further comprising comparing the conformational feature of the tau amyloid with a reference conformational feature of tau amyloid formed *in vitro* in the presence of an amyloidogenic tau protein from a patient suffering from a neurological disease or condition, wherein the reference conformation feature is absent from tau amyloid formed *in vitro* in the presence of an amyloidogenic tau protein from a healthy control.
5. The method of claim 1, wherein the subject is a human being.
6. The method of claim 4, wherein the subject is a patient who suffers from the neurological disease or condition.
7. The method of claim 4, wherein the neurological disease or condition is selected from the group consisting of Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, Chronic Traumatic Encephalopathy (CTE), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Lewy body dementia, frontotemporal dementia, traumatic brain injury, and multiple sclerosis (MS).
8. The method of claim 4, wherein the neurological disease or condition is selected from the group consisting of Alzheimer's disease (AD), Cortical Basal Degeneration (CBD) and Progressive Supranuclear Palsy (PSP).
9. The method of claim 1, wherein the bodily fluid is selected from the group consisting of blood, cerebrospinal fluid (CSF), lymphatic fluid, saliva, tear and urine.
10. The method of claim 1, wherein the bodily fluid is cerebrospinal fluid (CSF).

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11. The method of claim 1, wherein the monomeric tau protein is a polypeptide having an amino acid sequence at least 90% identical to a sequence selected from SEQ ID NOs: 1-47, or a functional fragment thereof.

5 12. The method of claim 1, wherein the amyloidogenic tau protein comprises a polypeptide having an amino acid sequence at least 90% identical to a sequence selected from SEQ ID NOs: 1-47, or a functional fragment thereof.

13. The method of claim 1, wherein the amyloidogenic tau protein comprises an amino acid sequence at least 90% identical to that of the monomeric tau protein or a functional fragment thereof.

10 14. The method of claim 1, wherein the concentration of the monomeric tau protein in step (a) is greater than 0.08 mg/ml.

15. The method of claim 1, wherein the amount of the amyloidogenic tau protein in step (a) is less than 1 pg.

15 16. The method of claim 1, further comprising purifying the amyloidogenic tau protein from the sample before step (a).

17. The method of claim 1, further comprising determining the concentration of the amyloidogenic tau protein in the sample.

18. The method of claim 3, wherein the conformational feature is selected from the group consisting of fibril width, twist periodicity, and a combination thereof.

20 19. The method of claim 4, wherein the reference conformational feature is predetermined.

25 20. The method of claim 4, further comprising administering to the subject a therapy effective for treating the disease, wherein the conformational feature of the tau amyloid is substantially similar to the reference conformational feature, and wherein the disease is selected from the group consisting of Alzheimer's disease (AD), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD).

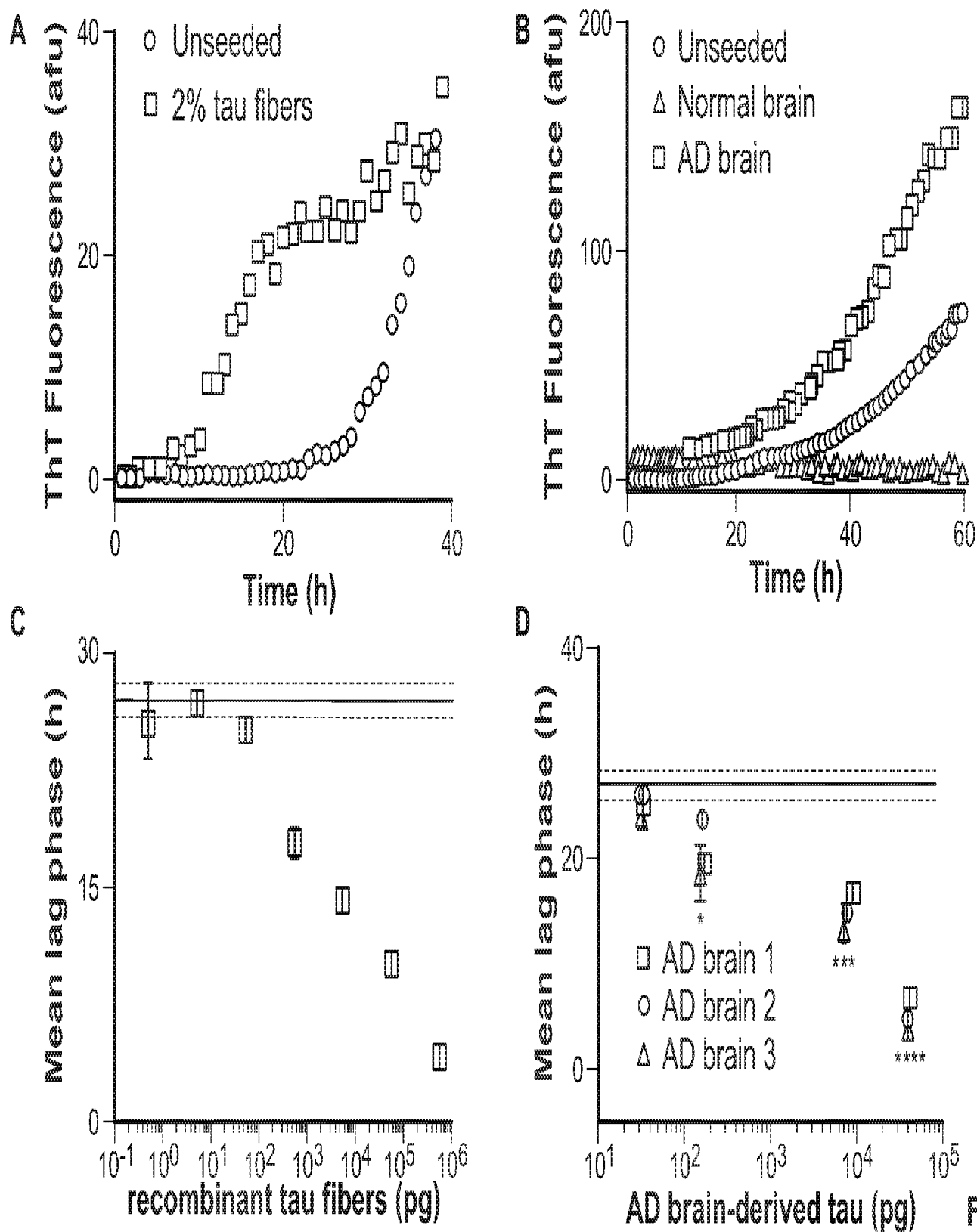


FIG. 1

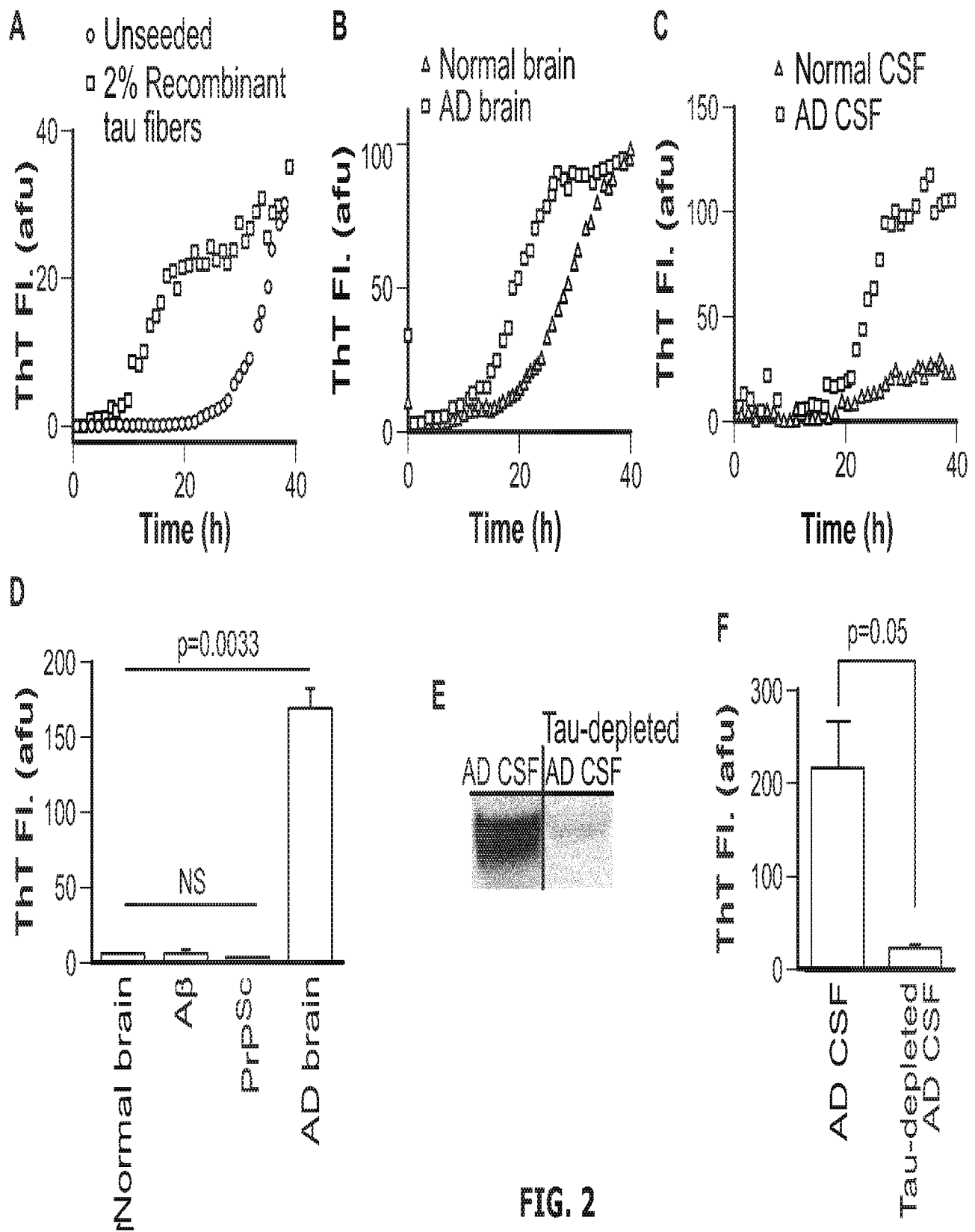


FIG. 2

FIG. 3

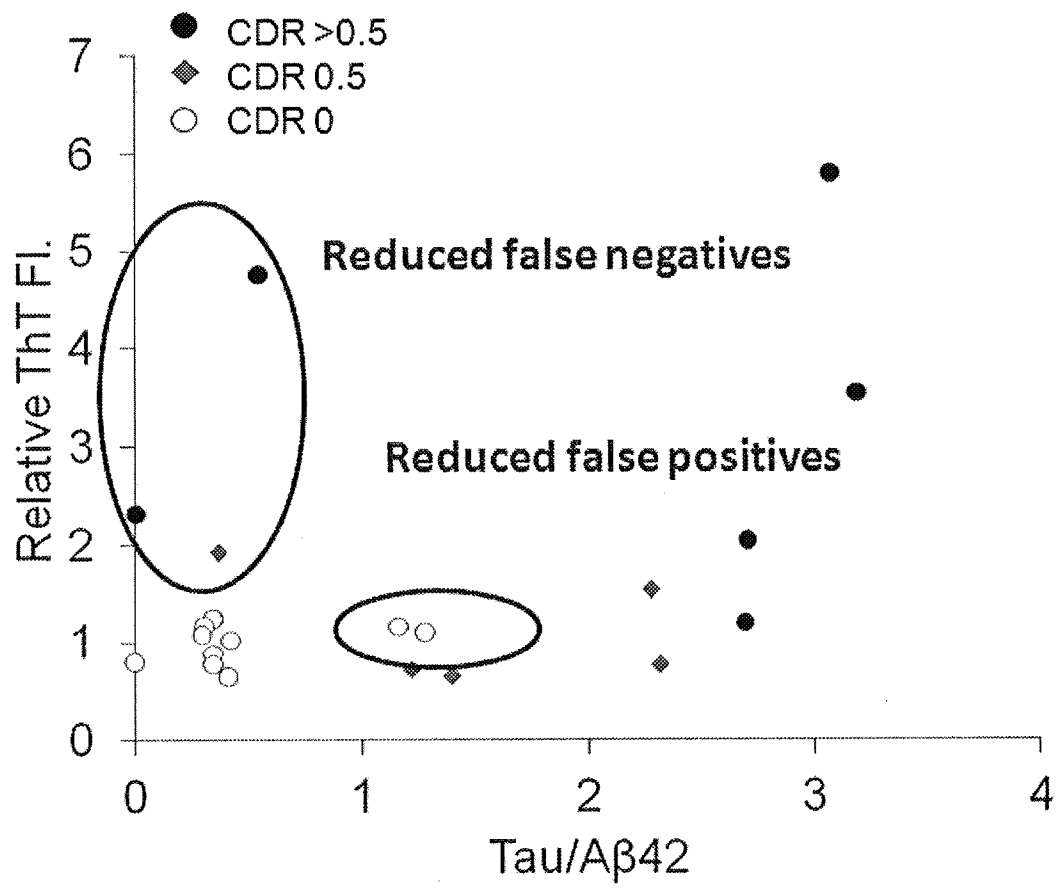
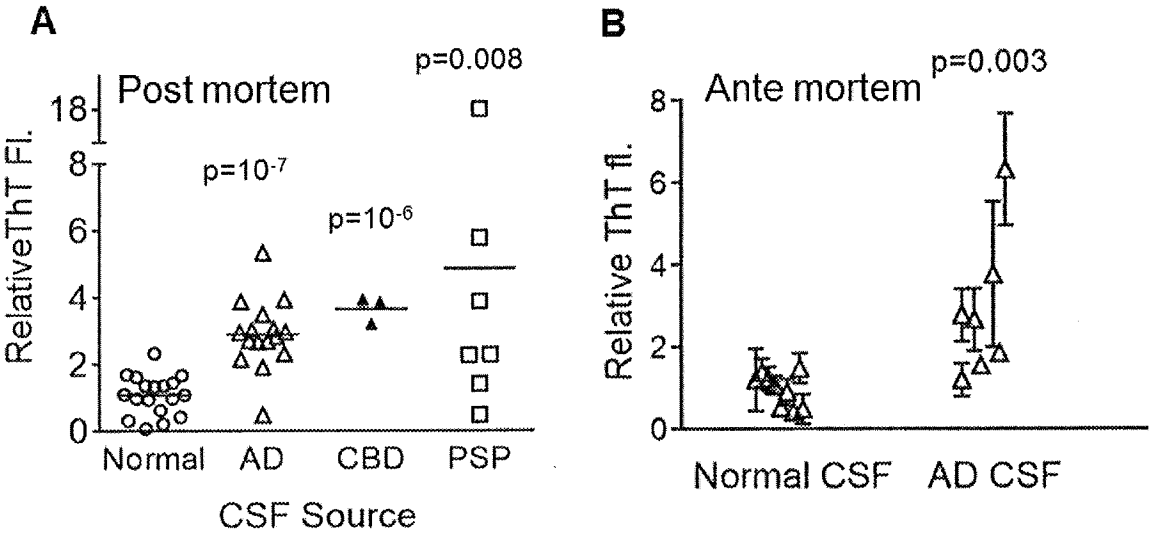
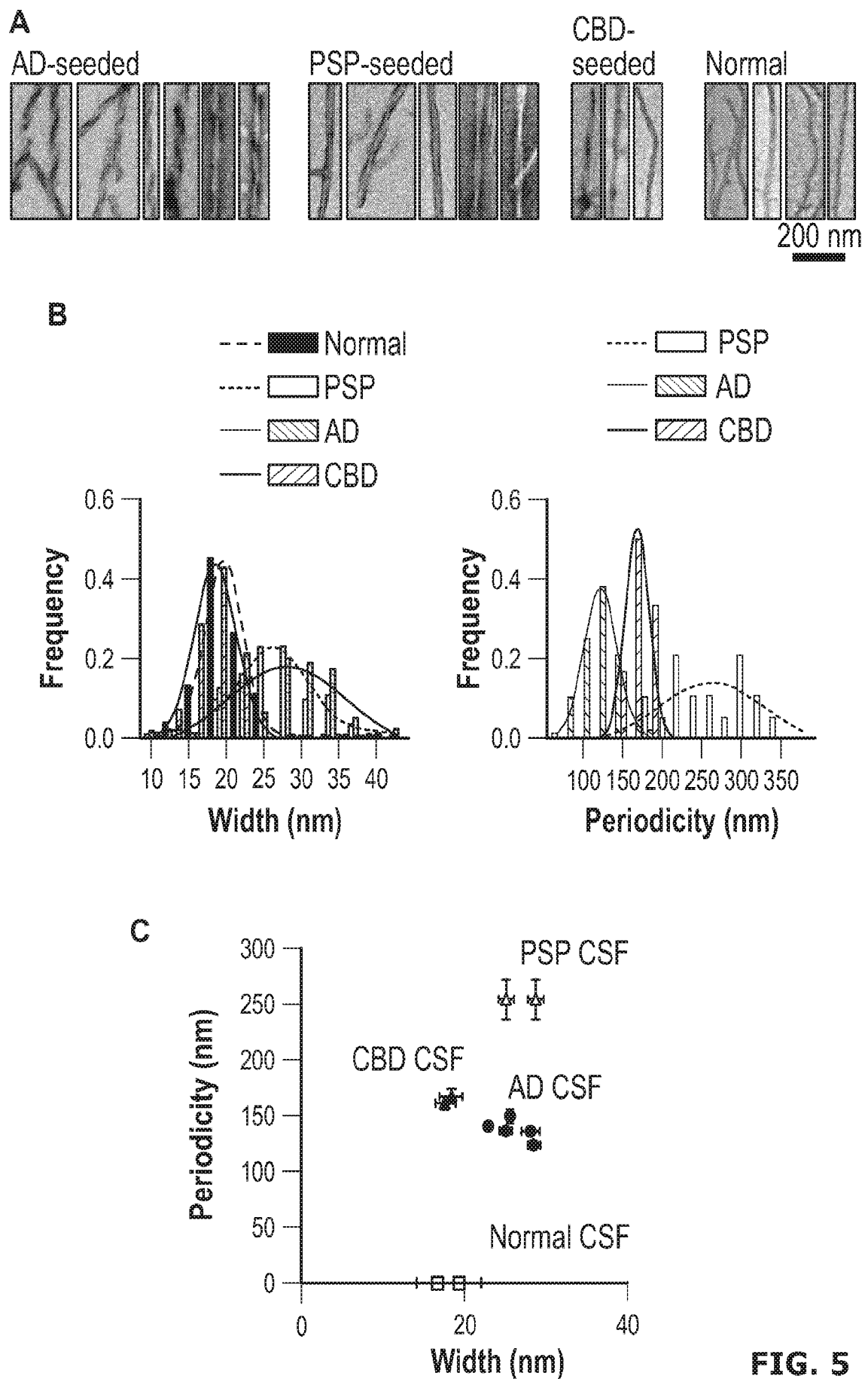
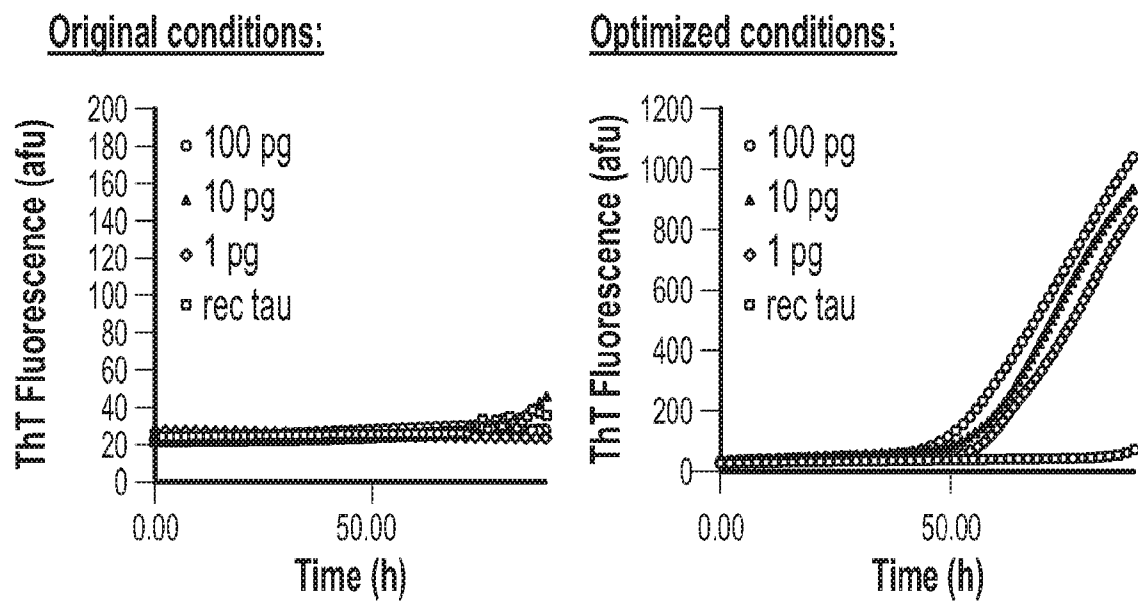
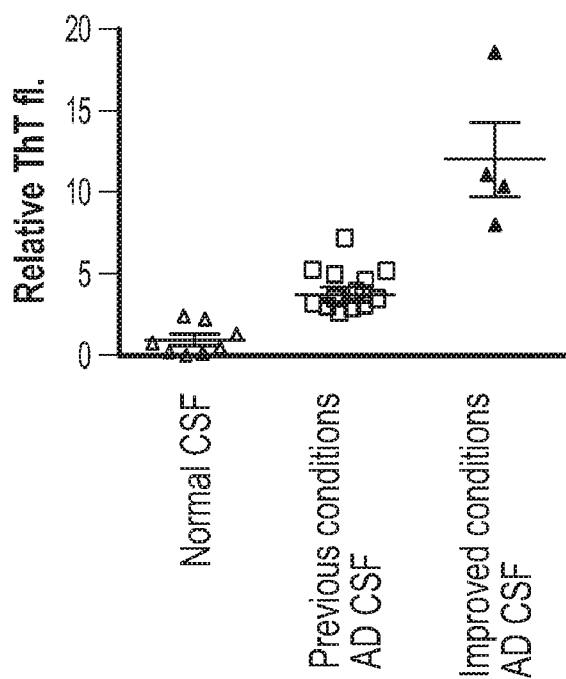


FIG. 4



**FIG. 5**

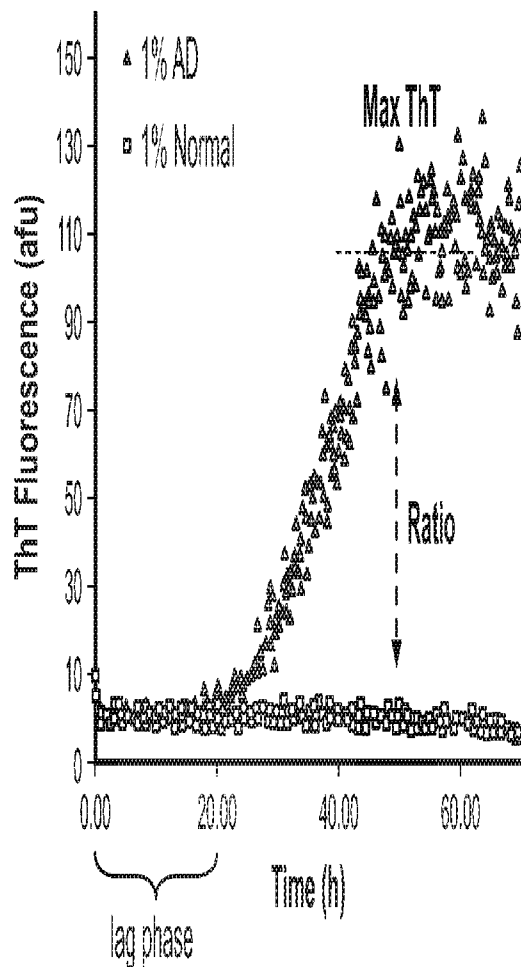
A**B****FIG. 6**

A**Simple Analysis**

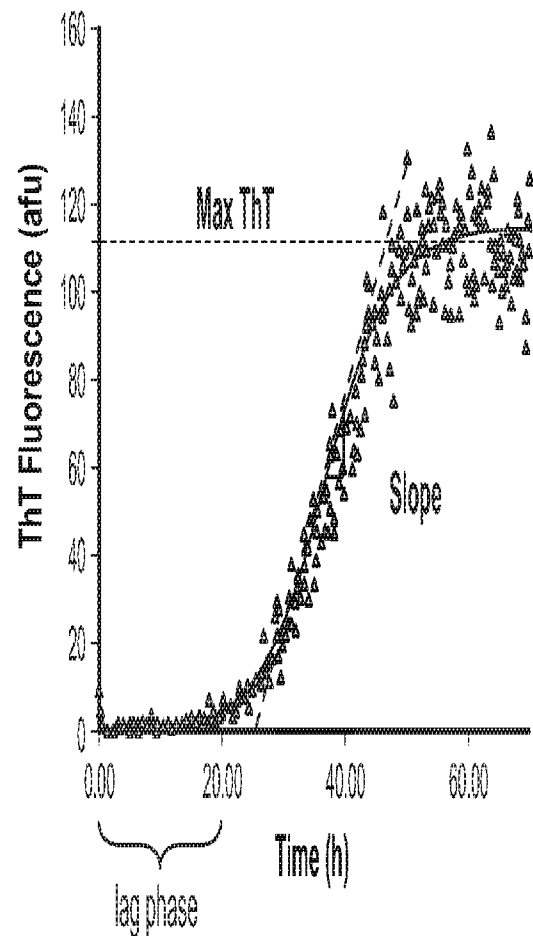
Max ThT = average(last 10 measurements)

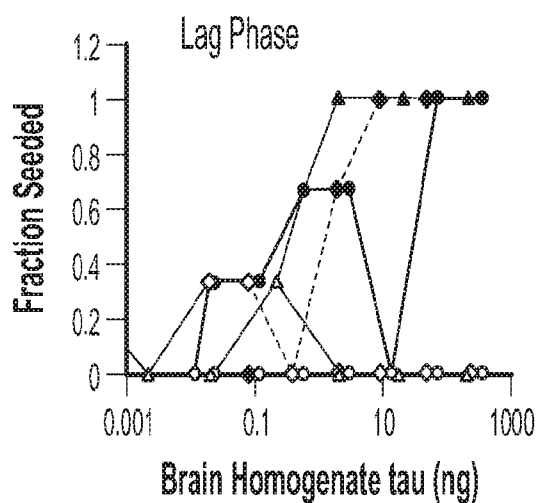
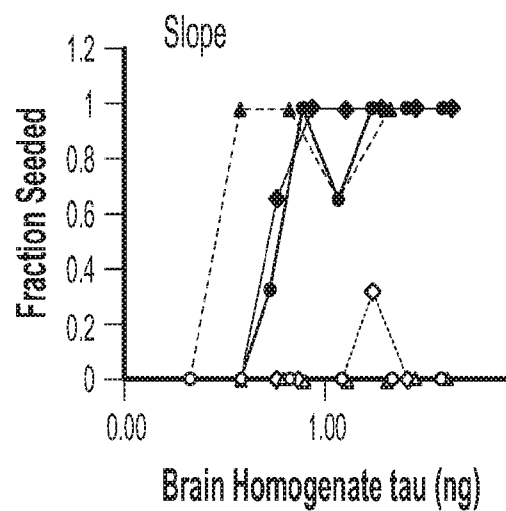
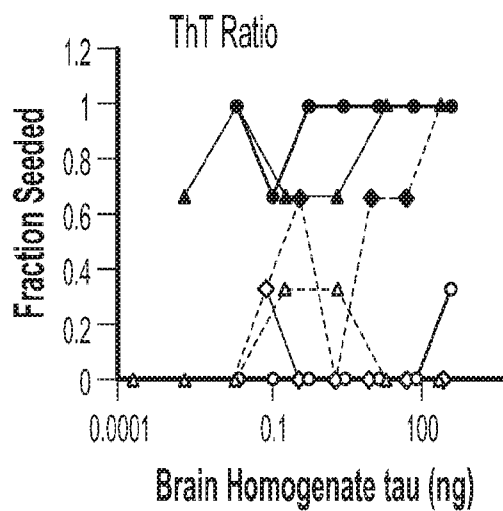
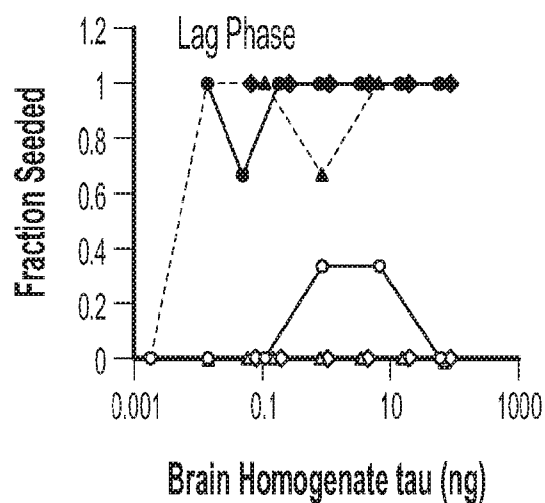
Ratio = $t @ \max(AD(t)/Normal(t))$

Lag phase = t_{avg} @ 5 measurements > background ($p < 0.01$)

**B****Sigmoidal Fit**

$$y(t) = \frac{\text{Max}}{1 + e^{\frac{4 \text{slope}}{\text{Max}}(\text{lag} - t) + 2}}$$

**FIG. 7**

A Simple Analysis**B Sigmoidal Fit****FIG. 8**

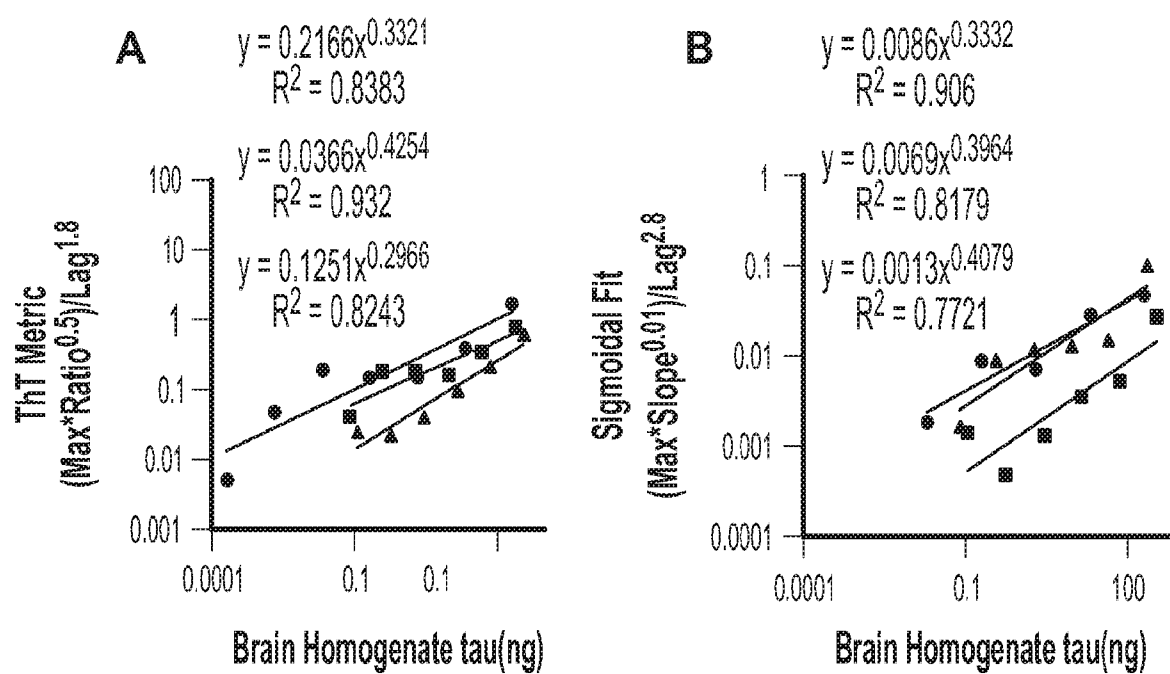
**FIG. 9**

FIG. 10

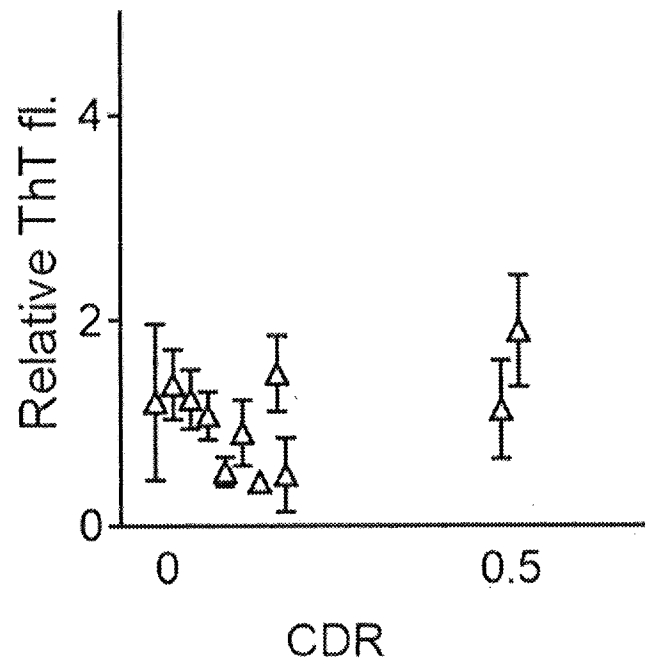
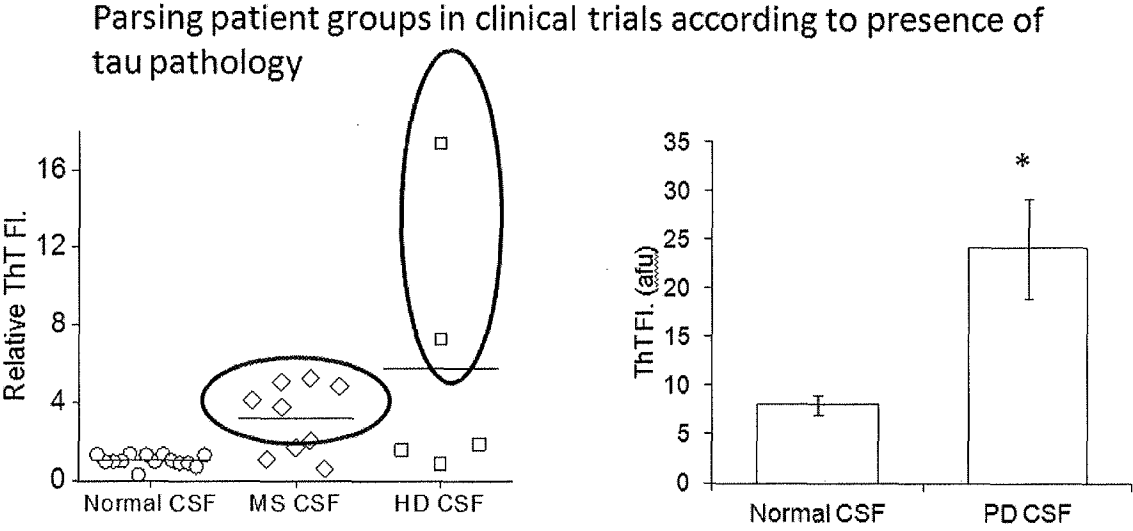


FIG. 11



Device/kit/method of detecting altered tau protein
conformation in cerebrospinal fluid

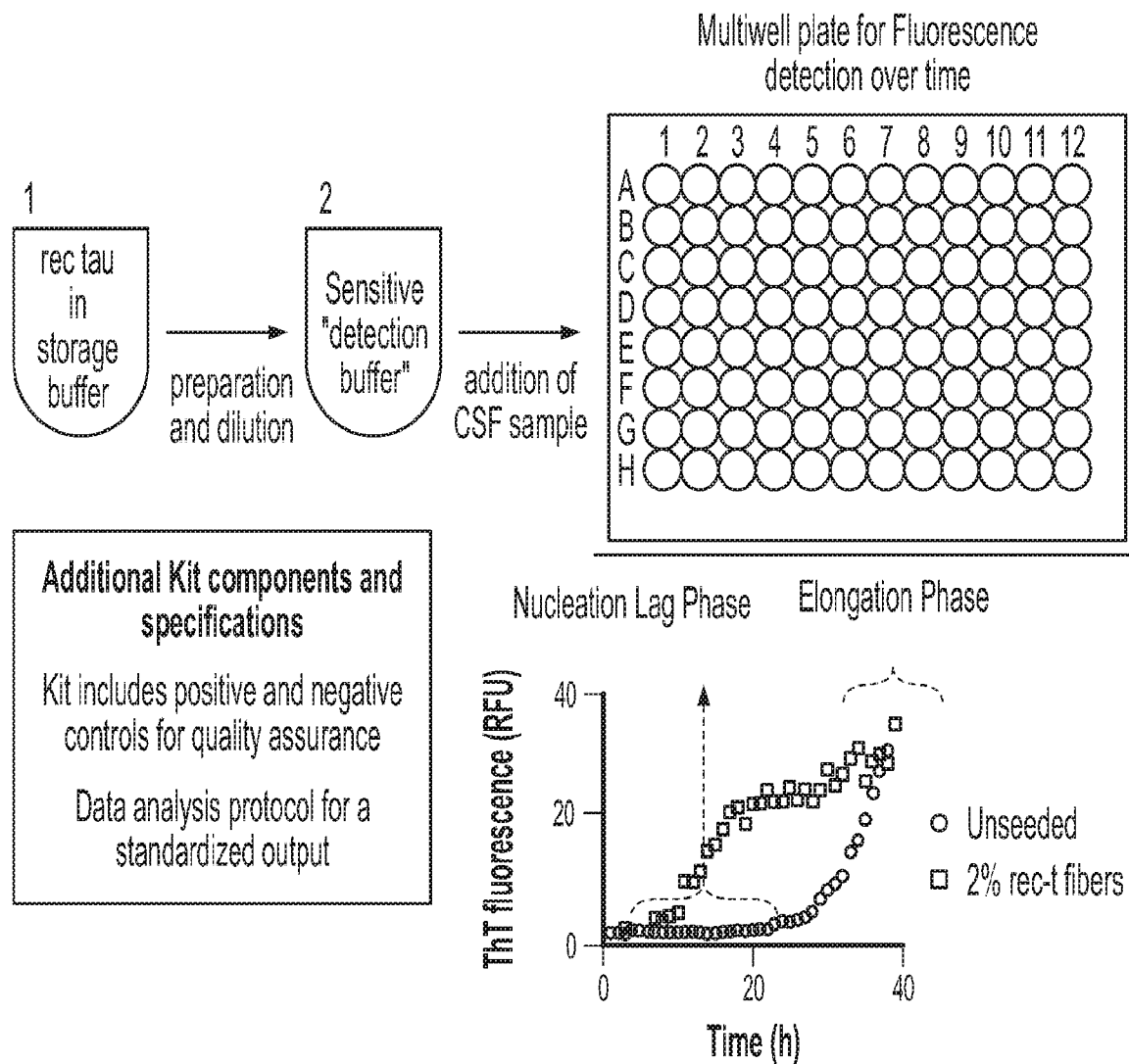


FIG. 12

FIG. 13

Human Tau Protein 381 aa NP_001190180 (SEQ ID NO: 1)

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 61 setsdakstp taeaeagig dtpsledeaa ghvtqarmvs kskdgtgsdd kkakgadgk
 121 kiatprgaap pgqkgqanat ripaktpap ktpssgepp ksgdrsgyss pgspgtpgsr
 181 srtpslptpp trepkkvavv rtpkspssa ksrlqtapvp mpdlknvksk igstenlkhq
 241 pgggkvqivy kpvdlskvt kcgslgnihh kpgggqvevk sekldfkdrv qskigsldni
 301 thvpgggnkk iethklfre nakaktdhga eivykspvvs gdtsprrhln vsstgsidmv
 361 dspqlatlad evsaslakq l

Human Tau Protein 412 aa NP_001116539 (SEQ ID NO: 2)

1 maepqrqefev medhagtygl gdrkdqggyt mhqdqegdd aglkesplqt ptedgseepg
 61 setsdakstp taeaeagig dtpsledeaa ghvtqarmvs kskdgtgsdd kkakgadgk
 121 kiatprgaap pgqkgqanat ripaktpap ktpssgepp ksgdrsgyss pgspgtpgsr
 181 srtpslptpp trepkkvavv rtpkspssa ksrlqtapvp mpdlknvksk igstenlkhq
 241 pgggkvqiin kkldlsnvqs kcgskdnikh vpgggsvqiv ykpvdlskvt skcgslgnih
 301 hkpgggqvev ksekldfkdr vqskigsldn ithvpgggnk kiethklfr enakaktdhg
 361 aeivykspvv sgdtsprrhln nvsstgsidm vdspqlatla devsaslakq gl

Human Tau Protein 352 aa NP_058525 (SEQ ID NO: 3)

1 maepqrqefev medhagtygl gdrkdqggyt mhqdqegdd aglkaeeagi gdtpsledea
 61 aghvtqarmv skskdgtgsd dkkakgadgk tkiatprgaa ppgqkgqana tripaktpa
 121 pktppssgep pkgdrsgys spgspgtpgs srtpslptp ptrepkkvav vrtppkspss
 181 aksrlqtapv pmpdlknvks kigstenlkh qpggkvqiv ykpvdlskvt skcgslgnih
 241 hkpgggqvev ksekldfkdr vqskigsldn ithvpgggnk kiethklfr enakaktdhg
 301 aeivykspvv sgdtsprrhln nvsstgsidm vdspqlatla devsaslakq gl

Human Tau Protein 383 aa NP_058518 (SEQ ID NO: 4)

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 121 pktppssgep pkgdrsgys spgspgtpgs srtpslptp ptrepkkvav vrtppkspss
 181 aksrlqtapv pmpdlknvks kigstenlkh qpggkvqii nkkldlsnvq skcgskdnik
 241 hvpgggsvqi vykpvdlskv tskcgslgni hhkpgggqve vksekldfkdr rvqskigsld
 301 nithvpgggn kkiethklf renakaktdh gaeivykspv vsdtsprrhl snvsstgsid
 361 mvdspqlatl adevsaslak qgl

Human Tau Protein 441 aa NP_005901 NP_776088 (SEQ ID NO: 5)

1 maepqrqefev medhagtygl gdrkdqggyt mhqdqegdd aglkesplqt ptedgseepg
 61 setsdakstp taedvtaplv degapgkqaa aqphteipeg ttaeeagigd tpsledeaag
 121 hvtqarmvsk skdgtgsddk kakgadgk iatprgaapp gqkgqanatr ipaktpap
 181 tppssgeppk sgdrsgysp gspgtpgsrs rtpslptppt repkkvavv rtpkspssak
 241 srlqtapvpm pdlnvkski gstenlkhq pggkvqiink kldlsnvqsk cgskdnhkv
 301 pgggsvqivy kpvdlskvt kcgslgnihh kpgggqvevk sekldfkdrv qskigsldni
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 421 dspqlatlad evsaslakq l

Human Tau Protein 410 aa NP_001190181 (SEQ ID NO: 6)

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 61 setsdakstp taedvtaplv degapgkqaa aqphteipeg ttaeeagigd tpsledeaag
 121 hvtqarmvsk skdgtgsddk kakgadgk iatprgaapp gqkgqanatr ipaktpap
 181 tppssgeppk sgdrsgysp gspgtpgsrs rtpslptppt repkkvavv rtpkspssak
 241 srlqtapvpm pdlnvkski gstenlkhq pggkvqivykp vdlkskvt skcgslgnihh

301 pgggqvevks ekldfkdrvq skigsldnit hvpggggnkki ethkltfren akaktdhgae
361 ivykspvvsg dtsprhlsnv sstgsidmvd spqlatlade vsaslakqgl

FIG. 14

Rat Tau Protein 343 aa (SEQ ID NO: 7)

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1 maepqrqefdt medqagdytm lqdqegdmhd glkaeeagig dtpnmedqaa ghvtqarvag
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121 ppksrgsgy sspgspgtpg srsrtpslpt pptrepkkva vvrtppkspk asksrqtatp
181 vmpdlnkvr skigstenlk hqpgggkvqi vykpvdlskv tskcgslgni hhpkggggqve
241 vksekldfkd rvqskigsld nithvpgggg kkiethkltf renakaktdh gaeivykspv
301 vsqgdtsprrhl snvsstgsid mvdspqlatl adevsaslak qgl

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Rat Tau Protein 374aa (SEQ ID NO: 8)

```

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121 ppksrgsgy sspgspgtpg srsrtpslpt pptrepkkva vvrtppkspk asksrqtatp
181 vmpdlnkvr skigstenlk hqpgggkvqi inkklslsnv qskcgskdni khvpggggsvq
241 ivykpvdlsk vtskcgslgn ihhpkgggqv evkseklfdk drvqskigs dnithvpggg
301 nkkiethklf frenakaktd hgaeivyksp vvsgdtsprrhl snvsstgsi dmvdspqlat
361 ladevsasla kqgl

```

Rat Tau Protein 403 aa XP_008766500.1 (predicted) (SEQ ID NO: 9)

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121 tpgqkgtsna tripakttt ptktpgsgp pksrgsgys spgspgtpgs srsrtpslpt
181 ptreppkvav vrtppkspk sksrqtatp vmpdlnkvr skigstenlk hqpgggkvqi
241 nkklslsnv qskcgskdni hvpkgggsvqi vykpvdlskv tskcgslgni hhpkggggqve
301 vksekldfkd rvqskigsld nithvpgggg kkiethkltf renakaktdh gaeivykspv
361 vsqgdtsprrhl snvsstgsid mvdspqlatl adevsaslak qgl

```

Rat Tau Protein 432 aa XP_008766499 (predicted) (SEQ ID NO: 10)

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1 maepqrqefdt medqagdytm lqdqegdmhd glkesppqpp addgseepgs etsdakstpt
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121 kdrtgndekk akgadgktg kiatprgaat pgqkgtsnat ripaktttsp ktpgsggepp
181 ksgsgysy psgpgtppg srtpslptt treppkvavv rtpkspkask srqtatpvp
241 mpdlnkvrsk igstenlk hqpgggkvqi inkklslsnv qskcgskdni hvpkgggsvqi
301 ykpvdlskv tskcgslgni hhpkgggqvev ksekldfkd rvqskigsld nithvpgggg
361 kiethkltf renakaktdh aeivykspvv sgdtsprrhl snvsstgsid mvdspqlatla
421 devsaslak qgl

```

Rat Tau Protein 469 aa XP_008766497.1 (predicted) (SEQ ID NO: 11)

```

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121 ptrtpgssd plikpsspav cpepatpky vssvtrngs pgtkqmkkg adgktgakia
181 tprgaatpgg kgtatnatp aktstpsktp psggeppksg ersgysspgs pgtppgsr
241 slptptpre pkkvavvrt pkspsaskr lqtatpmpd lnkvrskigs tenlkhpqpg
301 gkvqiinkkl dlnvqskcg skdnkhvpg ggsqvivyp vdlkvtskc gslgnihhk
361 gggqvkvkse kldfkdqvq kigsldnith vpggggnkkie thkltfrena kaktthgaei
421 vykspvvsgd tsprhl snvs stgsidmvd spqlatladev saslakqgl

```

Rat Tau Protein 498 aa XP_008766496.1 (predicted) (SEQ ID NO: 12)

```

1 maepqrqefdt medqagdytm lqdqegdmhd glkesppqpp addgseepgs etsdakstpt
61 aedvtaplve erapdkqata qshteipegt taeeagigdt pmedqaagh vtqarvagv
121 kdrtgndekk aktstpscak tpsnrpcls trtpgssdp likpsspavc pepatpky

```

181 ssvtprngsp gtkqmkllkga dgktgakiat prgaatpgqk gtsnatripa kttpspktp
241 gsgeppksge rsgysspssp gtpgsrsrtp sltpptrep kkavvrtpp kpsasksl
301 qtapvpmpdl knvrsigst enlkhqpggg kvqiinkld lsnvskcgs kdnikhvpgg
361 gsvqivykpvl dskvtskcg slgnihhkpg ggqvevksek ldfkdrvqsk igsldnithv
421 pgggnkkiet hkltfrenak aktdhgaeiv ykspvvsgdt sprhlsnvss tgsidmvdsp
481 qlatladevs aslakqgl

FIG. 15

Mouse Tau Protein 372 aa NP_034968.3 (SEQ ID NO: 13)

1 madprqefdt medhagdytl lqdqegdmhd glkaeeagig dtpnqedqaa ghvtqarvas
61 kdrtgndekk akgadgktga kiatprgaas paqkgtsnat ripakttsp ktpgsggepp
121 ksgersgyss pgspgtpgsr srtpslptpp trepkkvavv rtpkspas ksrlqtapvp
181 mpdlknvrsk igstenlkhq pgggkvqiin kkldlnvqs kcgskdnikh vpgggsqviv
241 ykpvdlskvt skcgslgnih hkpgggqvev ksekldfkdr vqskigsldn ithvpgggnk
301 kiethkltfr enakaktdhg aeivykspvv sgdtsprrhs nvsstgsidm vdsqqlatla
361 devsaslakq gl

Mouse Tau Protein 341 aa NP_001272385.1 (SEQ ID NO: 14)

1 madprqefdt medhagdytl lqdqegdmhd glkaeeagig dtpnqedqaa ghvtqarvas
61 kdrtgndekk akgadgktga kiatprgaas paqkgtsnat ripakttsp ktpgsggepp
121 ksgersgyss pgspgtpgsr srtpslptpp trepkkvavv rtpkspas ksrlqtapvp
181 mpdlknvrsk igstenlkhq pgggkvqviv kpvdlskvts kcgslgnihh kpgggqvevk
241 sekldfkdrv qskigsldni thvpgggnkk iethkltfre nakaktdhga eivykspvvs
301 gdtsprrhsn vsstgsidmv dspqlatlad evsaslakqg l

Mouse Tau Protein 430 aa NP_001033698.1 (SEQ ID NO: 15)

1 madprqefdt medhagdytl lqdqegdmhd glkesppqpp addgaeepgs etsdakstpt
61 aedvtaplvd erapdkqaaa qphtepegi taeagigdt pnqedqaagh vtqarvaskd
121 rtgndekkak gadgktgaki atprgaaspa qkgtsnatri pakttspkt ppgsggeppks
181 gersgysspg spgtpgsrsr tpslptpptr epkkvavvrt ppkspasaks rlqtapvpmp
241 dlknvrskig stenlkhqpg ggkvqiinkk ldlnvqskc gskdnikhvp gggsqvivyk
301 pvdlskvtsk cgslnihhk pgggqvevks ekldfkdrvq skigsldnit hvpgggnkki
361 ethkltfren akaktdhgae ivykspvvsg dtsprrrhsn sstgsidmvd spqlatlade
421 vsaslakqgl

Mouse Tau Protein 350 aa NP_001272384.1 (SEQ ID NO: 16)

1 madprqefdt medhagdytl lqdqegdmhd glkarvaskd rtgndekkak gadgktgaki
61 atprgaaspa qkgtsnatri pakttspkt ppgsggeppks gersgysspg spgtpgsrsr
121 tpslptpptr epkkvavvrt ppkspasaks rlqtapvpmp dlknvrskig stenlkhqpg
181 ggkvqiinkk ldlnvqskc gskdnikhvp gggsqvivyk pvdlskvtsk cgslnihhk
241 pgggqvevks ekldfkdrvq skigsldnit hvpgggnkki ethkltfren akaktdhgae
301 ivykspvvsg dtsprrrhsn sstgsidmvd spqlatlade vsaslakqgl

Mouse Tau Protein 364 aa NP_001272383.1 (SEQ ID NO: 17)

1 madprqefdt medhagdytl lqdqegdmhd glkaeeagig dtpnqedqaa ghvtqarvas
61 kdrtgndekk akgadgktga kiatprgaas paqkgtsnat ripakttsp ktpgsggepp
121 ksgersgyss pgspgtpgsr srtpslptpp trepkkvavv rtpkspas ksrlqtapvp
181 mpdlknvrsk igstenlkhq pgggkvqviv kpvdlskvts kcgslgnihh kpgggqvevk
241 sekldfkdrv qskigsldni thvpgggnkk iethkltfre nakaktdhga eivykspvvs
301 gdtsprrhsn vsstgsidmv dspqlatlad evsaslakqg kaallssqvw nyshdlatit
361 dlgl

FIG. 16

Cattle Tau Protein 448 aa NP_776531 (SEQ ID NO: 18)

1 maepreqfdv medhaqgdyt lqdqegdmdp glkesplqtp addgseepgs etsdakstpt
61 aedataplvd egapgeqaaa qapaeipegt aeeeagigdt snledqaagh vtqarmvskg
121 kdgtgpddkk tkgadgkpgt kiatprgaap pgqkgqanat ripakttpt ktspatmqvq
181 kkpppagaks ergesgksd rsgysspgsp gtpgsrstrp sltpptrep kkavavrtpp
241 kspsaaksl qaapgmpdl knvskigst enlkhqpggg kvqiinkld lsnvskcgs
301 kdnikhvpgg gsvqivykpvl dskvtskcg slgnihhkpg ggqvevksek ldfkdrvqsk
361 igslidnithv pgggnkkiet hkltfrenak aktdhgaeiv ykspvsgdt sprhlnvss
421 tgsidmvdsp qlatladevs aslakqgl

Cattle Tau Protein 370 aa AAI09942 (SEQ ID NO: 19)

1 maepreqfdv medhaqgdyt lqdqegdmdp glkesplqtp addgseepgs etsdakstpt
61 aeaeagigdt tsnlledqaag hvtqarmvsk gkdgtgpddk ktkgadgkpg tkiatprgaa
121 ppgqkgqana tripakttpt pktspgesgk sgdrsgysp gspgtpgsrs rtpsltppt
181 repkkavavr tppkspsaak srlqaapgpm pdlknvskski gstenlkhqpg gggkvqivyk
241 pvdlskvtsk cgslnihhk pgggqvevks ekldfkdrvq skigslidnit hvpgggnkki
301 ethkltfren akaktdhgae ivykspvsg dtsprhlnv sstgsidmvd spqlatlade
361 vsalakqgl

FIG. 17

Tree Frog Tau Protein 403 aa NP_001072465.1 (SEQ ID NO: 20)

1 madhyqdyds vgdhtrdgsa qqiytgsgd hilessqesv irlptaahgd dvasvqedla
 61 ngeavrsqgh agipegttah eqekhetdsq ekgaqeaggp esaksqslas rnatsasrip
 121 aktssipktp psavrrdqrk pppsgakpdr espksgersg ysspgspgtp tgrssstpp
 181 trepkkiavi rtpkspasa ksrlqpvtsa aampdlknvr skigsidnir hqpgggkvqi
 241 vhhkidlssv qskcgskdnl khmpgggtiq ithkpidlth itskcgsgfn ihhrpgggnv
 301 evkselefd kiqskigsld nithtpggga kkieshklmf renakaktdh gaeivykspg
 361 qsgetsprrl snvsssgsin mtdspqlstl adqvsaslak qgl

Tree Frog Tau Protein 421 aa XP_012808026 (predicted) (SEQ ID NO: 21)

1 madhyqdyds vgdhtrdgsa qqiytgsgd hilessqesv irlptaahgd dvasvqedla
 61 ngeavrsqgh agipegttae eagvghtpsq qdraaaheqe khetdsqekg aqeaggpesa
 121 ksqsasrna tsasripakt ssipktpsa vrrdqrkppp sgakpdraes pksrgersgys
 181 spgspgtpgt rssstpptr epkkiavirt ppspasaks rlqvtsapa mpdlknvrsk
 241 igsidnirhq pgggkvqivh kkidlssvqs kcgskdnlkh mppgggtiqit hkipidlthit
 301 skcgsgfnih hrpgggnvev kselefdki qskigsldni thtpgggakk ieshklmfre
 361 nakaktdhga eivykspgqs getsprrlsn vsssgsinmt dspqlstlad qvsaslakqg
 421 I

Tree Frog Tau Protein 465 aa XP_012808025.1 (predicted) (SEQ ID NO: 22)

1 madhyqdyds vgdhtrdgsa qqiytgsgd hilessqesv irlptaahgd dvasvqedla
 61 ngeavrsqgh agipegttah eqekhetdsq ekgaqistkh ppaknaksrl ytvpqkpsi
 121 tskspspaa ssvrsiqrts lgavqpgvt arlhrakeag gpesaksqsl asrnatsasr
 181 ipaktssipk tpsavrrdq rkpppsgakp drespksger sgysspgspg tptgrssst
 241 pptrepkkia virtppkspa saksrlqvvt spaampdlkn vrskigsidn irhqpgggkv
 301 qivhkkidls svqskcgskd nlkhmpgggt iqithkpidl thitskcgsgfn ihhrpggg
 361 nvevksele fdkiqskigs ldnithtpgg gakkieshkl mfrenakakt dhgaeivyks
 421 pgqsgetspr rlnvsssgs inmtdspqls tladqvsasl akqgl

Tree Frog Tau Protein 482 aa XP_012808024 (predicted) (SEQ ID NO: 23)

1 madhyqdyds vgdhtrdgsa qqiytgsgd hilessqesv irlptaahgd dvasvqedla
 61 ngeavrsqgh agipegttae eagvghtpsq qdraaaheqe khetdsqekg aqistkhppa
 121 knaksrlytv pqkpsitsk spsspaassv rsiqrtslga vgqpgvtarl hrakeagge
 181 saksqslasr natsasripa ktssipktp savrrdqrk ppsgakpdre spksrgersg
 241 sspgspgtp grssstppt repkkiavir tppkspasak srlqvtsapa ampdlknvr
 301 kigsidnirh qpggkvqiv hkkidlssvq skcgskdnlk hmpgggtiqi thkpidlthi
 361 tskcgsgfni hrpgggnve vkselefdk iqskigsldn ithtpgggk kieshklmf
 421 enakaktdhg aeivykspgq sgetsprrls nvsssgsinm tdspqlstla dqvasslakq
 481 gl

Tree Frog Tau Protein 483 aa XP_012808023 (predicted) (SEQ ID NO: 24)

1 madhyqdyds vgdhtrdgsa qqiytgsgd hilessqesv irlptaahgd dvasvqedla
 61 ngeavrsqgh agipegttae eagvghtpsq qdraaaheqe khetdsqekg aqistkhppa
 121 knaksrlytv pqkpsitsk spsspaassv rsiqrtslga vgqpgvtarl hrakeagge
 181 saksqslasr natsasripa ktssipktp savrrdqrk ppsgakpdra espksgersg
 241 ysspgspgtp tgrssstpp trepkkiavi rtpkspasa ksrlqvtsa aampdlknvr
 301 skigsidnir hqpgggkvqi vhhkidlssv qskcgskdnl khmpgggtiq ithkpidlth
 361 itskcgsgfn ihhrpgggnv evkselefd kiqskigsld nithtpggga kkieshklmf
 421 renakaktdh gaeivykspg qsgetsprrl snvsssgsin mtdspqlstl adqvsaslak
 481 qgl

Tree Frog Tau Protein 324 aa XP_011544655.1 (predicted) (SEQ ID NO: 25)
1 mwakqrtpm vskskdgtgs ddkkakgadg ktkiatprga appgqkgqan atripaktp
61 apktpssge ppksgrsgy sspgspgtpg srsrtplpt pptrepkkva vvrtpksp
121 saksrlqtap vpmpdlknvk skigstenlk hqpggkvqi inkkldlsnv qskcgskdni
181 khvpgggsvq ivykpvdlsk vtskcgslgn ihhkpgggqv evkseklfdk drvqskigsl
241 dnithvpggg nkkiethklt frenakaktd hgaeivyksp vvsgdtsprh lsnvsstgsi
301 dmvdspqlat ladevsasla kqgl

FIG. 18

Japanese Medaka Tau Protein 483 aa XP_011486741.1 (predicted) (SEQ ID NO: 26)

```

1 mdfmnnasns ysgdtmsss lanmtindqh hqengvqmgh rnagdakmke eeaalsengl
61 ksvselcqpe sshcddeapp aesspaekee aevsqsene sitseekgqp aaggavstaq
121 gktenevksp ggakpqvrga kpptktaapd arsgqsspgt pkspasqals aaeankvkkv
181 avvrstsksp aslksrspap laaaampmdl knvrskigst enikhqpggg kvqileqkld
241 lsnvqakcgs kdnikhqpgg gkvqildkkl dlssvqsrcg skdnikhtpg ggkvkileqr
301 ldfsnnvqskc gskdnikhvp ggggnvqiyek kldlssvqsr cgskdnlghv pgggnvqivh
361 kkidlstvts kcgskanihh kpgggnieik seklefkvqs kigsldnikh vpgggakrie
421 shkltfreta kartdhgaei vsledsphql stvsssgsin madspqlstl adqvsaslak
481 qgl

```

Japanese Medaka Tau Protein 486 aa XP_011486740.1 (predicted) (SEQ ID NO: 27)

```

1 mdfmnnasns ysgdtmsss lanmtindqh hqengvqmgh rnagdakmke eeaalsengl
61 ksvselcqpe sshcddeapp aqgktenevk tqataspvss pkrpsvttta strnghssip
121 vktsrsgpgh aspggkpvrg rgakpptaap apdarsgqss pgtpkspasq alsaeeankv
181 kkavvrsts kspaslsrs paplaaaapm pdlknvrski gstenikhqp gggkvqileq
241 kldlssvqak cgskdnikh qpgggkvqild kldlssvqs rcgskdnikh tpgggkvkil
301 eqrldfsnnv skcgskdnik hvpgggnvqi yekldlssv qsrcgskdnl ghvpggggnvq
361 ivhkkidlst vtskcgskan ihhkpgggni eikseklef vqskigsldn ikhvpgggk
421 rieshkltrf etakartdhg aeivsledsp hqlstvsstg sinmadspql stladqvas
481 lakqgl

```

Japanese Medaka Tau Protein 523 aa XP_011486739.1 (predicted) (SEQ ID NO: 28)

```

1 mdfmnnasns ysgdtmsss lanmtindqh hqengvqmgh rnagdakmke eeaalsengl
61 ksvselcqpe sshcddeapp aesspaekee aevsqsene sitseekgqp aaggavstaq
121 ktenevktqt atspvsspk rpsvttast rnhssipvkt srsgpgghas ggakpqvrga
181 kpptktaapd arsgqsspgt pkspasqals aaeankvkkv avvrstsksp aslksrspap
241 laaaampmdl knvrskigst enikhqpggg kvqileqkld lsnvqakcgs kdnikhqpgg
301 gkvqildkkl dlssvqsrcg skdnikhtpg ggkvkileqr ldfsnnvqskc gskdnikhvp
361 ggggnvqiyek kldlssvqsr cgskdnlghv pgggnvqivh kkidlstvts kcgskanihh
421 kpgggnieik seklefkvqs kigsldnikh vpgggakrie shkltfreta kartdhgaei
481 vsledsphql stvsssgsin madspqlstl adqvsaslak qgl

```

Japanese Medaka Tau Protein 524 aa XP_011486737.1 (predicted) (SEQ ID NO: 29)

```

1 mdfmnnasns ysgdtmsss lanmtindqh hqengvqmgh rnagdakmke eeaalsengl
61 ksvselcqpe sshcddeapp aesspaekee aevsqsene sitseekgqp aaggavstaq
121 gktenevktq tatspvssp rpsvttast rnhssipvk tsrsgpgghas pggakpqvrg
181 akpptaap darsgqsspg tpkspasqal saaeankvkk vavvrstsksp paslksrspa
241 plaaaampmd lknvrskigs tenikhqpgg gkvqileqkl dlnvqakcg skdnikhqpg
301 ggvqildkkl dlssvqsrc gskdnikhtpg gggkvkileq rldfsnnvqsk cgskdnikh
361 pgggnvqiye kldlssvqs rcgskdnlgh vpggggnvqiv hkkidlstvt skcgskanih
421 hkpgggniei kseklefvq skigsldnik hvpgggkrie eshkltfret akartdhgae
481 ivsledsphq lsvsssgsi nmadspqlst ladqvasla kqgl

```

FIG. 19

Black rockcod Tau Protein 483 aa XP_010782995.1 (predicted) (SEQ ID NO: 30)

1 mdytnnssns ytsghdmsss lanitisdqh hqengimkep eaavseneaq pvseldcdkv
61 hpvetsekag veaagselse testscggeg qlaaaaaagg ggaastaqak idnasadkqp
121 glaksqtqgv ksaaqaasak kpptpknqkd gsgghsspgtk spssqlsaka aaeankvkkv
181 avvrstpksp gslksrppvp laaaampdl knvrskvgst dnlkhqpggg kvqileqkld
241 fssvqskcgs kgnmkhvpvg gnvqildkkl elpnvssrcg skdnikhtpg ggvqvivekk
301 ldlssvqsrc gskdnmkhva ggggnvqivek kdlssvqsr cgsdnikhv agggnvqivh
361 kkvdltvnqa kcgsksnlhh kpgggnieik nekvefkgqs kvsglnigh vpgggqrrie
421 shklsfresa kartdhgaei vsledsphpl stvsssgsln iadspqlstl adqvsaslak
481 qgl

Black rockcod Tau Protein 529 aa XP_010782994.1 (predicted) (SEQ ID NO: 31)

1 mdytnnssns ytsghdmsss lanitisdqh hqengimkep eaavseneaq pvseldcdkv
61 hpvetsekag veaagselse testscggeg qlaaaaagg gaastaqaki dnasadtpt
121 aikptsspk pspvtggnt nspgngpss ipikssspg rpgggpglak sqtqgvksaa
181 qaasakkpnt pknqkdsgs sspgkspss qlsakaaea nkvvkvavvr stpkspgslk
241 srppvplaaa amppdlknvr skvgstdnlk hqpgggkvqi leqkldfss qskcgskgm
301 khvpgggnvq ildkklelpn vssrcgskd ikhtpgggkv qivekkldls svqsrcgskd
361 nmkhvagggn vqivekkldl ssvqsrcgsk dnkhvagg nvqivhkkvd ltnvqakcgs
421 ksnlhhkpgg gnieiknekv efkgqskvgs lgnighvpgg gqrrieshkl sfresakart
481 dhgaeivsl dsphplstvs ssgslniads pqlstladq vsalakqgl

Black rockcod Tau Protein 530 aa XP_010782992.1 (predicted) (SEQ ID NO: 32)

1 mdytnnssns ytsghdmsss lanitisdqh hqengimkep eaavseneaq pvseldcdkv
61 hpvetsekag veaagselse testscggeg qlaaaaaagg ggaastaqak idnasadtpt
121 taikptsspk rpsvtggnt tnspsngps sipikssspg prpgggpgla ksqtqgvksa
181 aqaasakkpp tpknqkdsgs hsspgtkspss qlsakaeee ankvvkvavvr rtpkspgsl
241 ksppvpplaa aampdlknv rskvgstdnl khqpgggkvq ileqkldfss vqskcgskgm
301 mkhvpgggnv qildkklelp nvssrcgskd nikhtpgggk vqivekkldl ssvqsrcgsk
361 dnmkhvagg nvqivekkld lssvqsrcgs kdnkhvagg gnvqivhkkv dltvqakcgs
421 sksnlhhkpg ggnieiknekv vefkgqskvg slgnighvpg gqqrrieshk lfresakar
481 tdhgaeivsl edsphplstvs ssgslniad spqlstladq vsalakqgl

FIG. 20

Chicken Tau Protein 432 aa NP_001186122.1 (SEQ ID NO: 33)

1 maephqditt veeqerqhvp sgyplqipvd dgsdepvset sdakstptte dataplveeg
 61 dqedqhgeip egttaeeagi gatpsledha agdaaqarid skdkegaetd ekkpkgqemr
 121 ggtkpatprs aagqaqrns natripaktp tapktppgsg rkeqkkpppa aakpekgeqp
 181 ksgdrsgyss pgspgtpgsr srtpslptpp arepkkvavv rtpkspasa ksrpqsaap
 241 mpdlknvksk igstenlkhq pgggkvqiin kkldfssvqs kcgskdnikh ipgggsvqiv
 301 ykpvdishvt skcgslgnih hkpqqgqvev ksekldfkdk vqskigsldn ishvpgggnk
 361 kiethkltfr enakaktdhg aeivykspti sgdasprrls nvsstgsinm vdspqlatla
 421 devsaslakq gl

Chicken Tau Protein 345 aa ABY86222 (SEQ ID NO: 34)

1 maephqditt veeqerqhvp saeeagigat psledhaagd aaqaridskd kegaetdekk
 61 pkgqemrggt kpatprsaag qaqrnsnat ripaktptap ktppgsgrke qkkpppaaak
 121 pekarepkkv avvrtpksp asaksvqps aapmpdlknv kskigstenl khqpgggkvq
 181 iinkkldfss vqskcgskdn ikhipgggsv qivykpvdls hvtskcgslg nihhkpqqgq
 241 vevkseklfd kdkvqskigs ldnishvpgg gnkkiethkl tfrenakakt dhgaeivyks
 301 ptisgdaspr rlnvsvstgs inmvdsplqla tladevsasl akqgl

Chicken Tau Protein 348 aa ABY86221 (SEQ ID NO: 35)

1 maephqditt veeqerqhvp saeeagigat psledhaagd aaqaridskd kegaetdekk
 61 pkgqemrggt kpatprsaag qaqrnsnat ripaktptap ktppgsgrke qkkpppaaak
 121 pekgeqpksg drsgyspgs pgtpgsrsrt psptppare pkkvavvrtp pkspasaksr
 181 vqpsaampmd lknvskigs tenlkhqpgg gkvqivypv dlshvtskcg slgnihhkp
 241 ggqvevksek ldfkdkvqsk igslndishv pgggnkkiet hkltfrenak aktldhgaeiv
 301 ykspstisgda sprrlsnvss tgsinmvdsp qlatladevs aslakqgl

Chicken Tau Protein 400 aa XP_015154842 (predicted) (SEQ ID NO: 36)

1 maephqditt veeqerqhvp sdatplvee gdqedqhgei pegttaeeag igatpsledh
 61 aagdaaqari dskdkegaet dekkpkgqem rggtkpatpr saagqaqrns natripakt
 121 ptapktppgs grkeqkkppp aaakpekare pkkvavvrtp pkspasaksr vqpsaampmd
 181 lknvskigs tenlkhqpgg gkvqiinkkl dfssvqskcg skdnikhipg ggsvqivnqk
 241 ldfssvqsrc gskdnikhip gggsqvivyp pvdishvtsk cgslgnihhk pgggqvevks
 301 ekldfkdkvq skigsldnis hvpgggnkki ethkltfren akaktdhgae ivyksptisg
 361 dasprrlsnv sstgsinmvd spqlatlade vsaslakqgl

Chicken Tau Protein 410 aa XP_015154838 (predicted) (SEQ ID NO: 37)

1 maephqditt veeqerqhvp saeeagigat psledhaagd aaqaridskd kegaetdekk
 61 pkgqemrggt kpatprsaag qaqrnsnat ripaktptap ktppgsgrke qkkpppaaak
 121 pekgeqpksg drsgyspgs pgtpgsrsrt psptppare pkkvavvrtp pkspasaksr
 181 vqpsaampmd lknvskigs tenlkhqpgg gkvqiinkkl dfssvqskcg skdnikhipg
 241 ggsvqivnqk ldfssvqsrc gskdnikhip gggsqvivyp pvdishvtsk cgslgnihhk
 301 pgggqvevks ekldfkdkvq skigsldnis hvpgggnkki ethkltfren akaktdhgae
 361 ivyksptisg dasprrlsnv sstgsinmvd spqlatlade vsaslakqgl

Chicken Tau Protein 429 aa XP_015154837.1 (predicted) (SEQ ID NO: 38)

1 maephqditt veeqerqhvp sgyplqipvd dgsdepvset sdakstptte dataplveeg
 61 dqedqhgeip egttaeeagi gatpsledha agdaaqarid skdkegaetd ekkpkgqemr
 121 ggtkpatprs aagqaqrns natripaktp tapktppgsg rkeqkkpppa aakpekarep
 181 kkavavvrtp kspasaksv qpsaampmd knvskigst enlkhqpggg kvqiinkkl
 241 fssvqskcgs kdnikhipg gsvqivnqkl dfssvqsrcg skdnikhipg ggsvqivyp
 301 vdlshvtskc gslgnihhk pgggqvevkse kldfkdkvqs kigsldnish vpggggnkkie

361 thkltfrena kaktdhgaei vyksptisgd asprrlsnvs stgsinmvds pqlatladev
421 saslakqgl

Chicken Tau Protein 463 aa XP_015154833.1 (predicted) (SEQ ID NO: 39)

1 maephqditt veeqerqhvp sgyplqipvd dgsdepvset sdakstptte dataplveeg
61 dqedqhgeip egttaeeagi gatpsledha agdaaqarid skdkegaetd ekkpkqgemr
121 ggtpatprs aagqaqrnss natripaktp tapktpgsg rkeqkkpppa aakpekgeqp
181 ksgdrsgyss pgspgtpgsr srtpslptpp arepkkvavv rtpkspasa ksrqvpsaap
241 mpdlknvksk igstenlkhq pgggkvqiin kkldfssvqs kcgskdnikh ipgggsvqiv
301 nqkldfssvq srcgskdnikh hipgggsvqi vykpvdlsbv tskcgslgni hhhpggggqve
361 vksekldfkd kvqskigsld nishvpgggn kkiethkltf renakaktdh gaeivykspt
421 isgdasprrl snvsstgsin mvdspqatl adevsaslak qgl

Chicken Tau Protein 467 aa XP_015154832 (predicted) (SEQ ID NO: 40)

1 maephqditt veeqerqhvp saeeagigat psledhaagd aaqaridskd kegaetdekk
61 pkksspctak ppgdrasipp hrhtssstap pkspsspast skrvtrpas tgthetkakk
121 qemrggtkpa tpsaaggaq rnssnatrip aktptaktp pgsgskeqkk ppaaakpek
181 geqpksgdrs gysspgspgt pgsrsrtpsl ptpparepkk vavvrtppks pasaksvqp
241 saapmpdlkn vkskigsten lkhqpgggkv qiinkkldfs svqskcgskd nikhipgggs
301 vqivnqkldf ssvqsrcgsk dnikhpggg svqivypvd lshvtskcgslgnihhkpgg
361 gqvevksekl dfkdqvski gsldnshvp ggggnkkieth kltfrenaka ktdhgaeiv
421 ksptisgdas prrlsnvsst gsinmvdspq latladevsa slakqgl

Chicken Tau Protein 520 aa XP_015154831.1 (predicted) (SEQ ID NO: 41)

1 maephqditt veeqerqhvp sgyplqipvd dgsdepvset sdakstptte dataplveeg
61 dqedqhgeip egttaeeagi gatpsledha agdaaqarid skdkegaetd ekkpkksspc
121 takppgdras ipphrhtsss tappkspssp astskrvtrp pastgthetk akqemrggt
181 kpatprsaag qaqrnssnat ripaktptap ktpgsgske qkkpppaaak pekgeqpksg
241 drsgyspgs pgtpgsrsl psldtppare pkkvavvrt pksasaksv vqpsaapmpd
301 lknvskigs tenlkhqpgg gkvqiinkkl dfssvqskcg skdnikhpg ggsqvivnqk
361 ldfssvqsrc gskdnikhpg ggsqvivvyk pvdshvtsk cgslgnihhk pgggqvevks
421 ekldfkdqv skigsldnis hvpggggnkki ethkltfren akaktdhgae ivyksptisg
481 dasprrlsnv sstgsinmvd spqatlade vsaslakqgl

FIG. 21

R1	QTAPVPMPDLKN-VKSKIGSTENLKHQPGGG--KV-	(SEQ ID NO: 42)
R2	QIINKKL-DLSN-VQSKCGSKDNIKHVPGGG--SV-	(SEQ ID NO: 43)
R3	QIVYKPV-DLSK-VTSKCGSLGNIHHKPGGG--QV-	(SEQ ID NO: 44)
R4	EVKSEKL-DFKDRVQSKIGSLDNITHVPGGGNKKIE	(SEQ ID NO: 45)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/15461

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - G01N 33/53; G01N 23/00; G02B 21/06; G01J 4/00 (2016.01) CPC - C07K 16/18; G01N 33/6896; H01J 37/244; H01J 37/26; G01N 21/19; G02B 21/16 According to International Patent Classification (IPC) or to both national classification and IPC																							
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC(8) - G01N 33/53; G01N 23/00; G02B 21/06; G01J 4/00 (2016.01); USPC - 435/7.1; 436/811; 250/311; 359/386; 356/364 CPC - C07K 16/18; G01N 33/6896; H01J 37/244; H01J 37/26; G01N 21/19; G02B 21/16 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched IPC(8) - G01N 33/53; G01N 23/00; G02B 21/06; G01J 4/00 (2016.01); USPC - 435/7.1; 436/811; 250/311; 359/386; 356/364 CPC - C07K 16/18; G01N 33/6896; H01J 37/244; H01J 37/26; G01N 21/19; G02B 21/16 - see keyword below Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PubWEST(USPT,PGPB,EPAB,JPAB); PatBase; Medline, Google: amyloidogenic, amyloid, tau, conformation, fiber, fibril, twist, width, purifying, conformational feature, seed, sequence, similarity, neurological, Alzheimer's, Parkinson's, Huntington, Chronic Traumatic Encephalopathy, CTE, progressive supranuclear palsy, PSP, corticobasal degeneration, CBD																							
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>Y</td> <td>MOROZOVA et al., Conformational features of tau fibrils from Alzheimer's disease brain are faithfully propagated by unmodified recombinant protein. Biochemistry. 2013, Vol. 52(40), p. 6960-7. Abstract; pg 6960, col 1, lower para; pg 6961, col 1, para 1-3, and col 2, para 2-3; pg 6962, col 1, para 3, col 2, para 1, and Fig 1; and pg 6963, Fig 2 and Fig 3</td> <td>1-20</td> </tr> <tr> <td>Y</td> <td>US 2014/0154180 A1 (EISENBERG et al.) 05 June 2014 (05.06.2014), para [0004], [0041], [0043], [0055], [0058], [0072], [0096], [0098], [0119], and [0122]</td> <td>1-10, 18-20</td> </tr> <tr> <td>Y</td> <td>US 2008/0220449 A1 (VASAN et al.) 11 September 2008 (11.09.2008), Abstract; para [0012], [0054], [0062], [0066], [0070], [0084], [0123], and SEQ ID NO: 2</td> <td>1, 11-17</td> </tr> <tr> <td>A</td> <td>SARELL et al., Assessing the causes and consequences of co-polymerization in amyloid formation. Prion. 2013, Vol. 7(5), p. 359-68. Entire documentation, especially Abstract; and pg 361, col 1, middle para, and col 3, lower para</td> <td>1-20</td> </tr> <tr> <td>A</td> <td>KARDOS et al., Direct Measurement of the Thermodynamic Parameters of Amyloid Formation by Isothermal Titration Calorimetry. J Biol Chem. 2004, Vol. 279(53), p. 55308-14. Entire documentation, especially Abstract; and pg 55309, col 1, para 2-4, and Fig 1</td> <td>1-20</td> </tr> <tr> <td>A</td> <td>US 2012/0029169 A1 (MOE et al.) 02 February 2012 (02.02.2012), Abstract, para [0010], [0011], [0034], [0035], [0068], [0081], and [0084]</td> <td>1-20</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	Y	MOROZOVA et al., Conformational features of tau fibrils from Alzheimer's disease brain are faithfully propagated by unmodified recombinant protein. Biochemistry. 2013, Vol. 52(40), p. 6960-7. Abstract; pg 6960, col 1, lower para; pg 6961, col 1, para 1-3, and col 2, para 2-3; pg 6962, col 1, para 3, col 2, para 1, and Fig 1; and pg 6963, Fig 2 and Fig 3	1-20	Y	US 2014/0154180 A1 (EISENBERG et al.) 05 June 2014 (05.06.2014), para [0004], [0041], [0043], [0055], [0058], [0072], [0096], [0098], [0119], and [0122]	1-10, 18-20	Y	US 2008/0220449 A1 (VASAN et al.) 11 September 2008 (11.09.2008), Abstract; para [0012], [0054], [0062], [0066], [0070], [0084], [0123], and SEQ ID NO: 2	1, 11-17	A	SARELL et al., Assessing the causes and consequences of co-polymerization in amyloid formation. Prion. 2013, Vol. 7(5), p. 359-68. Entire documentation, especially Abstract; and pg 361, col 1, middle para, and col 3, lower para	1-20	A	KARDOS et al., Direct Measurement of the Thermodynamic Parameters of Amyloid Formation by Isothermal Titration Calorimetry. J Biol Chem. 2004, Vol. 279(53), p. 55308-14. Entire documentation, especially Abstract; and pg 55309, col 1, para 2-4, and Fig 1	1-20	A	US 2012/0029169 A1 (MOE et al.) 02 February 2012 (02.02.2012), Abstract, para [0010], [0011], [0034], [0035], [0068], [0081], and [0084]	1-20
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☐ Further documents are listed in the continuation of Box C. ☐																							
<table border="0"> <tr> <td> * Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed </td> <td> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family </td> </tr> </table>			* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family																			
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Date of the actual completion of the international search 04 April 2016		Date of mailing of the international search report 10 JUN 2016																					
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774																					

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/15461

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-20, directed to a method for detecting an amyloidogenic tau protein in a bodily fluid sample from a subject, comprising: (a) incubating the sample or a purified fraction thereof in vitro with a monomeric tau protein to form tau amyloid. The monomeric tau protein will be searched to the extent that the monomeric tau protein encompasses wherein the monomeric tau protein is a polypeptide having an amino acid sequence at least 90% identical to the sequence of SEQ ID NO: 1; and the amyloidogenic tau protein will be searched to the extent that the amyloidogenic tau protein encompasses wherein the amyloidogenic tau protein comprises a polypeptide having an amino acid sequence at least 90% identical to the sequence of SEQ ID NO: 1. It is believed that claims 1-10, 11-12(in part), 13-20 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass wherein the monomeric tau protein is a polypeptide having an amino acid sequence at least 90% identical to the sequence of SEQ ID NO: 1; and wherein the amyloidogenic tau protein comprises a polypeptide having an amino acid sequence at least 90% identical to the sequence of SEQ ID NO: 1. *****Continued in the extra sheet*****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-10, 11-12(in part), 13-20, limited to SEQ ID NO: 1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/15461

Continuation of:
Box No III (unity of invention is lacking)

(Continuation of Groups I+) Additional monomeric tau protein and amyloidogenic tau protein will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected monomeric tau protein and amyloidogenic tau protein. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be wherein the monomeric tau protein or the amyloidogenic tau protein is a polypeptide having an amino acid sequence at least 90% identical to the sequence of SEQ ID NO: 2 [claims (1-10), 11-12(in part), (13-20)].

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Feature

Among Groups I+, each SEQ ID NO represents a structurally different protein sequence.

Common Technical Features

The inventions of Groups I+ share the technical feature of a method for detecting an amyloidogenic tau protein in a bodily fluid sample from a subject, comprising: (a) incubating the sample or a purified fraction thereof in vitro with a monomeric tau protein to form tau amyloid, and (b) detecting the presence of the tau amyloid (claim 1).

However, these shared technical features do not represent a contribution over prior art as being obvious over an article entitled 'Conformational features of tau fibrils from Alzheimer's disease brain are faithfully propagated by unmodified recombinant protein' by MOROZOVA et al. (hereinafter 'Morozova'; Biochemistry. 2013, Vol. 52(40), p. 6960-7), in view of US 2014/0154180 A1 to EISENBERG et al. (hereinafter 'Eisenberg') as follows:

Morozova discloses a method for detecting an amyloidogenic tau protein in a sample from a subject (Abstract - 'Alzheimer's disease (AD)... recombinant tau protein is seeded with paired helical filaments (PHFs) isolated from AD brain...AD-isolated PHFs act as a conformational template for the formation of recombinant tau fibrils. ...use of AD PHFs as seeds to stimulate... tau fibers that closely resemble those associated with AD pathology and provides a biochemical model of tau misfolding', wherein 'use of AD PHFs as seeds to stimulate... tau fibers' is 'detecting an amyloidogenic tau protein from a subject' based on the definition; pg 6961, col 1, para 3 - 'Human brain tissue samples ...tissue from the frontal cortex of AD patients...brain homogenates'; pg 6962, Fig 1; Specification: pg 1, ln 30-33 - 'assays rely on the seeded conversion of a monomeric protein substrate into an amyloidogenic protein, also called a seed protein, to increase the amount of misfolded protein in a sample'; pg 11, last para - 'term "amyloidogenic tau protein" used herein refers to a tau protein that is capable of stimulating conversion of monomeric tau protein into an amyloid conformation (i.e., an aggregate of tau proteins with a fiber-like morphology)), comprising:

(a) incubating the sample or a purified fraction thereof in vitro with a monomeric tau protein to form tau amyloid (pg 6961, col 2, para 2 - 'Recombinant monomeric tau protein ... was then incubated ... with an appropriate inducer...partially purified 1% brain homogenate'; pg 6961, col 1, para 3 - 'Human brain tissue samples ...tissue from the frontal cortex of AD patients...brain homogenates'; pg 6962, Fig 1, Legend - 'Misfolded tau in AD brain homogenate seeds the conversion of monomeric recombinant tau into amyloid'; Fig 1; pg 6961, col 1, para 2 - 'Recombinant 0N4R tau (rec tau) protein,... was purified...Monomeric recombinant tau protein ... with no signs of misfolding'; pg 6961, col 1, para 1 - 'an improved biochemical model of PHF propagation...for tau monomers to become incorporated into fibrils'; Abstract - 'Alzheimer's disease (AD)... recombinant tau protein is seeded with paired helical filaments (PHFs) isolated from AD brain'), and

(b) detecting the presence of the tau amyloid (Abstract - 'Alzheimer's disease (AD)... recombinant tau protein is seeded with paired helical filaments (PHFs) isolated from AD brain ...use of AD PHFs as seeds to stimulate recombinant tau amyloid formation'; pg 6962, Fig 1, Legend - 'Misfolded tau in AD brain homogenate seeds the conversion of monomeric recombinant tau into amyloid'; Fig 1; pg 6963, Fig 2).

Morozova further discloses wherein the sample is a brain sample from a subject suffering Alzheimer's disease (Abstract - 'Alzheimer's disease (AD)... recombinant tau protein is seeded with paired helical filaments (PHFs) isolated from AD brain'; pg 6961, col 1, para 3 - 'Human brain tissue samples ...tissue from the frontal cortex of AD patients...brain homogenates'; pg 6961, col 2, para 2 - 'Recombinant monomeric tau protein ... was then incubated ... with an appropriate inducer...purified 1% brain homogenate), and the method is an improved model for detecting Alzheimer's disease and drug screening (pg 6961, col 1, para 1 - 'an improved biochemical model of PHF propagation...for tau monomers to become incorporated into fibrils'; Abstract - 'Alzheimer's disease (AD)... recombinant tau protein is seeded with paired helical filaments (PHFs) isolated from AD brain... tau fibers that closely resemble those associated with AD pathology and provides a biochemical model of tau misfolding ... structural studies and drug screening').

Morozova does not specifically teach wherein the sample is a bodily fluid from a subject. Eisenberg discloses a method for detecting tau oligomers or mistolded fibers in a sample of a subject (para [0055] - 'a method for determining the presence of A.beta. or tau oligomers or fibers (particularly fibers) in a sample .. determination is carried out on an in vitro sample ... tau oligomers or fibers is in a sample from a subject'; para [0041] - 'the amyloid protein is A.beta'; para [0004] - 'amyloid-beta (A.beta)', by detecting the tau protein in a bodily fluid sample from a subject for diagnosing a disease such as Alzheimer's disease (para [0055] - 'determining the presence of A.beta. or tau oligomers or fibers (particularly fibers) in a sample ... the determination is carried out on an in vitro sample ... blood or cerebral spinal fluid (CSF)... determination ...tau oligomers or fibers is in a sample from a subject, the method can be a method for diagnosing the presence of an amyloid-mediated disease or condition, such as Alzheimer's disease'; para [0122] - 'Suitable samples' include tissues and bodily fluids, such as blood, cerebral spinal fluid (CSF)').

*****Continued in the next extra sheet*****

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/15461

Continuation of:

The previous extra sheet - Box No III (unity of invention is lacking)

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Morozova and Eisenberg, to obtain a method for detecting an amyloidogenic tau protein from a subject, comprising: (a) incubating the sample or a purified fraction thereof in vitro with a monomeric tau protein to form tau amyloid, and (b) detecting the presence of the tau amyloid, based on the teaching of Morozova, and further wherein the sample is a bodily fluid from a subject, based on the combination of Eisenberg and Morozova, in order to apply the method taught by Morozova for facilitating diagnosing disorders associated with the presence of an amyloidogenic tau protein with an expected success and without undue experimentation.

Without a shared special technical feature, the inventions lack unity with one another.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.