DETECTION OF CONFORMATIONALLY ALTERED PROTEINS AND PRIONS

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Abstract
The invention provides methods and kits for detecting conformationally altered proteins and prions in a sample. In one embodiment, the conformationally altered proteins and prions are associated with amyloidogenic diseases.
Transmissible spongiform encephalopathies:
TSE Conformers

Labeled peptide fragment

β-sheet Toxic form

α-helix Non-toxic form

Figure 1
**TSE Detection Schema**

- **Target**: TSE protein
- **Unknown conformation**
- **Receptor**: Labeled Peptide fragment
- **Signal**: Aggregates, Light scattering, Fluorescence, CD
- **Catalytic propagation**
- **Disaggregated**
- **β-sheet conformation**
- **Toxic conformation**
- **α-helix conformation**
- **Non-toxic conformation**

**Figure 2**
Circular Dichroism Indicating Conformational Change
Poly-L-Lysine 20 μM 52,000 MW
Initial test peptide system

- pH 7 25°C Random coil
- pH 7 25°C + pH 7 50°C +
- pH 11 50°C 1:1 pH 11 50°C 1:1

β-sheet β-sheet

Wavelength, nm

Figure 3
<table>
<thead>
<tr>
<th>Temperature</th>
<th>pH 7 alone</th>
<th>pH 11 alone</th>
<th>pH 7 + pH 11 25°C</th>
<th>pH 11 50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>Random coil</td>
<td>α-helix</td>
<td>Random coil</td>
<td></td>
</tr>
<tr>
<td>50°C</td>
<td>β-sheet</td>
<td>β-sheet</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Experiments with fluorescent probes for detection.

The data are from previous FRET experiments for proximal and distal locations in an α-helical bundle structure undergoing conformational change.

The spectra shown are for pyrene excimer formation at 480 nm, but other probes (FITC, etc.) can be used.

Figure 6
Engineering considerations for sensor design

The driving force must be commensurate with the energetic difference between the two conformational states. The process is driven by a differential interaction of the target peptide E, with the two conformations of the test PrP molecule.

Figure 7
A. 33mer palindrome

\[
\text{VVAGAAAAAGAVHKLNTPKLKHVAGAAAAGAVV}
\]

19 mer  \[\text{KPKTNLKHVAGAAAAGAVV}\]

14 mer  \[\text{LKHVAGAAAAGAVV}\]

B. \[\text{TNLKHVAGAAAAGAVV}\]

\[\text{PKLKHVAGAAAAGAVV}\]

Figure 10. Palindromic 33mer peptide probe.

A. Linear sequence of 33mer, 19mer and 14mer with palindromic sequences underlined and murine substituted V's and L's replace human/hamster sequence M's.

B. Folded sequence demonstrating parallel palindrome and diagram showing pyrene molecules present on both ends of the peptide forming an excimer structure.
FIGURE 11
FIGURE 12

1 - 14_mer
2 - 19_mer
3 - 33_mer

Water, pH 7
$C_{\text{peptide}} = 30 \, \mu\text{M}$
FIGURE 14

0.1 M TRIS pH 9.85 + 300

Fluorescence

Wavelength (nm)
DETECTION OF CONFORMATIONALLY ALTERED PROTEINS AND PRIONS

RELATED APPLICATIONS


FIELD OF THE INVENTION

[0002] The invention provides methods and kits for detecting conformationally altered proteins and prions in a sample.

[0003] In one embodiment, the conformationally altered proteins and prions are associated with amyloidogenic diseases.

BACKGROUND OF THE INVENTION


[0005] The conversion of normally soluble proteins into conformationally altered insoluble proteins is thought to be a causative process in a variety of other diseases. Structural conformational changes are required for the conversion of a normally soluble and functional protein into a defined, insoluble state. Examples of such insoluble proteins include: A. beta. peptide in amyloid plaques of Alzheimer’s disease (AD) and cerebral amyloid angiopathy (CAA); alpha-synuclein deposits in Lewy bodies of Parkinson’s disease, tau in neurofibrillary tangles in frontal temporal dementia and Pick’s disease; superoxide dismutase in amyotrophic lateral sclerosis; huntingtin in Huntington’s disease; and prions in Creutzfeldt-Jakob disease (CJD). (for reviews, see Glenner et al. (1989) J. Neurol. Sci. 94:1-28; Haan et al. (1990) Clin. Neurol. Neurosurg. 92(4):305-310).


[0007] In both AD and CAA, the main amyloid component is the amyloid beta protein (A. beta.). The A. beta. peptide, which is generated from the amyloid beta precursor protein (APP) by the action of two putative secretases, is present at low levels in the normal CNS and blood. Two major variants, A. beta.42 and A. beta.40, are produced by alternative carboxy-terminal truncation of APP (Selkoe et al.(1988) Proc. Natl. Acad. Sci. USA 85:7341-7345; Selkoe, (1993) Trends Neurosci 16:403-409). A. beta.142 is the more fibrillogenic and more abundant of the two peptides in amyloid deposits of both AD and CAA. In addition to the amyloid deposits in AD cases described above, most AD cases are also associated with amyloid deposition in the vascular walls (Hardy (1997), supra; Haan et al. (1990), supra; Terry et al., supra; Vinters (1987), supra; Ihle et al (1993), supra; Yamada et al. (1993), supra; Greenberg et al. (1993), supra; Levy et al. (1990), supra). These vascular lesions are the hallmark of CAA, which can exist in the absence of AD.

[0008] Human transthyretin (TTR) is a normal plasma protein composed of four identical, predominantly beta-sheet structured units, and serves as a transporter of the hormone thyroxin. Abnormal self assembly of TTR into amyloid fibrils causes two forms of human diseases, namely senile systemic amyloidosis (SSA) and familial amyloid polyneuropathy (FAP) (Kelly (1996) Curr Opin Struct Biol 6(1):11-7). The cause of amyloid formation in FAP is point mutations in the TTR gene; the cause of SSA is unknown. The clinical diagnosis is established histologically by detecting deposits of amyloid in situs in biopsy material.

[0009] To date, little is known about the mechanism of TTR conversion into amyloid in vivo. However, several laboratories have demonstrated that amyloid conversion may be simulated in vitro by partial denaturation of normal human TTR (McCulchen, Colon et al. (1993) Biochemistry 32(45):12119-27; McCulchen and Kelly (1993) Biochem Biophys Res Commun 197(2) 415-21). The mechanism of conformational transition involves a monomeric conformational intermediate which polymerizes into linear beta-sheet structured amyloid fibrils [Lai, Colon et al. (1996) Biochemistry 35(20):6470-82]. The process can be mitigated by binding with stabilizing molecules such as thyroxin or triiodophenol (Miroy, Lai et al. (1996) Proc Natl Acad Sci USA 93(26):10501-6).

[0010] The precise mechanisms by which neuritic plaques are formed and the relationship of plaque formation to the disease-associated neurodegenerative processes are not well-defined. The amyloid fibrils in the brains of Alzheimer’s and prion disease patients are known to result in the inflammatory activation of certain cells. For example, primary microgial cultures and the THP-1 monocytic cell line are stimulated by fibrillar beta-amyloid and prion peptides to activate identical tyrosine kinase-dependent inflammatory signal transduction cascades. The signaling response elicited by beta-amyloid and prion fibrils leads to the production of neurototoxic products, which are in part responsible for the neurodegeneration. C. K. Combs et al, J Neurosci 19:928-39 (1999).


[0012] Prions are infectious pathogens that cause central nervous system spongiform encephalopathies in humans and animals. Prions are distinct from bacteria, viruses and viroids. A potential prion precursor is a protein referred to as PrP 27-30, a 28 kdalton hydrophobic glycoprotein that polymerizes (aggregates) into rod-like filaments found as plaques in infected brains. The normal protein homologue differs from prions in that it is readily degradable, whereas prions are highly resistant to proteases. It has been suggested that prions may contain extremely small amounts of highly infectious nucleic acid, undetectable by conventional assay methods Benjamin Lewin, Genes IV (Oxford Univ. Press,
New York, 190 at p.1080. The predominant hypothesis at present is that no nucleic acid component is necessary for the infectivity of prion protein.

[0013] Complete prion protein-encoding genes have since been cloned, sequenced and expressed in transgenic animals. PrPC is encoded by a single-copy host gene and is normally found at the outer surface of neurons. During a post-translational process, PrPsc is formed from the normal, cellular PrP isoform ((PrPc)), and prion diseases result from conversion of PrPc into a modified isoform called PrPsc. PrPc is necessary for both the transmission and pathogenesis of the transmissible neurodegenerative diseases of animals and humans.

[0014] The normal prion protein (PrP) is a cell-surface metallo-glycoprotein that is mostly an α-helix and coiled-loop structure as shown in FIG. 8, and is usually expressed in the central nervous and lymph systems. It is believed to serve as an antioxidant and is thought to be associated with cellular homeostasis. The abnormal form of PrP, however, is a conformation which is resistant to proteases and is predominantly beta-sheet in its secondary structure, as shown in FIG. 9. It is believed that this conformational change in secondary structure leads to aggregation and eventual neurotoxic plaque deposition in the prion-disease process.

[0015] Prion-associated diseases include scrapie of sheep and goats, chronic wasting disease of deer and elk, and bovine spongiform encephalopathy (BSE) of cattle (Wilesmith, J. and Wells, Microbiol. Immunol. 172:21-38 (1991)). Four prion diseases of humans have been identified: (1) kuru, (2) Creutzfeldt-Jakob disease (CJD), (3) Gerstmann-Strassler-Scheinker Disease (GSS), and (4) fatal familial insomina (FFI) (Gajdusek, D.C., Science 197:943-960 (1977); Medori et al., N. Engl. J. Med. 326:444-449 (1992)).

[0016] Prion diseases are transmissible and insidious. For example, the long incubation times associated with prion diseases will not reveal the full extent of iatrogenic CJD for decades in millions of people treated with cadaver-sourced HGH worldwide. The importance of detecting prions in biological products has been heightened by the possibility that bovine prions have been transmitted to humans who developed new variant Creutzfeldt-Jakob disease (nCJD) (G. Chazot et al., Lancet 347:1181 (1996); R. G. Will et al. Lancet 347:921-925 (1996)).

[0017] Diseases caused by prions are hard to diagnose: the disease may be latent or subclinical (abnormal prions are detectable but symptoms are not). Moreover, normal homologues of a prion-associated protein exist in the brains of uninfeeced organisms, further complicating detection. Ivan Roitt, et al., Immunology (Mosby-Year Book Europe Limited, 1993), at 15.1.

[0018] Current techniques used to detect the presence of prion-related infections rely on gross morphological changes in the brain and immunochemoanical techniques that are generally applied only after symptoms are manifest. Many of the current detection methods rely on antibody-based assays or affinity chromatography that use brain tissue from dead animals, and in some cases capillary immuno-electrophoresis of blood samples.

[0019] Brain-tissue-based assays can lead to late detection and require slaughtering the animal to be tested. Prion-specific Check also entails slaughtering an animal to obtain a liquefied-brain tissue sample, which is subjected to an antibody using Western Blot. Although results are obtained in six to seven hours, the test does not account for the six-month lag time between PrPs accumulation in the brain and the onset of clinical symptoms. Tonsillar biopsy sampling, and blood and cerebrospinal sampling, while accurate, can require surgical intervention and take weeks to obtain results. Electrospray ionization mass spectrometry (ESI-MS), nuclear magnetic resonance NMR, circular dichroism (CD) and other non-amplified structural techniques require large amounts of sample and expensive equipment that is typically located a substantial distance from the sample source.

[0020] Detection methods for conformationally altered proteins associated with the aforementioned disorders such as Alzheimer’s disease and CAA are also inadequate in that, like the previously mentioned prion detection techniques, they often require post-mortem tissue sampling. Accordingly, the need exists for reliable and affordable detection methods for conformationally altered proteins and prions. Such methods should be applicable during the life of the subject at issue in order to obtain rapid diagnoses and facilitate prophylactic or remedial treatments.

SUMMARY OF THE INVENTION

[0021] The invention provides reliable, affordable, and safe methods for the detection of conformationally altered proteins and prions associated with a variety of diseases. Methods of the invention can be applied to obtain rapid diagnoses and facilitate prophylactic or remedial treatments. Significantly, the methods of the invention use small amounts of sample and are therefore less invasive and more readily applied than known diagnostic techniques. Further, methods of the invention can be used to analyze samples from a living subject and are not limited to samples obtained post mortem; and may be utilized in a manner that ensures that infectious material is not propagated during testing.

[0022] The invention overcomes many of the problems associated with prior art diagnostic techniques by using catalytic propagation to exploit conformational changes in conformationally altered protein or prions associated with a particular disease process, such as transmissible spongiform encephalopathy (TSE). Catalytic propagation may be used to amplify the number of existing conformationally altered protein fragments or prions in a sample and causes detectable aggregates to form as follows:

[0023] Upon interaction of a sample containing conformationally altered protein or prions with a conformational probe as defined hereinafter, the probe undergoes a conformational change and adopts the conformation of, and aggregates with, the conformationally altered protein (which may be soluble or insoluble) or prions. The resulting aggregates which exhibit β-sheet formation, may be readily detected using standard analytical techniques. As a result, the invention facilitates rapid and cost-effective analysis of small sample sizes and is widely applicable to tissues and bodily fluids from a variety of sources including, but not limited to, the brain.

[0024] The invention enables detection of small amounts of disease-associated conformationally altered proteins such as low-density lipoprotein receptor, cystic fibrosis transmembrane regulator, Huntington, A-beta peptide, prions,
insulin-related amyloid, hemoglobin, alpha synuclein, rhodopsin, crystallins, and p53. In a preferred embodiment, methods of the invention use palindromic probes as otherwise described herein, preferably, for example, a palindromic 33_mer probe containing amino acid sequences 126-104 and 109-126 of the PrP[^30] protein to detect prions in a sample. In a preferred embodiment, the probes are bound at each end to moieties that are optically distinct and detectable upon conformational conversion of the probes to a β-sheet structure.

[0025] In one embodiment, the invention provides a method for detecting conformationally altered proteins or prions in a sample comprising:

[0026] (a) reacting the sample with one or more α-helix or random coil conformational probes that interact with the β-sheet conformation insoluble proteins or prions in the sample and thereby (i) undergo a conformational conversion to a predominantly β-sheet conformation, and (ii) form detectable aggregates with the β-sheet conformation insoluble proteins or prions in the sample; and

[0027] (b) detecting levels of detectable aggregates, wherein levels of detectable aggregates correlate to the levels of β-sheet conformation insoluble proteins or prions in the sample and the infectiousness of the sample.

[0028] The invention also provides kits that use these methods as well as methods of diagnosing whether a subject suffers from, or is predisposed to, a disease associated with conformationally altered proteins or prions.

[0029] A kit of the instant invention comprises one or more α-helix or random coil conformational probes that interact with β-sheet conformation insoluble proteins or prions in the sample and thereby (i) undergo a conformational conversion predominantly to β-sheet conformation, and (ii) form detectable aggregates with the β-sheet conformation insoluble proteins or prions in the sample. The kit may further include moieties that bind to, or are bound to, probe termini and that are optically detectable upon conformational conversion of the probe to a predominantly β-sheet conformation, as well as instructions for using the kit, and solutions for suspending or fixing samples.

[0030] A method of diagnosing whether a subject suffers from, or is predisposed to, a disease associated with conformationally altered proteins or prion comprises:

[0031] (a) obtaining a sample from the subject;

[0032] (b) reacting the sample with one or more α-helix or random coil conformational probes that interact with the β-sheet conformation of insoluble proteins or prions in the sample and thereby (i) undergo a conformational conversion preferably to a predominantly β-sheet conformation, and (ii) form detectable aggregates with the β-sheet conformation insoluble proteins or prions in the sample; and

[0033] (c) detecting levels of detectable aggregates, wherein levels of detectable aggregates correlate to the amount of the β-sheet conformation insoluble proteins or prions in, and level of infectiousness of, the sample and indicate whether the subject suffers from, or is predisposed to, a disease associated with β-sheet conformation insoluble proteins or prions.

[0034] These and other aspects of the invention are described further in the following detailed description.

**BRIEF DESCRIPTION OF THE FIGURES**

[0035] FIG. 1 illustrates the alpha-helical monomer 10 and beta-sheet dimer 12 of a TSE conformer. The normal wild-type (wt) form of prion protein (PrP[^30]) prefers a monomeric state, while the abnormal, disease-causing form (PrP[^31]) prefers the multimeric state.

[0036] FIG. 2 illustrates a diagnostic analysis of a sample containing TSE protein comprised of beta-sheets 12.

[0037] FIG. 3 illustrates a circular dichroism graph of a diagnostic analysis that was performed in accordance with the invention and that used a poly-L-lysine 20 micromolar (μM) 52,000 molecular weight (MW) as a peptide-model.

[0038] FIG. 4 illustrates a absorbance graph of a diagnostic analysis that was performed using poly-L-lysine, 70 micromolar (μM) 52,000 molecular weight (MW), as a peptide model.

[0039] FIG. 5 illustrates the results from FIG. 3, that used a poly-L-lysine, 70 micromolar (μM) 52,000 molecular weight (MW) as a peptide model and the effect of pH and temperature on conformational change.

[0040] FIG. 6 illustrates a spectroscopic analysis that used pyrene as a fluorescent probe in proximal and distal locations in an alpha helical bundle structure that underwent conformational change.

[0041] FIG. 7 illustrates energy changes associated with conformational changes in proteinaceous material or prions.

[0042] FIG. 8 illustrates the alpha-helix and loop structure of PrP.

[0043] FIG. 9 illustrates the predominantly beta-sheet secondary structure of PrP[^35].

[0044] FIG. 10 illustrates a palindromic 33_mer probe used in the methods of the instant invention.


[0046] FIG. 12 illustrates a circular dichroism graph of a diagnostic analysis that was performed in aqueous conditions in accordance with the invention and that used a palindromic 33_mer probe and the 14_mer and the 19_mer amino acid sequences which make it up (these three sequences are set forth in FIG. 10).

[0047] FIG. 13 illustrates a variation of the spectroscopic analysis of FIG. 6, in which a spectrophotometric data of a diagnostic analysis that was performed using a palindromic 33_mer probe (SEQ ID NO: 1, SEE FIG. 10) that had pyrene attached to both ends. The spectral scans in the monomer (open) conformation yielded a strikingly fluorescent spectrum that had a maximum emission between 370
and 385 nm, while the excited dimer or excimer state of the pyrene-labeled peptide has an emission max between 475 and 510 nm.

[0048] FIG. 14 illustrates a spectroscopic analysis in which pyrene was used as a fluorophor, the excitation wavelength was around 350 nm, and the observation wavelength was around 365-600 nm. The normal emission of monomer pyrene following excitation (simple fluorescence) was recorded as the maximum wavelength at about 370-385 nm.

[0049] FIG. 15 illustrates the ratio of excimer formation (IIID) to monomer formation (IM) in a diagnostic analysis that used a palindromic 33 mer probe of sequence shown in FIG. 10 under various conditions. We expect to see minimum solubility of a protein when the conditions are near its isoelectric point and that is what we observed where conditions (2) approach the isoelectric point of the 33 mer peptide—it aggregates with itself since it has dramatically reduced solubility under these conditions as compared to (1). In this example, electrostatic interactions (pH=10) trigger self-association under extremely low concentrations (10 μM) at the isoelectric point of the peptide. The following legend applies to FIG. 15.

[0050] 1. pH 6-8, KCl (100-500 mM)
[0051] 2. pH 10-11, KCl (100-500 mM)

[0052] FIG. 16 illustrates an associative curve for conformation changes in a diagnostic analysis that used a palindromic 33 mer probe (SEQ ID NO: 1), the 19 mer (SEQ ID NO: 2) and 14 _mer (SEQ ID NO: 3) (See FIG. 10) under various conditions to determine the optimal parameters associated with the transformation from coiled to β-sheet.

[0053] FIG. 17 shows the results from the experiment described in Example 6 wherein the fluorescence of a complex of prion protein and 33 mer probe was measured as a function of time. The complex substantially dissociated over time (1 hour-24 hours). The expected fluorescence of the target peptide [520 nM] in the presence of infected brain homogenate (1), healthy brain homogenate (2), and peptide alone (3) in TRIS:TFE (1:1) solvent. The data were obtained for 0.01% brain homogenate from hamster (A), sheep (B), and elk (C) (hamster [270 pg/ml], sheep [60 pg/ml], and elk [6 pg/ml]).

[0054] FIG. 18(a)-(c) illustrate fluorescence spectra of target peptide [520 nM] in the presence of infected brain homogenate (1), healthy brain homogenate (2), and peptide alone (3) in TRIS:TFE (1:1) solvent. The data were obtained for 0.01% brain homogenate from hamster (A), sheep (B), and elk (C) (hamster [270 pg/ml], sheep [60 pg/ml], and elk [6 pg/ml]).

[0055] FIG. 19 illustrates a preliminary calibration curve of a fluorescent diagnostic analysis conducted in accordance with the invention. The data illustrated in this figure evidences that the present invention is more than two orders of magnitude more sensitive than the validated tests in use in Europe today without any optimization. Prion Infection: 1 IU=3M=200,000 PrP

[0056] The prion protein concentration was determined using the capillary immunoelectrophoresis method of Dr. Schmerr. See, Schmerr, et al., J Chromatogr. A., 853 (1-2), 207-214 (Aug. 20, 1999). The sensitivity of the diagnostics with the present invention appears to the left of the green bar, whereas the sensitivity of more conventional diagnostics appears to the right of the green bar. The data are taken from FIG. 18.

[0057] As used herein, the following terms have the following respective meanings. “Amyloidogenic diseases” are diseases in which amyloid plaques or amyloid deposits are formed in the body. Amyloid formation is found in a number of disorders, such as diabetes, Alzheimer’s Disease (AD), scrapie, Gerstmann-Straussler-Scheinker (GSS) Syndrome, bovine spongiform encephalopathy (BSE), Creutzfeldt-Jakob disease (CJD), chronic wasting disease (CWD), and related transmissible spongiform encephalopathies (TSEs).

[0058] TSE’s are fatal neurodegenerative diseases that include such human disorders as CJD, kuru, fatal familial insomnia, and GSS. Animal forms of TSE include scrapie in sheep, CWD in deer and elk, and bovine spongiform encephalopathy in cattle. These diseases are characterized by the formation and accumulation in the brain of an abnormal proteinase K resistant isofrom (PrP-res) of a normal protease-sensitive host-encoded prion protein (PrP sen). PrP-res is formed from PrP-sen by a post-translational process involving conformational changes that convert the PrP-sen into a PrP-res molecular aggregate having a higher beta-sheet content. The formation of these macromolecular aggregates of PrP-res is closely associated with TSE-mediated brain pathology in which amyloid deposits of PrP-res are formed in the brain, which eventually becomes “spongiform” (filled with holes).

[0059] TSE diseases appear to be transmitted by exposure to an unusual agent, for example by ritual cannibalism in the Fore people of New Guinea, or feeding of animal parts to cattle in bovine spongiform encephalopathy (BSE), iatrogenic CJD has also been caused by administration of human growth hormone derived from cadaveric pituitaries, transplanted dura mater and corneal grafts, as well as exposure of surgeons to affected tissue during neurological procedures.

[0060] The presence of a native prion protein (PrP) has been shown to be essential to pathogenesis of TSE. The cellular protein PrP-sen is aialoglycoprotein encoded by a gene that in humans is located on chromosome 20. The PrP gene is expressed in neural and non-neural tissues, with the highest concentration of its mRNA being in neurons. The translation product of the PrP gene consists of 253 amino acids in humans, 254 in hamsters and mice, 264 amino acids in cows, and 256 amino acids in sheep (all of these sequences are disclosed in U.S. Pat. No. 5,565,186, which describes methods of making transgenic mice that express species specific PrP). In prion protein related encephalopathies, the cellular PrP-sen is converted into the altered PrP-res that is distinguishable from PrP-sen in that PrP-res aggregates (Caughey and Chesebro, 1997, Trends Cell Biol. 7, 56-62); are protease K resistant in that only approximately the N-terminal 67 amino acids are removed by proteinase K digestion under conditions in which PrP-sen is completely degraded (Prusiner et al., 1986, Proc. Natl. Acad. Sci. USA 90, 10962-10966).

[0061] If PrP-sen is not expressed in the brain tissue of animal recipients of scrapie-infected neurografts, no pathology occurs outside the graft, demonstrating that PrP-res and
PrP-sen are both required for the pathology (Brander et al., Nature 379:339-343, 1996). The long latency period between infection and the appearance of disease (months to decades depending on species) has prompted the development of a cell-free in vitro test, in which PrP-res induces the conversion of PrP-sen to PrP-res (Kocisko et al., Nature 370:471-474, 1994). See also Prusiner et al., WO 97/16728 published May 9, 1997. These in vivo and in vitro observations indicate that direct interactions between PrP-res and PrP-sen form PrP-res and promote TSE pathogenesis.

[0062] Small synthetic peptides containing certain PrP sequences have previously been shown to spontaneouly aggregate to form fibrils with a high degree of beta-sheet secondary structure of the type seen in the insoluble deposits in TSE afflicted brains (Gasse et al., 1992, Proc. Natl. Acad. Sci. USA 89, 10940-10944; Come et al., 1993, Proc. Natl. Acad. Sci. USA 90, 5959-5963; Forloni et al., 1993, Nature 362, 543-546; Hope et al., 1996, Neurodegeneration 5, 1-11). Moreover, other synthetic PrP peptides have been shown to interact with PrP-sen molecules to form an aggregated complex with increased protease-resistance (Kaneko et al., Proc. Natl Acad. Sci. USA 92, 11160-11164, 1995; Kaneko et al., J. Mol. Biol. 270, 574-586, 1997).

[0063] “Conformationally altered proteins” include any protein which has a three dimensional conformation associated with a disease. The conformationally altered protein may cause the disease, may be a factor in a symptom of the disease, or may appear in a sample or in vivo as a result of other factors. A conformationally altered protein appears in another conformation which has the same amino acid sequence. These conformationally altered proteins are generally in the form of insoluble proteins exhibiting beta-sheet formation which are analyzed in the present invention.

[0064] The following is a non-limiting list of diseases followed parenthetically by associated insoluble proteins which assemble into two or more different conformations wherein at least one conformation is an example of a conformationally altered protein: Alzheimer’s Disease (APP, A beta peptide, alpha 1-antichymotrypsin, tau, non-A beta component, presenilin 1, presenilin 2 apos); Prion diseases, Creutzfeldt Jakob disease, scrapie and bovine spongiform encephalopathy (PrPSc); ALS (SOD and neurofilament); Pick’s disease (Pick body); Parkinson’s disease (alpha-synuclein in Lewy bodies); Frontotemporal dementia (tau in fibrils); Diabetes Type II (Amylin); Multiple myeloma—plasma cell dyscrasias (IgG1-chain); Familial amyloidotic polyneuropathy (Transhyretin); Medullary carcinoma of thyroid (Procalcitonin); Chronic renal failure (beta2-microglobulin); Congestive heart failure (Atrial natriuretic factor); Senile cardiac and systemic amyloidosis (Transhyretin); Chronic inflammation (Serum amyloid A); Atherosclerosis (ApoA1); Familial amyloidosis (Gelsolin); Huntington’s disease (Huntingtin).

[0065] An “insoluble protein” includes any protein associated with an amyloidogenic disease, including but not limited to any of the proteins identified in the preceding paragraph. Insoluble proteins generally exhibit beta-sheet formation in the aggregate.

[0066] “PrP protein”, “PrP” and like are used interchangeably herein and shall mean both the infectious particle form PrPSc known to cause diseases (spongiform encephalopathy) in humans and animals and the noninfectious form PrP, which, under appropriate conditions is converted to the infectious PrPSc form.

[0067] The terms “prion”, “prion protein”, “PrPSc protein” and the like are used interchangeably herein to refer to the infectious PrPSc form of a PrP protein. “Prion” is a contraction of the words “protein” and “infection.” Particles are comprised largely, if not exclusively, of PrPSc molecules encoded by a PrP gene. Prions are distinct from bacteria, viruses and viroids. Known prions infect animals and cause scrapie, a transmissible, degenerative disease of the nervous system of sheep and goats, as well as bovine spongiform encephalopathy (BSE), or “mad cow disease”, and feline spongiform encephalopathy of cats. Four prion diseases known to affect humans are (1) kuru, (2) Creutzfeldt-Jakob Disease (CJD), (3) Gerstmann-Strassler-Scheinker Disease (GSS), and (4) fatal familial insomnia (FFI). As used herein “prion” includes all forms of prions causing all or any of these diseases or others in any animals used—and in particular in humans and domesticated farm animals.

[0068] The term “PrP gene” is used herein to describe genetic material which expresses proteins including known polymorphisms and pathogenic mutations. The term “PrP gene” refers generally to any gene of any species which encodes any form of a prion protein. The PrP gene can be from any animal, and includes all polymorphisms and mutations thereof, it being recognized that the terms include other such PrP genes that are yet to be discovered. The protein expressed by such a gene can assume either a PrPc (non-disease) or PrPSc (disease) form.

[0069] A “peptidomimetic” is a biomolecule that mimics the activity of another biologically active peptide molecule.

[0070] “Protein” refers to any polymer of two or more individual amino acids (whether or not naturally occurring) linked via a peptide bond, and occurs when the carboxyl atom of the carboxylic acid group bonded to the alpha-carbon of one amino acid (or amino acid residue) becomes covalently bound to the amino nitrogen atom of amino group bonded to the alpha-carbon of an adjacent amino acid. These peptide bond linkages, and the atoms comprising them (i.e., alpha-carbon atoms, carboxylcarbon atoms (and their substituent oxygen atoms), and amino nitrogen atoms (and their substituent hydrogen atoms)) form the “polypeptide backbone” of the protein. In simplest terms, the polypeptide backbone shall be understood to refer to the amino nitrogen atoms, alpha-carbon atoms, and carboxylcarbons atoms of the protein, although two or more of these atoms (with or without their substituent atoms) may also be represented as a pseudoatom. Indeed, any representation of a polypeptide backbone that can be used in a functional site descriptor as described herein will be understood to be included within the meaning of the term “polypeptide backbone.”

[0071] The term “protein” is understood to include the terms “polypeptide” and “peptide” (which, at times, may be used interchangeably herein) within its meaning. In addition, proteins comprising multiple polypeptide subunits (e.g., DNA polymerase III, RNA polymerase II) or other components (for example, an RNA molecule, as occurs in telomerase) will also be understood to be included within the meaning of “protein” as used herein. Similarly, fragments of proteins and polypeptides are also within the scope of the invention and may be referred to herein as “proteins.”
“Conformation” or “conformational constraint” refers to the presence of a particular protein conformation, for example, an alpha-helix, parallel and antiparallel beta strands, leucine zipper, zinc finger, etc. In addition, conformational constraints can include amino acid sequence information without additional structural information. As an example, “—C—X—X—C—” is a conformational constraint indicating that two cysteine residues must be separated by two other amino acid residues, the identities of each of which are irrelevant in the context of this particular constraint. A “conformational change” is a change from one conformation to another.

The exact mechanism by which the sequence of a protein encodes the proper fold is unknown. In order to achieve the native state encoded by the fold, the protein molecule must convert to a unique conformation selected from many alternatives. Functional proteins are typically soluble and can adopt a variety of structures including coils and ordered elements. Ordered elements include the alpha helix predominant in proteins such as myoglobin and hemoglobin. During the human aging process, in some proteins the soluble structure (e.g. alpha helical regions) becomes conformationally altered into beta sheet structures that undergo aggregation associated with loss of function.

There are at least twenty proteins that are associated with human disease when they adopt a conformationally altered state, and some of these have been described previously. FIG. 1 illustrates both the alpha-helical monomer 10 and the beta-sheet dimer 12 forms of a TSE conformer. The normal wild-type (wt) form of prion protein (PrP\textsuperscript{C}) prefers a monomeric state, while the abnormal, disease-causing form (PrP\textsuperscript{Sc}) more readily takes on a multimeric state.

Protein structures can be determined by a variety of experimental or computational methods, several of which are described below. Protein structure can be assessed experimentally by any method capable of producing at least low resolution structures. Such methods currently include X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. X-ray crystallography is one method for protein structural evaluation, and is based on the diffraction of X-ray radiation of a characteristic wavelength by electron clouds surrounding the atomic nuclei in the crystal. X-ray crystallography uses crystals of purified biomolecules (but these frequently include solvent components, co-factors, substrates, or other ligands) to determine near atomic resolution of the atoms making up the particular biomolecule. Techniques for crystal growth are known in the art, and typically vary from biomolecule to biomolecule. Automated crystal growth techniques are also known.

Nuclear magnetic resonance (NMR) currently enables determination of the solution conformation (rather than crystal structure) of biomolecules. Typically only small molecules, for example proteins of less that about 100-150 amino acids, are amenable to these techniques. However, recent advances have lead to the experimental elucidation of the solution structures of larger proteins, using such techniques as isotopic labeling. The advantage of NMR spectroscopy over X-ray crystallography is that the structure is determined in solution, rather than in a crystal lattice, where lattice neighbor interactions can alter the protein structure. The disadvantage of NMR spectroscopy is that the NMR structure is not as detailed or as accurate as a crystal structure. Generally, biomolecule structures determined by NMR spectroscopy are of moderate resolution compared relative to those determined by crystallography.

Other techniques useful in studying biomolecule structure include circular dichroism (CD), fluorescence, and ultraviolet-visible absorbance spectroscopy. See, for example, Physical Biochemistry: Applications to Biochemistry and Molecular Biology, 2nd ed., W.H. Freeman & Co., New York, N.Y., 1982 for descriptions of these techniques.

“Equivalent” refers to amino acid sequences that are similar in sequence to the amino acid sequence of the protein to be analyzed but have at least one, but fewer than 5, (e.g., 3 or fewer) differences, substitutions, additions, or deletions. Thus, the substitution of one or more amino acid in a given sequence which does not substantially change the basic function of that amino acid within its use in context, is an equivalent for purposes of describing the present invention.

“Homology”, “homologs of”, “homologous”, or “identity” or “similarity” refers to sequence similarity between two polypeptides, with identity being a more strict comparison. Homology and identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same amino acid, then the molecules are identical at that position. A degree of identity of amino acid sequences is a function of the number of identical amino acids at positions shared by the amino acid sequences. A degree of homology or similarity of amino acid sequences is a function of the number of amino acids, i.e., structurally related, at positions shared by the amino acid sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences used in the present invention. Related sequences share more than 40% identity, preferably at least about 50% identity, more preferably at least about 70% identity, even more preferably at least about 90% identity, more preferably at least about 99% identity.

The term “percent identical” refers to sequence identity between two amino acid sequences. Identity can each be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same amino acid, then the molecules are identical at that position; when the equivalent site occupied by the same or a similar amino acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology, similarity, or identity refers to a function of the number of identical or similar amino acids at positions shared by the compared sequences. Various alignment programs and/or programs may be used, including FASTA, BLAST, or ENTREZ. FASTA and BLAST are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be used with, e.g., default settings. ENTREZ is available through the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Md. In one embodiment, the percent identity of
two sequences can be determined by the GCG program with a gap weight of 1, e.g., each amino acid gap is weighted as if it were a single amino acid mismatch between the two sequences. Other techniques for determining sequence identity are well-known and described in the art.

[0081] The term “interact” as used herein is meant to include detectable interactions (e.g., biochemical interactions) between molecules, such as interaction between protein-protein, protein-nucleic acid, nucleic acid-nucleic acid, and protein-small molecule or nucleic acid-small molecule in nature.

[0082] The term “homolog of an insoluble protein” includes all amino acid sequences that are encoded by a homolog of an insoluble protein gene, and all amino acid sequences that are equivalent or homologous to such sequences. Therefore, “homolog of an insoluble protein” includes proteins that are scored as hits in the Pfam family. To identify the presence of an “insoluble protein” domain in a protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against one of several databases (SwissProt, PIR, for example) using various default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsearch program, which is available as part of the HMMER package of search programs, is a family specific default program for MLOPA00563 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sontam _mer et al. (1997) _Proteins_ 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov et al.(1987) _Proc. Natl. Acad. Sci. USA_ 84:4355-4358; Krogh et al.(1994) _J. Mol. Biol._ 235:1501-1531; and Sultzl et al.(1993) _Protein Sci._ 2:305-314, the contents of which are incorporated herein by reference.

[0083] “Test specimen” is a sample of material to be tested. The sample may be prepared from tissue (e.g., a portion of ground meat, an amount of tissue obtained by a biopsy procedure) by homogenization in a glass homogenizer. The amount of material should be about 1 mg and 1 gm, preferably between 10 mg and 250 mg, ideally between 20 and 100 mg. The material to be sampled may be suspended in a suitable solvent, preferably phosphate-buffered saline at a pH between 7.0 and 7.8. The solvent may contain a detergent such as Triton X-100, SDS, or sarcosyl. Homogenization is performed for a number of excursions of the homogenizer, preferably between 10 and 25 strokes; ideally between 15 and 20 strokes. The suspended sample is preferably centrifuged at between 100 and 1,000 g for 5-to 10 minutes and the supernatant material sampled for analysis. In some samples, it may be preferable to treat the supernatant material with an additional reagent such as phosphotungstic acid according to the procedure described by Safar et al., _Nature Medicine_ 4, pp.1157-1165 (1998) and as modified by Wadsworth _et al._ _The Lancet_ , 558, pp.171-180 (2001).

[0084] The amount of sample to be tested is based on a determination of the protein content of the supernatant solution as measured by the procedure described by Bradford (1976). Preferably, this corresponds to between 0.5 and 2 mg of protein.

[0085] In addition to the procedure described above for tissue material, test samples may be obtained from serum, pharmaceutical formulations that may contain products of animal origin, spinal fluid, saliva, urine or other bodily fluids. Liquid samples may be tested directly or may be subjected to treatment with agents such as phosphotungstic acid as described above.

[0086] “Conformational probes” are preferably peptides that have amino acid sequences that are similar to, and more preferably identical to, some of those in the target protein and that also have the potential to undergo conformational alteration to produce β-sheet formation when complexed with the target protein (insoluble protein). Such alteration typically leads to a β-sheet structure not normally evidenced by the probe. Ideally, a probe has a palindromic structure with two amino acid sequences derived from the target protein. Preferred α-helix or random coil conformational probes (i.e., probes that exhibit α-helix or random coil conformation in solution) useful in the instant invention include the following:

[0087] a palindromic 33mer comprising amino acid sequences that are identical to amino acids 122-104 and 109-122 of the PrPSIC protein (SEQ ID NO: 13 and 14) (SwissProt PO4156 (Pfam ID Prion P100377 & P3991))

[0088] a palindromic 33mer comprising amino acid sequences that are equivalent to amino acids 122-104 and 109-122 of the PrPSIC protein SEQ ID NO: 13 and 14) (SwissProt PO4156 (Pfam ID Prion P100377 & P3991))

[0089] a palindromic 33mer comprising amino acid sequences that are between about 70% to about 90% identical to amino acids 122-104 and 109-122 of the PrPSIC protein SEQ ID NO: 13 and 14) (SwissProt PO4156 (Pfam ID Prion P100377 & P3991))

[0090] a probe comprising amino acid sequences that are identical to amino acids 1-40 of the Abeta peptide (Nref 0011747 (human))
[0091] a probe comprising amino acid sequences that are equivalent to amino acids 1-40 of the Abeta peptide (Nref 00111747 (human))

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLM (SEQ ID NO: 4)

[0092] a probe comprising amino acid sequences that are between about 70% to about 90% identical to amino acids 1-40 of the Abeta peptide (Nref 00111747 (human))

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLM (SEQ ID NO: 4)

[0093] a probe comprising amino acid sequences that are identical to amino acids 11-34 of the Abeta peptides (Nref 00111747 (human))

EYHQLVVFAEDVGSNKGAIGLM; (SEQ ID NO: 5)

[0094] a probe comprising amino acid sequences that are identical to amino acids 11-34 of the Abeta peptides (Nref 00111747 (human)) but with residue H13 substituted with R to reduce metal ion interactions and to increase the solubility of the peptide

EYHQLVVFAEDVGSNKGAIGLM; (SEQ ID NO: 6)

[0095] a probe comprising amino acid sequences that are identical to amino acids 25-35 of the Abeta peptides (Nref 00111747 (human))

GSKGAIGLM; (SEQ ID NO: 7)

[0096] a probe that has a helix-loop-helix conformation found in polylysine and an amino acid sequence that is at least 10 amino acid residues in length and is equivalent or homologous to SEQ ID NO:8

SEQ ID NO:8 XXXXXXXXXXXXXXXXXXXXXXXXX (27:mar);

[0097] a probe that has a conformation found in polyglutamine and an amino acid sequence that is equivalent or homologous to SEQ ID NO:9

SEQ ID NO:9 SQXXXXXXXXXXXXXXXXXXXXXXXXXX; (SEQ ID NO:10)

[0098] a probe comprising amino acid sequences that are homologous to amino acids 104-122 of wild-type (wt) TSE (Human NREF 00130350)

KEFKTNLEHVGAAAGAVV; (SEQ ID NO:10)

[0099] a probe comprising amino acid sequences that are equivalent to amino acids 104-122 of wild-type (wt) TSE (Human NREF 00130350)

MAEHLLQWLLLLLPTLCPGTARW; (SEQ ID NO:11)

[0100] a probe comprising amino acid sequences that are between about 70% to about 90% identical to amino acid sequences 104-122 of wild-type (wt) TSE (Human NREF 00130350)

KEFKTNLEHVGAAAGAVV; (SEQ ID NO:10)

[0101] a probe that comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and (c) has an amino acid sequence that is homologous to amino acid sequences 104-122 of wild-type (wt) TSE (Human NREF 00130350)

KEFKTNLEHVGAAAGAVV; (SEQ ID NO:10)

[0102] a probe that comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and (c) has an amino acid sequence that is equivalent to amino acid sequences 104-122 of wild-type (wt) TSE (Human NREF 00130350)

KEFKTNLEHVGAAAGAVV; (SEQ ID NO:10)

[0103] a probe that comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and (c) has an amino acid sequence that is between about 70% to about 90% identical to amino acid sequences 104-122 of wild-type (wt) TSE (Human NREF 00130350)

KEFKTNLEHVGAAAGAVV; (SEQ ID NO:10)

[0104] a probe comprising amino acid sequences that are identical to amino acids 1-38 of the human islet amyloid polypeptide precursor (amylin) protein (Accession # NP_000406 (human) implicated in human diabetes

MGILKLVFLIVLSVALNHKATPIESQVEKRK (SEQ ID NO:11)

[0105] a probe comprising amino acid sequences that are identical to at least 10 contiguous residues within the sequence corresponding to amino acids 1-38 of the human polypeptide precursor (amylin) protein (Accession # NP_000406 (human) human diabetes

MGILKLVFLIVLSVALNHKATPIESQVEKRK (SEQ ID NO:11)

[0106] a probe comprising amino acid sequences that are identical to amino acids 1-25 of the human lung surfactant protein (NCBI Accession # AAH32785 (human) implicated in human infant SIDS

MAEHLLQWLLLLLPTLCPGTARW; (SEQ ID NO:11)
[0107] a probe comprising amino acid sequences which include at least 10 contiguous amino acid residues of amino acids 104-122 of the human or amino acids 103-121 of the murine PrP<sup>Sc</sup> protein (SEQ ID NO: 13 and 14) (Swiss-Prot PO<sub>4156</sub> (Pfam ID Prion Pfo0377 & 03991))

[0108] Human prion protein

<table>
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<tr>
<th>Accession: PO4156 (SEQ ID NO: 13)</th>
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<tr>
<td>10 20 30 40 50 60</td>
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# Mouse prion protein

[0109] Mouse prion protein

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</tr>
<tr>
<td>250</td>
</tr>
</tbody>
</table>

VILLISFLIF IVG

[0110] a probe comprising amino acid sequences which include at least 10 contiguous amino acid residues of amino acids 235-269 (emphasized below) of the human plasma gelsolin (SEQ ID NO: 15) (PO<sub>4390</sub>), Maury, et al. FEBS Lett., 260(1), pp. 85-87 (1990);
[0111] a probe comprising amino acid sequences which include at least 10 contiguous amino acid residues of amino acids 27-146 (emphasized below) of the cystatin C protein sequence depicted below (SEQ ID NO: 17) (P01034), Levy, et al. J. Exp. Med., 169(S), pp.771-8 (1989). The amyloid forming version of the peptide is 120 amino acids corresponding to amino acid residues 27-146 below. An appropriate probe is any portion thereof of at least 10 amino acids, numerous probes can be posited accordingly;

[0112] Palindromic probe of cystatin C protein (from amino acids 39-47 of the above sequence with a four unit proline linker)


[0114] exemplary probe:

[0115] a probe comprising amino acid sequences which include at least 6 contiguous amino acid residues of amino acid residues 12-17 and 15-20 (emphasized below) of the (8-20) domain of the human islet amyloid polypeptide
Exemplary probes contain the following sequences which are minimal sequences within the sequence 8-20 of the above peptide sequence, which may be used without modification or may be used to form palindromic probes of the present invention:

LANFY; (SEQ ID NO: 22)

VPHALPPPLANPFLV (palindromic probe); (SEQ ID NO: 23)

FLVHSS; (SEQ ID NO: 24)

SSHULFPSLFVHSS (palindromic probe); (SEQ ID NO: 25)


A palindromic probe based upon the above referenced sequence (amino acid residues 10-19):

KSVLFGALPPPLAGUVFSE. (SEQ ID NO: 27)

Numerous other probes may be readily produced without undue experimentation using standard laboratory techniques and peptide and related chemical syntheses.

The native conformation of the probe is determined by one or more spectroscopic methods such as circular dichroism, Fourier transform infra-red, ultra-violet, nuclear magnetic resonance, or fluorescence, among others. This conformation in a solvent as described below should correspond to that of an alpha-helix or random coil (in circular dichroism, for example, the nature of the spectrum is indicative of the conformation).

The probe is modified to contain substituents that are detectable by optical means. Such substituents may include tryptophan (an amino acid), pyrene or similar fluorophores, all attached at or near the terminal positions of the peptide probes. Attachment of such fluorophores proceeds according to conventional chemical methods which are well-known in the art, preferably, but not necessarily through covalent attachment of the fluorophore to the probe. Ideally, the substituents have the capability to interact in such a manner as to produce a species known as an excimer. An excimer represents the interaction of two fluorophores that, upon excitation with light of a specific wavelength, emits light at a different wavelength which is also different in magnitude from that emitted by either fluorophore acting alone. Thus, structural alterations of the conformational probe that allow for the formation of such excimers can be detected by a change in optical properties. Such changes can be measured by known fluorometric techniques, including UV, IR, CD, NMR, or fluorescence, among numerous others, depending upon the fluorophore attached to the probe. The magnitude of these changes is related to the degree to which the probe has undergone the conformational alteration.

In another embodiment, the probe may be substituted with a radioactive material. Ideally, this should be positron emission of a sufficient energy to be detected by machines currently employed for this purpose. Such an entity would preferably contain oxygen-15 (an isotope of oxygen that decays by positron emission) or other radionuclide. In this embodiment, the radiolabeled probe may be injected into a patient and the binding of the probe to the protein target monitored externally.

A probe may comprise a peptide or peptidomimetic of at least five, preferably about ten or more amino acid residues that exhibits a random coil or alpha-helical conformation in solution. A peptide or peptidomimetic probe solvent may be aqueous and have a pH of between about 4 and about 10, preferably between about 5 and about 8, and may have an ionic strength of between about 0.05 and about 0.5 (when typically prepared with a chloride salt such as sodium chloride or potassium chloride). The solvent may also contain a percentage of a water-miscible organic material such as trifluoroethanol in amounts between about 30 to about 70% by volume, preferably between about 45 to about 60%. The solvent may be prepared with a suitable buffering system such as acetate/acetic acid, Tris, or phosphate.

The sequence of probe amino acids is determined from the nature of the target protein to be analyzed and usually comprises a region of the target that is known to undergo a structural transition from either an alpha-helix or coil to a beta-sheet. This latter structure is associated with the pathogenic form of the target protein. The conformational probe sequence ideally contains two repeats of the target sequence of interest, preferably between about 10 and 25 amino acids in length; more preferably between about 14 and 20 amino acids in length. These are arranged preferably in the probe to form a palindrome as illustrated in FIG. 10.

Preferred probes used in methods and kits of the invention have amino acid sequences corresponding to β-sheet regions of the protein to be analyzed. These probes are preferably at least 5 amino acids units in length and can be about 300-400 amino acid units in length (mer) or more, although, preferably these are about 10 amino acids to about 50 amino acids in length. In certain aspects of the invention, preferred probes which correspond to the α-sheet region are about 15 to about 100 mer, in others preferred probes may
range from about 20 to about 50 mer. The preferred length of a given probe will be a function of the probes ability to complex and produce β-sheet formation with the target protein.

0126 Probes for use in the present invention are readily determined from existing information in sequence databases already in existence or alternatively, may be readily determined experimentally. Thus, the probe will generally correspond to a minimum number of amino acids, preferably at least 10, and more preferably about 10 to 25 amino acids, which correspond to at least a portion of a peptide sequence of a target protein which undergoing a conformational transition from alpha-helix or random coil to β-sheet formation in the insoluble protein.

0127 Noted that within the experimental information which will guide the presentation and synthesis of an appropriate probe, there are some constraints which can guide the practitioner in making use of the present invention. Because there are only a few kcal difference separating a population in the initial conformation state from a population predominantly in the transformed conformational state (in complex). This transformation is provided by the driving force due either to the Kd of association between the probe molecule and its natural associate to form β-sheet complex, or due to changes in the electrostatic interactions between the molecules (for example, by lowering the ionic strength of the solution. If metal ions such as Al are involved, or the binding of another ligand, other electrostatic or steric effects could contribute. The size of the probe peptide can vary, but should be of sufficient length to have “reasonably” well-defined secondary structure under detection conditions and to have sufficient recognition specificity for the prion selected. The probe peptide should also accommodate single-site mutations in order to be generally applicable to mutated strains, recognizing that these changes and/or heterogeneities affect the thermodynamic stability of the molecule. Moreover, the probe must be non-toxic to the patient population, whether that population is a human patient population, a domesticated animal population or other mammalian population.

0128 Once a peptide sequence is established for a probe (which corresponds to at least a portion of a target protein responsible for β-sheet formation as described above), the peptide sequence may be adorned (at one, but preferably both ends of the peptide) with a moiety or chemical entity which can facilitate analysis of the peptide probe. Preferably, this moiety is a fluorophore, such as pyrene, but may vary widely, depending upon the analytical technique to be used for analysis. The moiety or chemical entity may be complexed or covalently bonded at or near the amino or carboxy end of the peptide, which is preferably encased with a short, hydrophobic peptide sequence. In preferred aspects of the present invention, both the amino and carboxy ends of the probe peptides are encased with small hydrophobic peptides ranging in size from about 1 to about 5 amino acids. These may be natural or synthetic, but are preferably natural (i.e., derived from a β-sheet formation region of a target protein. The fluorophore are preferably attached at or near the amino and/or carboxy end of the probe (preferably both) and may be, for example, pyrene, tryptophan, fluorescein, rhodamine, among numerous others and is preferably pyrene. It is preferable that the fluorophores form excimers when in the correct geometric orientation.

0129 Conformational probes according to the present invention are preferably palindromic in nature. This refers to the organization of a given conformational probe sequence such that it will contain first and second peptide sequences corresponding to a portion of the target protein responsible for β-sheet formation, but which peptide sequences are presented in a palindromic manner, i.e., from the carboxy end to the amino end (or amino end to carboxy end) in the first peptide sequence, and from the amino end to the carboxy end (or carboxy end to amino end) in the second peptide sequence. The first and second peptide sequence in the palindromic conformational probe do not have to be identical in length, although this may be preferred in certain embodiments, but should be at least roughly equivalent (the two peptide sequences (“arms” of the probe) should not be more than 15, preferably no more than 10 and even more preferably no more than 5 amino acids different in length). Preferably, the first and second peptide sequences within a palindromic probe sequence are separated by a linker comprising between 1 and 5 amino acids, preferably between 1 and 3 amino acids, which preferably contain at least one proline amino acid and more preferably comprise primarily proline amino acids. FIG. 10 presents an exemplary palindromic 33 mer conformation probe useful in the present invention.

0130 Preferably, conformational probes according to the present invention contain a hydrophobic amino acid sequence which is preferably derived from the relevant peptide sequence of the target protein (i.e., the peptide sequence responsible for α-sheet formation), and which may vary in length from 1 amino acid to 20 or more amino acids, preferably about 2-10 amino acids in length and appears at or near one of the two ends of the conformation probe. In the case of palindromic conformation probes, these hydrophobic amino acid sequences appear at the ends of the two peptide arms of the probe. Optionally, the probe also may contain a synthetic hydrophobic amino acid sequence (i.e., not natural to the peptide sequence of the target protein responsible for α-sheet formation) at at least one end of the probe and in the case of palindromic probes, at or near each end of the probe, which may vary in length from as few as one amino acid to 20 or more amino acids, preferably about 3-10 amino acids in length.

0131 By way of example and without limitation, if a desired peptide sequence in a target protein contains the sequence, reading from amino end to carboxy end, QRSTV-VURLKAAAV (where AAAV is a hydrophobic amino acid sequence) then the palindrome would contain a first peptide sequence which is VAAKLRLUVYTVSRQ and a second peptide sequence which is QRTSVVURALAAAV (or a close variation to that sequence), with the two sequences separated by a linker comprising from 1 to 5 amino acids, with at least one of those amino acids, and preferably most, if not all, of those amino acids, being proline amino acids. The probe would therefore be:

VAAKLRLUVYTVSRQQPPFQQRSTVVRULKAAAV

(hypothetical palindromic probe)
Preferably, the palindromic probe would contain a hydrophobic amino acid sequence obtained from the relevant sequence of the target protein. Conformational probes according to the present invention may be readily obtained.

The following rules may be used to guide the formation of an appropriate preferred conformational probe according to the present invention. These rules apply generally to conformational probes according to the present invention without limitation, but are more specifically used in context to produce the preferred palindromic conformational probes according to the present invention.

The following rules may be applied to the instant invention to produce preferred conformational peptide probes:

1. Each “arm” of the peptide palindrome should have a minimum of five, and preferably at least 10-12 amino acids and, ideally, not more than about 25 amino acids.

2. The amino acid sequence is selected from a region of a larger protein that is known to undergo a conformational transition from alpha-helix or random coil to beta sheet.

3. One or more of the following additional criteria:
   a) A high proportion of hydrophobic amino acids—generally not less than about 75% (based upon the number of amino acids), ideally 80% or greater
   b) Amino acid repeats of at least 20 and preferably 25 (such as is present in huntingtin)
   d) A linker sequence between each of the peptide arms that has 1 or more amino acids, preferably less than five and that contains one or more proline residues

Test criteria for peptide probe:

1. The conformation of the palindromic peptide probe should be that of an alpha helix or random coil but not a beta sheet.

2. Determination of the conformation of the peptide is ideally accomplished by circular dichroism measurements that can identify solution conformations. These are performed using a CD spectrometer in one or more solvents that can include aqueous buffers and/or organic agents such as trifluoroethanol—see FIG. 11.

Applying the general rules obtained above and using readily available methods in the art, one of ordinary skill can produce large numbers of conformational peptide probes having favorable characteristics to be useful in the present invention. “Circular dichroism” (CD) is observed when optically active matter absorbs L and R hand circular polarized light slightly differently, as measured by a CD spectropolarimeter. Differences are very small and represent fractions of degrees in ellipticity. FIG. 11 depicts an associative CD curve representative of the three distinct common conformational forms that proteins and peptides can assume. CD spectra for distinct types of secondary structure present in peptides and proteins are distinct. Measuring and comparing CD curves of complexed vs uncomplexed protein represents an accurate measuring means of practicing the instant invention.

Unexpectedly, we have determined that under near physiological conditions, the palindromic, 33-mer (SEQ ID NO: 1 or 29), which covalently connects two peptides—the 14-mer (SEQ ID NO: 3 and the 19-mer (SEQ ID NO:2) exhibits a largely coil conformation despite the proximity of two hydrophobic chains resembling the 14-mer structure, as illustrated in FIG. 12. The addition of a pyrene at each end of the palindromic 33-mer peptide allows for spectral observation of the conformational change, as illustrated in FIG. 13. The spectral scans for pyrene attached to the ends of the 33-mer in the monomer (open) conformation gives a strikingly different fluorescent spectrum, having a maximum emission between 370 and 385 nm, while the excited dimer or excimer state of the pyrene-labeled peptide has an emission max between 475 and 510 run.

Although it is possible to follow conformational changes by any of the several optical methods described above, a preferred embodiment of the invention utilizes fluorescence spectroscopy since that technique provides sensitivity, rapidity and simplicity of operation. The probe is modified by attachment at both termini of a fluorophore that has specific optical properties. It is preferred that these include the ability to fluoresce upon irradiation with light of a specific wavelength (defined by the absorption and emission spectra of the chromophore itself). Thus, irradiation with light of a wavelength near that of the absorption maximum and emission of light at a sufficiently higher wavelength so as to be distinguished from the excitation wavelength—this measurement is well known to those versed in the art. Examples of such fluorophores include, but are not limited to, pyrene, tryptophan, fluorescein, rhodamine. It is also preferred that the attached fluorophores have the capacity to form excimers when in the correct geometric orientation.

An “excimer” is an adduct that is not necessarily covalent and that is formed between a molecular entity that has been excited by a photon and an identical unexcited molecular entity. The adduct is transient in nature and exists until it fluoresces by emission of a photon. It is possible to recognize an excimer (or the formation of an excimer) by the production of a new fluorescent band at a wavelength that is longer than that of the usual emission spectrum. An excimer can be distinguished from fluorescence resonance energy transfer since the excitation spectrum is identical to that of the monomer.

The formation of the excimer is dependent on the geometric alignment of the fluorophores and is heavily influenced by the distance between them. In a preferred embodiment, fluorophores are present at each probe terminus and excimer formation between fluorophores is negligible as long as the overall probe conformation is alpha-helix or random coil. This is readily determined by
measurement of the fluorescent behavior of the probe in the solvent to be used for analysis in the absence of the target protein to be measured.

[0150] Preferred conformational transition following interaction with an analyte target is achieved by measuring fluorescence spectra under conditions where excimer formation can be analyzed. Typically, using pyrene as an exemplary fluorophor, the excitation wavelength would be about 350 nm and the observation wavelength 365-600 nm. The normal emission of monomer pyrene following excitation (simple fluorescence) is recorded as the maximum wavelength between about 370-385 nm. Representative data is shown in FIG. 14.

[0151] As shown in FIG. 14, the excimer or excited dimer state is recorded at a maximum of between 475-510 nm. The formation of the excited dimer state can also be encouraged through the addition of high salt and by conducting measurements at pH approaching the pI of the peptide (e.g., in the illustrated case, a pH of around 10).

[0152] Therefore, in a preferred method of the invention, interaction of the probe with the specific protein to be analyzed causes a conformational change in the probe such that excimer formation occurs. This is readily measured by the procedures described herein. Conversion of the probe structure from that exhibited in the absence of analyte (alpha-helix or random coil) to a beta-sheet structure enables fluorophores attached to the probe to form excimers that can be readily identified. Further, the magnitude of excimer formation is directly related to the amount of protein analyte present.

[0153] Proteins or prions may be detected in aggregated form or in the presence of other cellular constituents such as lipids, other proteins or carbohydrates. A sample preparation for analysis is preferably homogenized or subjected to a similar disruption of tissue or aggregate structures, and cellular debris is preferably removed by centrifugation. This process is ideally performed in the presence of a buffered salt solution and may utilize one of several detergents such as SDS, Triton X-100, or sarcosyl. Further concentration of the sample may be achieved by treatment with any of several agents; one preferred agent is phosphotungstate, which is employed according to the method of Safar et al Nature Medicine 4:1157-1165 (1998).

[0154] In a preferred embodiment of the invention, peptide probes are selected in order for addition to an unknown or test sample. The peptide probes are preferably proteins or peptide sequences that have secondary structures of predominately alpha-helix or random coil, but which are preferably, but not necessarily derived from portions of a target peptide responsible for beta-sheet formation. In a particularly preferred embodiment, the peptide probes are peptide fragments consisting of a helix-loop-helix structure found in polylysine. In another particularly preferred embodiment, the peptide probes can be made of a peptide sequence chosen from wild-type (wt) TSE, from a desired species-specific TSE peptide sequence, or even from a selectively mutated TSE sequence that has been mutated in such a manner as to render it destabilized and noninfectious. Additionally, extrinsic fluoros such as pyrene can be added or designed into the peptide probe to allow detection of anticipated conformational changes using common fluorescence detection techniques.

[0155] Once a peptide probe is selected, it is added to a test sample. Prior to the addition of the peptide probe, however, it is preferred to have the sample subjected to disaggregation techniques commonly known in the art, such as sonication. The disaggregation step allows any potentially aggregated sample material to break apart so that these disaggregated sample materials are free to combine with the newly introduced peptide probe, thereby facilitating the anticipated catalytic propagation.

[0156] After the test sample or disaggregated test sample is allowed to interact with the peptide probes, the resulting mixture is then subjected to analytical methods commonly known in the art for the detection of aggregates and to fluorescence measurements in cases where fluorescent peptide probes are used. Unknown or test samples containing any dominant beta-sheet formation characteristic of abnormally folded or disease-causing proteins result in an increase in beta-sheet formation and consequently aggregate formation in the final mixture containing both the test sample and the peptide probes. Conversely, unknown or test samples which lack a predominantly beta-sheet secondary structure will neither catalyze a transition to beta-sheet structure nor will propagate the formation of aggregates.

[0157] The initial conformational change can be triggered in the test samples in a number of ways. Without intending to be bound by any theory, the binding of a metal ligand could direct a change in the protein conformation and favor aggregation. The expression or cleavage of different peptide sequences can promote advanced aggregation leading to fibril and plaque formation. Genetic point mutations can also alter the relative energy levels required of the two distinct conformations, resulting in midpoint shifts in structural transitions. Furthermore, an increase in concentration levels could be sufficient to favor the conformational transition. Regardless of the initial trigger mechanism, however, the disease process in many of the abnormal protein conformations such as in prion-related diseases involves the catalytic propagation of the abnormal conformation, resulting in structural transformation of the previously normal protein.

[0158] Optical detection techniques useful in the instant invention include but are not limited to light scattering, or hydrophobicity detection using extrinsic fluoros such as 1-anilino-8-naphthalene sulfonate (ANS) or Congo Red stain, fluorescence resonance energy transfer (FRET) and quenching of intrinsic tryptophan fluorescence through either conformational change of monomer or binding at an interface in an alpha-beta heterodimer.

[0159] Other structural techniques include equilibrium ultracentrifugation or size-exclusion chromatography.

[0160] The instant invention uses propagated conformational change to correlate directly levels of abnormal proteins or prions with levels of infectivity. For this reason, it is preferable to utilize the methods of the invention in a manner in which there is no increase in infectious products as a result of the propagation. This can be achieved by placing a "break" in the links between the chain of infection, transmission, and propagation of the abnormal form. Such a "break" must occur at the transitional stage between the dimer and multimer forms of the aggregate. The physical formation of the multimer form can be blocked by simply impeding the step which leads to its formation. This may be achieved by using a probe in which the sequence of interest
is attached to a non-relevant peptide, or by a neutral “blocker” segment, with the understanding that probes on linkers or “tethers” are more likely to encounter each other and thus result in amplifying the signal.

[0161] The invention is described further in the following examples, which are illustrative and in no way limiting.

EXAMPLE 1

[0162] Materials and Methods

[0163] A sample may be obtained for testing and diagnosis through use of the instant invention as follows. A sample may be prepared from tissue (e.g. a portion of ground meat, or an amount of tissue obtained by a biopsy procedure) by homogenization in a glass homogenizer or by mortar and pestle in the presence of liquid nitrogen. The amount of material should be between about 1 mg and 1 gm, preferably between 10 mg and 250 mg, ideally between 20 and 100 mg. The material to be sampled may be suspended in a suitable solvent, preferably phosphate-buffered saline at a pH between 7.0 and 7.8. The addition of Rnase inhibitors is preferred. The solvent may contain a detergent (e.g., Triton X-100, SDS, or sarkosyl). Homogenization is performed for a number of excursions of the homogenizer, preferably between 10 and 25 strokes; ideally between 15 and 20 strokes. The suspended sample is preferably centrifuged at between 100 and 1,000 g for 5-10 minutes and the supernatant material sampled for analysis. In some samples, it may be preferable to treat the supernatant material with an additional reagent such as phosphotungstic acid according to the procedure described by Safar et al., Nature Medicine, 4, 1157-1165 (1998) and as modified by Wadsworth, The Lancet, 358, 171-180 (2001). Eight prion strains have PrPres molecules with different conformations. Soc. Safar, et al. and Wadsworth, ibid. Tissue distribution of protease resistant prion protein in variant Creutzfeldt Jakob disease has been reported using a highly sensitive immunoblotting assay as described in Wadsworth, et al., ibid.

[0164] The amount of sample to be tested is based on a determination of the protein content of the supernatant solution as measured by the procedure described by Bradford, Anal. Biochem. 72-248-254 (1976). A rapid and sensitive method for determining microgram quantities of protein utilizes the principle of protein-dye binding. Preferably, this corresponds to between about 0.5 and 2 mg of protein.

[0165] In addition to the procedure described above for tissue material, test samples may be obtained from serum, pharmaceutical formulations that may contain products of animal origin, spinal fluid, saliva, urine or other bodily fluids. Liquid samples may be tested directly or may be subjected to treatment with agents such as phosphotungstic acid as described above.

[0166] Illustrative Analysis

[0167] A sample containing TSE may be analyzed in accordance with the invention as follows. Referring to FIG. 2, the top row of the schematic illustrates an unknown sample of TSE protein represented as containing beta-sheets 12. The beta-sheets are disaggregated by sonication. Labeled peptide probes 14 are added and are allowed to bind to the sample 12. The beta-sheet conformation in sample 12 induces the peptide probes to conform to beta-sheet conformation 16. Beta-sheet propagation among the peptide probes 14 forms aggregates 18. The resulting transition to a predominately beta-sheet form and amplified aggregate formation is detected by techniques such as light scattering and circular dichroism (CD). In a particularly preferred embodiment, the peptide probe is fluorescently labeled and fluorescence detection is used.

[0168] The bottom row of FIG. 2 shows an alternative example in which the unknown sample of TSE protein is represented in its normal alpha-helical form 10. For consistency, the sample is subjected to the same disaggregation process described above. Upon addition of the labeled peptide probes 14, neither a transition to beta-sheet form nor binding to the unknown samples occurs. As a result, there is no aggregate fluorescence signal in the case of a labeled peptide probe and there is no detection of aggregate formation by other analytical tools. Based on this schematic, unknown samples can be tested for the presence or absence of such abnormal protein conformations or sequences.

EXAMPLE 2

[0169] Poly-lysine was used as a model peptide. Experiments were performed using model systems to illustrate the conformational changes involved in the transition from a predominately alpha-helix to a beta-rich form. The model system chosen used non-neurotoxic polyamino acid poly-lysine. The polyamino acid was chosen because of availability and safety; and normally evidences random coil conformation at pH values between 5 and 9.

[0170] FIG. 3 depicts a CD graph of an experiment in which poly-L-lysine 20 micro Molar (μM) 52,000 molecular weight (MW) was used as a peptide model.

[0171] As also illustrated in FIG. 3:

[0172] Sample 24, which was maintained at pH 7, 25°C., exhibited a minimum at approximately 205 nanometers (nm), indicating a random coil structure;

[0173] Sample 26 which was maintained at pH 11 (near the isoelectric point), at 50°C., resulted in a minimum at approximately 216 nanometers (nm) indicating a β-sheet structure (see FIG. 11 for exemplary CD spectra of protein conformations);

[0174] Sample 28, which was a 1:1 combination of samples maintained at pH7, 25°C. and at pH11, 50°C., resulted in a minimum at approximately 216 nanometers (nm) indicating β-sheet structure;

[0175] Sample 30, which was a 1:1 combination of samples maintained at pH 7, 50°C. and at pH 11, 50°C., resulted in a minimum at approximately 216 nanometers (nm), indicating β-sheet structure.

EXAMPLE 3

[0176] FIG. 4 illustrates general CD results of experiments that were conducted: (1) using poly-L-lysine; and (2) at varying temperatures and pH, to observe the effect of random coil to beta-sheet conformational changes under varying environmental conditions. The results indicate that both temperature and pH play an important role in the transition. The results also indicate that the addition of a relatively small amount of β-sheet peptide to a random coil sample can result in a shift towards a β-rich conformation.
and that such changes can be accelerated depending on the temperature and pH environment of the samples.

[0177] More specifically, FIG. 4 illustrates an absorbance graph generated using a poly-L-lysine 52,000 molecular weight (MW) at 70 micromolar (µM) as a peptide probe in accordance with the experimental technique described in Examples 1-3. FIG. 4 illustrates that:

[0178] Sample 32 (pH 11, 25° C.) evidenced a plateau at approximately 0.12, indicating a pre-dominantly α-helical structure;

[0179] Sample 34 (maintained at pH 7, 50° C.) evidenced a plateau at approximately 0.22, which indicated a predominantly random coil structure;

[0180] Sample 36 (a 1:10 combination of samples maintained at pH 7, 50° C. and at pH 11, 50° C.) resulted in a steeper incline from approximately 0.22 to 0.33, indicating an accelerated transition from random coil to β-sheet structure;

[0181] Sample 38 (a 1:10 combination of samples maintained at pH 7, 25° C. and at pH 11, 50° C.) resulted in a gradual incline from approximately 0.22 to 0.26, indicating a transition from random coil to β-sheet structure.

[0182] The observations based on all of the experiments described above show that the addition of a relatively small amount of β-sheet peptide to random coil sample can result in a shift towards a beta-rich conformation and that such changes can be accelerated depending on the temperature and pH environment of the samples.

EXAMPLE 4

[0183] The experiment that led to the results illustrated in FIG. 15 involved use of the 33_mer target peptide (SEQ ID NO: 1 and 29)

\[
\text{VVAGAAAAAGAVLHNTKPKLKIVGAGAAAGAV} \quad \text{(murine)}
\]

\[
\text{VVAGAAAAAGAMKNTKPKEKIVGAGAAAGAV} \quad \text{(human)}
\]

[0184] alone, and probing peptide association through the observation of excimer formation. The 33_mer target peptide (SEQ ID NO: 1 or 29) used was a murine amino acid sequence which differed from a corresponding human sequence in the substitution of methionine for valine and leucine at positions M11V, M14L, M20L, and M23V, as illustrated in FIG. 10B. We compared the results we observed using CD (in which peptides were unlabeled) and spectrophotometric studies (using pyrene-labeled peptides). No homogenate was used. The experiment that lead to the results illustrated in FIG. 15 was a detailed study undertaken to understand what triggered the 33_mer target peptide (SEQ ID NO: 1 or 29) to conformationally change from predominately monomeric to dimeric (excimeric) and become aggregated. Conditions were found that encouraged 33_mer labeled-peptide association in the µM-range.

[0185] Conditions that screened the electrostatic interactions of the 33_mer target peptide and thereby minimized its solubility (pI=10) triggered self-association of the peptide under extremely low concentrations (10 µM). This self association is evident in the formation of dimers or excimers and the concomitant far red shift in fluorescence by virtue of the pyrene fluorophor on the ends of the peptides. As an example, Curve 1 of FIG. 15 represents the conditions of pH 6-8, KCl (100-500 µM) where the predominant peptide conformer is monomeric; while Curve 2 of FIG. 15 represents the conditions of pH 10-11, KCl (100-500 µM), where at very low concentrations of peptide, we observed strong excimer formation (aggregation of the monomers).

EXAMPLE 5

[0186] The experiment that led to the results illustrated in FIG. 16 involved use of various individual peptides, and the 33_mer probe (comprising 19_mer and 14_mer) target peptide (SEQ ID NO: 1, 29 or 31) VVAGAAAAAGAVLHNTKPKLKLNVGAGAAAGAV (murine) VVAGAAAAAGAMKNTKPKEKIVGAGAAAGAV (human) The assay conditions were changed to observe the effect on conformation as monitored by CD. The goal was to determine what thermodynamic conditions result in one step transition from monomeric random coil to aggregated β sheet and avoid the associative ‘X’ state that is probably micelle formation of the peptides.

[0187] In the experiment that lead to the results illustrated in FIG. 16, a specific λ (205 nm) wavelength was used to monitor peptide association by CD to obtain detailed conformational information over a range of solvent conditions and across a range of peptide concentrations (peptide concentrations are presented in log scale and also refer to the standard diagram for CD—Figure 11).

[0188] The associative curve (θ202) recovered for the target peptides showed two conformational transitions at the 50 µM and 3 mM range, respectively, moving from a coil through to ‘X’ state and to β-sheet.

[0189] Referring to FIG. 16, for solvent conditions above 50% (far left dashed line), the 33_mer target peptide (SEQ ID NO: 1 and 29)

\[
\text{VVAGAAAAAGAVLHNTKPKEKIVGAGAAAGAV} \quad \text{(murine)}
\]

\[
\text{VVAGAAAAAGAMKNTKPKEKIVGAGAAAGAV} \quad \text{(human)}
\]

[0190] transitioned from the coil state to a β-sheet state at 3 µM, while the component 19_mer or 14_mer were able to transition, but at nearly 10-fold higher peptide concentration (middle line). Under aqueous conditions, (thick line) none of the peptides were able to self associate into a α-sheets structure.

[0191] The 33_mer palindromic peptide target peptide (SEQ ID NO: 1 and 29) exhibited unique properties at very low concentrations (ie. 1 µM) under 50% solvent (acetone- trile or trifluoroethanol) conditions in that it avoided the “cloud-end” associative state (as exhibited by the plateauing effect under aqueous conditions).

[0192] FIG. 16 shows that a variation in solvent and temperature does not significantly affect the associative behavior of target peptides and that all of the peptides follow the same curve, indicating that sequence specificity is not an important feature in this kind of molecular assembling.

EXAMPLE 6

[0193] The experiment that led to the results illustrated in FIG. 17 was conducted as follows.

[0194] One gram of scrapie infected (strain 293) hamster brain material was homogenized in liquid nitrogen in sterile
phosphate buffered saline. Ten-fold serial dilutions were made into sterile PBS. The concentration of protease resistant prion protein (PrPSc) in the brain homogenates was determined by capillary electrophoresis antibody-capture. Brain homogenate equivalent to 10 ng of protease resistant prion protein (PrPSc) was mixed with 1.5 μM of 33-mer target peptide in 50% TFE (trifluoroethanol) and incubated for 1 hour at room temperature prior to excitation at 350 nm in a dual fluorometer spectrophotometer and emission from 350 to 600 nm recorded, the excitation and emission scan were repeated at 5 hours and 24 hours. The 33-mer peptide alone was used as a control.

Addition of the infectious prion protein led to the significant increase in the fluorescence of the 33-mer target peptide, which was found to be in near β-sheet conformation by CD data under conditions of 50% Tris:50% TFE. This increase of fluorescence indicated the formation of 33-mer aggregates. The 33-mer aggregates were found to be unstable and dissociate irreversibly with time.

Following the emission of fluorescence for the complex versus the peptide over time illustrated that the complex dissociated with time, while the peptide fluorescence remained stable monitoring at two different wavelengths, 377 nm (triangle) and 475 nm (square).

EXAMPLE 7

The experiment that led to the results illustrated in FIG. 18 was conducted as follows.

One gram of scrapie infected and healthy hamster brain, sheep brain and elk brain were homogenized in liquid nitrogen in sterile phosphate buffered saline. Ten-fold serial dilutions were made into sterile PBS. The concentration of protease resistant prion protein (PrPSc) in the brain homogenates was determined by capillary electrophoresis antibody-capture. Brain homogenates, infected and healthy, were mixed with 0.52 μM of 33-mer target peptide in 50% TFE (trifluoroethanol):50% TRIS and incubated for 1 hour at room temperature prior to excitation at 350 nm in a dual fluorimeter spectrophotometer and emission at 350 to 600 nm recorded. The 33-mer peptide alone in 50% TFE:50% TRIS was used as an additional control.

Fluorescence spectra of the target peptide [520 nM] in the presence of infected brain homogenate (graph line 1), healthy brain homogenate (graph line 2), and peptide alone (graph line 3) in TRIS:TFE (1:1) solvent are shown in FIG. 18. The data are for 0.01% brain homogenate from hamster (panel A), sheep (panel B), and elk (panel C). hamster [270 pg/ml], sheep [60 pg/ml], and elk [6 pg/ml].
Leu Lys His Val Ala Gly Ala Ala Ala Gly Ala Val Val

Asp Ala Glu Phe Arg His Asp Gly Ser Gly Tyr Glu Val His His Gln

Lys Leu Val Phe Phe Ala Glu Val Gly Ser Asn Lys Gly Ala

Ile Ile Gly Leu Met Val Gly Gly Val Val

Glu Val His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly

Ser Asn Lys Gly Ala Ile Ile Gly Leu

Glu Val Arg His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly

Ser Asn Lys Gly Ala Ile Ile Gly Leu

Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met

Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu
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<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Peptide

<400> SEQUENCE: 9
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Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln 20

<210> SEQ ID NO 10
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Peptide

<400> SEQUENCE: 10
Lys Pro Lys Thr Asn Leu Lys His Val Ala Gly Ala Ala Ala Ala Ala Ala Ala Ala Ala 1 5 10 15
Gly Ala Val Val

<210> SEQ ID NO 11
<211> LENGTH: 38
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Peptide

<400> SEQUENCE: 11
Met Gly Ile Leu Lys Leu Gln Val Phe Leu Ile Val Leu Ser Val Ala Leu Asn His Lys Ala Thr Pro Ile Glu Ser His Gln Val 1 5 10 15 20 25 30
Glu Lys Arg Lys Cys Asn Thr Ala 35

<210> SEQ ID NO 12
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Peptide

<400> SEQUENCE: 12
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<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE: 
<223> OTHER INFORMATION: Synthetic Peptide

<400> SEQUENCE: 13

Met Ala Asn Leu Gly Cys Trp Met Leu Val Leu Phe Val Ala Thr
1   5  10   15

Trp Ser Asp Leu Gly Leu Cys Lys Arg Pro Lys Pro Gly Gly
20   25   30

Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gin Gly Ser Pro Gly
35   40   45

Gly Asn Arg Tyr Pro Pro Gly Gly Gly Gly Trp Gly Gln Pro
50   55   60

His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln
65   70   75

Pro His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly
80   85   90

Gly Gly Gly Gly Thr His Ser Glu Trp Asn Lys Pro Ser Lys Pro
95  100  105

Lys Thr Asn Met Lys His Met Ala Gly Ala Ala Ala Ala Gly Ala
110  115  120

Val Val Gly Gly Tyr Gly Tyr Met Leu Gly Ser Ala Met Ser
125  130  135

Arg Pro Ile Ile His Phe Gly Ser Asp Tyr Glu Asp Arg Tyr Tyr
140  145  150

Arg Glu Asn Met His Arg Tyr Pro Asn Gln Val Tyr Tyr Arg Pro
155  160  165

Met Asp Glu Tyr Ser Asn Gln Asn Asn Phe Val His Asp Cys Val
170  175  180

Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr Lys Gly
185  190  195

Glu Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg Val Val
200  205  210

Glu Gln Met Cys Ile Thr Gln Tyr Glu Arg Glu Ser Gln Ala Tyr
215  220  225

Tyr Gln Arg Gly Ser Ser Met Val Leu Phe Ser Ser Pro Pro Val
230  235  240

Ile Leu Leu Ile Ser Phe Leu Leu Ile Val Gly
245  250

<210> SEQ ID NO 14
<211> LENGTH: 254
<212> TYPE: PRT
<213> ORGANISM: murine
<220> FEATURE: 

<400> SEQUENCE: 14

Met Ala Asn Leu Gly Tyr Trp Leu Leu Ala Leu Phe Val Thr Met
1   5  10   15

Trp Thr Asp Val Gly Leu Cys Lys Arg Pro Lys Pro Gly Gly
20   25   30

Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
---continued---

35 40 45
Gly Asn Arg Tyr Pro Pro Gln Gly Gly Thr Trp Gly Gln Pro His 50 55 60
Gly Gly Trp Gly Gln Pro His Gly Gly Ser Trp Gly Gln Pro 65 70 75
His Gly Gly Ser Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln 80 85 90
Gly Gly Gly Thr His Asn Gln Trp Asn Lys Pro Ser Lys Pro Lys 95 100 105
Thr Asn Leu Lys His Val Ala Gly Ala Ala Ala Gly Ala Val 110 115 120
Val Gly Gly Leu Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg 125 130 135
Pro Met Ile His Phe Gly Asn Asp Trp Glu Asp Arg Tyr Tyr Arg 140 145 150
Glu Asn Met Tyr Arg Tyr Pro Asn Gln Val Tyr Tyr Arg Pro Val 155 160 165
Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val His Asp Cys Val Asn 170 175 180
Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr Lys Gly Glu 185 190 195
Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg Val Glu 200 205 210
Gln Met Cys Val Thr Gln Tyr Gln Gly Ser Gln Ala Tyr Tyr 215 220 225
Asp Gly Arg Arg Ser Ser Ser Thr Val Leu Phe Ser Ser Pro Pro 230 235 240
Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly 245 250

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Peptide

<400> SEQUENCE: 15

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Ser Arg Gly Ala Ser Gln Ala Gly Ala Pro Gln Gly Arg Val Pro 35 40 45
Glu Ala Arg Pro Asn Ser Met Val Val Glu His Pro Glu Phe Leu 50 55 60
Lys Ala Gly Lys Glu Pro Gly Leu Gln Ile Trp Arg Val Glu Lys 65 70 75
Phe Asp Leu Val Pro Val Pro Thr Asn Leu Tyr Gly Asp Phe Phe 80 85 90
Thr Gly Asp Ala Tyr Val Ile Leu Lys Thr Val Glu Leu Arg Asn 95 100 105
Gly Asn Leu Gln Tyr Asp Leu His Tyr Trp Leu Gly Asn Glu Cys
---continued---

Ser Gln Asp Glu Ser Gly Ala Ala Ala Ile Phe Thr Val Gln Leu
110 115 120

Asp Asp Tyr Leu Asn Gly Arg Ala Val Gin His Arg Glu Val Gin
125 130 135

Gly Phe Glu Ser Ala Thr Phe Leu Gly Tyr Phe Lys Ser Gly Leu
140 145 150

Lys Tyr Lys Asn Gly Gln Val Ala Ser Gly Phe Lys His Val Val
155 160 165

Pro Asn Glu Val Val Val Gin Arg Leu Phe Gin Val Lys Gly Arg
170 175 180

Arg Val Val Arg Ala Thr Gln Val Pro Val Ser Trp Glu Ser Phe
185 190 195

Asn Asn Gly Asp Cys Phe Ile Leu Asp Leu Gly Asn Asn Ile His
200 205 210

Gln Trp Cys Gly Ser Asn Ser Ser Arg Tyr Glu Arg Leu Lys Ala
215 220 225

Thr Gin Val Ser Lys Gly Ile Arg Asp Asn Glu Arg Ser Gly Arg
230 235 240

Arg Ala Gin Val Gin Ser Glu Thr Val Gin Ser Gin Met
245 250 255

Gln Gin Val Gin Pro Lys Gin Ala Leu Pro Ala Gin Thr Gin
260 265 270

Asp Thr Ala Lys Gin Ala Asp Ala Asn Arg Lys Leu Ala Lys Leu
275 280 285

Tyr Lys Val Ser Asn Gly Ala Gly Thr Met Ser Val Ser Leu Val
290 295 300

Ala Asp Glu Asn Pro Phe Ala Gin Gly Ala Leu Lys Gin Gin Ser Gin
305 310 315

Cys Phe Ala Leu Asp His Gly Lys Asp Gin Ile Phe Val Thr
320 325 330

Lys Gly Lys Gin Ala Asn Thr Gin Glu Gin Gin Lys Ala Gin Thr
335 340 345

Thr Gin Ser Ser Asp Phe Ile Thr Gin Met Gin Thr Gin Gin Gin
350 355 360

Gln Val Ser Val Leu Pro Gin Gly Gin Thr Pro Leu Gin Thr
365 370 375

Gln Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
380 385 390

Gly Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
395 400 405

Pro Phe Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
410 415 420

Gln His Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
425 430 435

Arg Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
440 445 450

Gly Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
455 460 465

Gly Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
470 475 480

Arg Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
485 490 495
Arg Glu Ser Thr Glu Val Ala Ala Ser Ala Ile Leu Thr
500 505 510
Ala Glu Leu Asp Glu Leu Glu Glu Thr Pro Val Glu Ser Arg
515 520 525
Val Val Glu Gly Lys Glu Pro Ala His Leu Met Ser Leu Phe Gly
530 535 540
Gly Lys Pro Met Ile Ile Tyr Gly Thr Ser Arg Glu Gly
545 550 555
Gly Glu Thr Ala Pro Ala Ser Thr Arg Leu Phe Glu Val Arg Ala
560 565 570
Asn Ser Ala Gly Ala Thr Arg Ala Val Glu Val Leu Pro Lys Ala
575 580 585
Gly Ala Leu Asn Ser Asn Asp Ala Phe Val Leu Lys Thr Pro Ser
590 595 600
Ala Ala Tyr Leu Trp Val Gly Thr Gly Ala Ser Ala Glu Lys
605 610 615
Thr Gly Ala Gln Glu Leu Leu Arg Val Leu Arg Ala Gln Pro Val
620 625 630
Gln Val Ala Glu Gly Ser Glu Pro Asp Gly Phe Trp Glu Ala Leu
635 640 645
Gly Gly Lys Ala Ala Tyr Arg Thr Ser Pro Arg Leu Lys Asp Lys
650 655 660
Lys Met Asp Ala His Pro Pro Arg Leu Phe Ala Cys Ser Asn Lys
665 670 675
Ile Gly Arg Phe Val Ile Glu Glu Val Pro Gly Glu Leu Met Gln
680 685 690
Glu Asp Leu Ala Thr Asp Asp Val Met Leu Leu Asp Thr Trp Asp
695 700 705
Gln Val Phe Val Trp Val Gly Lys Asp Ser Gln Glu Glu Glu Lys
710 715 720
Thr Glu Ala Leu Thr Ser Ala Lys Arg Tyr Ile Glu Thr Asp Pro
725 730 735
Ala Asn Arg Asp Arg Arg Thr Pro Ile Thr Val Val Lys Gln Gly
740 745 750
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770 775 780
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Asn Glu Arg Ser Gly Arg Ala Arg Val His Val Ser Glu Glu Gly
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Thr Glu Pro Glu Ala
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<210> SEQ ID NO 17
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Ala Val Ala Leu Ala Val Ser Pro Ala Ala Gly Ser Ser Pro Gly
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Lys Pro Pro Arg Leu Val Gly Gly Pro Met Asp Ala Ser Val Glu
35 40 45

Glu Glu Gly Val Arg Arg Ala Leu Asp Phe Ala Val Gly Glu Tyr
50 55 60

Asn Lys Ala Ser Asn Met Tyr His Ser Arg Ala Leu Gln Val
65 70 75

Val Arg Ala Arg Lys Gln Ile Val Ala Gly Val Tyr Phe Leu
80 85 90

Asp Val Glu Leu Gly Arg Thr Thr Cys Thr Lys Thr Gln Pro Asn
95 100 105

Leu Asp Asn Cys Pro Phe His Asp Gln Pro His Leu Lys Arg Lys
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Asp Phe Cys Ser Phe Gln Ile Tyr Ala Val Pro Trp Gln Gly Thr
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Met Thr Leu Ser Lys Ser Thr Cys Gln Asp Ala
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<400> SEQUENCE: 18

Glu Glu Glu Val Ser Ala Asp Met Pro Pro Pro Pro Met Asp Ala
1  5 10 15

Ser Val Glu Glu Glu
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Met Ala Thr Leu Glu Lys Leu Met Lys Ala Phe Glu Ser Leu Lys
1  5 10 15

Ser Phe Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln
20 25 30

Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Pro Pro Pro Pro

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1 5 10 15

Gln Gln

<210> SEQ ID NO 21
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Methionine Glycine Isoleucine Leucine Lysine Leucine Glutamine Leucine Valine Isoleucine Leucine Leucine Lysine Leucine Glutamine
1  5  10  15

Alanine Leucine Asparagine His Leucine Lysine Alanine Threonine Proline Isoleucine Glutamine Serine Leucine Lysine Leucine Glutamine
20  25  30

GluLysArgLysCysAsparagineThreonineAlaThrCysAlaThrGlutamineArgLeucine
35  40  45

AlaAsparaginePheLeucineValineSerineSerineAsparaginePheGlyAlaIsoleucineLeucine
50  55  60

SerineSerineThrTyrGlySerineAsparagineThreonineTyrGlyLysineAsparagineAlanine
65  70  75

ValineGlutamineValineLysineArginineGlutamateLeucineAsparagineTyrLeucineProlineLeucine
80  85

<210> SEQ ID NO 22
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Peptide

<400> SEQUENCE: 22

Leucine Alanine Asparagine Phenylalanine Valine
1  5

<210> SEQ ID NO 23
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

<220> FEATURE:
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<400> SEQUENCE: 23

Valine Phenylalanine Alanine Leucine Proline Proline Proline Leucine Alanine Asparagine Phenylalanine Valine
1  5  10

<210> SEQ ID NO 24
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<212> TYPE: PRT
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Phenylalanine Valine Serine Serine
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<210> SEQ ID NO 25
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Serine Serine Valine Leucine Phenylalanine Proline Proline Phenylalanine Valine Serine Serine
1  5  10  15
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Met Ala Ser His Arg Leu Leu Leu Cys Leu Ala Gly Leu Val 1   5   10   15

Phe Val Ser Glu Ala Gly Pro Thr Gly Thr Gly Glu Ser Lys Cys 20  25  30

Pro Leu Met Val Lys Val Leu Asp Ala Val Arg Gly Ser Pro Ala 35  40  45

Ile Asn Val Ala Val His Val Phe Arg Lys Ala Ala Asp Asp Thr 50  55  60

Trp Glu Pro Phe Ala Ser Gly Thr Ser Glu Ser Gly Glu Leu 65  70  75

His Gly Leu Thr Thr Glu Glu Phe Val Glu Gly Ile Tyr Lys 80  85  90

Val Glu Ile Asp Thr Lys Ser Tyr Trp Lys Ala Leu Gly Ile Ser 95 100 105

Pro Phe His Glu His Ala Glu Val Val Phe Thr Ala Asn Asp Ser 110 115 120

Gly Pro Arg Arg Tyr Thr Ile Ala Ala Leu Leu Ser Pro Tyr Ser 125 130 135

Tyr Ser Thr Thr Ala Val Val Thr Asn Pro Lys Glu 140 145

<210> SEQ ID NO 27
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Glu Ser Val Phe Val Leu Gly Ala Leu Pro Pro Pro Leu Ala 1   5   10   15

Gly Leu Val Phe Val Ser Glu 20

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<212> TYPE: PRT
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<220> FEATURE:
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<400> SEQUENCE: 28

Val Ala Ala Ala Lys Leu Arg Xaa Val Val Thr Ser Arg Glu Pro 1   5   10  15

Pro Pro Pro Glu Arg Ser Thr Val Val Xaa Arg Leu Lys Ala Ala 20  25  30  35

Ala Val
What is claimed is:

1. A method for detecting β-sheet conformation of insoluble proteins or prions in a sample comprising:

(a) reacting the sample with one or more α-helix or random coil conformational probes that interact with β-sheet conformation insoluble proteins or prions in the sample and thereby (i) undergo a conformational conversion to a predominantly β-sheet conformation, and (ii) form detectable aggregates with the β-sheet conformation insoluble proteins or prions in the sample; and

(b) detecting levels of detectable aggregates, wherein levels of detectable aggregates correlate to the levels of β-sheet conformation insoluble proteins or prions in the sample.

2. A method of claim 1, wherein probe termini are bound to moieties that are optically detectable when the probes form detectable aggregates with the β-sheet conformation insoluble proteins or prions in the sample.

3. A method of claim 2, wherein the moieties are fluorophores.

4. A method of claim 1, wherein probe termini are bound to radiolabeled moieties that are detectable when the probes form detectable aggregates with the β-sheet conformation insoluble proteins or prions in the sample.

5. A method of claim 1, wherein the probes comprise at least two amino acid sequences that are complementary to amino acid sequences of the β-sheet conformation insoluble proteins or prions.

6. A method of claim 1, wherein one or more of the probes comprise at least two amino acid sequences that are homologous to amino acid sequences of the β-sheet conformation insoluble proteins or prions.

7. A method claim 6, wherein one or more of the probes is a palindromic probe.

8. A method of claim 1, wherein the β-sheet conformation insoluble proteins or prions are selected from the group consisting of low-density lipoprotein receptor, cystic fibrosis transmembrane regulator, Huntingtin, Abeta peptide, prions, insulin-related amyloid, hemoglobin, alpha synuclein, rhodopsin, crystallins, and p53.

9. A method of claim 1, where one or more probes is a palindromic 33-mer comprising amino acid sequences that are homologous to amino acids 122-104 and 109-122 of the PrP<sup>Sc</sup> protein (SEQ ID NO: 1 or 29).33-mer palindrome

10. A method of claim 1, wherein one or more probes is a palindromic 33-mer comprising amino acid sequences that are equivalent to amino acids 122-104 and 109-122 of the PrP<sup>Sc</sup> protein (SEQ ID NO: 1 or 29).33-mer palindrome

11. A method of claim 1, wherein one or more probes is a palindromic 33-mer comprising amino acid sequences that are between about 70% to about 90% identical to amino acids 122-104 and 109-122 of the PrP<sup>Sc</sup> protein (SEQ ID NO: 1 or 29).33-mer palindrome

12. A method of claim 1, wherein one or more probes is a probe comprising amino acid sequences that are homologous to amino acids 1-40 of the Abeta peptide Nref 00111747 (human)

13. A method of claim 1, wherein one or more probes comprise amino acid sequences that are equivalent to amino acids 1-40 of the Abeta peptide (SEQ ID NO:4)

14. A method of claim 1, wherein one or more probes comprise amino acid sequences that are between about 70% to about 90% identical to amino acids 1-40 of the Abeta peptide (SEQ ID NO:4)
15. A method of claim 1, wherein one or more probes comprise an amino acid sequence that has a helix-loop-helix conformation found in polylysine and that is equivalent to

SEQ ID NO: 8. KKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK.

16. A method of claim 1, wherein one or more probes comprise an amino acid sequence that has a helix-loop-helix conformation found in polylysine and that is homologous to

SEQ ID NO: 8. KKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK.

17. A method of claim 1, wherein one or more probes comprise an amino acid sequence that has a helix-loop-helix conformation found in polylysine and that is equivalent to

SEQ ID NO: 8. KKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK.

18. A method of claim 1, wherein one or more probes comprise an amino acid sequence that has a helix-loop-helix conformation found in polylysine and that is between about 70% to about 90% identical to SEQ ID NO: 8.

SEQ ID NO: 8. KKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK.

19. A method of claim 1, wherein one or more probes comprise amino acid sequences that are homologous to amino acids 104-122 of wild-type (wt) TSE (SEQ ID NO:10)

(SEQ ID NO:10) KPKTNLKHVAGAAAAAGAVV.

20. A method of claim 1, wherein one or more probes comprise amino acid sequences that are equivalent to amino acids 104-122 of wild-type (wt) TSE (SEQ ID NO:10).

(SEQ ID NO:10) KPKTNLKHVAGAAAAAGAVV.

21. A method of claim 1, wherein one or more probes comprise amino acid sequences that are between about 70% to about 90% identical to amino acids 104-122 of wild-type

(SEQ ID NO:10) KPKTNLKHVAGAAAAAGAVV.

22. A method of claim 1, wherein one or more probes comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious;

and (c) has an amino acid sequence that is homologous to

SEQ ID NO: 10 KPKTNLKHVAGAAAAAGAVV.

23. A method of claim 1, wherein one or more probes comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious;

and (c) has an amino acid sequence that is equivalent to SEQ ID NO: 10

SEQ ID NO: 10 KPKTNLKHVAGAAAAAGAVV.

24. A method of claim 1, wherein one or more probes comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious;

and (c) has an amino acid sequence that is between about 70% to about 90% identical to SEQ ID NO: 10

SEQ ID NO: 10 KPKTNLKHVAGAAAAAGAVV.

25. The method of claim 1, wherein the probes comprise an extrinsic fluor.

26. The method of claim 25, wherein the extrinsic fluor is pyrene.

27. A method of claim 1, further comprising reacting the sample and probes prior to detecting with a probe that limits the formation of detectable aggregates to detectable but non-infectious levels.

28. A method of claim 1, wherein levels of detectable aggregates are compared to levels of β-sheet conformation insoluble proteins or prions associated with amyloidogenic diseases.

29. A method of claim 1, wherein the β-sheet conformation insoluble proteins or prions form amyloid plaques or amyloid deposits associated with amyloidogenic diseases.

30. A method of claim 1, wherein the sample is disaggregated prior to reaction with the probe.

31. A method of claim 1, wherein the sample is a tissue sample or is a liquid biological material obtained from spinal fluid, saliva, urine or other bodily fluids.

32. A method of claim 1, wherein excimers are formed by reacting one or more α-helix or random coil conformational probes with β-sheet conformation insoluble proteins or prions in the sample.

33. A kit comprising one or more α-helix or random coil conformational probes that interact with β-sheet conformation insoluble proteins or prions in a sample and thereby (a) undergo a conformational conversion to a predominately β-sheet conformation, and (b) form detectable aggregates with the β-sheet conformation insoluble proteins or prions in the sample, wherein levels of detectable aggregates correlate to the levels of β-sheet conformation insoluble proteins or prions in the sample.

34. A kit of claim 33, wherein probe termini are bound to moieties that are optically detectable when the probes form detectable aggregates with β-sheet conformation insoluble proteins or prions in a sample.

35. A kit of claim 34, wherein the moieties are fluorophores.

36. A kit of claim 33, wherein probe termini are bound to radionuclide moieties that are detectable when the probes form detectable aggregates with β-sheet conformation insoluble proteins or prions in a sample.
37. A kit of claim 33, wherein the probes comprise at least two amino acid sequences that are complementary to amino acid sequences of β-sheet conformation insoluble proteins or prions.

38. A kit of claim 33, wherein one or more of the probes comprise at least two amino acid sequences that are homologous to amino acid sequences of β-sheet conformation insoluble proteins or prions.

39. A kit of claim 33, wherein one or more of the probes comprise an amino acid sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 20, 22, 23, 24, 25 or 27.

40. A kit of claim 33, wherein the β-sheet conformation insoluble proteins or prions are selected from the group consisting of low-density lipoprotein receptor, cystic fibrosis transmembrane regulator, Huntingtin, Abeta peptide, prions, insulin-related amyloid, hemoglobin, alpha synuclein, rhodopsin, crystallins, and p53.

41. A kit of claim 33, wherein one or more probes is a palindromic 33-mer comprising amino acid sequences that are homologous to amino acids 122-104 and 109-122 of the human or murine PrPsc protein (SEQ ID NO: 1 or 29)

\[
\text{VYAGAAAAGAVHKLNPXKLLHVGAGAAAAGAVV} \quad \text{(murine)}
\]

\[
\text{VYAGAAAAGAVHKLNPXKLLHVGAGAAAAGAVV} \quad \text{(human)}
\]

42. A kit of claim 33, wherein one or more probes is a palindromic 33-mer comprising amino acid sequences that are equivalent to amino acids 122-104 and 109-122 of the PrPsc protein (SEQ ID NO: 1 or 29).

\[
\text{VYAGAAAAGAVHKLNPXKLLHVGAGAAAAGAVV} \quad \text{(murine)}
\]

\[
\text{VYAGAAAAGAVHKLNPXKLLHVGAGAAAAGAVV} \quad \text{(human)}
\]

43. A kit of claim 33, wherein one or more probes is a palindromic 33-mer comprising amino acid sequences that are between about 70% to about 90% identical to amino acids 122-104 and 109-122 of the PrPsc protein (SEQ ID NO: 1 or 29).

\[
\text{VYAGAAAAGAVHKLNPXKLLHVGAGAAAAGAVV} \quad \text{(murine)}
\]

\[
\text{VYAGAAAAGAVHKLNPXKLLHVGAGAAAAGAVV} \quad \text{(human)}
\]

44. A kit of claim 33, wherein one or more probes is a probe comprising amino acid sequences that are homologous to amino acids 1-40 of the Abeta peptide (SEQ ID NO: 4).

\[
\text{DAEFRHDSGYEVHHQKLVFFAEDVGSNK-GAILGLVAGV} \quad \text{}\]

45. A method of claim 1, wherein one or more probes comprise amino acid sequences that are equivalent to amino acids 1-40 of the Abeta peptide (SEQ ID NO: 4).

\[
\text{DAEFRHDSGYEVHHQKLVFFAEDVGSNK-GAILGLVAGV} \quad \text{}\]

46. A kit of claim 33, wherein one or more probes comprise amino acid sequences that are between about 70% to about 90% identical to amino acids 1-40 of the Abeta peptide (SEQ ID NO: 4).

\[
\text{DAEFRHDSGYEVHHQKLVFFAEDVGSNK-GAILGLVAGV} \quad \text{}\]

47. A kit of claim 33, wherein one or more probes comprise an amino acid sequence that is equivalent or homologous to SEQ ID NO: 9 or 20.

48. A kit of claim 33, wherein one or more probes comprise an amino acid sequence that has a helix-loop-helix conformation found in polylysine and that is homologous to SEQ ID NO: 8.

49. A kit of claim 33, wherein one or more probes comprise an amino acid sequence that has a helix-loop-helix conformation found in polylysine and that is equivalent to SEQ ID NO: 8.

50. A kit of claim 33, wherein one or more probes comprise an amino acid sequence that has a helix-loop-helix conformation found in polylysine and that is between about 70% to about 90% identical to SEQ ID NO: 9.

51. A kit of claim 33, wherein one or more probes comprise amino acid sequences that are homologous to amino acid sequences 104-122 of wild-type (wt) TSE (SEQ ID NO: 10).

\[
\text{KPEFNYHVGAGAAAAGAVV} \quad \text{}\]

52. A kit of claim 33, wherein one or more probes comprise amino acid sequences that are equivalent to amino acid sequences 104-122 of wild-type (wt) TSE (SEQ ID NO: 10).

\[
\text{KPEFNYHVGAGAAAAGAVV} \quad \text{}\]

53. A kit of claim 33, wherein one or more probes comprise amino acid sequences that are between about 70% to about 90% identical to amino acid sequences 104-122 of wild-type (wt) TSE (SEQ ID NO: 10).

\[
\text{KPEFNYHVGAGAAAAGAVV} \quad \text{}\]

54. A kit of claim 33, wherein one or more probes comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and

(c) has an amino acid sequence that is homologous to SEQ ID NO: 10.

55. A kit of claim 33, wherein one or more probes comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and

(c) has an amino acid sequence that is equivalent to SEQ ID NO: 10.

56. A kit of claim 33, wherein one or more probes comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and

(c) has an amino acid sequence that is between about 70% to about 90% identical to SEQ ID NO: 10.

57. A kit of claim 33, wherein the probes comprise an extrinsic fluor.

58. A kit of claim 57, wherein the extrinsic fluor is pyrene.

59. A kit of claim 33, further comprising a pendant probe that limits the formation of detectable aggregates to detectable but non-infectious levels.

60. A method of diagnosing whether a subject suffers from, or is predisposed to, a disease associated with conformationally altered proteins or prion comprising:
(a) obtaining a sample from the subject;
(b) reacting the sample with one or more α-helix or random coil conformational probes that interact with β-sheet conformation insoluble proteins or prions in the sample and thereby (i) undergo a conformational conversion to a predominately β-sheet conformation, and (ii) form detectable aggregates with the β-sheet conformation insoluble proteins or prions in the sample; and
(c) detecting levels of detectable aggregates, wherein levels of detectable aggregates correlate to the amount of β-sheet conformation insoluble proteins or prions in the sample, and level of infectiousness of, the sample and indicate whether the subject suffers from, or is predisposed to, a disease associated with β-sheet conformation insoluble proteins or prions.

60. A method of claim 59, wherein one or more probes is a palindromic 33_mer comprising amino acid sequences that are between about 70% to about 90% identical to amino acids 122-104 and 109-122 of the PrPSC protein (SEQ ID NO: 1 or 29).

(SEQ ID NO:4) DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGV

61. A method of claim 59, wherein one or more probes is a probe comprising amino acid sequences that are homologous to amino acids 1-40 of the Abeta peptide (SEQ ID NO:4)

(SEQ ID NO:4) DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGV

62. A method of claim 60, wherein one or more of the probes comprise at least two amino acid sequences that are complementary to amino acid sequences of the β-sheet conformation insoluble proteins or prions.

63. A method of claim 60, wherein probe termini are bound to m moieties that are optically detectable when the probes form detectable aggregates with the β-sheet conformation insoluble proteins or prions in the sample.

64. A method of claim 60, wherein the probes comprise at least two amino acid sequences that are homologous to amino acid sequences of the β-sheet conformation insoluble proteins or prions.

65. A method of claim 60, wherein one or more of the probes comprise at least two amino acid sequences that are homologous to amino acid sequences of the β-sheet conformation insoluble proteins or prions.

66. A method claim 60, wherein one or more of the probes comprise an amino acid sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 18, 20, 22, 23, 24, 25 or 27.

67. A method of claim 60, wherein the β-sheet conformation insoluble proteins or prions are selected from the group consisting of low-density lipoprotein receptor, cystic fibrosis transmembrane regulator, Huntington, Abeta peptide, prions, insulin-related amyloid, hemoglobin, alpha synuclein, rhodopsin, crystallins, transthyretin, gelsolin, cystatins and p53.

68. A method of claim 60, wherein one or more probes is a palindromic 33_mer comprising amino acid sequences that are homologous to amino acids 122-104 and 109-122 of the PrPSCI protein (SEQ ID NO: 1 or 29).

VVGAAAAGAVHKLMTKPLHVGAAAAGAVVV (murine)

VVGAAAAGAVKHMNTKPKHMVGAAAAGAVVV (human)

69. A method of claim 60, wherein one or more probes is a palindromic 33_mer comprising amino acid sequences that are equivalent to amino acids 122-104 and 109-122 of the PrP SM protein (SEQ ID NO:1 or 29).

VVGAAAAGAVHKLMTKPLHVGAAAAGAVVV (murine)

VVGAAAAGAVKHMNTKPKHMVGAAAAGAVVV (human)

70. A method of claim 60, wherein one or more probes is a palindromic 33_mer comprising amino acid sequences that are between about 70% to about 90% identical to amino acids 122-104 and 109-122 of the PrPSC protein (SEQ ID NO: 1 or 29).

VVGAAAAGAVHKLMTKPLHVGAAAAGAVVV (murine)

VVGAAAAGAVKHMNTKPKHMVGAAAAGAVVV (human)

71. A method of claim 60, wherein one or more probes is a probe comprising amino acid sequences that are homologous to amino acids 1-40 of the Abeta peptide (SEQ ID NO:4)

(SEQ ID NO:4) DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGV

72. A method of claim 60, wherein one or more probes comprise amino acid sequences that are equivalent to amino acids 1-40 of the Abeta peptide (SEQ ID NO:4)

(SEQ ID NO:4) DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGV

73. A method of claim 60, wherein one or more probes comprise amino acid sequences that are between about 70% to about 90% identical to amino acids 1-40 of the Abeta peptide

(SEQ ID NO:4) DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGV

74. A method of claim 60, wherein one or more probes comprise amino acid sequence that is an oligo or polylysine.

75. A method of claim 74, wherein said probe is homologous to SEQ ID NO: 8.

76. A method of claim 60, wherein one or more probes are equivalent to SEQ ID NO: 8.

77. A method of claim 60, wherein one or more probes comprise an amino acid sequence that has a helix-loop-helix conformation found in lysine and that is between about 70% to about 90% identical to oligo- or polylysine.

78. A method of claim 61, wherein one or more probes comprise amino acid sequences that are homologous or equivalent to amino acids 104-122 of wild-type (wt) TSE (SEQ ID NO:10).

79. A method of claim 60, wherein one or more probes comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and

(c) has an amino acid sequence that is homologous or equivalent to SEQ ID NO: 10.

80. A method of claim 61, wherein one or more probes comprise an amino acid sequence that: (a) is a selectively mutated TSE sequence; (b) is destabilized and noninfectious; and

(c) has an amino acid sequence that is between about 70% to about 90% identical to SEQ ID NO: 10.
81. A method of claim 60, wherein the probes comprise an extrinsic fluor.

82. The method of claim 60, wherein the extrinsic fluor is pyrene.

83. A method of claim 60, further comprising reacting the sample and probes prior to detecting with a pendant probe that limits the formation of detectable aggregates to detectable but non-infectious levels.

84. A method of claim 60, wherein levels of detectable aggregates are compared to levels of β-sheet conformation insoluble proteins or prions associated with amyloidogenic diseases.

85. A method of claim 60, wherein the β-sheet conformation insoluble proteins or prions form amyloid plaques or amyloid deposits associated with amyloidogenic diseases.

86. A method of claim 60, wherein the sample is disaggregated prior to reaction with the probe.

87. A method of claim 60, wherein the sample is a tissue sample or is a liquid biological material obtained from spinal fluid, saliva, urine or other bodily fluids.

88. A method of claim 60, wherein the probes are formed by reacting one or more α-helix or random coil conformational probes with β-sheet conformation insoluble proteins or prions in the sample.

89. A palindromic peptide probe comprising three peptide sections, a first peptide section, a second peptide section and a third peptide section, said first and said third sections comprising peptide sequences each of which comprises at least 5 amino acids identical to a peptide fragment from a target insoluble protein which is responsible for β-sheet formation in said target insoluble protein and wherein at least a portion of said first peptide section is a palindrome of at least a portion of said third peptide section, said first peptide section or said third peptide section being identical to at least a five amino acid peptide sequence in said peptide fragment from said target insoluble protein, said second peptide sequence comprising between 1 and 10 amino acid units one of which is a proline residue.

90. The probe according to claim 89 wherein said first and said third sections are endcapped with hydrophobic amino acids which can be chemically modified or complexed to accommodate a chemical moiety capable of being measured.

91. The probe according to claim 90 wherein said chemical moiety is a chromophore and both said first and third peptide sections of said probe comprise said chromophore.

92. The probe according to claim 90 wherein said chromophore is selected from the group consisting of pyrene, tryptophan, fluorescein rhodamine.

93. The probe according to claim 92 which is in the form of an excimer.

94. The probe according to claim 89 wherein said second proline section comprises between 1 and 5 amino acid residues all of which are proline residues.

95. The probe according to claim 89 wherein said target peptide is selected from the group consisting of low-density lipoprotein receptor, cystic fibrosis transmembrane regulator, Huntington, Abeta peptide, prions, insulin-related amyloid, hemoglobin, alpha synuclein, rhodopsin, crystallins, transthyretin, gelatin, cystatins and p53.

96. The probe according to claim 89 wherein said first peptide section and said third peptide section consist of identical amino acids.

97. The probe according to claim 89 wherein said first and said second peptide sections each comprise about 10 to about 25 amino acid residues.

98. The palindromic probe according to claim 89 selected from the group consisting of SEQ ID NO: 1, 18, 23, 25, 27 and 29.

99. The method according to claim 60 wherein said disease is Alzheimer’s Disease, Prion diseases, Creutzfeldt Jakob disease, scrapie and bovine spongiform encephalopathy (PrPsc); ALS (SOD and neurofilament); Pick’s disease; Parkinson’s disease, Frontotemporal dementia; Diabetes Type II (Amylin); Multiple myeloma—plasma cell dyscrasias; Familial amyloidotic polyneuropathy; Medullary carcinoma of thyroid;

Chronic renal failure, Congestive heart failure, Senile cardiac and systemic amyloidosis (transhyretin), Chronic inflammation, Atherosclerosis, Familial amyloidosis, or Huntington’s disease.

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