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(54) Title: ASSEMBLIES OF OLIGOMERIC AMYLOID BETA PROTEIN AND USES THEREOF

(57) Abstract: The present invention provides assemblies of oligomeric amyloid beta protein and uses thereof.



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5 ASSEMBLIES OF OLIGOMERIC AMYLOID BETA PROTEIN
AND USES THEREOF

CONTINUING APPLICATION DATA

10 This application claims the benefit of U.S. Provisional Application Serial
No. 60/621,547, filed October 22, 2004, U.S. Provisional Application Serial No.
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20 BACKGROUND

The amyloid- β protein (A β) is implicated in the pathogenesis of
Alzheimer's disease (AD). Functional imaging and neuropathological data support
the fact that brain dysfunction in AD precedes neuron loss, leading to the
prediction that specific forms of A β could disrupt memory before there is
25 significant structural brain damage. The A β peptides are the major amyloid
protein deposited in AD brains and both natural and synthetic forms have
devastating effects on the viability and function of neurons. See, for example,
Yankner et al., Science 250, 279-82 (1990); Pike et al., Brain Res 563, 311-4
(1991); Pike et al., J Neurosci 13, 1676-87 (1993); Lambert et al., Proc Natl Acad
30 Sci U S A 95, 6448-53 (1998); Walsh et al., Nature 416, 535-9 (2002); and Kaye
et al., Science 300, 486-9 (2003). However, the identification and isolation of
endogenous A β species causing brain dysfunction in AD has remained elusive
because of the difficulty of correlating memory loss with distinct isolates of A β
from subcellular compartments of the brain. There has been a long-standing
35 debate as to whether the fibrillar, insoluble A β that is deposited in amyloid plaques

disrupts memory in AD. The early studies showed an inverse relationship between amyloid load and cognitive function (Blessed et al., Br J Psychiatry 114, 797-811 (1968)). Nevertheless, the hypothesis that fibrillar A β is the cause of memory deficits has been undermined by the observation that some individuals with very
5 high plaque loads have normal cognitive function (Crystal et al., Neurology 38, 1682-7 (1988); Arriagada et al., Neurology 42, 1681-8 (1992); Giannakopoulos et al., Arch Neurol 52, 1150-9 (1995); Lue et al., J Neuropathol Exp Neurol 55, 1083-8 (1996); and Davis et al., J Neuropathol Exp Neurol 58, 376-88 (1999)). In addition, several groups have recently demonstrated correlations between memory
10 loss and soluble, non-fibrillar A β (Kuo et al., J Biol Chem 271, 4077-81 (1996); McLean et al., Ann Neurol 46, 860-6 (1999); and Lue et al., Am J Pathol 155, 853-62 (1999)). Yet, the precise form of the soluble A β responsible for the deficits in AD has not been established.

15

SUMMARY OF THE INVENTION

The present invention includes isolated, soluble amyloid- β protein (A β) assemblies having more than one detergent stable oligomer of amyloid- β proteins. In some embodiments, the amyloid- β protein assembly disrupts cognitive function.
20 In some embodiments, the amyloid- β protein assembly has a molecular weight of about 40 kilodaltons (kDa) as measured by SDS polyacrylamide gel electrophoresis. In some embodiments, the amyloid- β protein assembly has a molecular weight of about 56 kDa as measured by SDS polyacrylamide gel electrophoresis. In some embodiments, the amyloid- β protein assembly includes
25 detergent stable dimers of amyloid- β protein. In some embodiments, the amyloid- β protein assembly includes detergent stable trimers of amyloid- β protein. In some embodiments, the amyloid- β protein assembly includes detergent stable tetramers of amyloid- β protein. In some embodiments, the amyloid- β protein assembly includes detergent stable hexamers of amyloid- β protein.
30 The present invention includes isolated, soluble amyloid- β protein assemblies having a dodecamer of amyloid- β proteins. In some embodiments, the dodecamer of amyloid- β proteins includes six detergent stable dimers of amyloid- β protein. In some embodiments, the dodecamer of amyloid- β proteins includes four

detergent stable trimers of amyloid- β protein. In some embodiments, the dodecamer of amyloid- β proteins includes three detergent stable tetramers of amyloid- β protein. In some embodiments, the dodecamer of amyloid- β proteins includes two detergent stable hexamers of amyloid- β protein. In some
5 embodiments, the dodecamer of amyloid- β proteins has a molecular weight of about 56 kDa as measured by SDS polyacrylamide gel electrophoresis.

The present invention includes isolated, soluble amyloid- β protein assemblies having more than one detergent stable trimer of amyloid- β proteins. In some embodiments, the amyloid- β protein assemblies include three detergent
10 stable amyloid- β protein trimers. In some embodiments, the amyloid- β protein assemblies include four detergent stable amyloid- β protein trimers. In some embodiments, the amyloid- β protein assemblies have a molecular weight of about 40 kDa as measured by SDS polyacrylamide gel electrophoresis. In some
embodiments, the amyloid- β protein assemblies have a molecular weight of about
15 56 kDa as measured by SDS polyacrylamide gel electrophoresis.

The present invention includes isolated, soluble amyloid- β protein assemblies having more than one detergent stable tetramer of amyloid- β proteins. In some embodiments, the amyloid- β protein assemblies include three detergent
stable amyloid- β protein tetramers. In some embodiments, the amyloid- β protein
20 assemblies have a molecular weight of about 56 kDa as measured by SDS polyacrylamide gel electrophoresis.

The isolated, soluble amyloid- β protein assemblies of the present invention may disrupt cognitive function.

The present invention includes compositions including isolated, soluble
25 amyloid- β protein assemblies.

The present invention includes vaccines including isolated, soluble amyloid- β protein assemblies.

The present invention includes antibodies that bind to amyloid- β protein assemblies. In some embodiments, antibodies that bind to amyloid- β protein
30 assemblies do not bind to monomeric amyloid- β protein. In some embodiments, antibodies that bind to amyloid- β protein assemblies do not bind to dimeric amyloid- β protein. In some embodiments, antibodies that bind to amyloid- β protein assemblies do not bind to trimeric amyloid- β protein. In some

embodiments, antibodies that bind to amyloid- β protein assemblies do not bind to tetrameric amyloid- β protein.

The present invention includes a method of disrupting memory of learned behavior in a mammal, the method including administering amyloid- β protein assemblies intracranially. In some embodiments of the method, the mammal may be a mouse, rat, dog, or non-human primate. In some embodiments of the method, the amyloid- β protein assembly includes four detergent stable amyloid- β protein trimers. In some embodiments of the method, the amyloid- β protein assembly includes three detergent stable amyloid- β protein tetramers. In some embodiments of the method, the mammal serves an animal model system for a cognitive deficit. In some embodiments of the method, the mammal serves an animal model system for Alzheimer's disease. In some embodiments of the method, the mammal demonstrates cognitive deficits consistent with presymptomatic or early Alzheimer's disease.

The present invention includes an animal model, the animal model including a mammal wherein an amyloid- β protein assembly has been administered intracranially. In some embodiments of the animal model, the mammal is a mouse, rat, dog, or non-human primate. In some embodiments of animal model, the mammal demonstrates disruption of complex learned behaviors. In some embodiments of animal model, the mammal demonstrates cognitive deficits consistent with presymptomatic or early Alzheimer's disease.

The present invention includes a method of screening for an agent effective for the treatment of a cognitive disorder, the method including administering a test agent to a first animal to which a soluble amyloid- β protein assembly having more than one detergent stable oligomer of amyloid- β proteins has been intracranially administered; measuring cognitive function of the first animal; comparing the cognitive function of the first animal to the cognitive function of a second animal to which a soluble amyloid- β protein ($A\beta$) assembly having more than one detergent stable oligomer of amyloid- β proteins has been intracranially administered, but no test agent has been administered; wherein an improvement in the cognitive function of the first animal compared to the cognitive function of the second animal indicates the test agent is an effective agent for the treatment of a cognitive disorder. In some embodiments of the method, the cognitive disorder is a mild cognitive impairment. In some embodiments of the method, the cognitive

disorder is an age related memory decline or an age associated memory impairment. In some embodiments of the method, the cognitive disorder is Alzheimer's disease.

5 The present invention includes a method of treating a cognitive disorder in a subject, the method including administering to the subject an agent that inhibits the assembly of monomers of amyloid β protein into soluble amyloid- β protein assemblies having more than one detergent stable oligomer of amyloid- β proteins. In some embodiments of the method, the cognitive disorder is a mild cognitive impairment. In some embodiments of the method, the cognitive disorder is an age
10 related memory decline or an age associated memory impairment. In some embodiments of the method, the cognitive disorder is Alzheimer's disease.

The present invention includes agents that inhibit the assembly of detergent-stable oligomers of amyloid β protein into soluble amyloid- β protein assemblies. The present invention includes agents that promote the clearance of
15 soluble amyloid- β protein assemblies from neurological tissue.

The present invention includes a method of treating a cognitive disorder in a subject, the method including administering to the subject an agent that inhibits the assembly of detergent-stable oligomers of amyloid β protein into a soluble amyloid- β protein assembly including more the one detergent stable oligomer of
20 amyloid- β proteins. In some embodiments of the method, the cognitive disorder is a mild cognitive impairment. In some embodiments of the method, the cognitive disorder is an age related memory decline or an age associated memory impairment. In some embodiments of the method, the cognitive disorder is Alzheimer's disease.

25 The present invention includes a method of treating a cognitive disorder in a subject, the method including administering to the subject an agent that promotes the clearance from neurological tissue of a soluble amyloid- β protein assembly including more than one detergent-stable oligomer of amyloid- β proteins. In some embodiments of the method, the cognitive disorder is a mild cognitive impairment.
30 In some embodiments of the method, the cognitive disorder is an age related memory decline or an age associated memory impairment. In some embodiments of the method, the cognitive disorder is Alzheimer's disease.

The present invention includes a method of detecting a cognitive disorder in a subject, the method including detecting in a fluid or tissue sample taken from

the subject soluble amyloid- β protein assemblies including more than one detergent stable oligomer of amyloid- β proteins. In some embodiments of the method, the cognitive disorder is a mild cognitive impairment. In some embodiments of the method, the cognitive disorder is an age related memory decline or an age associated memory impairment. In some embodiments of the method, the cognitive disorder is Alzheimer's disease.

The present invention includes a method of detecting a presymptomatic cognitive disorder in a subject, the method including detecting in a fluid or tissue sample taken from the subject soluble amyloid- β protein assemblies including more than one detergent stable oligomer of amyloid- β proteins. In some embodiments of the method, the cognitive disorder is a mild cognitive impairment. In some embodiments of the method, the cognitive disorder is an age related memory decline or an age associated memory impairment. In some embodiments of the method, the cognitive disorder is Alzheimer's disease.

The present invention includes a method for assaying the effects of soluble oligomers of the amyloid β protein on cognitive function, the method including administering a soluble amyloid- β protein assembly comprising more than one detergent-stable oligomer of amyloid- β proteins intracranially into an animal and measuring cognitive function to determine the disruption of cognitive behavior. In some embodiments of the method, the disruption of cognitive behavior of the animal is compared to the long-term disruption of cognitive behavior of another animal treated in the same fashion except saline rather a soluble amyloid- β protein assembly comprising one or more one detergent-stable oligomer of amyloid- β proteins is administered intracranially. In some embodiments of the method, the cognitive disorder is a mild cognitive impairment. In some embodiments of the method, the cognitive disorder is an age related memory decline or an age associated memory impairment. In some embodiments of the method, the cognitive disorder is Alzheimer's disease.

The present invention includes methods of isolating soluble amyloid- β protein assemblies including more than one detergent-stable oligomer of amyloid- β proteins, the method including homogenizing neuronal tissue in a lysis buffer; size fractionating amyloid- β protein ($A\beta$) assemblies; and isolating an amyloid- β protein ($A\beta$) assembly of a desired size.

Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.

BRIEF DESCRIPTION OF THE FIGURES

5 Figures 1A-1C. Temporal patterns of memory decline and soluble, extracellular A β oligomers in Tg2576 mice. In Fig. 1A spatial memory in mice from 4 to 17 months shows a progressive but irregular decline with periods of stability. Tg2576^{+/+} (filled circles, ●), Tg2576^{-/-} (open circles, ○). ANOVA, $p < 0.01$, followed by t test, $*p < 0.01$. In Fig. 1B a temporal analysis of spatial
10 memory shows three stages of performance. Tg2576^{+/+} (filled bars), Tg2576^{-/-} (open bars). ANOVA, $*p < 0.01$, $**p < 0.001$. Fig. 1C is an identification of A β oligomers in soluble, extracellular-enriched extracts of proteins from brains of 5-, 6-, and 7-month mice, assessed by western blot (WB) with or without immunoprecipitation (IP). The intensity of the 40 kDa band co-migrating with
15 nonamers was $33.92 \pm 12.5\%$ ($n = 4$) of the intensity of the 56 kDa band corresponding to dodecamers.

 Figures 2A-2H. Water maze performance correlates inversely with A β nonamer and dodecamer levels. A lack of significant correlations between spatial memory and monomeric (Fig. 2A and Fig. 2D), trimeric (Fig. 2B and Fig. 2E), or
20 hexameric (Fig. 2C and Fig. 2F) soluble A β species was detected in the extracellular-enriched fractions of 5-month (open circles, ○) and 6-month (filled circles, ●) Tg2576^{+/+} mice. Nonameric (Fig. 2G) and dodecameric (Fig. 2H) A β levels correlate inversely with spatial memory at 6 months (ANOVA).

 Figures 3A-3C. A β *56 disrupts cognitive function. In Fig. 3A soluble
25 proteins in extracellular-enriched extracts from Tg2576^{+/+} or Tg2576^{-/-} mice were fractionated by SEC to generate fractions with (19^{+/+}) or without (17^{+/+} and 19^{-/-}) the 56 kDa A β species. Bands at 75 and 150 kDa (arrows) present in non-Tg littermates and IP19^{+/+} were considered to be non-specific (ns). Fig. 3B presents Perseveration Errors, presented as a percentage of baseline rates ($\pm$ SEM), in the
30 ALCR paradigm two hours after rats received injections of the indicated fractions into the lateral ventricles. Fig. 3C presents Switching Errors, presented as a percentage of baseline rates ($\pm$ SEM), in the ALCR paradigm two hours after rats received injections of the indicated fractions into the lateral ventricles. Wilcoxon matched-pairs signed-ranks test, $*p < 0.05$.

Figures 4A-4E. Elimination of A β *56 coincides with an interlude of normal spatial memory. Fig. 4A shows A β *56 levels decline in Tg2576^{+/-} mice between 12.0-12.4 months of age. Fig. 4B shows levels of A β *56 between 10.7-13.0 months of age. Values represent band intensities (mean $\pm$ SD) relative to the intensities observed at 10.7 months. ANOVA, $p < 0.02$, followed by t test, * $p < 0.01$, $n = 4$ animals per age group. In Fig. 4C the dip in A β *56 levels coincides with a transient recovery of spatial memory (target quadrant occupancy $\pm$ SEM in Day 5 probe trial). ANOVA, $p < 0.01$, followed by t test, * $p < 0.01$, ** $p < 0.001$, and by Fisher's protected least significant difference (PLSD) test, ‡ $p < 0.001$, # $p < 0.05$. Tg2576^{+/-} (filled bars), Tg2576^{-/-} (open bars). Fig. 4D demonstrates that the kinetics of the rate of change in A β (x-42) levels (SDS-soluble A β (x-42) [open circles, ○] and SDS-insoluble A β (x-42) [filled squares, ■]) in Tg2576^{+/-} mice. Abbreviations: FA = SDS-insoluble/formic acid-soluble; Δt = time interval. Fig. 4E presents a hypothetical dynamic relationship between soluble and insoluble pools of A β .

Figures 5A-5C. Fidelity of the technique for measuring soluble, extracellular A β oligomers in vivo. Fig. 5A illustrates the procedures used to extract A β . The forebrain is subjected to a four step extraction protocol generating four fractions (extracellular-enriched soluble (EC), intracellular-enriched soluble (IC), membrane-enriched (MB), and insoluble (Insol)). Fig. 5B presents SDSPAGE analyses of various protein markers in the four collected fractions (extracellular-enriched soluble (EC), intracellular-enriched soluble (IC), membrane-enriched (MB), and insoluble (Insol)). Partial characterization and validation of the fractionation procedure was achieved using the NMDA receptor subunit NR2 as a marker for membrane-enriched proteins, APP as a marker for both soluble (sAPP α) and membrane-enriched (full-length APP = fl-APP) proteins, and the extracellular serine protease tissue-type plasminogen activator t-PA, as a marker for soluble proteins. Other protein markers used were microtubule-associated proteins MAP-2 and tau (cytoskeleton), the protein kinases, ERKs and JNK (cytosol), and flotillin-2 (lipid rafts). Fig. 5C demonstrates a validation of the use of immunoprecipitation to study A β species quantitatively. To ensure that immunoprecipitated A β species were a faithful index of brain A β levels, two sequential immunoprecipitations (IP1 and IP2) with either 6E10 or 4G8 monoclonal antibodies were performed and revealed negligible amounts ($1 \pm$

0.64%, n = 4) of 6E10 immunoreactive material in western blot after the first immunoprecipitation in extracts from the oldest animals (25 months).

Figures 6A-6D. Biochemical and structural properties of A β assemblies in Tg2576 mice. Fig. 6A shows soluble, extracellular-enriched A β species purified using affinity columns packed with 200 μ g of 6E10 or 4G8 antibodies. Captured proteins were eluted in acidic buffer (pH 3), fractionated by SDS-PAGE, and WB were revealed with 6E10. Fig. 6B shows multimers are resistant to the strong chaotropic agent, 8M urea. Soluble, extracellular-enriched extracts from 12- to 20-month Tg2576+/- brains were loaded onto 8M urea containing SDS-PAGE gels, electrotransferred, and probed with 6E10. The presence of urea did not alter electrophoretic migration patterns, indicating that A β oligomers are probably not associated with large globular proteins. Fig. 6C shows soluble HMW A β oligomers are not resistant to treatment with $\geq 15\%$ hexafluoroisopropanol (HFIP). Monomeric A β levels increased with rising HFIP concentrations. Trimers were exceptionally resistant to HFIP. Fig. 6D is an evaluation of A β multimers with the anti-oligomer antibody, A11.

Figures 7A-7D. Characterization of native A β oligomer size and expression levels in Tg2576 mice. Fig. 7A is a SDS-PAGE analysis of soluble brain extracts fractionated by SDS-free-size-exclusion chromatography (SEC) showed that all A β oligomers migrated at expected molecular weights using globular protein standards. Bands revealed at 75 and 150 kDa are non-specific bands which are also present in blots of extracts from non-transgenic mice. Fig. 7B shows soluble, extracellular-enriched A β species assessed by western blot using 6E10 in mice between 9 and 25 months of age. Fig. 7C represents a semi-quantification of A β species levels (relative to β -tubulin levels) expressed as percentage of respective averaged signals observed in 9- month-old animals (n = 6/age group) for 1-mer, 3-mer, 4-mer, 6-mer, and 9-mer. Fig. 7D 7C represents a semi-quantification of A β species levels (relative to β -tubulin levels) expressed as percentage of respective averaged signals observed in 9- month-old animals (n = 6/age group) for 12-mers. ANOVA, followed by t test, *p < 0.01, # p < 0.05.

Figures 8A and 8B. Absence of correlation between A β oligomer levels and swimming speed or path length during the cued (or visible) phase of water maze testing. Fig. 8A shows the relationship between swimming speed and A β levels in 5-month-old Tg2576+/- mice. Fig. 8B shows the relationship between

swimming speed and A β levels in 6-month-old Tg2576+/- mice. ANOVA p values are displayed in graphs alongside r² values.

Figures 9A-9C. No change in levels of intracellular A β levels and in CTFs in 5- and 6-month Tg2576 mice. In Fig. 9A soluble, intracellular-enriched A β species in 5-, 6- and 7-month mice was evaluated by western blot (WB) using 6E10. Fig. 9A shows levels of sAPP α . Fig. 9B demonstrates compartmentalization of APP-CTFs in Tg2576 mouse brain. CTF- β s were immunoprecipitated (IP'd) from soluble or membrane extracts with 6E10 and identified in WB with APPCter-C17. No immunoreactive bands were detected in the soluble fraction, but a doublet of bands around approximately 12 kDa was present in the membrane-enriched fraction. As a control, both CTF- β and CTF- α were detected with APPCter-C17. In Fig. 9C IPs of CTFs using APPCter- C17 confirmed no overall change in CTFs between 5 to 7 months of age. Full-length APP also captured by the antiserum is displayed on top and shows no variation in levels.

Figures 10A-10E. Trimers are the predominant oligomeric A β species secreted from Tg2576 cultured primary brain cells. Fig. 10A shows levels of naturally secreted A β species in the conditioned media (CM) of 7- or 14-DIV (days in vitro)-old neurons evaluated by IP followed by WB with 6E10 antibodies. Fig. 10B shows cortical astrocytes modulate the levels of neuron-derived A β species in the CM. Fig. 10C shows boiling the membranes enhances the detection of monomeric A β with 6E10, which constitutes the major A β species secreted in the CM. However, boiling did not significantly enhance the ability to detect the oligomeric A β species. Fig. 10D shows intracellular protein preparations from Tg2576+/- primary neurons devoid of APP-CTFs were IP'd with 6E10, revealing trimers but not tetramers. Fig. 10E shows membrane-associated APP-derived molecules in Tg2576+/- primary neurons. IPs using 6E10 captures CTF- β s and A β monomers. The blot was denatured and re-probed with APPCter-C17 to confirm the nature of the approximately 13 kDa bands, revealing both phosphorylated (pCTF) and nonphosphorylated CTF- β s.

Figure 11. Overall response rates. Response rates of rats were compared at baseline and after receiving fractions from Tg Pos Lane 19 containing A β *56 from aTg2576 transgene positive mouse (corresponding Tg Neg Lane 19 from a

transgene negative mouse), and Tg Control Lane 17 without A β *56 from a transgene positive mouse.

Figure 12. Running response rates of rats compared at baseline and after receiving fractions containing A β *56 from a Tg2576 transgene positive mouse and Tg2576 Control Ln 17 without A β *56 from a transgene positive mouse.

Figure 13. Post reinforcement pause as a function of ratio size. Post reinforcement pause was compared as a function of ratio size in rats at baseline and after receiving fractions containing A β *56 from a Tg2576 transgene positive mouse and Tg2576 Control Ln 17 without A β *56 from a transgene positive mouse

Figure 14. Extracellular-enriched Tg2576 extracts from the B6SJL and 129FVBF1 strains of mice.

Figure 15. Methods for screening candidate monoclonals for antibodies that specifically detect A β *56.

Figures 16A-16C. Human-derived A β *56 physically binds NMDA receptors. Fig. 16A demonstrates that A β *56 coimmunoprecipitates with NR1 NMDA receptor subunits in brain tissue from Alzheimer (AD) patients but not from control subjects with no cognitive impairment (NCI), or extracts containing no brain proteins (NP). Fig. 16B demonstrates that A β *56 co-immunoprecipitates with NR2A, but much less readily with NR2B, NMDA receptor subunits in brain tissue from subjects with AD but not from control subjects (NCI). Fig. 16C demonstrates that A β *56 does not co-immunoprecipitate with α 7 nicotinic acetylcholine receptors (α 7nAChR). Panels below each blot confirm the ability of the various receptor antibodies to immunoprecipitate the respective receptors or receptor subunits.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS OF THE INVENTION

The present invention shows, for the first time, that cognitive deficits occur as a result of the accumulation of soluble assemblies of oligomers of amyloid- β proteins. The soluble amyloid- β protein assemblies of the present invention are made up of one or more detergent-stable oligomers of amyloid- β protein. In some embodiments, the soluble amyloid- β protein assemblies are made up of more than

one detergent-stable oligomer of amyloid- β protein. The soluble amyloid- β protein ($A\beta$) assemblies of the present invention may also be referred to herein as $A\beta^*$ assemblies, $A\beta^*$ molecules, $A\beta$ star assemblies, $A\beta$ star molecules, A-beta* assemblies, A-beta* molecules, A-beta star assemblies, A-beta star molecules, $A\beta^*$, $A\beta^*56$, or $A\beta$ star 56.

The soluble amyloid- β protein assemblies of the present invention may be isolated. The soluble amyloid- β protein assemblies of the present invention may be purified. The soluble amyloid- β protein assemblies of the present invention may be isolated and purified. As used herein, the term "isolated" means that a polypeptide, oligomer of polypeptides, or assembly of oligomers is either removed from its natural environment or synthetically derived, for instance by recombinant techniques, or chemically or enzymatically synthesized. As used herein, the term "purified" means that a polypeptide, oligomer of polypeptides, or assembly of oligomers is essentially free from any other polynucleotides or polypeptides and associated cellular products or other impurities.

As used herein an "amyloid-beta protein," also referred to as an "amyloid-beta polypeptide," an "amyloid-beta peptide," an amyloid-beta molecule," an "amyloid- β protein," an "amyloid- β polypeptide," an "amyloid- β peptide," and "amyloid- β molecule," an " $A\beta$ protein," an " $A\beta$ polypeptide," an " $A\beta$ peptide," an " $A\beta$ molecule," "amyloid-beta," "amyloid β ," or " $A\beta$," is the major constituent of amyloid plaques in the brains of individuals afflicted with Alzheimer's disease, a polypeptide of 39 to 43 amino acid residue first identified by Glenner and Wong (see, for example, Glenner et al., (1984) *Biochem Biophys. Res Commun* 120, 885-890; and Glenner and Wong (1984) *Biochem Biophys Res Commun* 122, 1131-1135) and Masters et al. (See Masters et al., (1985) *Embo J* 4, 2757-2764; and Masters et al., (1985) *Proc Natl Acad Sci USA* 82, 4245-4249). The gene for the amyloid precursor protein (APP) of the amyloid-beta protein has been cloned and sequenced (see, for example, Kang et al., (1987) *Nature* 325, 733; Tanzi et al., (1987) *Science* 235, 880-884; and Selkoe (1994) *Annual Review of Neuroscience* Vol, 17, 489-517). As used herein, an amyloid-beta protein may be any of the various known allelic variants and mutations of the amyloid-beta protein.

Amyloid beta peptide is generated from the beta-amyloid precursor protein (beta APP) in a two-step process. The first step involves cleavage of the extracellular, amino-terminal domain of beta APP. Protein cleavage is performed

by an aspartyl protease termed beta-secretase (BACE). This enzyme is synthesized as a propeptide that must be modified to the mature and active form by the prohormone convertase, furin. Beta APP cleavage by the mature form of BACE results in the cellular secretion of a segment of beta APP and a membrane-bound remnant. This remnant is then processed by another protease termed gamma-secretase. Gamma-secretase cleaves an intra-membrane site in the carboxyl-terminal domain of beta APP, thus generating the amyloid beta peptide. Gamma-secretase is believed to be a multi-subunit complex containing presenilin-1 and 2 as central components. Found associated with the presenilins is the transmembrane glycoprotein nicastrin. Nicastrin has been found to bind to the carboxyl-terminus of betaAPP and helps to modulate the production of the amyloid beta peptide. Also found in the neurofibrillary lesions in Alzheimer's disease is the protein termed Tau. Tau is a neuronal microtubule-associated protein found predominantly on axons. The function of tau is to promote tubulin polymerization and stabilize microtubules. Tau, in its hyperphosphorylated form, is the major component of paired helical filaments (PHF), which is the building block of neurofibrillary lesions in Alzheimer's disease brain. See, for example, J. Neurosci. 18:1743-1752, 1998 and Neuron, 19:939-945, 1997.

As used herein, an amyloid-beta protein is a monomeric polypeptide, made up of one polypeptide chain. A monomeric polypeptide is also referred to herein as a "monomer."

As used herein an oligomer of amyloid β , also referred to an oligomeric form of amyloid β , is a detergent-stable configuration of more than one amyloid-beta protein. An oligomer is not necessarily polymerized. An oligomer of amyloid β may be soluble. As used herein a "dimer" is a detergent-stable configuration of two amyloid-beta proteins. As used herein a "trimer" is a detergent-stable configuration of three amyloid-beta proteins. As used herein a "tetramer" is a detergent-stable configuration of four amyloid-beta proteins. As used herein a "pentamer" is a detergent-stable configuration of five amyloid-beta proteins. As used herein a "hexamer" is a detergent-stable configuration of six amyloid-beta proteins.

As used herein, an "assembly" is a configuration of one or more oligomers of A β proteins. In a preferred embodiment, an assembly is a configuration of more than one A β protein oligomers. An assembly of oligomers of A β proteins may be,

for example, an assembly of two oligomers of A β proteins, three oligomers of A β proteins, four oligomers of A β proteins, five oligomers of A β proteins, six oligomers of A β proteins, or more oligomers of A β proteins. In some embodiments, an assembly of oligomers of A β proteins may be, for example, a nanomer of nine amyloid β proteins or a dodecamer of twelve amyloid β proteins. In some embodiments, an assembly of oligomers of A β proteins may be, for example, an assembly of more than one hexamer of amyloid β proteins, more than one pentamer of amyloid β proteins, more than one tetramer of amyloid β proteins, more than one trimer of amyloid β proteins, or more than one dimer of amyloid β proteins. In some embodiments, an assembly of oligomers of A β proteins may be, for example, an assembly of two hexamers of amyloid β proteins, three hexamers of amyloid β proteins, two tetramers of amyloid β proteins, three tetramers of amyloid β proteins, four tetramers of amyloid β proteins, two trimers amyloid β proteins, three trimers amyloid β proteins, four trimers of amyloid β proteins, five trimers amyloid β proteins, two dimers of amyloid β proteins, three dimers of amyloid β proteins, four dimers of amyloid β proteins, five dimers of amyloid β proteins, six dimers of amyloid β proteins, seven dimers of amyloid β proteins, or eight dimers of amyloid β proteins.

In some embodiments, amyloid- β protein assemblies may include detergent-stable dimers of amyloid- β protein. In some embodiments, amyloid- β protein assemblies may include detergent-stable trimers of amyloid- β protein. In some embodiments, amyloid- β protein assemblies may include detergent-stable tetramers of amyloid- β protein. In some embodiments, amyloid- β protein assemblies may include detergent-stable pentamers of amyloid- β protein. In some embodiments, amyloid- β protein assemblies may include detergent-stable hexamers of amyloid- β protein.

The present invention also includes isolated, soluble amyloid- β protein assemblies having one or more amyloid- β protein trimers. As used herein an "amyloid- β protein trimer" is a detergent-stable configuration of three A β molecules. In some embodiments, a soluble amyloid- β protein assembly has more than one amyloid- β protein trimer. In some embodiments, the amyloid- β protein assembly includes three amyloid- β protein trimers. In some embodiments, the amyloid- β protein assembly is a nonamer of amyloid- β proteins. In some embodiments, an amyloid- β protein assembly has a molecular weight of about 40

kDa as measured by SDS polyacrylamide gel electrophoresis. In some embodiments, the amyloid- β protein assembly includes four amyloid- β protein trimers. In some embodiments, the amyloid- β protein assembly has a molecular weight of about 56 kDa as measured by SDS polyacrylamide gel electrophoresis.

5 In some embodiments, amyloid- β protein assemblies may be a dodecamer of amyloid- β proteins. Such dodecamers of amyloid- β proteins may be six dimers of amyloid- β protein, four trimers of amyloid- β protein, three tetramers of amyloid- β protein, or two hexamers of amyloid- β protein. In some embodiments, the dodecamer of amyloid- β proteins has a molecular weight of about 56 kDa as
10 measured by SDS polyacrylamide gel electrophoresis.

 As used herein, a detergent-stable, also referred to herein as “detergent stable,” configuration does not disassemble or disassociate into its component subunits in a detergent solution. Such a detergent solution may be, for example, a 1% solution Triton X-100 or a 2% solution of SDS. Thus, a detergent stable
15 oligomer of amyloid- β protein does not disassociate into separate amyloid- β protein monomers in a detergent solution. Likewise, a detergent stable assembly of oligomers of amyloid- β protein does not disassociate into separate oligomers of amyloid- β proteins in a detergent solution.

 The assemblies of amyloid β protein of the present invention are soluble.
20 As used herein, the term “soluble” means remaining in aqueous solution. In some embodiments, soluble assemblies of amyloid β protein remain in the supernatant after centrifugation, including, for example, ultracentrifugation. Soluble assemblies of amyloid β protein may remain in solution in a wide range of solutions, including, but not limited to, water, in an isotonic solution, tissue culture
25 medium, a buffered solution, a detergent buffer, an organic buffer, or a body fluid, including, for example, plasma or cerebrospinal fluid. Assemblies of amyloid β protein may remain in solution in a physiological buffer.

 Assemblies of amyloid β protein may remain in solution in range of temperatures. For example, the assemblies of amyloid β protein may remain in
30 solution at a temperature greater than 0°C. Assemblies of amyloid β protein may remain in solution, for example, at a temperature of at least about 4°C, at a temperature of at least about 10°C, at a temperature of at least about 15°C, at a temperature of at least about 25°C, at a temperature of at least about 37°C, at a temperature of at least about 42°C, at a temperature of at least about 50°C, at a

temperature of at least about 55°C, at a temperature of at least about 60°C, at a temperature of at least about 70°C, at a temperature of at least about 75°C, at a temperature of at least about 80°C, at a temperature of at least about 85°C, at a temperature of at least about 90°C, at a temperature of at least about and/or at a temperature of at least about 95°C.

Assemblies of amyloid β protein may remain in solution, for example, at a temperature of less than about 4°C, at a temperature of less than about 10°C, at a temperature of less than about 15°C, at a temperature of less than about 25°C, at a temperature of less than about 37°C, at a temperature of less than about 42°C, at a temperature of less than about 50°C, at a temperature of less than about 55°C, at a temperature of less than about 60°C, at a temperature of less than about 70°C, at a temperature of less than about 75°C, at a temperature of less than about 80°C, at a temperature of less than about 85°C, at a temperature of less about 90°C, at a temperature of less than about 95°C, and/or at a temperature of less than about 100°C.

Assemblies of amyloid β protein may remain in solution, for example, at a temperature of about 4°C, at a temperature of about 10°C, at a temperature of about 15°C, at a temperature about 25°C, at a temperature of about 37°C, at a temperature of about 42°C, at a temperature of at about 50°C, at a temperature of about 55°C, at a temperature of about 60°C, at a temperature of about 70°C, at a temperature of about 75°C, at a temperature of at about 80°C, at a temperature of about 85°C, at a temperature of about 90°C, and/or at a temperature of about 95°C.

Assemblies of amyloid β protein may remain in solution in a range of any of the various temperatures discussed above.

In some embodiments, the oligomers or assemblies of amyloid- β protein are non-fibrillar. As used herein, a “non-fibrillar” protein, also referred to herein a “globular” protein, has little alpha helical or beta sheet structure. As used herein, a fibrillar protein has extensive alpha helix or beta sheet structure. The oligomers or assemblies of amyloid β protein may be preparations from which the fibrillar form of amyloid β is absent or has been removed. See, for example, U.S. Patent No. 6,218,506 and Walsh et al., (2002) Nature 416, 535-539 for a more complete discussion of the non-fibrillar structure of amyloid beta.

Both assemblies of amyloid β protein and oligomers of amyloid β protein may be obtained from a wide variety of sources. Assemblies of amyloid β protein

and oligomers of amyloid β protein may be obtained from natural sources; for example, from natural fluids, cells, or tissues, including, but not limited to, plasma, brain tissue, and cerebrospinal fluid. Assemblies of amyloid β protein and oligomers of amyloid β may be isolated from the culture medium of cells
5 expressing endogenous or transfected amyloid β protein precursor genes. For example, assemblies amyloid β or oligomers of amyloid β protein may be obtained from the culture medium of Chinese hamster ovary (CHO) cells stably transfected to express amyloid β protein (Podlinsky et al., J Biol. Chem., 1995, 270(16):9564-9570). Assemblies of amyloid β protein and oligomers of amyloid β protein may
10 be synthetically produced. Assemblies of amyloid β protein and oligomers of amyloid β protein may be produced recombinantly

Amyloid- β protein assemblies of the present invention disrupt cognitive functioning, representative of a cognitive disorder. Such cognitive disorders include, but are not limited to, mild cognitive impairment, memory deficits, age
15 related memory decline, age associated memory impairment, and Alzheimer's disease, including, but not limited to presymptomatic Alzheimer's disease and early Alzheimer's disease. Disruptions of cognitive function may be representative of any phase of a neurological disorder, including, but not limited to, a presymptomatic phase, a preclinical phase, or an early phase of a neurological
20 disorder. The disruption of cognitive function may be representative of age-related memory decline or age-associated memory impairment (see Craik, F. I. in Handbook of the Psychology of Aging (eds. Birren, J. E. & Schall, K.) 384-420 (Van Nostrand-Reinhold, New York, 1977) and Morrison and Hof, Science 1997, 278;412-9).

25 Cognitive disruption may be assayed by any of a variety of methods. One means of assessing cognitive functioning is the Alternating Lever Cyclic Ratio (ALCR) test, which has proven to be sensitive for measuring cognitive function (O'Hare et al., Behav Pharmacol 1996, 7:742-753; and Richardson et al., Brain Res 2002, 954:1). Under ALCR, rats learn a complex sequence of lever-pressing
30 requirements for food reinforcement in a two-lever experimental chamber. Rats must alternate between the two levers, switching to the other lever after pressing the first lever enough to get a food pellet. The number of presses required for each food reward proceeds from low (2 presses) to high (56 presses), incorporating intermediate values based on the quadratic function, $x^2 - x$. One cycle is an entire

ascending and descending sequence of these response requirements (for example, 2, 6, 12, 20, 30, 42, 56, 56, 42, 30, 20, 12, 6, and 2 presses per food reward). Six such full cycles are presented during each session. Errors are scored when the subject perseveres on a lever after reward, that is, does not alternate (a
5 perseveration error), or when a subject switches levers before completing the response requirement on that lever (a switching error).

Other procedures that may be used to assess cognitive functioning, include, but are not limited to, a delayed non-matching to place test, a morris water maze (commonly used to assess working memory in rats and mice), a delayed matching
10 to sample test (an operant procedure for testing working memory), and a fixed-interval operant responding test (a sensitive procedure to assess non-specific cognitive effects, for example, when the type and anatomical location of the cognition being tested is unknown), a delayed conditioning procedure (representing a variety of operant or non-operant tests under which animals are
15 exposed to stimuli paired with a reward or punishment and, after a delay, their ability to respond appropriately to the stimulus-reward combination is assessed), or a repeated acquisition procedure (an operant test, under which subjects are required to repeatedly learn a new stimulus sequence).

With the present invention, the accumulation of assemblies of oligomers of
20 amyloid β is associated with in one or more neurological functional deficiencies. Such functional deficiencies may be transient or permanent. Such functional deficiencies may be observed in the absence of neuropathological damage. Such neuropathologies may include, for example, amyloid plaque formation, amyloid deposits, oxidative stress, astrogliosis, microgliosis, cytokine production,
25 dystrophic neurons, formation of neurofibrillary tangles, neurodegeneration, gross neuronal atrophy, neuronal loss, synaptic loss, and other manifestations of neuropathology.

Also included in the present invention are compositions including one or more of the soluble amyloid- β protein assemblies described herein. A composition
30 may include one or more accessory ingredients including, but not limited to, diluents, buffers, binders, disintegrants, surface active agents, thickeners, lubricants, preservatives (including antioxidants), solvents, diluents, antibacterial and antifungal agents, absorption delaying agents, carrier solutions, suspensions, colloids, and the like. A composition may further include additional therapeutic

agents. The preparation and use of such compositions is well known in the art.

A composition may be a pharmaceutical acceptable composition, meaning that the composition is not biologically or otherwise undesirable, i.e., the material may be administered to an individual without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. Also included in the present invention are vaccines including one or more of the amyloid- β protein assemblies described herein. The preparation and use of such vaccines is well known in the art.

The present invention includes antibodies that bind to the amyloid- β protein assemblies described herein. In some embodiments, an antibody binds to an amyloid- β protein assembly and does not bind to monomeric amyloid- β protein. In some embodiments, an antibody binds to an amyloid- β protein assembly and does not bind to dimeric amyloid- β protein. In some embodiments, an antibody binds to an amyloid- β protein assembly and does not bind to trimeric amyloid- β protein. In some embodiments, an antibody binds to an amyloid- β protein assembly and does not bind to tetrameric amyloid- β protein. In some embodiments, an antibody binds to an amyloid- β protein assembly and does not bind to the amino-terminal region of the amyloid- β protein. In some embodiments, an antibody binds to an amyloid- β protein assembly and does not bind to the mid-region of the amyloid- β protein. In some embodiments, an antibody binds to an amyloid- β protein assembly and does not bind to the carboxyl-terminal region of the amyloid- β protein. Also included in the present invention are compositions including one or more of the antibodies as described herein.

As used herein, the terms "antibody" or "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments thereof, such as F(ab')₂ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. The term "polyclonal antibody" refers to an antibody produced from more than a single clone of plasma cells; in contrast "monoclonal antibody" refers to an antibody produced from a single clone of plasma cells. Polyclonal antibodies may be obtained by immunizing a variety of warm-blooded animals such as horses, cows, goats, sheep,

dogs, chickens, rabbits, mice, hamsters, guinea pigs and rats as well as transgenic animals such as transgenic sheep, cows, goats or pigs, with an immunogen. The resulting antibodies may be isolated from other proteins by using an affinity column having an Fc binding moiety, such as protein A, or the like. Monoclonal antibodies can be obtained by various techniques familiar to those skilled in the art. Briefly, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell [see, Kohler and Milstein (1976) *Eur. J. Immunol.* 6, 511-519; J. Goding (1986) In "Monoclonal Antibodies: Principles and Practice," Academic Press, pp 59-103]. Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and the yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host.

Isolated assemblies of oligomers of amyloid β protein, oligomers of amyloid β protein, or fragments thereof may serve as an antigen to immunize an animal to elicit an immune response. Immunization with antigen may be accomplished in the presence or absence of an adjuvant, e.g., Freund's adjuvant. Booster immunizations may be given at intervals, e.g., 2-8 weeks. Both polyclonal and monoclonal antibodies may be labeled with detectable label using methods known in the art. For example, fluorescent labels or peroxidase may be used as detectable labels. Various techniques useful in these arts are discussed, for example, in Harlow and Lane, (1988) "Antibodies: A Laboratory Manual," Cold Spring Harbor, New York.

A therapeutically useful antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring one or more CDRs from the heavy and light variable chains of a mouse (or other species) immunoglobulin into a human variable domain, then substituting human residues into the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with immunogenicity of murine constant regions. Techniques for producing humanized monoclonal antibodies can be

found, for example, in Jones et al., *Nature* (1986);321: 522 and Singer et al., *J. Immunol.*, (1993);150: 2844.

In addition, chimeric antibodies can be obtained by splicing the genes from a mouse antibody molecule with appropriate antigen specificity together with
5 genes from a human antibody molecule of appropriate biological specificity; see, for example, Takeda et al., *Nature* (1985);314: 544-546. A chimeric antibody is one in which different portions are derived from different animal species.

The phrase "specifically binds" or "specifically immunoreactive with," when referring to an antibody, refers to a binding reaction that is determinative of
10 the presence of a protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Typically a specific or selective reaction will be at least twice background signal or
15 noise and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein.

The present invention also includes a method for detecting the presence of assemblies of amyloid β protein in a sample taken from a subject by contacting a
20 sample with one of the antibodies as discussed herein and detecting binding of the antibody. The sample may be, for example, serum, blood, cerebrospinal fluid (CSF), or brain tissue.

The present invention includes a method of disrupting memory of learned behavior in a non-human mammal by administering an amyloid- β protein assembly
25 intracranially. Intracranial administration includes, for example, intracerebral or intracerebroventricular (ICV) administration. The non-human mammal may be a rat, mouse, dog, or primate. The mammal may serve as an animal model system for a cognitive disorder, including, but not limited to, Alzheimer's Disease. The mammal may demonstrate cognitive deficits consistent with presymptomatic or
30 early Alzheimer's disease. The amyloid- β protein assembly may be made up of six amyloid- β protein dimers, four amyloid- β protein trimers, three amyloid- β protein tetramers, or two amyloid- β protein hexamers.

The present invention includes an animal model for a cognitive disorder, the animal model being a non-human mammal having amyloid- β protein

assemblies administered intracranially. The non-human mammal may be a rat, mouse, dog, or primate. The mammal may demonstrate disruption of complex learned behaviors. The mammal may demonstrate cognitive deficits consistent with presymptomatic or early Alzheimer's disease.

5 Intracranial delivery of assemblies of amyloid β protein may be by any of a wide variety of means. For example, intracranial delivery may be accomplished by oral, subcutaneous, intraperitoneal, intravenous, and/or intrathecal administration. Delivery may be by local delivery or injection. Delivery may be pump or extended release composition. Intracranial administration may include, for example,
10 intracerebral or intracerebroventricular administration. Assemblies of amyloid β may be delivered to an animal, for example, a vertebrate animal, including a mammal. Mammals include, for example, a rodent, including, but not limited to, a mouse or a rat, a dog, a non-human primate, or other non-human mammals.

 Assemblies of amyloid β may be administered in a wide range of
15 concentrations. For example, assemblies of amyloid β may be administered at concentrations that are higher than the concentration of assemblies of amyloid β found in brain or cerebrospinal fluid (CSF); assemblies of amyloid β may be administered at concentrations that are the same or similar to the concentration of assemblies of amyloid β found in brain or CSF; or assemblies of amyloid β may be
20 administered at concentrations that are less than the concentration of assemblies of amyloid β found in brain or CSF.

 The present invention includes a method of screening for an agent effective for the treatment of a cognitive disorder by administering an agent to a first animal to which isolated soluble amyloid- β protein assemblies having one or more
25 detergent-stable oligomers of amyloid- β protein have been intracranially administered; measuring cognitive function of said first animal; comparing the cognitive function of said first animal to the cognitive function of a second animal treated in the same fashion except no agent was administered; wherein an improvement in the cognitive function of said first animal compared to the
30 cognitive function of said second animal indicates said agent is effective for the treatment of a cognitive disorder. The present invention also includes agents identified by the screening methods described herein and methods of treatment that include the administration of such agents.

The present invention includes a method of screening for agents that inhibit or prevent the assembly of monomers of amyloid β protein into a detergent-stable oligomer of amyloid- β proteins, agents identified by such screening methods, and methods of treatment that include the administration of such agents.

- 5 The present invention also includes agents that inhibit or prevent the assembly of one or more detergent-stable oligomers of amyloid β protein into a soluble amyloid- β protein assembly, agents identified by such a screening method, and methods of treatment that include the administration of such agents.

- 10 An agent may be administered by any of a wide variety of means. For example, an agent may be delivered orally, subcutaneously, intramuscularly, intravenously, intrathecally, and/or intracranially. Delivery may be by local delivery or injection. Delivery may be by pump or extended release composition. An agent may be delivered prior to, during, and/or after delivery of another therapeutic agent. An agent may be delivered prior to during, and/or after the measurement of cognitive functioning. One or more agents may be administered.
- 15 As used herein, "treating" a condition or a subject includes therapeutic, prophylactic, and/or diagnostic treatments. Treatment can be initiated before, during, and/or after the development of the condition to be treated.

- 20 Suitable agents include any of a wide variety of molecules, including, but not limited to, polypeptides, nucleic acids, antibodies, antisense molecules, ribozymes, small chemical molecules, and the like.

- 25 The present invention includes methods of detecting a cognitive disorder in a subject by detecting soluble amyloid- β protein assemblies having one or more detergent-stable oligomers of amyloid- β protein in a fluid or tissue sample taken from the subject.

The present invention includes methods of detecting presymptomatic Alzheimer's disease in a subject by detecting soluble amyloid- β protein assemblies having one or more detergent-stable oligomers of amyloid- β protein in a fluid or tissue sample taken from the subject.

- 30 The present invention includes methods for assaying the effects of soluble oligomers of the amyloid β protein on cognitive function by administering a soluble amyloid- β protein assembly having one or more detergent-stable oligomers of amyloid- β protein intracranially into an animal and measuring cognitive function to determine the long-term disruption of cognitive behavior. In some

embodiments, the disruption of cognitive behavior of the animal may be compared to the disruption of cognitive behavior of another animal treated in the same fashion except saline rather a soluble amyloid- β protein assembly having one or more one detergent-stable oligomers of amyloid- β protein administered

5 intracranially.

The present invention includes methods for isolating soluble amyloid- β protein assemblies having one or more one detergent-stable oligomers of amyloid- β protein by homogenizing neuronal tissue in a lysis buffer, size fractionating amyloid- β protein assemblies, and isolating an amyloid- β protein assembly of a
10 desired size. For example, about 100 to 200 mg of brain tissue may be harvested in 500 ml of a lysis solution containing 50 mM Tris-HCl (pH 7.6), 0.01% NP-40, 150 mM NaCl, 2mM EDTA, 0.1% SDS with 1 mM phenylmethylsulfonyl fluoride (PMSF) in the presence of a protease inhibitor cocktail (Sigma). Then, the lysate may be mechanically homogenized using a 1 ml syringe and needle, gage 20,
15 repeating ten times and centrifuged for 90 minutes at 13,000 rpm. The A β assemblies of different sizes may be separated by size-exclusion chromatography on Tricorn Superdex® 75 columns (Amersham Life Sciences, Piscataway, NJ, USA) run at a flow rate of 1 ml/minutes in 50 mM Ammonium Acetate, pH 8.5. Then eluted proteins may be concentrated by evaporation using a vacuum system
20 (SpeedVac®, Savant Technologies, USA).

The present invention is illustrated by the following examples. It is to be understood that the particular examples, materials, amounts, and procedures are to be interpreted broadly in accordance with the scope and spirit of the invention as set forth herein. All headings are for the convenience of the reader and should not
25 be used to limit the meaning of the text that follows the heading, unless so specified.

EXAMPLES

Example 1

Specific Amyloid- β Protein Assembly in the Brain

5 Impairs Cognitive Function

Cognitive function often declines with age and is believed to deteriorate initially because of changes in synaptic function rather than loss of neurons. Some individuals progress to develop Alzheimer's disease (AD) with neurodegeneration.

- 10 In this example Tg2576 mice were used to investigate the cause of cognitive decline in the absence of neurodegeneration or amyloid- β (A β) protein amyloidosis. Strong correlations were found between memory deficits and extracellular accumulation of a 56-kD soluble A β assembly, called A β *56 (A β star 56), which disrupts cognitive function when injected directly into healthy rats.
- 15 This example demonstrates that A β *56 disrupts cognitive function independently of plaques or neuronal loss, and contributes to cognitive deficits associated with AD.

- Age-related cognitive decline (ARCD) occurs in many mammalian species (F. I. Craik, in Handbook of the Psychology of Aging J. E. Birren, K. Schall, Eds. (Van Nostrand-Reinhold, New York, 1977) pp. 384-420; and Gallagher and Rapp, Annu Rev Psychol 48, 339 (1997), including humans, and appears to result from synaptic dysfunction (Morrison and Hof, Science 278, 412 (1997). Poor cognitive function can predict AD up to fifteen years before diagnosis (Kawas et al., Neurology 60, 1089 (2003), and non-demented individuals at risk genetically for
- 25 AD show abnormalities in functional brain imaging tests (Small et al., Proc Natl Acad Sci USA 97, 6037 (2000); and Bookheimer et al., N Engl J Med 343, 450 (2000)). These and other studies imply that AD has an insidious onset, which blurs the boundary between ARCD and AD (Albert and Drachman, Neurology 55, 166 (2000)).
- 30 Tg2576 mice express a human amyloid- β precursor protein (APP) variant linked to AD and develop many neuropathological features of AD, including amyloid plaques containing A β , dystrophic neurites and inflammatory changes (Hsiao et al., Science 274, 99 (1996); and Benzing et al., Neurobiol Aging 20, 581 (1999)), but lack neurofibrillary tangles, significant neuronal loss or gross atrophy

(Irizarry et al., J Neuropathol Exp Neurol 56, 965 (1997)). Young Tg2576 mice (less than six months) have normal memory and lack neuropathology, middle-aged mice (aged six to fourteen months) develop memory deficits without neuronal loss, and old mice (greater than fourteen months of age) form abundant neuritic plaques associated with minimal neuronal loss (Hsiao et al., Science 274, 99 (1996); Irizarry et al., J Neuropathol Exp Neurol 56, 965 (1997); Kawarabayashi et al., Journal of Neuroscience 21, 372 (2001); Westerman et al., J Neurosci 22, 1858 (2002); and Urbanc et al., Proc Natl Acad Sci U S A 99, 13990 (2002)). Tg2576 mice are therefore a good model to study pre-clinical stages of AD, prior to the diagnosis of dementia or the onset of neuronal loss.

In Tg2576 mice, as in other APP transgenic mice, there is strong evidence that A β is responsible for age-related deterioration in cognitive function (Westerman et al., J Neurosci 22, 1858 (2002); Janus et al., Nature 408, 979 (2000); and Chen et al., Nature 408, 975 (2000)). However, there are several paradoxical findings about the relationship between A β and cognitive decline that suggest a complex role of A β in cognitive impairment. For example, spatial reference memory in Tg2576 mice declines modestly but significantly at six months and remains stable for 7 to 8 months (Figs. 1A and 1B). However, no candidate A β species measured to date corresponds with the decline in memory observed at six months and the cognitive stability observed thereafter (see Table 2). Thus, the enigma that a rapidly increasing amount of A β , the molecule believed to be responsible for cognitive impairment, is associated with no change in cognitive function. One solution to this conundrum is to posit the existence of soluble A β assemblies that disrupt cognition (Hsia et al., Proc Natl Acad Sci U S A 96, 3228 (1999); Ashe, Learn Mem 8, 301 (2001); and Klein et al., Trends Neurosci 24, 219 (2001)). With this example, A β * (A β star) was identified in Tg2576 mice.

A major challenge in analyzing A β in the brain lies in reliably separating A β from various cellular compartments (for example, extracellular, intracellular, and membrane associated). This obstacle was overcome by developing an extraction procedure that separates proteins in known cellular compartments with high fidelity (Fig. 5A). The extraction method used in this example allowed the quantification and comparison of up to four different pools of transgene-derived A β species.

To resolve the paradox of a mismatch between A β levels and memory deficits, the extraction procedure was used to search for A β^* in Tg2576 mice between four and twenty-five months. A β^* molecules were required to satisfy the following two criteria: one) their appearance coincides with memory loss at six months; and two) their levels remain stable in middle-aged mice (aged six to fourteen months). By immunoblotting immunoglobulin-depleted brain extracts, a set of A β protein assemblies was found in the soluble, extracellular-enriched fraction from six-month mice (Fig. 1C). Besides a faint band corresponding to A β monomers, there were bands co-migrating with trimeric (14-kD), hexameric (27-kD), nonameric (40-kD) and dodecameric (56-kD) A β assemblies. Interestingly, these species represent multiples of trimeric A β oligomers, with high molecular weight (HMW) assemblies (greater than 20kDa) appearing in mice of six or more months of age. Identical bands were detected using 6E10 and 4G8 antibodies, excluding the possibility that they represented degradation products of soluble APP (Fig. 6A).

Although this result suggests that aging induces trimers to associate and form HMW assemblies, the possibility that the HMW assemblies represent A β tightly bound to other proteins cannot be excluded. However, this is unlikely based upon their stability and immunospecificity. First, the possibility that they would be disrupted by urea, a common denaturant of globular proteins (Gordon et al. J Biol Chem 243, 5663 (1968)), was considered. All A β oligomers were detected in 8M urea-containing SDS-PAGE (Fig. 6B), arguing against the presence of a globular protein. In contrast, when exposed to $\geq 15\%$ hexafluoroisopropanol (HFIP), all but trimers and, to a lesser extent, tetramers (which form at 13 months) disassembled (Fig. 6C), suggesting that the HMW complexes are held together by hydrogen, not covalent, bonds. The resistance of trimers to dissociation in greater than 25% HFIP supports the conclusion that trimers are the fundamental assembly unit. Second, the HMW complexes are recognized by the A11 antiserum (Fig. 6D), which specifically detects soluble A β assemblies that are distinct from A β fibrils in AD brain (Kayed et al., Science 300, 486 (2003)). This antiserum revealed HMW assemblies, but not trimers, in soluble, extracellular-enriched fractions, implying that the HMW complexes are soluble A β oligomers and confirming previous observations that A11 reacts only with A β oligomers larger than tetramers (Kayed et al., Science 300, 486 (2003)). Finally, since it is possible

that trimers might be fragments of HMW complexes released during the sample preparation or gel fractionation procedures, or HMW assemblies might be artificially generated during the biochemical procedures, the native size of soluble A β assemblies was examined under non-denaturing conditions. Soluble, extracellular-enriched extracts were fractionated by size-exclusion chromatography (SEC) and subjected to SDS-PAGE (Fig. 7A). High and low molecular weight (MW) A β assemblies were found in the collected fractions, at the appropriate intervals. Taken together, these results indicate that A β in six-month Tg2576 mice forms a ladder of stable, soluble, physiological assemblies comprised of trimers and multiples of trimers.

Trimers and hexamers were excluded as components of A β^* , because they were present prior to memory impairment (less than six months). However, a 56-kD band, corresponding to dodecamers, appeared at six months, along with lesser quantities of nonamers (Fig. 1C). The mean levels of the trimeric, hexameric, nonameric and dodecameric A β assemblies in Tg2576 mice up to twenty-five months remained stable throughout life, although there was considerable variability between animals of the same age (Figs. 7B-D). Because dodecamers and nonamers appeared at six months and remained stable throughout life, they fulfilled the criteria for being designated as A β^* . No A β assemblies were found in old mice corresponding to the second drop in memory function at fifteen months (Figs. 1A and 1B); by this age it is possible that the abundant plaques with prominent dystrophic neurites disrupt synaptic function sufficiently to impair memory further (Stern et al., J Neurosci 24, 4535 (2004)).

The variability in levels of A β assemblies between animals of the same age provided an opportunity to examine correlations between the different A β oligomers and memory impairment, which were assessed by comparing spatial memory and soluble A β species in two groups of five- and six-month-old Tg2576 mice (Fig. 2). Monomers, trimers and hexamers of A β did not correlate significantly with performance in five- or six-month mice (Figs. 2A-F). However, the nonamers and dodecamers, which were present in six-month but not five-month animals, correlated significantly with performance in an inverse relationship (Figs. 2G and 2H). Importantly, the appearance of the 56-kD A β species (putative dodecamers) at six months was not associated with deficits in the cued phase of water maze testing, indicating that this A β species is not sufficient to impair

sensorymotor function or to alter motivation, attention or activity levels. There were no correlations between the levels of any A β oligomers and performance in the cued phase of the water maze test, further arguing against their effects on non-cognitive aspects of behavior (Fig. 8).

5 Because intracellular A β has been proposed to disrupt memory in 3 X Tg-AD (Billings et al., Neuron 45, 675 (2005)), additional analyses was performed to evaluate the potential accumulation of A β species within brain cells. Only trimeric and monomeric A β species were detected in the soluble, intracellular-enriched fraction, with no modulation between five and six months (Fig. 9A). Full-length
10 APP and C-terminal fragments (CTFs) in membrane-associated fractions were also examined, and no change in the levels of APP, CTF- β s or CTF- α was found (Figs. 9B and 9C). In cytosolic extracts of cultured primary Tg2576 cortical neurons, monomers and trimers were the only A β species detected (Fig.10), supporting the in vivo findings. Thus, no intracellular or membrane-associated A β species or
15 CTFs correlating with the onset of memory deficits were found in six-month Tg2576 mice.

 The observations that the 56-kD A β assembly appears at six months when memory declines, that its levels are stable in aging mice, that it is more abundant than the 40-kD A β complex, and that it correlates most strongly with memory
20 impairment suggested that it was a likely candidate for A β *. To determine whether the 56-kD A β assembly directly disrupts cognitive function, the 56-kD A β assembly was isolated from all other A β species and its effect assayed in rats using the Alternating Lever Cyclic Ratio (ALCR) procedure, a test of executive cognitive function and reference memory function, which was previously proven to
25 be sensitive to the behavioral effects of A β oligomers secreted by Chinese hamster ovary (CHO) cells (Cleary et al., Nat Neurosci 8, 79 (2005)). In the ALCR paradigm, substances which disrupt cognitive function produce increases in switching and perseveration errors that can be dissociated from effects on motivation and activity levels. To isolate the 56-kD A β assembly, proteins were
30 fractionated from soluble, extracellular-enriched brain extracts of seven-month Tg2576 mice and non-transgenic littermates by SEC (Fig. 7), and injected selected fractions into the lateral cerebral ventricles of ALCR-trained rats. Fraction 19+/- containing the 56-kD A β assembly was found to significantly increase switching and perseveration errors measured two hours after the injections; increases were

not found with the corresponding fraction 19-/- from a non-transgenic littermate or fraction 19+/- after immunoprecipitation with 6E10 (Figs. 3B and 3C). The increase in errors cannot be attributed to changes in motivation or activity levels because the response rates were unaffected (Fig. 11 and Fig. 12), in keeping with the preservation of performance during the cued phase of water maze testing in Tg2576 mice. The data show that the effects of the 56-kD A β assembly were on cognitive function rather than on non-cognitive aspects of behavior.

To exclude the possibility that cognitive function was disrupted by 6E10-reactive proteins greater than 60-kD in fraction 19+/-, fraction 17+/- containing HMW 6E10-reactive proteins, but lacking the 56-kD protein, was tested and no significant increase in errors was found. In addition, fraction 26+/- containing trimers did not significantly increase errors, consistent with the failure of trimers to correlate significantly with memory impairment. Interestingly, the increases in errors as a result of the 56-kD A β assembly were transient; that is, they were not significantly increased when assessed the day after the ICV injection, following a time course similar to that of A β oligomers generated by CHO cells (Cleary et al., Nat Neurosci 8, 79 (2005)). These results indicate that an extracellular 56-kD assembly of soluble A β , herein named A β *56, directly and specifically disrupts cognitive function in healthy rats.

To further link A β *56 to memory loss, it was asked whether eliminating it would restore memory. Lacking specific antibodies targeting A β *56, an experiment of nature was utilized to examine the consequences on memory of removing it from the brain. A transient disappearance of A β *56 was found between 12.0 and 12.4 months of age in Tg2576 mice (Figs. 4A and 4B). To investigate the effects on memory of eliminating A β *56, spatial memory was tested in five separate groups of Tg2576 mice whose ages spanned an interval surrounding twelve months. Although the performance of Tg2576 mice at 10.7 and 11.8 months was lower than in non-transgenic (non-Tg) littermates, it improved significantly in 12.0- and 12.5-month animals and was similar to that of non-Tg littermates (Fig. 4C). However, this restoration of memory was brief. With the reappearance of A β *56, the performance of 12.6-month Tg2576 mice declined and was significantly lower than in non-Tg littermates. Thus, the elimination of A β *56 restored spatial reference memory, indicating that A β *56 specifically causes memory loss in Tg2576 mice.

Interestingly, when the rates of change of SDS-soluble and SDS insoluble A β (x-42) were calculated using previously published results (Kawarabayashi et al., Journal of Neuroscience 21, 372 (2001)), it was found that a brief dip in A β *56 coincided with the peak rate of accumulation of SDS-insoluble A β (x-42) occurring
5 between 11.6 and 12.6 months of age (Fig. 4D), when mature amyloid plaques consistently appear (Kawarabayashi et al., Journal of Neuroscience 21, 372 (2001)). This observation may be explained by soluble A β assemblies and fibrillar A β existing in a dynamic equilibrium with each other or competing for the same pool of monomeric A β (Fig. 4E), indicating that very high rates of deposition of
10 fibrillar A β create a "sink" leading to reduced levels of A β *56.

A β *56 may elucidate the functional significance of a soluble putative A β dodecamer in AD brain which is recognized by antibodies to A β -derived diffusible ligands (ADDLs) (Gong et al., Proc Natl Acad Sci U S A 100, 10417 (2003)). However, in Tg2576 mice, antibodies raised against ADDLs detect a 20- to 100-
15 fold increase in the cortical signal between 13 and 17 months (Chang et al., J Mol Neurosci 20, 305 (2003)), and a 130-fold increase in the hippocampal signal between 9 and 20 months. Since the levels of A β *56 do not change appreciably with age, these results indicate that anti-ADDL antibodies do not specifically detect A β *56. A β *56 impairs memory by inducing transient physiological, rather
20 than permanent neuropathological, alterations of the brain, as inferred by the transient effects following injections into rats and the interlude of normal memory in 12-month Tg2576 mice that occurs when A β *56 levels dip and mature plaques appear. Intriguingly, the coincidence of improved memory and the appearance of plaques suggests that amyloid deposition, at least in the earliest stages, may protect
25 the brain from the detrimental effects of A β *. This could explain why some cognitively intact individuals have high plaque loads (Crystal et al., Neurology 38, 1682 (1988)).

The accumulation of insoluble aggregates consisting of proteins such as tau, huntingtin, prion protein, ataxin, and A β occurs in many neurodegenerative
30 disorders. These aggregates often define the disorders neuropathologically, but their relative contribution to disease symptoms compared to other, hypothetical, intermediate protein assemblies is controversial (Orr, Nature 431, 747 (2004); Santacruz et al., Science 309, 476 (2005); and Duff and Planel, Nature Medicine 11, 826 (2005)), and the identity of the theoretical intermediates has been elusive.

The present discovery, that A β *56 is responsible for memory loss in plaqueforming Tg2576 mice, and causes cognitive deficits when injected directly into healthy rats, sets a precedent for identifying other “star” proteins inducing brain dysfunction; such as, for example, a tau* (Santacruz et al., Science 309, 476
 5 (2005)). That A β * is a highly specific form of A β offers the potential for developing precise diagnostic methods to detect its correlate in humans with pre-clinical AD, opening the possibility of targeting A β * and aborting the disease before permanent structural changes have developed.

10 Methods

Transgenic animals. Tg2576 mice (Hsiao et al., Science 274, 99 (1996)) were the offspring of mice backcrossed successively to B6SJLF1 breeders, except for mice used in the behavioral and biochemical experiments shown in Figs. 4A-4C, which were in the 129FVBF1 strain background.

15 Antibodies. The following primary antibodies were used: 6E10 and 4G8 [1:100- 10,000 dilution] (Signet Laboratories, USA), R1282 [1:75 dilution] (Walsh et al., Nature 416, 535 (2002)), R1736 [1:1000 dilution] (Haass et al., Nature 357, 500 (1992)), FCA3542 (Barelli et al., Mol Med 3, 695 (1997)), APPCter-C17 [1:5000 dilution] (Sergeant et al., J Neurochem 81, 663 (2002)), anti-Flotillin-2,
 20 anti-ERKs, anti-JNK and anti-c-Jun [all 1:200 dilution] (Santa Cruz Biotechnology, Inc., USA), Tau-5 [1:1,000 dilution] (Biosource International, USA), anti-MAP-2 [1:200 dilution] and anti-Actin [1:250] (Sigma) and anti-t-PA (American Diagnostica Inc., USA). The A11 anti-oligomer antibody [1:5,000 dilution] (Kayed et al., Science 300, 486 (2003)) was detected with a biotinylated
 25 anti-rabbit antibody [1:2,000,000 dilution] (Vector Laboratories) and ExtrAvidin® [1:5,000 dilution] (Sigma).

Morris water maze behavioral test. Spatial reference memory was assessed using a modified version of the Morris water maze (Westerman et al., J Neurosci 22, 1858 (2002)). Testing was tailored for Tg2576 transgene positive and negative
 30 mice in each background strain, since 129FVBF1 mice learn more rapidly than B6SJLTg2576 transgene positive and negative mice received visible platform training for three days, eight trials per day, followed by hidden platform training for nine days, four trials per day. Three probe trials were performed twenty hours after twelve, twenty-four, and thirty-six training trials, and the mean

% target quadrant occupancy for the three probe trials, was calculated.

129FVBF1-Tg2576 transgene positive and negative mice received visible platform training for three days, six trials per day, followed by hidden platform training for six days, four trials per day. Probe trials performed twenty hours after four, eight, sixteen, and twenty-four training trials lasted sixty seconds, but % target quadrant occupancy was calculated using the first thirty seconds because the 129FVBF1 mice exhibited extinction. The probe trial following sixteen training trials (Day 5 target quadrant occupancy) was determined to be the most sensitive to the effect of transgene on performance across the age range tested. To coordinate the timing of the assessment of memory with the brief dip in A β *56 levels, we used this probe trial to measure retention of spatial memory in 129FVBF1-Tg2576 mice.

Alternating Lever Cyclic Ratio (ALCR) Procedure.

Subjects. Thirty-eight male Sprague-Dawley rats, approximately 120 days old and weighing 250-300 grams (g) at the beginning of the experiment, were housed individually with free access to water in a temperature and humidity controlled environment. Rats were maintained at 90% of their free-feeding weights during the experimental studies. During the course of the study, four rats' data were removed due to illness from blocked cannulae.

Apparatus. Behavioral training and testing was conducted in two-lever rat test chambers (model E10, Coulbourn Instruments, Inc.) enclosed in sound attenuating compartments. Each food reinforcer consisted of a 45 milligram (mg) pellet (F0021, Bioserv Holton Ind., Frenchtown, NJ) delivered into a tray situated midway between the levers. A food tray light flash and an audible pellet dispenser click signaled food delivery. Control of experimental contingencies and data collection was accomplished using computer MED PC computer software and interface (Med Associates, Fairfield NJ).

Alternating Lever Cyclic Ratio (ALCR) behavioral procedures and testing. In the ALCR task, subjects learn a sequence of lever-pressing requirements for food reinforcement in a two-lever experimental chamber. Rats must alternate responding between the two levers, switching to the other lever after pressing enough to get a food pellet. The number of presses required for each food reinforcer varies from low (for example, two) to high (for example, fifty-six), incorporating intermediate values based on the quadratic function $[x^2 - x]$. One

response cycle is an entire ascending and descending sequence of these response requirements (2, 6, 12, 20, 30, 42, 56, 56, 42, 30, 20, 12, 6, and 2 presses per food reinforcer). Six cycles are presented during each session. Thus, the subject alternates responding on the two levers, with an increasing, and then a decreasing, response per reinforcement ratio, six times per session. Errors occur when the subject perseveres on a lever after reinforcement, i.e., does not alternate (perseveration error), or when a subject switches levers before completing the response requirement on that lever (switching error). Perseveration errors are accumulated until the subject presses the correct lever, while switching errors are counted as a single occurrence for each premature switch to the incorrect lever.

In addition to errors, dependent variables relevant to activity levels (Overall Response Rate and Running Response Rate) and the subjects' abilities to track the size of the current work requirement (Post Reinforcement Pause and relationship of Response Rate to Lever Press Requirement) are also collected. Overall Response Rate is calculated as lever presses per second over the entire session, while Running Response Rate is calculated as the response rate only during the time the rat is actively engaged in lever-pressing. Post Reinforcement Pause (PRP) is the pause time in seconds that typically occurs following reinforcement. PRP is directly related to the work required (presses) for each reinforcer. Similarly, lever press rates (responses per unit time) are known to vary directly with the presses per reinforcer requirement.

ALCR Training. Behavioral sessions were conducted five to seven days per week. Rats were first trained to press both levers for food reinforcement and subsequently reinforced for a lever press only if they switched levers after each reinforcer delivery. The ratio of required responses per reinforcer delivery was slowly increased to 10:1 across 26 daily sessions. At this point the ALCR was introduced using sequential response per reinforcer ratios of: 1 3 5 8 10 15 15 10 8 5 3 1. This cycle repeated six times during each daily session. The response per reinforcer ratio cycle was slowly increased to the terminal values of: 2 6 12 20 30 42 56 56 42 30 20 12 6 2, required responses per reinforcer, and the cycle repeated six times each session. Sessions ended when the rat completed six cycles or after two hours (h) (mean session time, approximately 40 minutes). Rats received forty sessions of training in the ALCR task prior to surgery.

Baseline errors in the ALCR task. After recovery from surgery, mean baseline error rates (switching and perseveration) in the ALCR task were established for individual rats. To establish baseline error rates, errors were averaged across sessions, with the restriction that the highest and lowest error rate was removed before calculating the mean. During baseline sessions, rats were injected ICV with saline and/or were subjected to 'sham' injections under which the entire injection procedure was performed but no injectate was given. To ensure accurate baseline comparisons over the entire course of the study, mean error baselines were re-established after every two or three injections.

ICV injections. During the injection procedure, rats were removed from their home cages, cannula cap stylets were removed, and a 33-gauge internal injection cannula was inserted into the guide cannula. The injection cannula tip extended into the lateral ventricle 0.5 millimeter (mm) past the end of the guide cannula tip. The injection cannula was connected with PE 20 plastic tubing to a 50 microliter (μ l) Hamilton syringe containing the injectate. Following the injection, the cannula was capped with a stylet and the rat was placed in a holding box for two hours prior to the ALCR. On non-injection days, rats were subjected to sham injections, under which the same procedure was followed but no injectate was actually given. In addition, 20 μ l of saline (0.9%) was injected at least once each week in order to help keep the cannula patent.

Statistics. Perseveration errors and switching errors were not normally distributed. Therefore, a non-parametric matched-pair t-test (Wilcoxon) was used to evaluate within-subject changes in performance from baseline to post-injection within each group. Due to the large differences between switching errors and perseveration errors, these error rates are analyzed separately.

Surgery. Rats were anesthetized using a combination of ketamine (60 milligram/ kilogram (mg/kg)) and xylazine (20 mg/kg) and placed in a rat stereotaxic instrument. A 26-gauge guide cannula (Plastic One, Roanoke, VA) was implanted in either the right or left lateral ventricle. For ICV cannula placement, the stereotaxic coordinates, with the incisor bar set 3.5 millimeter (mm) above the interaural line, were ± 1.5 mm lateral and 1.0 posterior to bregma, and 3.5mm below the surface of the skull. Half the rats received cannulae directed at the right lateral ventricle and half received cannulae directed at the left lateral ventricle. The rats were allowed to recover for five days following surgery.

Cognitive function in rats following injections of SEC fractions into the lateral ventricles was assessed using the ALCR assay (Richardson et al., Brain Res 954, 1 (2002)). Briefly, in the ALCR task rats learn a complex sequence of lever-pressing requirements for food reward. Rats must alternate between two levers, switching levers after pressing one lever enough to get a food pellet. The number of presses required for each food reward proceeds from low (e.g., 2) to high (e.g., 56). One cycle is an entire ascending and descending sequence of these response requirements (e.g., 2, 6, 12, 20, 30, 42, 56, 56, 42, 30, 20, 12, 6, and 2 presses reward). Six full cycles are presented during each session. Based upon response rates and post reinforcement pauses, subjects learn to track the ratio size. Errors are scored when the subject perseveres on a lever after reward, i.e., does not alternate (a perseveration error), or when a subject switches levers before completing the response requirement on that lever (a switching error).

Protein extractions. Hemi-forebrains were harvested in 500 µl of solution containing 50 millimolar (mM) Tris-HCl (pH 7.6), 0.01% NP-40, 150 mM NaCl, 2mM EDTA, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride (PMSF), and protease inhibitor cocktail (Sigma). Soluble, extracellular-enriched proteins were collected from mechanically homogenized lysates (1 ml syringe, gauge 20 needle [10 repeats]) following centrifugation for 5 minutes at 3,000 rpm. Cytoplasmic proteins were extracted from cell pellets mechanically dissociated with a micropipettor in 500 µl TNT-buffer (50 mM Tris-HCl [pH 7.6], 150 mM NaCl, 0.1% Triton X-100) following centrifugation for 90 minutes at 13,000 rpm. Membrane-associated proteins were extracted from pellets following gentle agitation on a rotating platform in 500 µl of buffer containing 50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 0.5% Triton X-100, 1 mM EGTA, 3% SDS, 1% deoxycholate, 1 mM PMSF, and protease inhibitor cocktail (Sigma) following centrifugation for 90 minutes at 13,000 rotations per minute (rpm). Insoluble material was incubated with 20 µl 80% formic acid, mechanically dissociated with a micropipette, gently agitated for one hour and buffered with 380 µl 1M Tris-HCl, pH 8.0. Samples were centrifuged for 90 minutes at 13,000 rpm and the supernatant was collected for analysis.

Fractions were immunodepleted by sequential incubation for one hour at 4°C with 40 µl of Protein A-Sepharose, Fast Flow® followed by 40 µl of Protein G-Sepharose, Fast Flow® (Amersham Pharmacia Biotech U.K., Ltd., Little

Chalfont, Buckinghamshire, U.K.). All supernatants were clarified by centrifuging for 90 minutes at 13,000 rpm prior to western blot analysis. Protein amounts were determined (BCA Protein Assay, Pierce).

Size-exclusion chromatography (SEC). Protein extracts were loaded on
5 Tricorn Superdex® 75 columns (Amersham Life Sciences, Piscataway, NJ, USA) and run at a flow rate of approximately 0.3 milliliter/minute (ml/min). Fractions of 500 µl of eluate in 50 mM Ammonium Acetate, pH 8.5, were evaporated using a vacuum system (VacuFuge™, Brinkmann-Eppendorf, USA).

Western blots and immunoprecipitations. SDS-PAGE: Pre-cast 10-20%
10 SDS-polyacrylamide Tris-Tricine gels (Bio-Rad) or 16% Tris-Tricine gels in the presence or absence of SDS or Urea 8M were used. 100-250 microgram (µg) of protein per sample was resuspended with 4X Tricine loading buffer. Proteins were transferred to PVDF membranes (Immobilon Psq membrane, Millipore) or 0.2 µm nitrocellulose membranes (Bio-Rad). Membranes were boiled for 10 minutes in
15 PBS and blocked in TBST (Tris-Buffered Saline-Tween®20) containing 5% bovine serum albumin (BSA) plus 5% Top-Block (Sigma), and probed with appropriate antisera/antibodies diluted in 5% BSA - 5% Top-Block TBST. Blots were developed with an ECL detection system (Supersignal Pico Western system, Pierce or Western Lightning™ Chemiluminescence Reagent Plus, PerkinElmer).

20 Immunoprecipitations. Aliquots (100-500 µg) of protein extracts were diluted to 500 µl with dilution buffer and incubated with appropriate antibodies, incubated overnight at 4°C, and mixed with 40 µl of Protein G-Sepharose, Fast Flow® (Amersham Pharmacia Biotech U.K., Ltd., Little Chalfont, Buckinghamshire, U.K.) for one hour. The beads were washed twice in Buffer A,
25 twice with Buffer B, and proteins were eluted in 10-40 µl of loading SDS-PAGE buffer by boiling.

In order to detect small changes in cognitive performance, a particularly sensitive assessment procedure was employed, a procedure capable of measuring transient cognitive changes under the extremely low dose conditions that represent
30 physiological levels of proteins such as oligomeric Aβ. To do this, a procedure originally developed to assess Food Rewarded Behavior (Ettinger and Staddon, Behav Neurosci 97, 639 (1983)) was modified so as to enable the assessment of the cognitive effects of very small doses of psychoactive drugs (Weldon et al., Pharmacol Biochem Behav 54, 753 (1996)). This procedure, the Alternating Lever

Cyclic Ratio (ALCR) test, has proven to be more sensitive than many previously published methods for measuring drug effects on cognitive function (Richardson et al., Brain Res 954, 1 (2002), O'Hare et al., Behav Pharmacol 7, 742 (1996)).

- Briefly, rats must learn a complex sequence of lever-pressing requirements in order to earn food reinforcement in a two-lever experimental chamber. Subjects must alternate between two levers by switching to the other lever after pressing the first lever enough to get food reward. The exact number of presses required for each food reward changes, first increasing from two responses per food pellet up to fifty-six presses per food pellet, then decreasing back to two responses per pellet. Intermediate values are based on the quadratic function, $x^2 - x$. One cycle is an entire ascending and descending sequence of these lever press requirements (e.g., 2, 6, 12, 20, 30, 42, 56, 56, 42, 30, 20, 12, 6, and 2 presses per food reward). Six such full cycles are presented during each daily session. Errors are scored when the subject perseveres on a lever after pressing enough to get the food reward, i.e., does not alternate (a perseveration error), or when a subject switches levers before completing the response requirement on that lever (a switching error).

- ALCR Development. The ALCR assessment procedure was developed over several years. Several potentially confounding variables that might affect the results in the ALCR task, such as sensitivity, motivation and activity, were systematically assessed in the various published studies listed in Table 1 (IH = intrahippocampal; PO = oral; ICV = intracerebroventricular; FOA = Forced Operant Alternation task; PR = Progressive Ratio task).

TABLE 1

	Reference	Assessments	Compounds Tested	Route	ALCR Cognitive Effect	ALCR Running Response Rate	Direct test of Motivation
5	Cleary et al. ¹	ALCR	A β oligomers	ICV	Increased errors	No effect	
	Richardson et al. ²	ALCR	A β	IH	Increased errors		
10		ALCR	ibuprofen	PO	Normalized errors	No effect	
	MacNabb et al. ³	ALCR	methimzole	PO	Slow learning	No effect	No effect
		FOA, PR	thyroxine	IP			
15	O'Hare et al. ⁴	ALCR	A β	IH	Increased errors	No effect	
	MacNabb et al. ⁵	ALCR	methimzole	PO	Slow learning	No effect	No effect
		FOA, PR					
20	Weldon et al. ⁶	ALCR	atropine	IP	Increased errors	No effect Reduced (1 mg/kg)	

1. Cleary et al., Nat. Neurosci. 8, 79 (2005).

2. Richardson et al., Brain Res. 954, 1 (2002).

25 3. MacNabb et al., Neurosci. Res. 36, 121 (2002).

4. O'Hare et al., Brain Res. 815, 1, (1999).

5. MacNabb et al., Brain Res. 847, 231 (1999).

6. Weldon et al., Pharmacol. Biochem. Behav. 54, 753 (1996).

30 Sensitivity. There is ample empirical evidence in the literature that the
ALCR: 1) is at least as sensitive to pharmacological challenge as many other
assays (see, for example, MacNabb et al., Brain Res 847, 231 (1999)); 2) produces
reliable (repeatable) results under daily testing for months (see, for example,
O'Hare et al., Brain Res 815, 1 (1999)); 3) has external validity through
35 comparison with results from other cognitive or memory assays using well
characterized pharmacological agents (see, for example, Weldon et al., Pharmacol
Biochem Behav 54, 753 (1996)); 4) has empirical evidence of internal validity (see
below for discussion of motivational, motoric, and activity issues); and 5) has
previously been shown to be affective in assessing several forms of the A β protein
40 (see, for example, Cleary et al., Nat Neurosci 8, 79 (2005) and Richardson et al.,
Brain Res 954, 1 (2002)).

In terms of the relative sensitivity of ALCR to other useful tests of
cognitive function and memory, Weldon and colleagues tested the effects of
atropine, a common anti-cholinergic agent known to affect memory and cognitive
45 function (Weldon et al., Pharmacol Biochem Behav 54, 753 (1996)). They found
that 1.0 mg/kg atropine IP increases switching errors significantly. The typical IP
atropine dose range in many maze studies reported in the literature is from 3 to 55
mg/kg.

Motivation. All non-reflexive behavioral assays are necessarily affected by manipulations of motivation, but concerns may arise about susceptibility of the task to uncontrolled motivational variables, ability of the task to differentiate or discriminate between motivational and cognitive effects, and potential motivational effects of small soluble A β oligomers that might be mistaken for cognitive effects.

Uncontrolled motivational variables. In the ALCR task rats respond for food (45 mg pellet) under mild food restriction (90% free feeding weight). Several ways to control variability due to unintended changes in the value of the food reinforcer are employed. Each rat is given an exact amount of (weighed) food, and the amount of food is adjusted daily based upon current weight. Under these circumstances the rat's weight is precisely controlled to within $\pm 2-3\%$ of the target weight prior to each session. On the rare occasions when weight exceeds norms, such as sometimes happens early in training or when rats are not run on weekends, experimental compounds are not injected and those data are not included in baseline calculations. Also, rats are fed their entire daily ration just after finishing the daily behavioral session so as to minimize any food-related variability on the next day's session.

Discrimination between motivational and cognitive effects. A good cognitive assay should demonstrate sensitivity to cognitive change under conditions where motivation is unaffected. Few cognitive/memory assays produce specific empirical evidence on this question in each publication, but some tests, like the Morris Water Maze, possess high face validity on this issue. Because the complexity of the ALCR task precludes overt face validity on this issue, an issue was addressed directly in two previously published reports using the classic motivational assessment assay, the Progressive Ratio (PR) procedure developed by Hodos in 1961 (Hodos, Science 134, 943 (1961)). The PR assay requires an increasing number of lever presses for each successive reinforcer. Eventually the number of presses required for a reinforcer exceeds the value of that reinforcer. At that point the subject stops responding and the number of responses in the last ratio completed is called the 'break point' and is considered a measure of reinforcer efficacy or motivation (Hodos and Kalman, J Exp Anal Behav 6, 387 (1963)). Break points under PR schedules have been shown to be sensitive to many typical factors influencing motivational level, such as reinforcer magnitude (Cleary et al., Brain Res 653, 210 (1994)), self-administered drug dose (Griffiths et al.,

Psychopharmacology (Berl) 65, 125 (1979)), or food deprivation level (Macenski et al., Psychopharmacology (Berl) 112, 204 (1993)). In one study, break points in the PR task and cognitive function in the ALCR task were both assessed in animals treated with methimazole in utero (MacNabb et al., Brain Res 847, 231 (1999)).

- 5 This treatment results in animals that exhibit congenital hypothyroidism. The hypothyroid rats showed significantly slower learning to criterion than controls in the ALCR task, but their motivation for food was not different from that of controls as measured in the PR task.

- 10 In a second study using congenitally hypothyroid rats with replacement thyroxin treatment, all treated groups showed normal learning to criterion and error rates, while untreated groups showed impaired acquisition and increased errors compared to controls (MacNabb et al., Neurosci Res 36, 121 (2000)). When motivation for food was assessed in the PR task, all groups (treated and untreated congenital hypothyroid) performed equally well. These data clearly show that the
- 15 ALCR is capable of detecting cognitive deficits without changes in motivational status. Potential effects of A β on motivation for food. The above research does not preclude a motivational effect of A β *56 in the current study. However, no published paper or presentation has proposed that any form of A β affects motivation for food. While all behaviours are eventually affected by progressive
- 20 neurodegenerative conditions like Alzheimer's disease, the present example deals specifically with initial A β oligomeric effects early in the disease process, long before the appearance of plaques and neurodegeneration. This issue with the response rate data described is also addressed under the heading "Activity." In addition, it is noted that animals never left pellets in the food receptacle under any
- 25 of the drug conditions.

- Activity. Drug-induced motoric effects can have profound effects on cognitive assays. This issue in the ALCR task has been addressed in previous published studies (see Table 1). It is worth noting that in the current study data from subjects that did not complete all ratio requirements in each cycle was not
- 30 included in the analysis. Thus, the number of opportunities to make errors is always the same under baseline and treatment conditions regardless of the rate of response and the number of reinforcers earned is constant across sessions and conditions. Each subject's error data under treatment is always compared to that

subject's error data under non-treatment (baseline) conditions in a within-subject design. Different forms of response rate data are addressed below.

Absolute responses are equal to the sum of the products of each ratio value RV (2, 6, . . . 56) times its occurrence, plus total errors (perseveration plus
5 switching errors). This is not a useful assessment of activity since compounds that increase errors will always have higher absolute responses.

Absolute response rates (or total responses per session length) is a better measure, but post reinforcement pause contributes significantly to session length. As demonstrated in several other published studies, drugs that increase lever
10 choice errors often disrupt the subject's ability to keep track of ratio size, resulting in a flattened slope for the PRP by Ratio Value function (see, for example, O'Hare et al., Behav Pharmacol 7, 742 (1996)). A flattened PRP function decreases session length while the associated increased errors drive total responses up. Under these circumstances absolute response rate is a less than perfect metric of
15 activity or motivation. Nevertheless, interpretation of ALCR error rates is stronger if absolute response rates (correct + incorrect responses divided by session length) are stable. To evaluate this effect, absolute response rates after infusion of A β *56 and control injectates were calculated (Fig. 11). No statistically significant differences in absolute response rate were found between these conditions.

20 Running Response Rate. The best indicator of changes in activity level, potential drug-induced motoric impairment, and motivation under this lever pressing task is the Running Response Rate (RRR). RRR measures the responses per second during the time the animal is actually responding on the lever. RRR, of course, has a direct relationship to the size of the ratio requirement. This measure
25 is easily affected by sedating drugs and drugs that deleteriously affect motor function. Fig. 12 shows RRR plotted across all ratio values for the current study. There were no statistically significant differences in RRR between any of the injection groups. Differences were tested using multiple RMANOVAs, at each ratio value and no significant differences in RRR were found across treatments.

30 Thus, no differences in response rates in either absolute (total) responses per minute or running response rates were found under treatment conditions that produced significant changes in errors. To the extent that activity and motivation are reflected in these response rates, A β *56-induced errors in the ALCR task are not confounded by changes in activity or motivation.

ALCR Cognitive Effects

Prefrontal Executive Function. It is likely the ALCR task can measure cognitive changes involving prefrontal executive function. In fact, empirical evidence of this effect was obtained using hypothyroid rats in the ALCR task. The results in the ALCR task relate to results seen in hypothyroid humans and monkeys (MacNabb et al., Brain Res 847, 231 (1999), MacNabb et al., Neurosci Res 36, 121 (2000)). Frontal lobe executive function affecting choice behavior in humans is often assessed by the Wisconsin Card Sorting Test, wherein subjects are rewarded for choosing cards based upon stimulus class or set (e.g., shape) and a rule relating classes (e.g., square then green). A key feature is that the rule changes during the task. Humans with frontal lobe damage typically perseverate and do not apply the new rule. The present data on increased perseveration errors in the ALCR task are consistent with this general paradigm of cognitive dysfunction. A discussion of these effects can be found in MacNabb et al., 1999 (MacNabb et al., Brain Res 847, 231 (1999)).

Hippocampus Involvement. Previously published research supports ALCR's sensitivity to direct disruption of hippocampal systems. In one study, A β was aggregated prior to injection at 10^{-4} M and injected bilaterally into rat hippocampal formations (O'Hare et al., Behav Pharmacol 7, 742 (1996)). Perseveration errors, i.e., errors resulting from the rat choosing the incorrect lever after the Post Reinforcement Pause (PRP), increased significantly after IH A β injection. The PRP is discussed more fully below. Histological examination of the brains ninety days post injection showed accurate injectate placement and revealed that aggregated A β was still present in the hippocampus. A recent study used ALCR to assess the effects of a non-steroidal anti-inflammatory drug, ibuprofen, on A β -induced deficits following IH injection of a suspension of pre-aggregated A β (Richardson et al., Brain Res 954, 1 (2002)). Results from this study replicated the findings of O'Hare et. al., 1999 (see O'Hare et al., Brain Res 815, 1 (1999)) in regard to A β -induced ALCR performance decrements and also demonstrated that ibuprofen mitigated these effects. These studies clearly show the ALCR is sensitive to direct hippocampal disruption.

Memory. ALCR was not specifically designed as a test of memory, however, some form(s) of memory are involved in this or any task that requires the

animal to learn and perform—such as learning to press a lever to earn food. An additional form of memory is involved in discriminating between response options or choosing (e.g., switching rules), dependent upon the past occurrence of some event (e.g., food delivery). These memory forms are typically captured under the general rubric of ‘reference’ memory, for memory of the ‘rules/requirements of the task.’ Whether ALCR was sensitive to this type of memory disruption was specifically addressed by comparing choice responding in the ALCR, Forced ALCR and Forced Operant Alternation (FAO) tasks with multiple fixed-ratio requirements (MacNabb et al., *Brain Res* 847, 231 (1999), MacNabb et al., *Neurosci Res* 36, 121 (2000)). In the FOA and Forced ALCR tasks, lever pressing still alternates but there is only one choice available after the PRP. In this study, only the ALCR task differentiated between hypothyroid and normal animals in their ability to reach a learning and accuracy proficiency criterion. Hypothyroid rats did not differ in reaching the learning criterion in the Forced ALCR or simple operant Forced Alternation tasks with multiple fixed ratio values, reflecting the added value of learning the switching rules.

To claim that ALCR incorporates a working memory component there must be a delay between the response choice and the absent temporally discrete discriminative stimulus upon which it is based. Usually, these delays are independent variables imposed by the experimental procedure. In the ALCR task there is a reliable delay between discriminative stimulus and response but it is a dependent variable, i.e., under the control of the subject. The delay, called a Post Reinforcement Pause (PRP), has been shown to occur reliably after completing a ratio response requirement, like those in the ALCR procedure. The PRP reliably occurs in many species and under a great diversity of conditions, from birds pecking lighted disks to humans playing slot machines (Schreiber and Dixon, *Psychol Rep* 89, 67 (2001)). The PRP is directly related to the size of the ratio requirement. As can be seen in Fig. 13, mean PRPs in the ALCR task are directly related to ratio size. It should be clear from Fig. 13 that PRP is not feeding behavior but is, as consistently reported in the operant literature, a direct function of ratio size. It should also be noted that mean PRPs after infusions of A β *56 are reduced relative to baseline under the higher ratio sizes. Even though this reduction is not statistically significant, it would if anything make the correct lever choice after infusions of A β oligomers easier and thus work against a significant

finding of increased perseveration errors reported in the present example. It is indicative of effects seen with several drugs known to affect cognitive function in the ALCR task and reflects the subjects' failure to track the size of the current ratio size. While the data in Fig. 13 suggests that rats given A β *56 were impaired in their ability to track the ratio requirement (see O'Hare et al., Behav Pharmacol 7, 742 (1996)), the effects did not reach statistical significance. In summary, there is a consistent pause after completing a response requirement and before the choice that might result in a perseveration error in the ALCR task. However, despite the contention that there is a consistent delay prior to the choice response, it is acknowledged that the situation in the ALCR task does not mimic typical procedures used to measure working memory.

Table 2.

Levels of various A β species in Tg2576 mice.

Type of A β	Citation	Ages Tested (months)	Appearance at 6 months	Fold-change in A β ±
ADDLs	Chang et al. ¹	13, 17	ND	~5x in septum, ~20 -100x in cortex
A β 42 _{SDS}	Kawarabayashi et al. ²	2-23	no	~7-8x
A β 42 _{FA}	Kawarabayashi et al. ²	2-23	yes	~500-1000x
A β 42 _{SDS}	Kawarabayashi et al. ²	2-23	no	~10x
A β 42 _{FA}	Kawarabayashi et al. ²	2-23	yes	~1000x
Lipid raft A β	Kawarabayashi et al. ³	4-12, 17, 24	yes	>500x
Intracellular A β	Takahashi et al. ⁴	3, 10	no	~2x

±Fold-change in A β between 6-13 months, except for ADDLs and intracellular A β , where fold-change is denoted for the ages tested. Abbreviations: ND, not determined; ADDLs: A β -derived diffusible ligands; A β SDS: SDS-extractable A β ; A β FA: SDSinsoluble, formic acid-extractable A β .

Three A β species first appear at six months: 1) SDS-insoluble A β 40; 2) SDS-insoluble A β 42; and 3) A β in lipid rafts (Kawarabayashi et al., J Neurosci 21, 372 (2001); and Kawarabayashi et al., J Neurosci 24, 3801 (2004)). However, all three A β species increase 500- to 1000-fold in the interval from six to fourteen

months of age when there is little or no further change in memory. Other A β species, including intracellular A β (Takahashi et al., J Neurosci 24, 3592 (2004)) and SDS-soluble A β (Kawarabayashi et al., J Neurosci 21, 372 (2001)) are already present prior to 6 months of age, and therefore are probably not involved in

5 memory loss occurring at six months. ADDL levels increase 20- to 100-fold in the cortex between 13 and 17 months of age (Chang et al., J Mol Neurosci 20, 305 (2003)), and 130-fold in the hippocampus between nine and twenty months of age.

In all figures, the ages of mice (in months) are indicated above each gel in bold characters below the corresponding genotype. Tg2576^{-/-}, Tg2576^{+/-}, and

10 Tg2576^{+/+} denote mice harboring zero (non-Tg), one and two transgene arrays, respectively. Numerals inside bars denote numbers of mice. Spatial memory refers to the retention of spatial information evaluated in the Morris water maze as measured by mean % target quadrant occupancy scores $\pm$ S.E.M during probe trials. When indicated, synthetic human A β ₁₋₄₂ peptide (hA β ₄₂) was loaded in

15 parallel as a size marker and positive control (right lane). Arrows indicate respective migration positions of monomers (1-mer), dimers (2-mer), trimers (3-mer), tetramers (4-mer), hexamers (6-mer), nonamers (9-mer) and dodecamers (12-mer), as well as sAPP α . Western blot (WB), immunoprecipitation (IP).

Fig. 1 shows temporal patterns of memory decline and soluble,

20 extracellular A β oligomers in Tg2576 mice. The spatial memory in mice from 4 to 17 months shows a progressive but irregular decline with periods of stability (Fig. 1A). Temporal analysis of spatial memory shows three stages of performance (Fig. 1B). In Fig. 1C the identification of A β oligomers in soluble, extracellular-enriched extracts of proteins from brains of 5-, 6-, and 7-month mice,

25 was assessed by western blot (WB) with or without immunoprecipitation (IP). The intensity of the 40 kDa band co-migrating with nonamers was $33.92 \pm 12.5\%$ (n = 4) of the intensity of the 56 kDa band corresponding to dodecamers.

Fig. 2 shows that water maze performance correlates inversely with A β nonamer and dodecamer levels. There is a lack of significant correlations between

30 spatial memory and monomeric, trimeric or hexameric soluble A β species detected in the extracellular-enriched fractions of 5-month and 6-month Tg2576^{+/-} mice (Fig2A-2F). Figs. 2G and 2H show that the nonameric and dodecameric A β levels correlate inversely with spatial memory at 6 months.

As shown in Fig. 3, A β *56 disrupts cognitive function. Soluble proteins in extracellular-enriched extracts from Tg2576+/- or Tg2576-/- mice were fractionated by SEC to generate fractions with (19+/-) or without (17+/- and 19-/-) the 56 kDa A β species (Fig. 3A). Fraction 19+/- was also subjected to three rounds of IP with 6E10 to remove the 56kDa A β species. Bands at 75 and 150 kDa present in non-Tg littermates and IP19+/- were considered to be non-specific (ns). Perseveration Errors (Fig. 3B) and Switching Errors (Fig. 3C), presented as a percentage of baseline rates ($\pm$ SEM), in the ALCR paradigm two hours after rats received injections of the indicated fractions into the lateral ventricles. Both types of errors increased significantly above baseline only when the 56kDa A β species was present. (Wilcoxon matched-pairs signed-ranks test, * $p < 0.05$).

Fig. 4 shows that the elimination of A β *56 coincides with an interlude of normal spatial memory. A β *56 levels decline in Tg2576+/- mice between 12.0-12.4 months of age (Fig. 4A). Levels of A β *56 between 10.7-13.0 months of age (Fig. 4B). Values represent band intensities (mean $\pm$ SD) relative to the intensities observed at 10.7 months. ANOVA, $p < 0.02$, followed by t test, * $p < 0.01$, $n = 4$ animals per age group. The dip in A β *56 levels coincides with a transient recovery of spatial memory (target quadrant occupancy $\pm$ SEM in Day 5 probe trial) (Fig. 4C). ANOVA, $p < 0.01$, followed by t test, * $p < 0.01$, ** $p < 0.001$, and by Fisher's protected least significant difference (PLSD) test, ‡ $p < 0.001$, # $p < 0.05$. Tg2576+/-, Tg2576-/- . Fig. 4D shows kinetics of the rate of change in A β (x-42) levels (SDS-soluble A β (x-42) and SDS-insoluble A β (x-42) in Tg2576+/- mice (adapted from Kawarabayashi et al., 2001(11)). Abbreviations: FA = SDS-insoluble/formic acid-soluble; Δt = time interval. And, Fig. 4E shows a hypothetical dynamic relationship between soluble and insoluble pools of A β .

Fig. 5 shows the fidelity of the technique for measuring soluble, extracellular A β oligomers in vivo. In Fig. 5A procedures used to extract various pools of A β are illustrated. Fig. 5B shows an SDSPAGE analysis of several protein markers in the collected fractions. The forebrain was subjected to a four step extraction protocol generating four fractions (extracellular-enriched soluble, intracellular-enriched soluble, membrane-enriched, and insoluble). Partial characterization and validation of the fractionation procedure was achieved using the NMDA receptor subunit NR2 as a marker for membrane-enriched proteins, APP as a marker for both soluble (sAPP α) and membrane-enriched (full-length

APP = fl-APP) proteins, and the extracellular serine protease tissue-type plasminogen activator t-PA, as a marker for soluble proteins. Other protein markers used were microtubule-associated proteins MAP-2 and tau (cytoskeleton), the protein kinases, ERKs and JNK (cytosol), and flotillin-2 (lipid rafts). Soluble microtubule-free tau, ERKs, JNK and c-Jun were mainly identified in the intracellular-enriched soluble fraction; cytoskeleton proteins MAP-2 and microtubule-bound tau were present within membrane-enriched fractions; and flotillin-2 was found in the insoluble pellet resuspended with Tris-buffered formic acid. Fig. 5C presents validation of the use of immunoprecipitation to study A β species quantitatively. To ensure that immunoprecipitated A β species were a faithful index of brain A β levels, two sequential immunoprecipitations (IP1 and IP2) with either 6E10 or 4G8 monoclonal antibodies were performed and revealed negligible amounts ($1 \pm 0.64\%$, $n = 4$) of 6E10 immunoreactive material in WB after the first IP in extracts from the oldest animals (25 months).

Fig. 6 presents the biochemical and structural properties of A β assemblies in Tg2576 mice. Fig. 6A demonstrates the purification of soluble, extracellular-enriched A β species using affinity columns packed with 200 μ g of 6E10 or 4G8 antibodies. Captured proteins were eluted in acidic buffer (pH 3), fractionated by SDS-PAGE, and WB were revealed with 6E10. Fig. 6B shows that A β multimers are resistant to the strong chaotropic agent, 8M urea. Soluble, extracellular-enriched extracts from 12- to 20-month Tg2576+/- brains were loaded onto 8M urea containing SDS-PAGE gels, electrotransferred, and probed with 6E10. The presence of urea did not alter electrophoretic migration patterns, indicating that A β oligomers are probably not associated with large globular proteins. Fig. 6C demonstrates that soluble HMW A β oligomers are not resistant to treatment with greater than or equal to 15% hexafluoroisopropanol (HFIP). Monomeric A β levels increased with rising HFIP concentrations. Trimers were exceptionally resistant to HFIP. Fig. 6D represents an evaluation of A β multimers with the anti-oligomer antibody, A11. Note that hAs42 standards are not detected with the A11 antibody (right lane).

Fig. 7 is a characterization of native A β oligomer size and expression levels in Tg2576 mice. Fig. 7A shows a SDS-PAGE analysis of soluble brain extracts fractionated by SDS-free size exclusion chromatography (SEC) showed that all A β oligomers migrated at expected molecular weights using globular protein

standards. These data confirm that high molecular weight (HMW) A β oligomers are not artificially generated during electrophoresis from monomeric or trimeric A β species, and trimers are not degradation products of HMW oligomers. Bands revealed at 75 and 150 kDa are non-specific bands which are also present in blots of extracts from non-transgenic mice. In Fig. 7B soluble, extracellular-enriched A β species are assessed by WB using 6E10 in mice between 9 and 25 months of age. Figs. 7C and 7D represent a semi-quantification of A β species levels (relative to β -tubulin levels) expressed as percentage of respective averaged signals observed in 9-month-old animals (n = 6/age group). ANOVA, followed by t test, *p < 0.01, # p < 0.05.

Fig. 8 shows the absence of a correlation between A β oligomer levels and swimming speed or path length during the cued (or visible) phase of water maze testing. Figs. 8A and 8B show the relationship between swimming speed and A β levels in 5- (Fig. 8A) and 6- (Fig. 8B) month-old Tg2576+/- mice. ANOVA p values are displayed in graphs alongside r² values. Fig. 9 shows there is no change in levels of intracellular A β levels and in CTFs in 5- and 6-month Tg2576 mice. In Fig. 9A soluble, intracellular-enriched A β species in 5-, 6- and 7-month mice evaluated by western blot (WB) using 6E10. The top insert shows levels of sAPP α . Fig. 9B demonstrates compartmentalization of APP-CTFs in Tg2576 mouse brain. CTF- β 's were IP'd from soluble or membrane extracts with 6E10 and identified in WB with APPCter-C17. No immunoreactive bands were detected in the soluble fraction, but a doublet of bands around approximately 2 kilodalton (kDa) was present in the membrane-enriched fraction. As a control, both CTF- β and CTF- α were detected with APPCter-C17. Fig. 9C presents IPs of CTFs using APPCter-C17 confirmed no overall change in CTFs between 5 to 7 months of age. Full-length APP also captured by the antiserum is displayed on top and shows no variation in levels. Trimers are the predominant oligomeric A β species secreted from Tg2576 cultured primary brain cells. Near pure primary cortical neurons or neurons cocultured with astrocytes immunolabelled with antibodies for MAP-2, GFAP and DNA intercalants DAPI or Propidium Iodine (Ipr). In Fig. 10A the levels of naturally secreted A β species in the conditioned media (CM) of 7- or 14-DIV (days in vitro)-old neurons were evaluated by IP followed by WB with 6E10 antibodies. In CM from 7-DIV neurons trimers are most prominent. In CM from 14-DIV neurons tetramers and trimers are present, but monomers are barely

detectable (without boiling the membrane). Fig. 10B shows that cortical astrocytes modulate the levels of neuron-derived A β species in the CM. When Tg2576+/+ neurons were co-cultured in the presence of Tg2576-/- astrocytes, the overall levels of A β diminished and trimers were the only species detected in the CM (without boiling the membrane). Fig. 10C shows that boiling the membranes enhances the detection of monomeric A β with 6E10, which constitutes the major A β species secreted in the CM. However, boiling did not significantly enhance the ability to detect the oligomeric A β species, presumably because the tertiary and quaternary structure of the trimers and tetramers readily exposes the 6E10 epitope without requiring further denaturation. Fig. 10D intracellular protein preparations from Tg2576+/- primary neurons devoid of APP-CTFs were IP'd with 6E10, revealing trimers but not tetramers, just as in the soluble, intracellular-enriched fractions of 5- to 6-month Tg2576+/- mice (see Fig. 9A). These data suggest that trimers are formed within neurons and subsequently secreted. HMW A β oligomers are assembled outside of neurons. Fig. 10E demonstrates membrane-associated APP-derived molecules in Tg2576+/- primary neurons. IPs using 6E10 captures CTF- β s and A α monomers. The blot was denatured and re-probed with APPCter-C17 to confirm the nature of the approximately 13 kDa bands, revealing both phosphorylated (pCTF) and nonphosphorylated CTF- β . When extracts were immunodepleted of APP-CTFs, only A β monomers remained.

Fig. 11 presents overall response rates. Response rates of rats were compared at Baseline and after receiving fractions from Tg Pos Lane 19 (Lane 19+/- in Fig. 3) containing A β *56 from a Tg2576 transgene positive mouse, corresponding Tg Neg Lane 19 (Lane 19-/- in Fig. 3) from a transgene negative mouse, and Tg Control Lane 17 (Lane 17+/- in Fig. 3) without A β *56 from a transgene positive mouse. There were no significant differences between the overall response rates.

Fig. 12 presents running response rates. Running response rates of rats were compared at Baseline and after receiving fractions containing A β *56 from a Tg2576 transgene positive mouse Lane 19+/- in Fig. 3) and Tg2576 Control Ln 17 (Lane 17+/- in Fig. 3) without A β *56 from a transgene positive mouse. There were no significant differences between the running response rates.

Fig. 13 presents post reinforcement pause as a function of ratio size. Post reinforcement pause was compared as a function of ratio size in rats at Baseline

and after receiving fractions containing A β *56 from a Tg2576 transgene positive mouse (Lane 19+/- in Fig. 3) and Tg2576 Control Ln 17 (Lane 17+/- in Fig. 3) without A β *56 from a transgene positive mouse. There were no significant differences between these values.

5

Example 2

The isolation of A β * from B6SJL and 129FVBF1 mice

Following the procedures detailed in Example 1, Fig. 14 demonstrates that
10 B6SJL and 129FVBF1 mice show identical patterns of soluble A β oligomers at various ages. This argues against the potential effects of strain background on A β formation.

Example 3

15

Antibodies to A β *

Binding specificities and screening methodology for antibodies directed against A β *

Candidate anti-A β * clones will be screened comprehensively using
20 methods that ensure that the anti-A β * monoclonals specifically recognize natively folded A β *56, and do not bind fibrillar or monomeric A β . Dot blot methods followed by confirmatory liquid-phase immunoprecipitation and immunoblotting experiments will be used for this purpose. Direct liquid phase ELISA methods will also be used. These dual methods are depicted in Fig. 15. The dot blot method is
25 advantageous due to its rapid throughput and minimal potential for steric hindrance preventing detection of suitable clones. The ELISA method is useful due to its ability to detect natively folded A β *56 directly.

Fig. 15 shows various methods for screening candidate monoclonals for antibodies that specifically detect A β *56. In the Dot Blot assay, A β *56, synthetic
30 monomeric A β (1-42), soluble A β (1-42) oligomers and fibrillar A β (1-42) will be spotted at known concentrations on nitrocellulose or nylon filters. The filters will be overlaid with candidate monoclonals. Clones that selectively stain A β *56 at low concentrations will be selected. In the Western Blot assay, which is a confirmatory test for the Dot Blot assay, A β *56, synthetic monomeric A β (1-42),

soluble A β (1-42) oligomers and fibrillar A β (1-42) will be size-fractionated by polyacrylamide gel electrophoresis and transferred to nitrocellulose or nylon filters. The filters will be overlaid with candidate monoclonals. Clones that selectively stain A β *56 but no other forms of A β will be selected. In the liquid
5 phase ELISA method, monoclonal anti-A β antibodies 6E10 or 4G8 will be immobilized onto the wells of plastic plates, overlaid with A β *56. Candidate monoclonals will be applied to wells. Clones that bind A β *56 will be detected with goat anti-mouse antibodies conjugated to a fluorescent marker.

10 Making antibodies directed against A β *

To generate anti-A β * monoclonals, mice will be immunized with purified A β *56 from the brains of Tg2576 mice greater than six months old, AD patients, or Down syndrome patients, or with synthetic A β oligomers that include species which are ~56 kDa. A β *56 will be purified by immunoaffinity chromatography
15 followed by size-exclusion chromatography so that it runs as a single band on silver stained gels, as we have previously shown. Biochemical methods will also be used to purify A β *56, taking advantage of the stability of A β *56 in 8M urea, which denatures most globular proteins.

To determine that the purified immunogen is biologically active, it will be
20 assayed for its ability to inhibit NMDA-evoked currents in cultured neurons, prior to injection as an immunogen. However, this is not an essential step, because the screening method described above will select only those monoclonals that specifically detect A β *56.

It is expected that these methods will successfully lead to the generation of
25 specific antibodies to A β *56, particularly since multimerized proteins tend to be better immunogens than monomeric proteins, because they crosslink immunoglobulins on B-cells.

A source of concern is that the use of an immunogen consisting of 56kDa A β oligomers generated from synthetic A β would theoretically yield fewer
30 monoclonals that target the specific conformation of natively folded A β *56. For this reason, the screening of candidate anti-A β * monoclonals using natively folded A β *56, which is shown in Fig. 15, is important.

It is possible that A β *56 in human brain (from AD patients) and mouse brain (from Tg2576 mice) may differ subtly in conformation. Therefore, both

natively folded A β *56 purified from AD and Tg2576 mouse brain tissue will be used to screen monoclonals. The results will generate a two by two catalogue of clones showing specific recognition of AD-A β *56, Tg2576-A β *56, both AD-A β *56 and Tg2576-A β *56, or neither protein complex. This catalogue will aid in
 5 selecting the most appropriate anti-A β * monoclonals for use in humans.

Assessing therapeutic efficacy of anti-A β * monoclonals in behavioral and electrophysiological assays

Three approaches will be used to assess the functional efficacy of anti-A β *
 10 monoclonals, which have been biochemically validated using the methods described above. Additional behavioral and electrophysiological assays will be used to determine whether the anti-A β * monoclonals are functionally effective in neutralizing the detrimental effects of A β *. Monoclonals with the highest binding affinities to purified, natively folded A β *56 will be used for functional assays.

15 In the first approach, intraperitoneally (IP) administered anti-A β * monoclonals will be assessed for their ability to prevent and to reverse spatial reference memory deficits in Tg2576 mice. In the second approach, anti-A β * monoclonals administered IP or injected directly into the lateral ventricles will be evaluated for their ability to block the disruption of cognitive function in healthy
 20 rats receiving A β *56. In the third approach, the response of A β *56 inhibition of NMDA-evoked currents to anti-A β * monoclonals will be studied. Monoclonals that show both selective biochemical binding to A β *56 and neutralizing effects on the deleterious functional actions of A β *56 will be important therapeutic and diagnostic tools.

25 Assessing ability of anti-A β * monoclonals to ameliorate spatial reference memory deficits in Tg2576 mice

To test the effects of IP administered anti-A β * monoclonals on spatial reference memory deficits in Tg2576 mice, a protocol previously employed to
 30 show reversal of pre-existing memory deficits in Tg2576 following IP administration of BAM-10 (Kotilinek et al., J Neurosci. 22(15):6331-5 (2002)), a monoclonal antibody raised against A β (1-10) which clears plaques *in vivo* (Bacsikai et al., J Neurosci. 22(18):7873-8 (2002) and Lombardo et al., J Neurosci. 26;23(34):10879-83 (2003)) and detects soluble A β monomers and oligomers as

well as A β *56, will be employed. In addition, anti-A β * monoclonals will be administered to young, unimpaired mice to assess the prophylactic potential of the anti-A β * monoclonals. BAM-10 will serve as a positive control and non-specific immunoglobulin G will serve as a negative control. These experiments will

5 determine the ability of the anti-A β * monoclonals to cross the blood brain barrier and to neutralize A β *56 or to act as “peripheral sinks” to extract A β *56 out of the brain.

Assessing the ability of anti-A β *monoclonals to block A β *-induced behavioral

10 deficits in healthy rats

The ALCR behavioral protocol (Cleary et al., Nat. Neuroscience 8, 79-84 (2005) and PCT /US2005/023070, filed June 30, 2005) may be used to assay A β *56 directly, and also to test the effects of anti-A β * monoclonals on AD-A β *56 mediated disruption of cognitive function in healthy rats. Anti-A β * monoclonals

15 will be administered directly into the lateral ventricles, prior to injecting Tg2576-A β *56 or AD-A β *56. These experiments will show that the monoclonals specifically inhibit the deleterious effects of Tg2576-A β *56 or AD-A β *56 on cognitive function.

A β * specifically disrupts cognitive function and antibodies directed against

20 A β * specifically target a this key causing cognitive deficits. Thus, antibodies directed against A β * will be therapeutically effective in ameliorating cognitive deficits. The effectiveness of such administration of antibodies is supported by the prior success of passive and active vaccine studies in mice in preventing and reversing memory loss and the encouraging preliminary reports with immunization

25 with AN-1792, (beta amyloid peptide 1-42) on cognitive function in a subset of Alzheimer’s disease (AD) patients (see, for example, Gilman et al., Neurology 64:1553-1562 (2005). The passive administration of such antibodies would avoid the risk of encephalitis caused by a cellular immune reaction associated with active immunization, such immunization with AN-1792.

30 A β * may be present at low levels in serum or CSF in the pre-clinical or very early stages of Alzheimer’s disease. Thus, then Alzheimer’s disease may be diagnosed by detecting A β * in serum or CSF, using antibodies specifically directed against it. For example, with highly specific polyclonal and monoclonal antibodies against A β *, nanotechnology may be used to detect quantities of A β * in the

attomolar (10^{-18} M) range (see, for example, Georganopoulou et al., Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2263-4). Thus, antibodies directed against A β * may be used in methods for the early diagnosis or prediction of cognitive disorders, including, but not limited to, AD.

5

Example 4

Human A β *56

It has been shown that A β *56 is a ligand of the NMDA receptors (U.S. Provisional Application 60/703,653, filed July 29, 2005). The interaction of A β assemblies and ionotropic glutamate receptors in brain tissue from patients with AD and control individuals without dementia was examined. NR1, NR2A and, to a significantly lesser extent, NR2B antibodies immunoprecipitated a 56-kD 6E10-immunoreactive protein co-migrating with A β *56 in brain tissue samples from all four patients with AD, but in neither of two samples from control individuals with no cognitive impairment (NCI) (Figs. 16A, 16B). The unequal levels of NR2A subunits were not due to inconsistencies in loading samples, and therefore reflected actual receptor subunit levels in the brain specimens. These data indicate that A β *56 or an A β *56-like molecule binds NMDA receptors selectively in AD patients. Since NR2B subunits are preferentially found in extra synaptic NMDA receptor complexes (Collingridge et al, Wang, Nat Rev Neurosci 5, 952 (2004)), it is possible that in AD brain, A β *56 binding of NMDA receptors is biased toward synaptic NMDA receptors. Larger sample sizes will be required to ascertain whether the binding of A β *56 or an A β *56-like molecule in human brain tissue to NMDA receptors can be used to define AD biochemically.

As shown in Fig. 16, human-derived A β *56 physically binds NMDA receptors. Fig. 16A demonstrates that A β *56 coimmunoprecipitates with NR1 NMDA receptor subunits in brain tissue from Alzheimer (AD) patients but not from control subjects with no cognitive impairment (NCI), or extracts containing no brain proteins (NP). Fig. 16B demonstrates that A β *56 co-immunoprecipitates with NR2A, but much less readily with NR2B, NMDA receptor subunits in brain tissue from subjects with AD but not from control subjects (NCI). Fig. 16C demonstrates that A β *56 does not co-immunoprecipitate with α 7 nicotinic acetylcholine receptors (α 7nAChR). Panels below each blot confirm the ability of

the various receptor antibodies to immunoprecipitate the respective receptors or receptor subunits.

Methods

5 Human brain tissue. Frozen specimens of cerebral cortex were obtained from three AD patients and two cognitively intact control subjects, and one AD patient.

Antibodies. The following primary antibodies were used: 6E10 [1:100-10,000 dilution] against A β 1-17 (Signet Laboratories, USA) and antibodies raised
10 against NR1 and NR2 subunits (A-D) [1:200 dilution] (Santa Cruz Biotechnologies Inc, USA).

Protein extractions. Soluble, extracellular-enriched fractions were generated from hemi-forebrains harvested in 500 μ l of solution containing 50 mM Tris-HCl (pH 7.6), 0.01% NP-40, 150 mM NaCl, 2mM EDTA, 0.1% SDS, 1 mM
15 phenylmethylsulfonyl fluoride (PMSF), and protease inhibitor cocktail (Sigma). Soluble, extracellular-enriched proteins were collected from mechanically homogenized lysates (1 ml syringe, gauge 20 needle [10 repeats]) following centrifugation for 5 minutes at 3,000 rpm.

Membrane-enriched fractions were generated from hemi-forebrains
20 harvested in 500 μ l of solution containing 50 mM Tris-HCl (pH 7.6), 0.1% NP-40, 150 mM NaCl, 2mM EDTA, 1% SDS, 1 mM PMSF, 2 mM 1,10-PTH and protease inhibitor cocktail (Sigma). Lysates were mechanically homogenized (1 ml syringe and needle, gauge 20 [10 repeats]) and centrifuged for 90 minutes at 13,000 rpm. Membrane-associated proteins were generated from the pellets re-suspended with
25 500 μ l of buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 0.5% Triton X-100, 1 mM EGTA, 3% SDS, 1% deoxycholate, 1 mM of PMSF) following centrifugation for 90 minutes at 13,000 rpm. All supernatants were clarified by centrifuging for 90 minutes at 13,000 rpm prior to western blot analysis. Protein amounts were determined (BCA Protein Assay, Pierce). Western blot and immunoprecipitations
30 were performed as described in Example 1.

The complete disclosure of all patents, patent applications, and publications, and electronically available material (including, for instance, nucleotide sequence submissions in, e.g., GenBank and RefSeq, and amino acid

sequence submissions in, e.g., SwissProt, PIR, PRF, PDB, and translations from annotated coding regions in GenBank and RefSeq) cited herein are incorporated by reference. The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood
5 therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

10

What is claimed is:

1. An isolated, soluble amyloid- β protein assembly comprising more than one detergent stable oligomer of amyloid- β proteins.
2. The isolated, soluble amyloid- β protein assembly of claim 1 wherein the amyloid- β protein assembly disrupts cognitive function.
3. The amyloid- β protein assembly of claim 1 wherein the amyloid- β protein assembly has a molecular weight of about 40 kDa as measured by SDS polyacrylamide gel electrophoresis.
4. The amyloid- β protein assembly of claim 1 wherein the amyloid- β protein assembly has a molecular weight of about 56 kDa as measured by SDS polyacrylamide gel electrophoresis.
5. An isolated, soluble amyloid- β protein assembly comprising a dodecamer of amyloid- β proteins.
6. The amyloid- β protein assembly of claim 5 wherein the dodecamer of amyloid- β proteins comprises six detergent stable dimers of amyloid- β protein.
7. The amyloid- β protein assembly of claim 5 wherein the dodecamer of amyloid- β proteins comprises four detergent stable trimers of amyloid- β protein.
8. The amyloid- β protein assembly of claim 5 wherein the dodecamer of amyloid- β proteins comprises three detergent stable tetramers of amyloid- β protein.
9. The amyloid- β protein assembly of claim 5 wherein the dodecamer of amyloid- β proteins comprises two detergent stable hexamers of amyloid- β protein.

10. The amyloid- β protein assembly of claim 5 wherein the amyloid- β protein assembly has a molecular weight of about 56 kDa as measured by SDS polyacrylamide gel electrophoresis.
11. The isolated, soluble amyloid- β protein assembly of claim 1 comprising more than one detergent stable trimer of amyloid- β proteins.
12. The amyloid- β protein assembly of claim 11 comprising three detergent stable amyloid- β protein trimers.
13. The amyloid- β protein assembly of claim 11 comprising four detergent stable amyloid- β protein trimers.
14. The amyloid- β protein assembly of claim 11 wherein the amyloid- β protein assembly has a molecular weight of about 40 kDa as measured by SDS polyacrylamide gel electrophoresis.
15. The amyloid- β protein assembly of claim 11 wherein the amyloid- β protein assembly has a molecular weight of about 56 kDa as measured by SDS polyacrylamide gel electrophoresis.
16. The isolated, soluble amyloid- β protein assembly of claim 1 comprising more than one detergent stable tetramer of amyloid- β proteins.
17. The amyloid- β protein assembly of claim 16 comprising three detergent stable amyloid- β protein tetramers.
18. The amyloid- β protein assembly of claim 16 wherein the amyloid- β protein assembly has a molecular weight of about 56 kDa as measured by SDS polyacrylamide gel electrophoresis.
19. A composition comprising the amyloid- β protein assembly of any one of claims 1-18.

20. A vaccine comprising the amyloid- β protein assembly of any of claims 1-18.
21. An antibody that binds to the amyloid- β protein assembly of claim 1.
22. An antibody that binds to the amyloid- β protein assembly of claim 1 and does not bind to monomeric amyloid- β protein.
23. An antibody that binds to the amyloid- β protein assembly of claim 1 and does not bind to dimeric amyloid- β protein.
24. An antibody that binds to the amyloid- β protein assembly of claim 1 and does not bind to trimeric amyloid- β protein.
25. An antibody that binds to the amyloid- β protein assembly of claim 1 and does not bind to tetrameric amyloid- β protein.
26. A method of treating a cognitive disorder in a subject, the method comprising administering an antibody of claim 21 to the subject.
27. A method of detecting a cognitive disorder in a subject, the method comprising contacting a fluid or tissue taken from the subject with an antibody of claim 21.
28. A method of detecting a presymptomatic cognitive disorder in a subject, the method comprising contacting a fluid or tissue sample taken from the subject with an antibody of claim 21.
29. A method of disrupting memory of learned behavior in a non-human mammal, the method comprising administering the amyloid- β protein assembly of claim 1 intracranially.
30. The method of claim 29 wherein the non-human mammal serves an animal model system for a cognitive deficit.

31. The method of claim 29 wherein the non-human mammal serves an animal model system for Alzheimer's disease.
32. The method of claim 29 wherein the non-human mammal demonstrates cognitive deficits consistent with presymptomatic or early Alzheimer's disease.
33. An animal model comprising a non-human mammal wherein an amyloid- β protein assembly of any of claim 1 has been administered intracranially.
34. The animal model of claim 33 wherein the non-human mammal is a mouse, rat, dog, or non-human primate.
35. The animal model of claim 33 wherein the non-human mammal demonstrates disruption of complex learned behaviors.
36. The animal model of claim 33 wherein the non-human mammal demonstrates cognitive deficits consistent with presymptomatic or early Alzheimer's disease.
37. A method of screening for an agent effective for the treatment of a cognitive disorder, the method comprising:
- administering a test agent to a first animal to which a soluble amyloid- β protein assembly of more than one detergent stable oligomer of amyloid- β proteins has been intracranially administered;
 - measuring cognitive function of the first animal;
 - comparing the cognitive function of the first animal to the cognitive function of a second animal to which a soluble amyloid- β protein assembly comprising more than one detergent stable oligomer of amyloid- β proteins has been intracranially administered, but no test agent has been administered;
 - wherein an improvement in the cognitive function of the first animal compared to the cognitive function of the second animal indicates the test agent is an effective agent for the treatment of a cognitive disorder.

38. A method of treating a cognitive disorder in a subject, the method comprising administering to the subject an agent that inhibits the assembly of monomers of amyloid β protein into a soluble amyloid- β protein assembly comprising more than one detergent stable oligomer of amyloid- β proteins.

39. An agent that inhibits the assembly of detergent-stable oligomers of amyloid β protein into a soluble amyloid- β protein assembly comprising more than one detergent stable oligomer of amyloid- β proteins.

40. An agent that promotes the clearance of a soluble amyloid- β protein assembly comprising a soluble amyloid- β protein assembly comprising more than one detergent stable oligomer of amyloid- β proteins from neurological tissue.

41. A method of treating a cognitive disorder in a subject, the method comprising administering to the subject an agent that inhibits the assembly of detergent-stable oligomers of amyloid β protein into a soluble amyloid- β protein assembly comprising more than one detergent-stable oligomer of amyloid- β proteins.

42. A method of treating a cognitive disorder in a subject, the method comprising administering to the subject an agent that promotes the clearance from neurological tissue of a soluble amyloid- β protein assembly comprising more than one detergent-stable oligomer of amyloid- β proteins.

43. A method of detecting a cognitive disorder in a subject, the method comprising detecting in a fluid or tissue taken from the subject soluble amyloid- β protein assemblies comprising more than one detergent-stable oligomer of amyloid- β proteins.

44. A method of detecting a presymptomatic cognitive disorder in a subject, the method comprising detecting in a fluid or tissue sample taken from the subject soluble amyloid- β protein assemblies comprising more than one detergent-stable oligomer of amyloid- β proteins.

45. A method for assaying the effects of soluble oligomers of amyloid β protein on cognitive function, the method comprising:
administering a soluble amyloid- β protein assembly comprising more than one detergent-stable oligomer of amyloid- β proteins intracranially into an animal;
measuring cognitive function to determine the disruption of cognitive behavior.
46. The method of claim 45, wherein the disruption of cognitive behavior of the animal is compared to the long-term disruption of cognitive behavior of another animal treated in the same fashion except saline rather a soluble amyloid- β protein assembly comprising more one detergent-stable oligomer of amyloid- β protein is administered intracranially.
47. The methods of any one of claims 38 or 41-44, wherein the cognitive disorder is an age related memory decline or an age associated memory impairment.
48. The methods of any one of claims 38 or 41-44, wherein the cognitive disorder is Alzheimer's disease.
49. The methods of any one of claims 38 or 41-44, wherein the cognitive disorder is mild cognitive impairment.
50. A method of isolating soluble amyloid- β protein assemblies, the method comprising more than one detergent-stable oligomer of amyloid- β proteins, the method comprising:
homogenizing neuronal tissue in a lysis buffer;
size fractionating amyloid- β protein assemblies; and
isolating an amyloid- β protein assembly of a desired size.

Fig. 1B

Fig. 1A

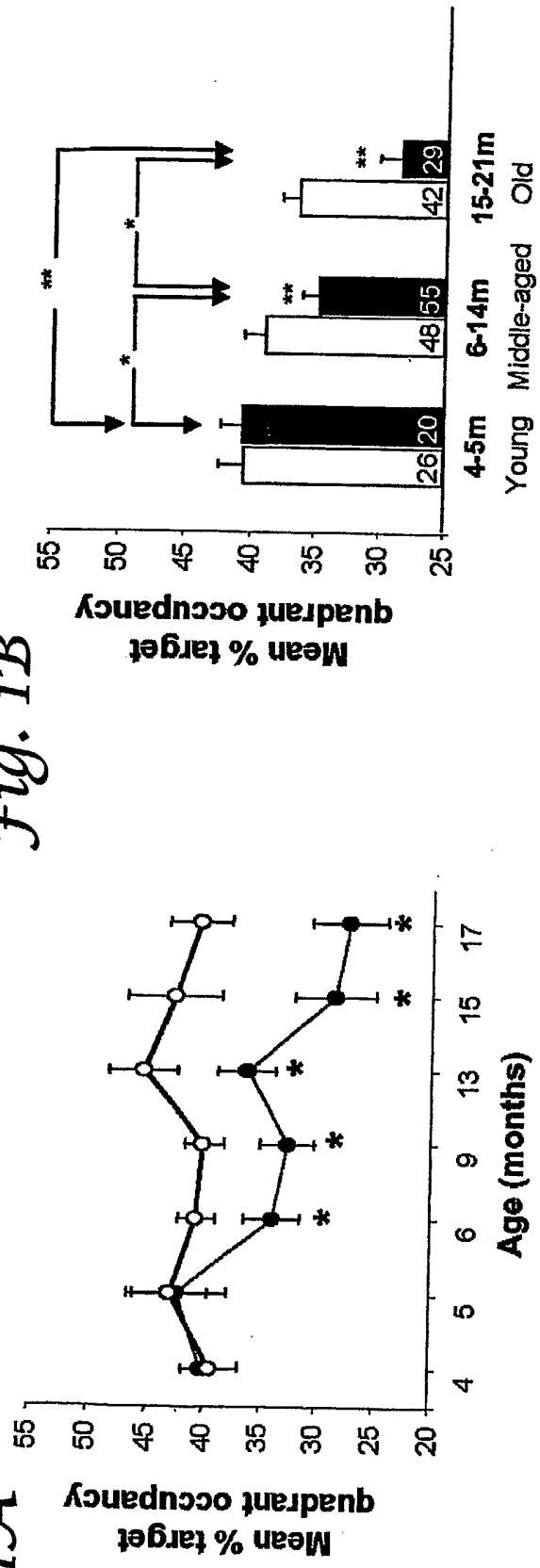


Fig. 1C

Extracellular-enriched

IP 6E10

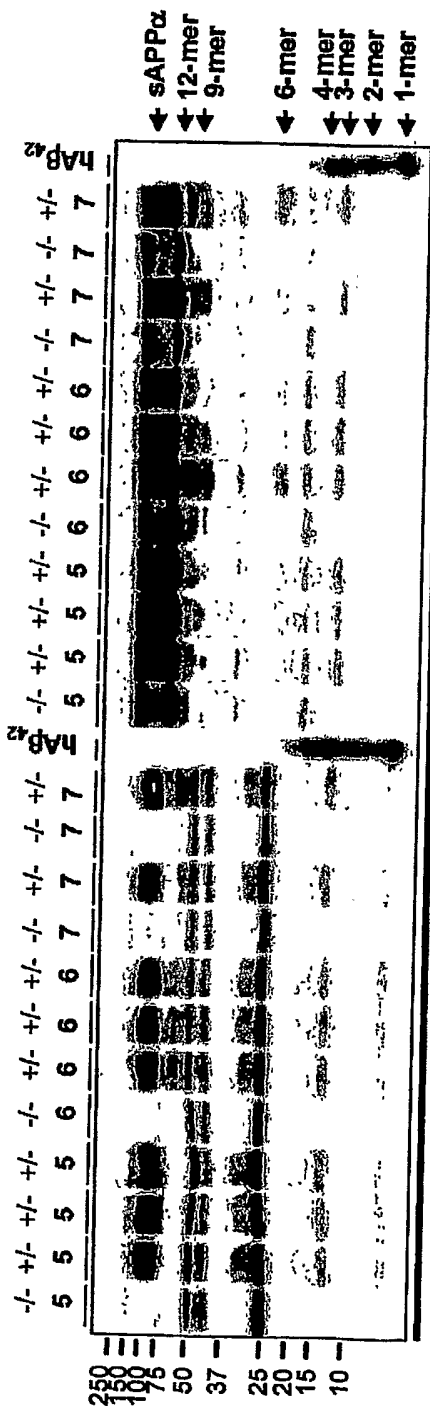


Fig. 2A

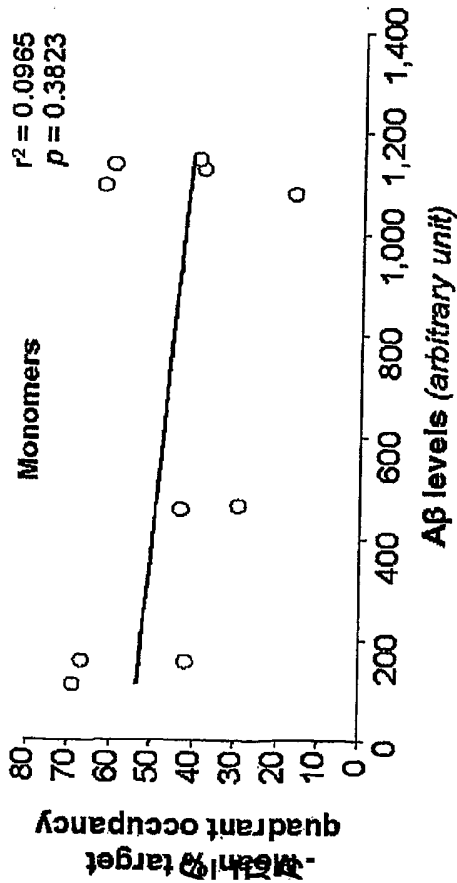


Fig. 2B

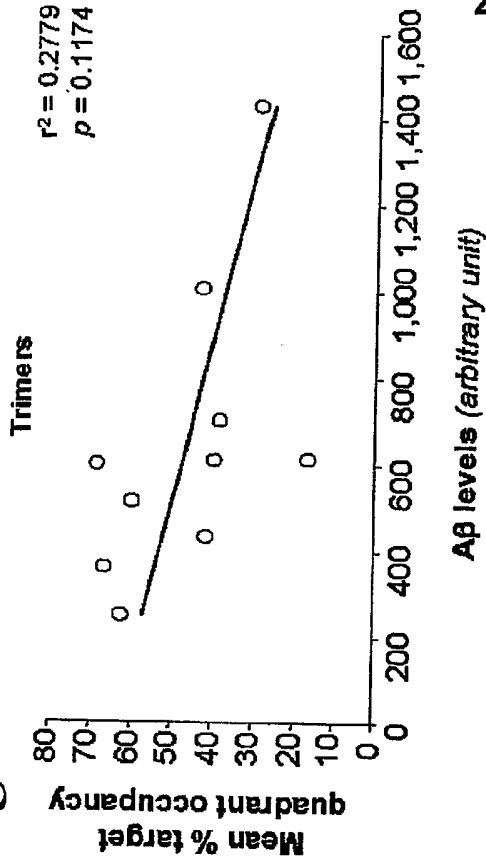


Fig. 2C

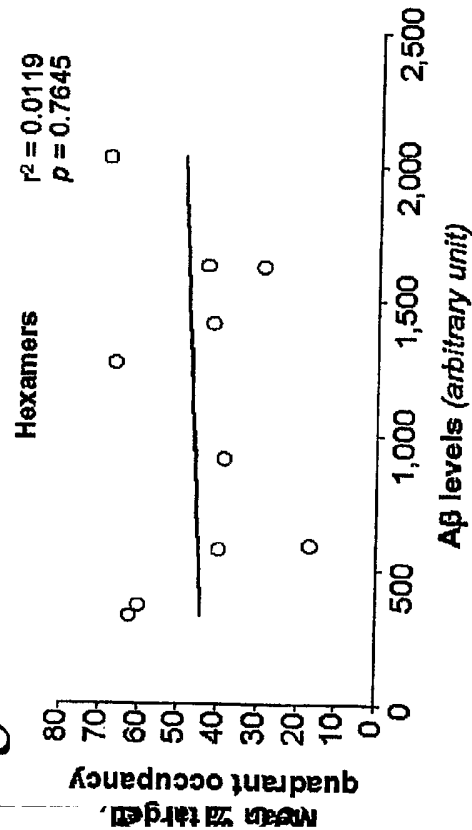


Fig. 2D

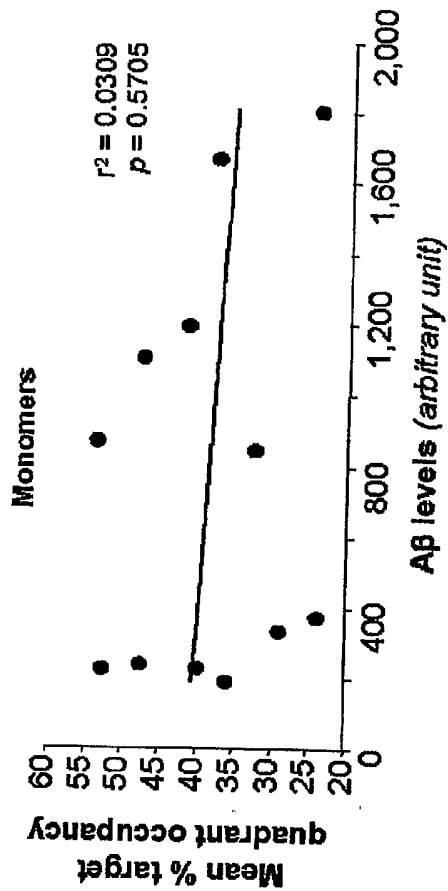


Fig. 2E

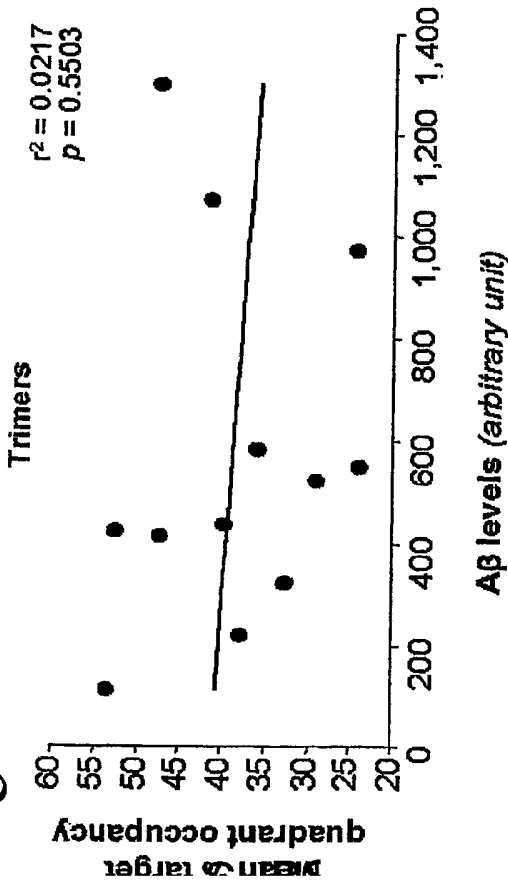


Fig. 2F

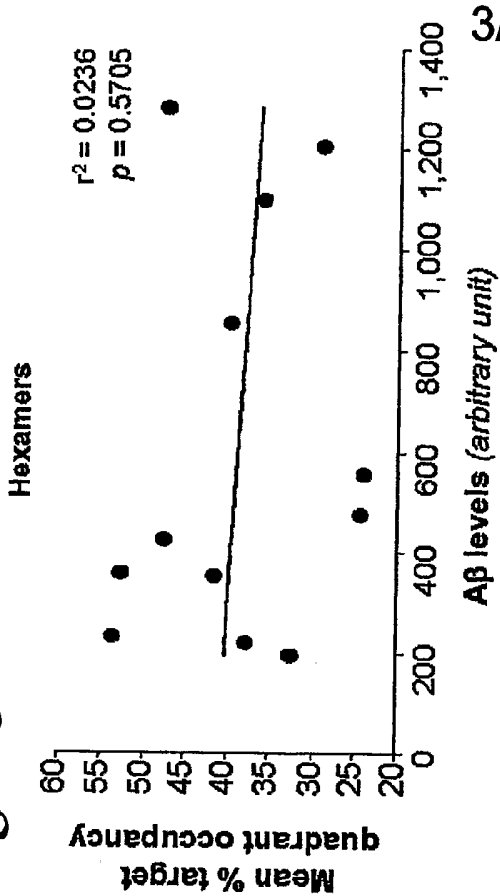


Fig. 2G

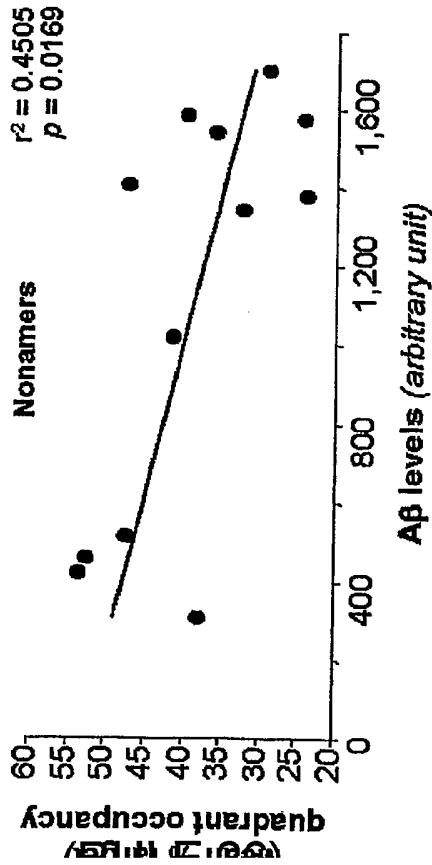


Fig. 2H

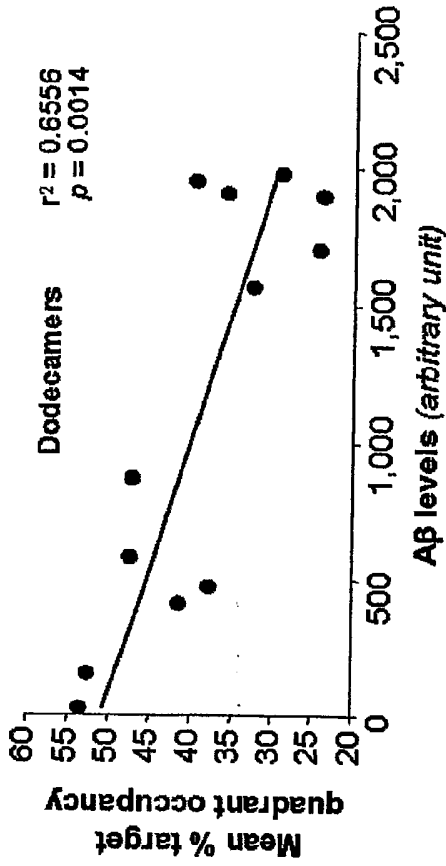


Fig. 3B

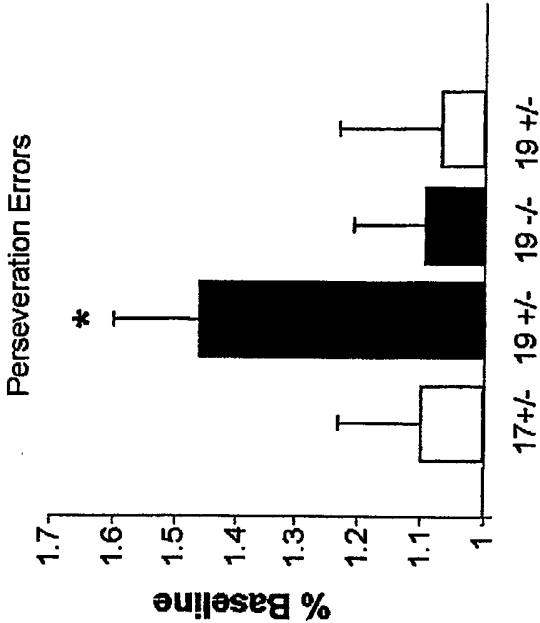


Fig. 3C

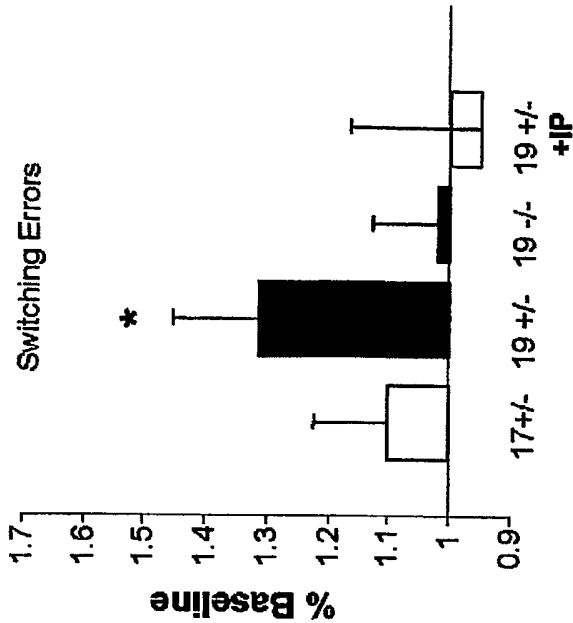
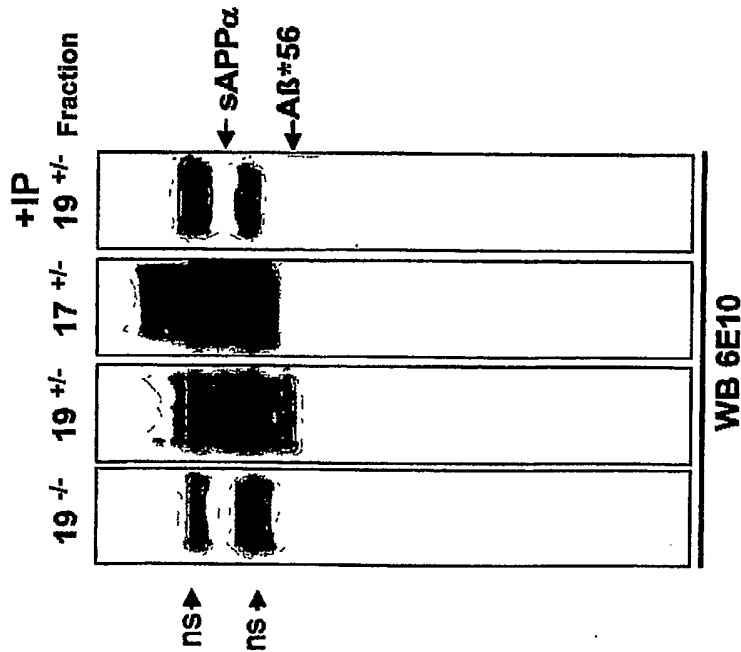
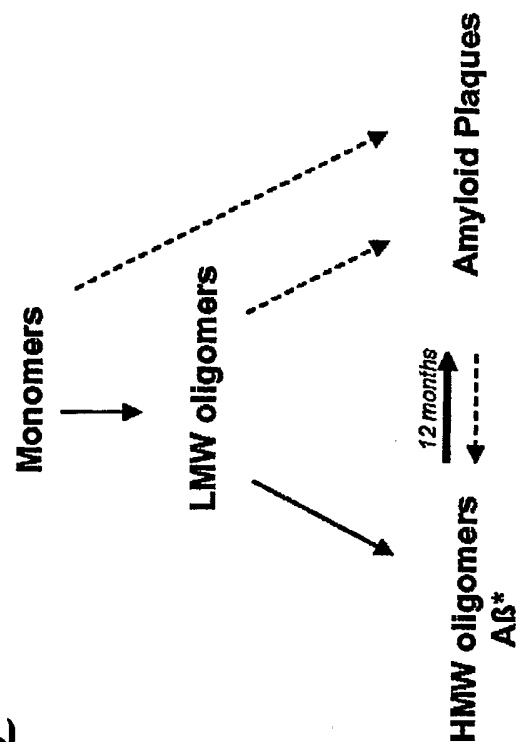
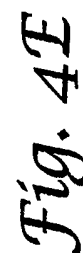
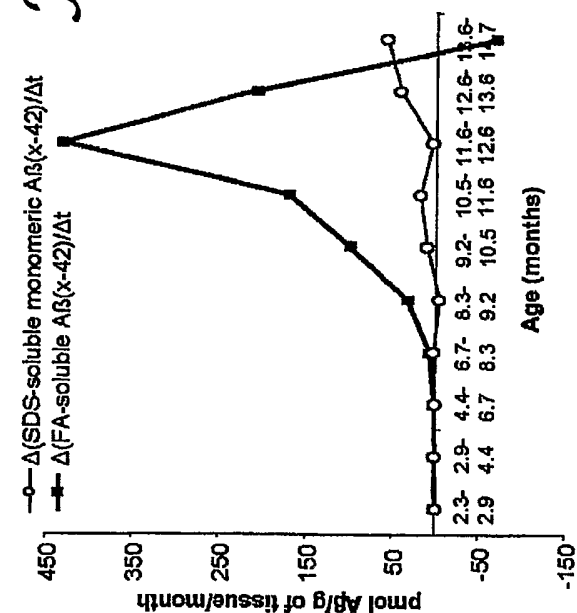
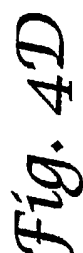
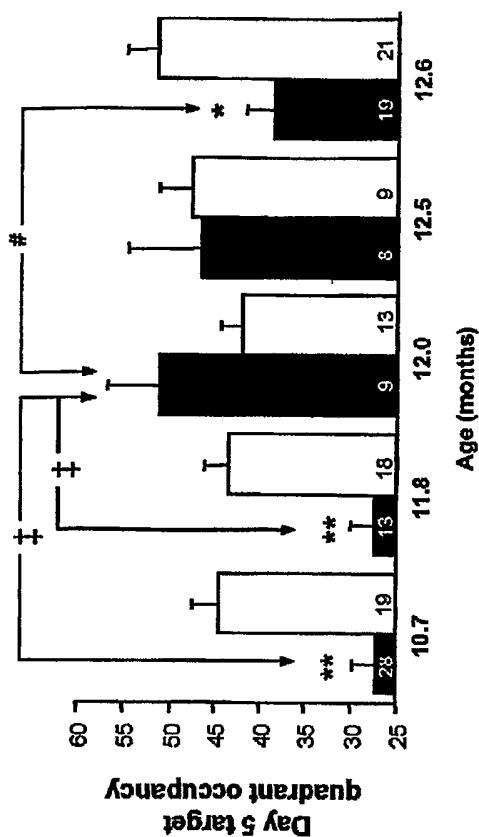
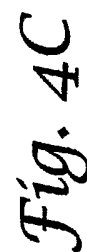
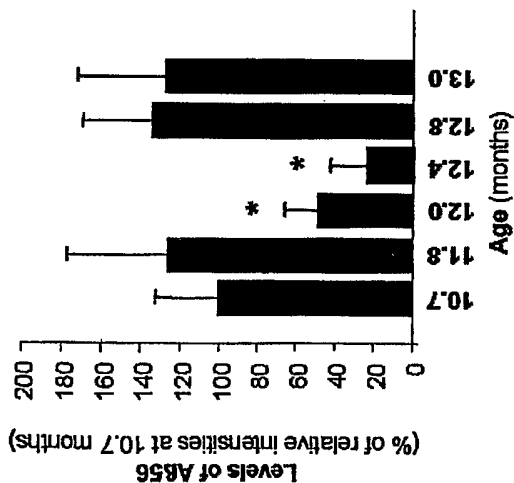
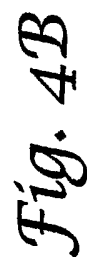
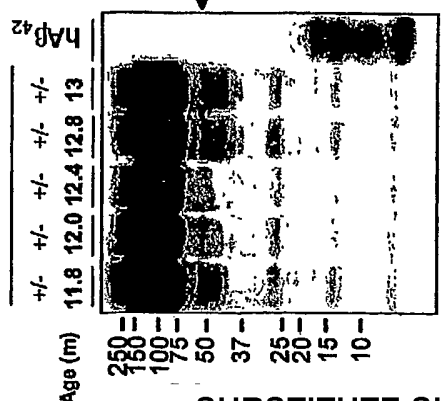
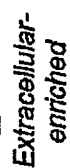
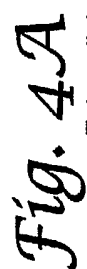
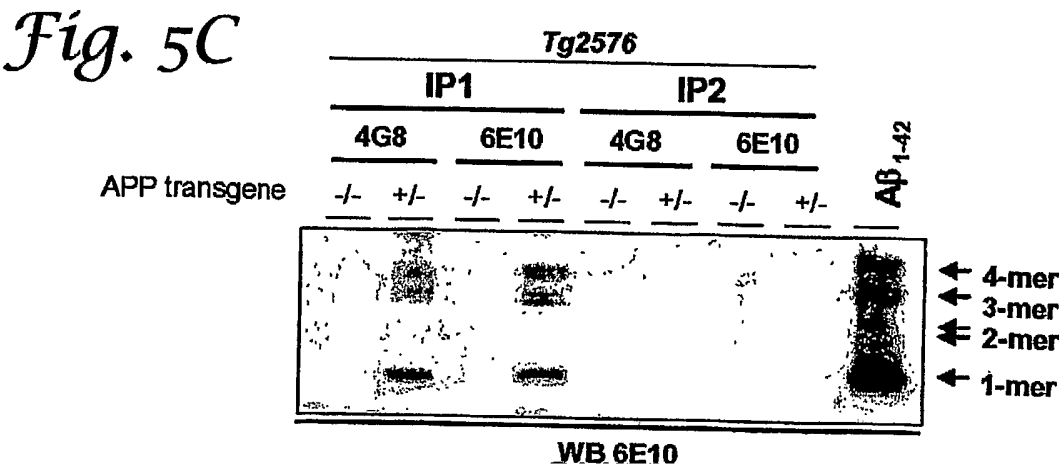
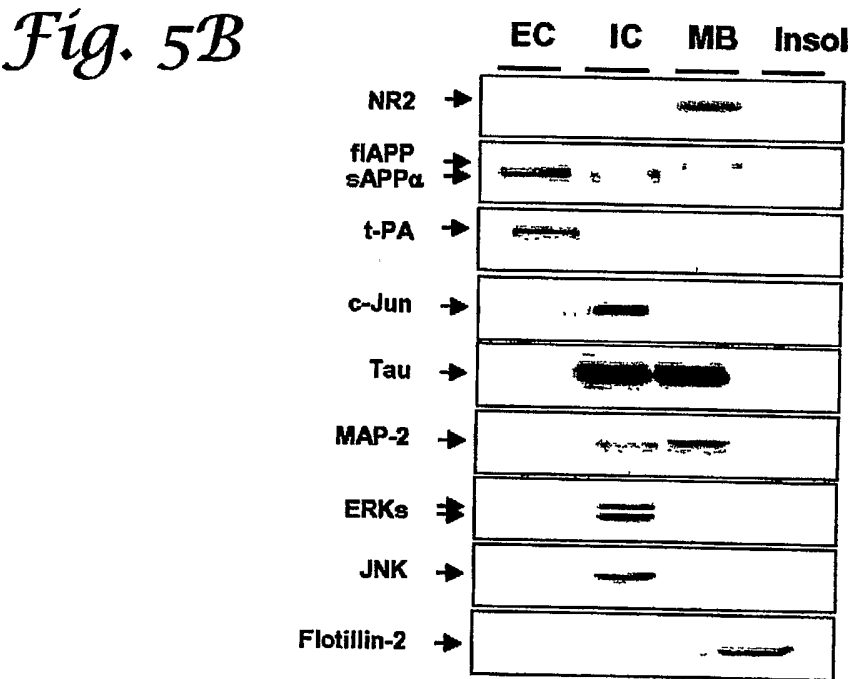
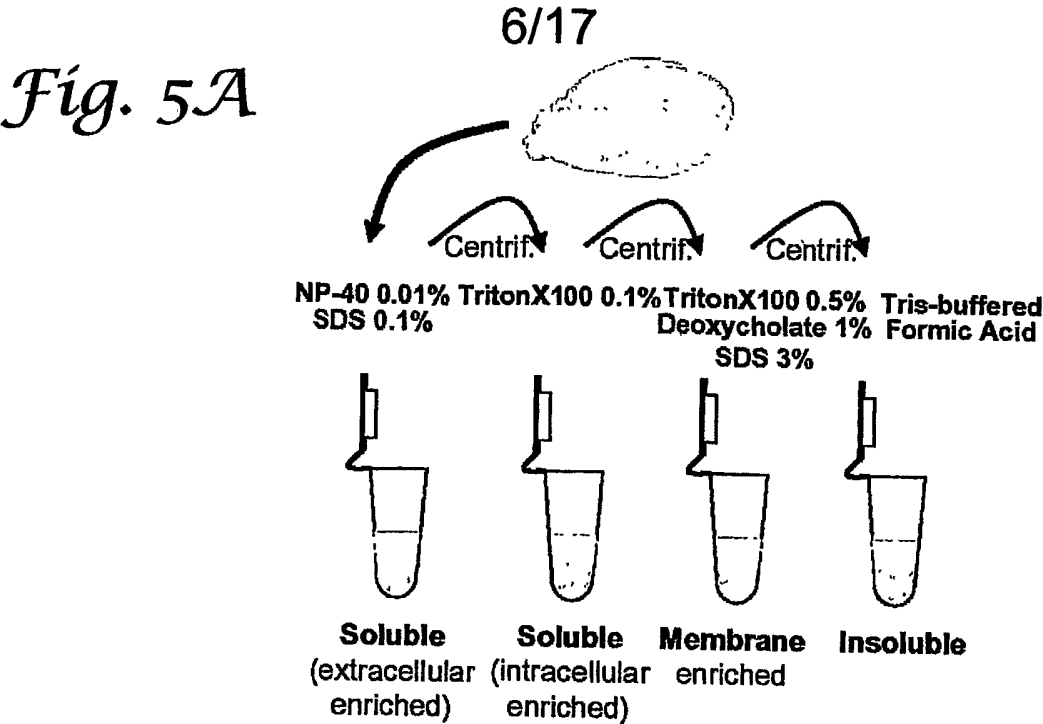


Fig. 3A







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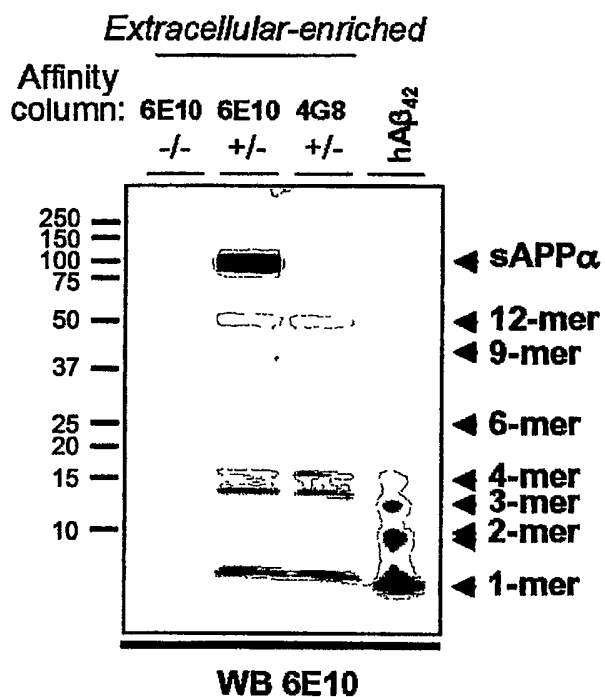
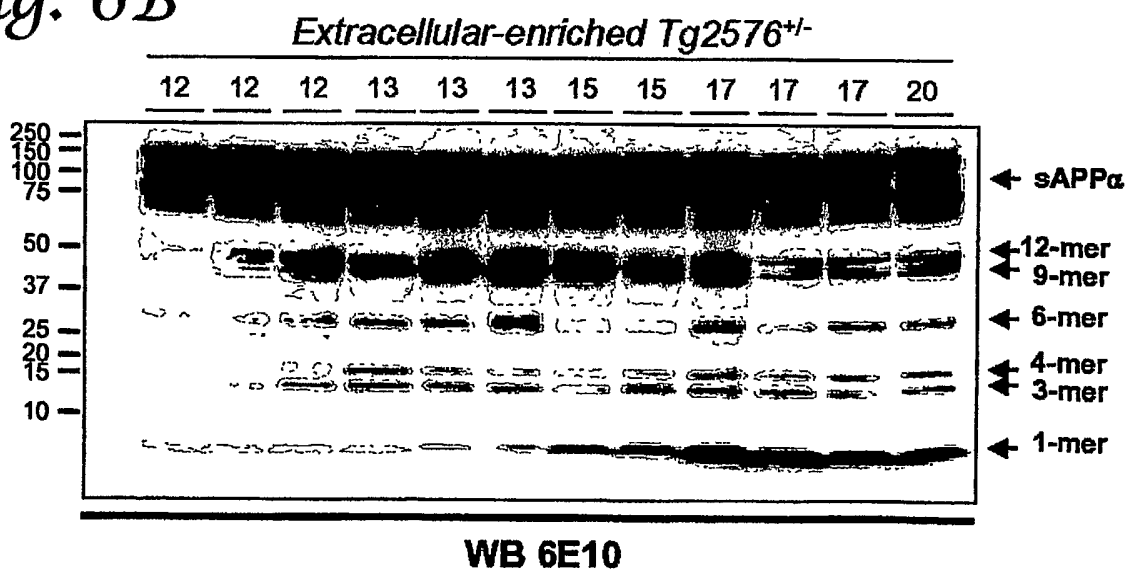
Fig. 6A*Fig. 6B*

Fig. 6C

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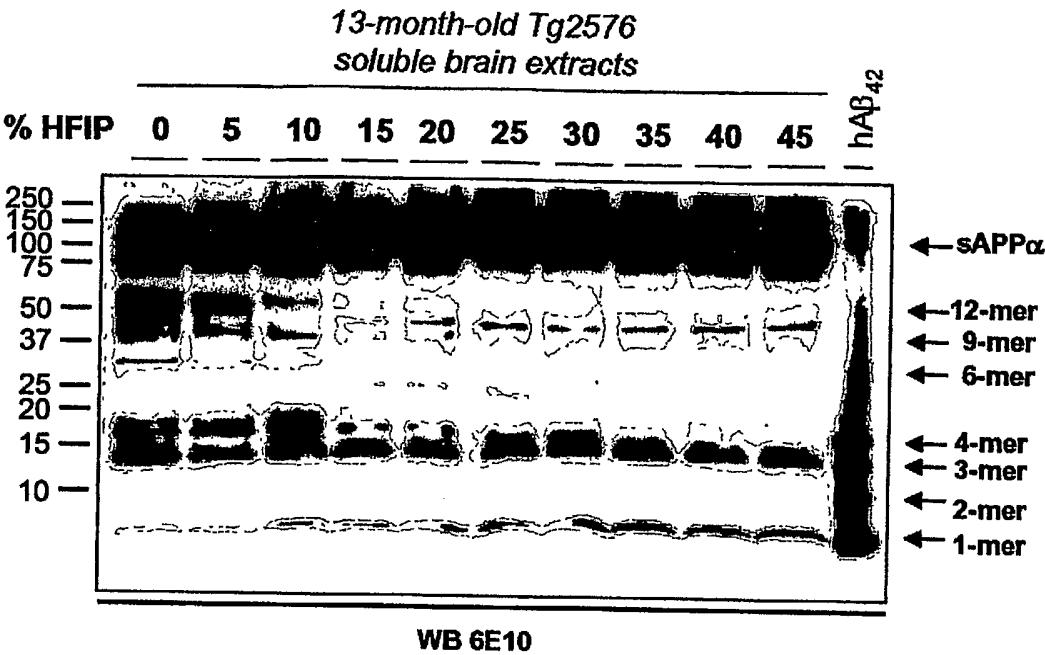


Fig. 6D

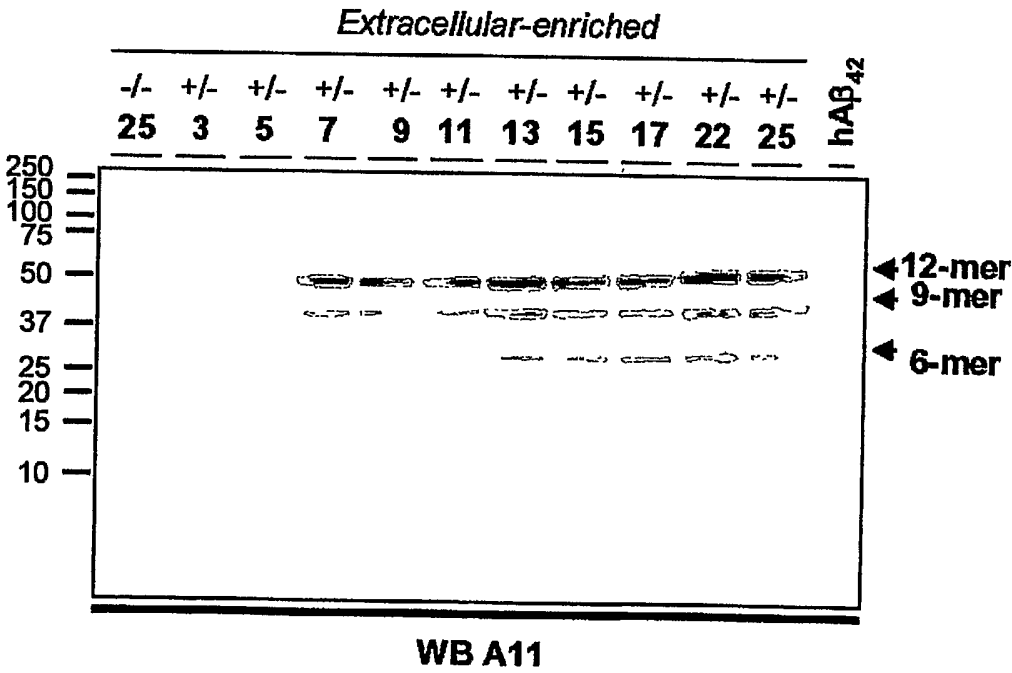


Fig. 7A

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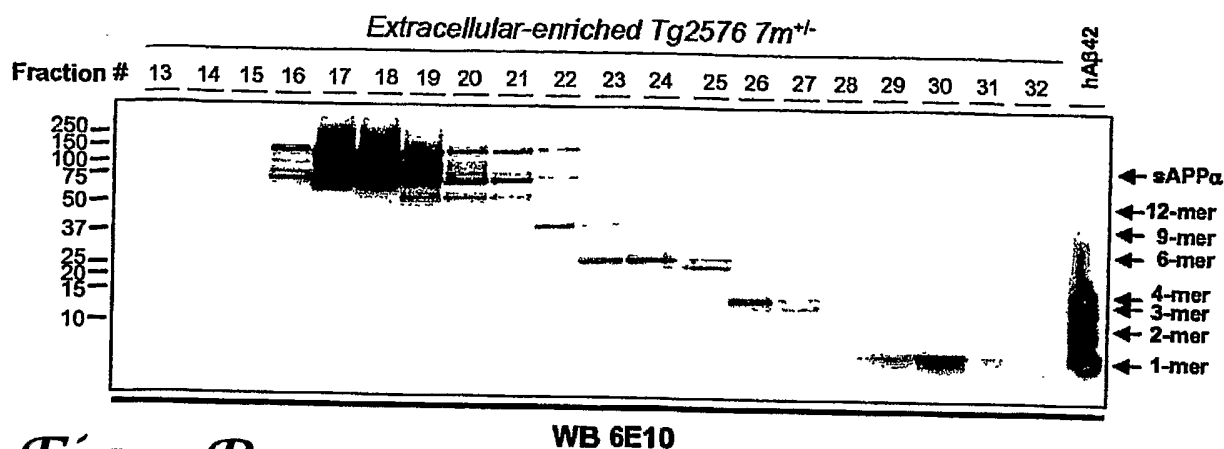


Fig. 7B

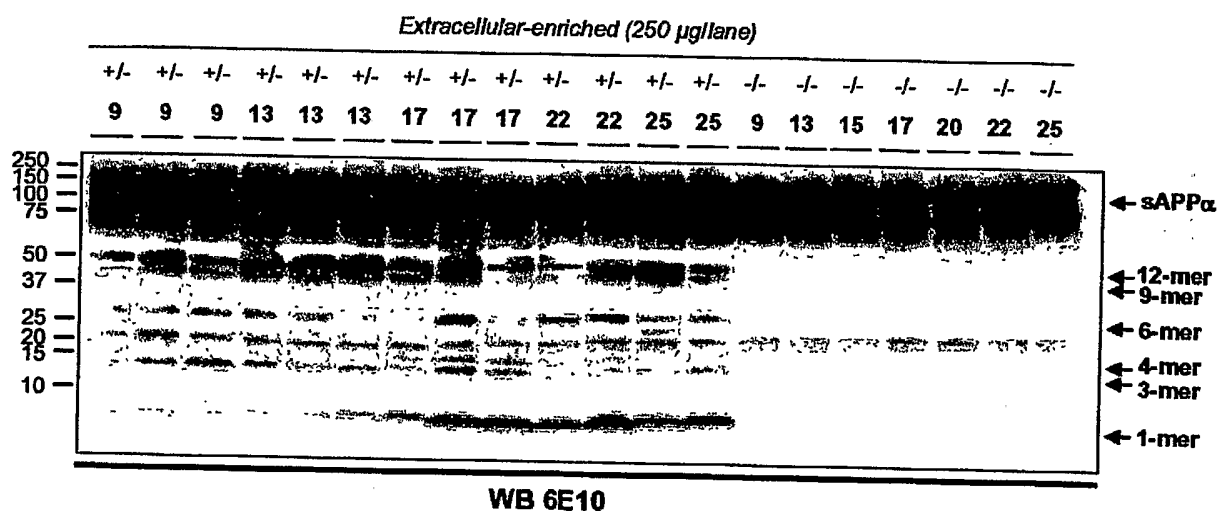


Fig. 7C

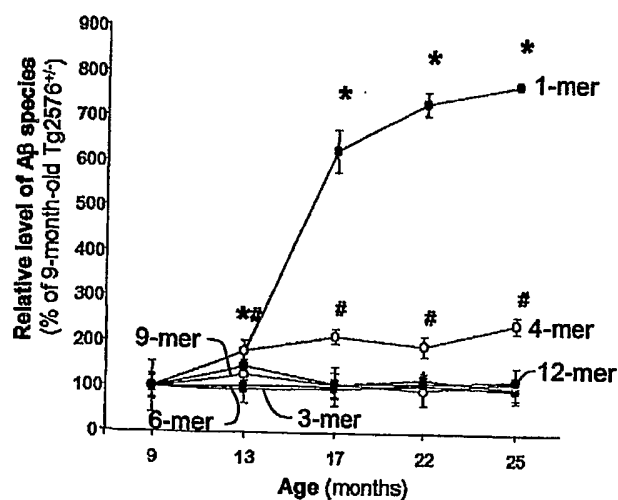
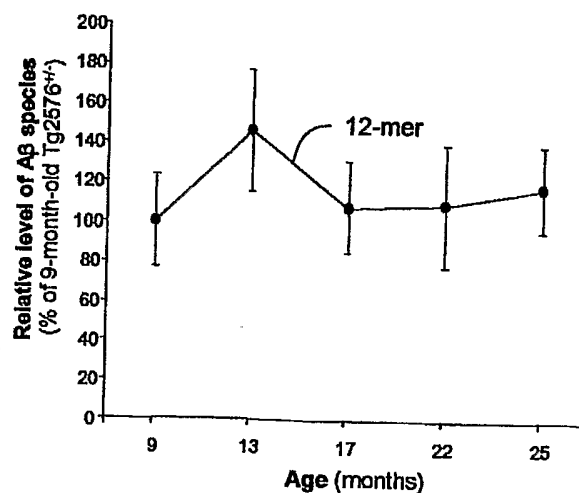


Fig. 7D



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Fig. 8A

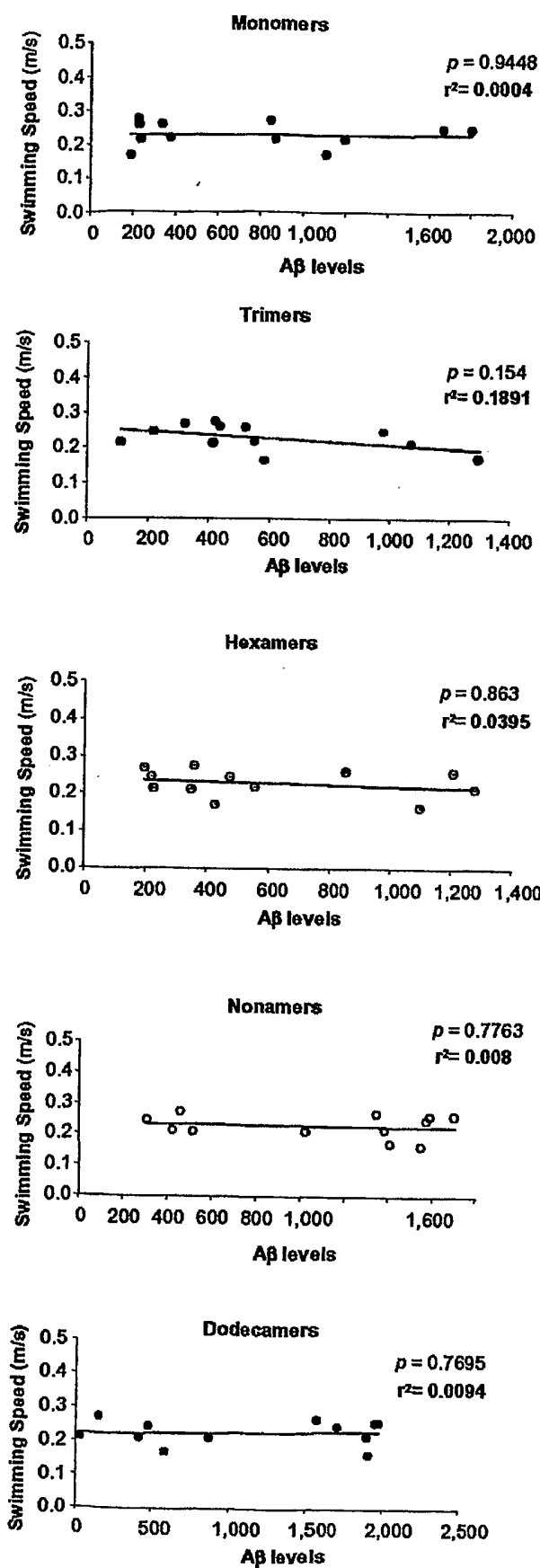


Fig. 8B

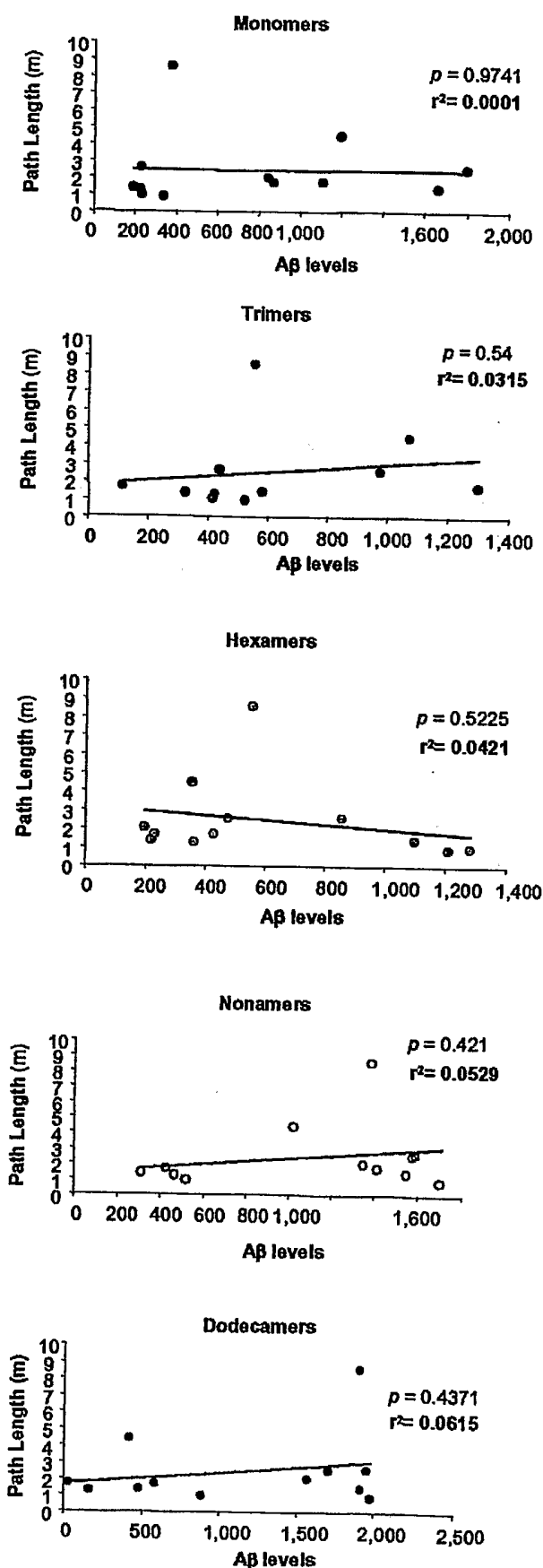


Fig. 9A

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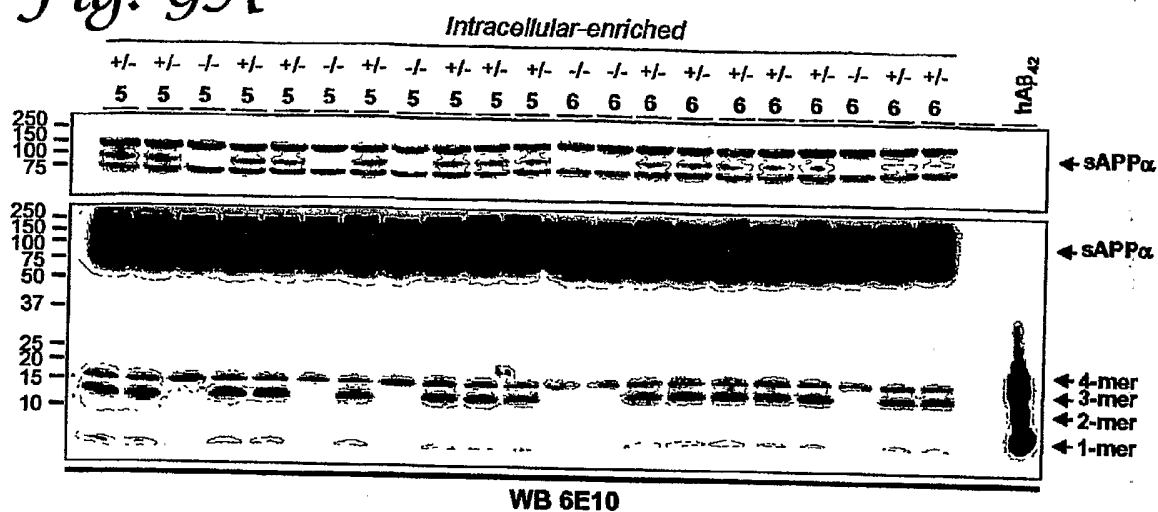


Fig. 9B

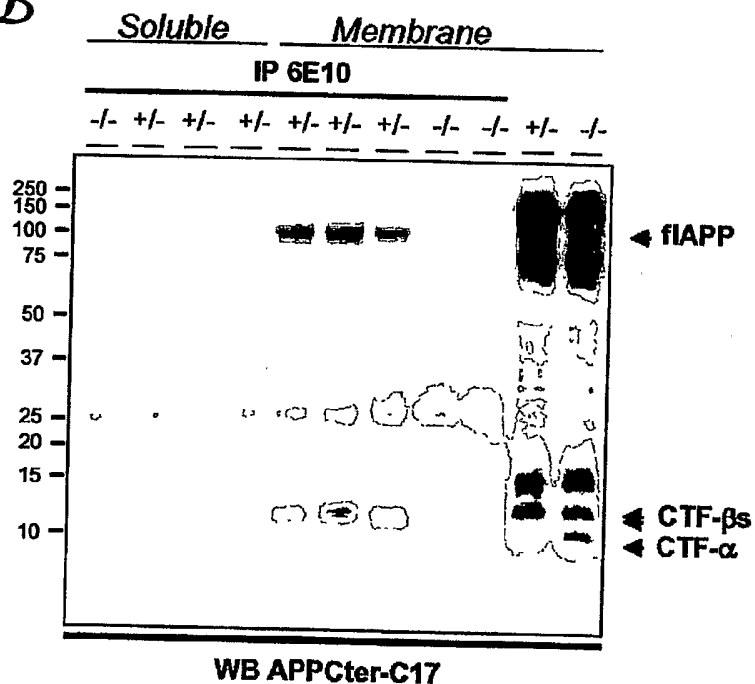
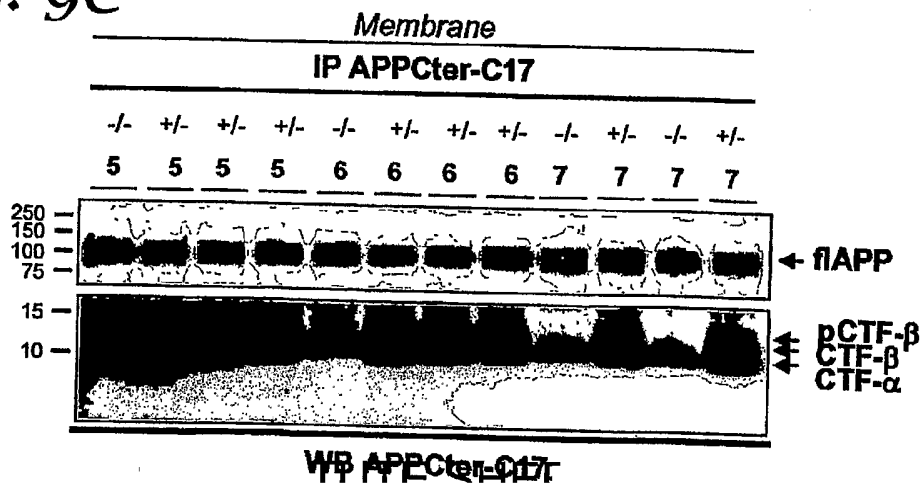
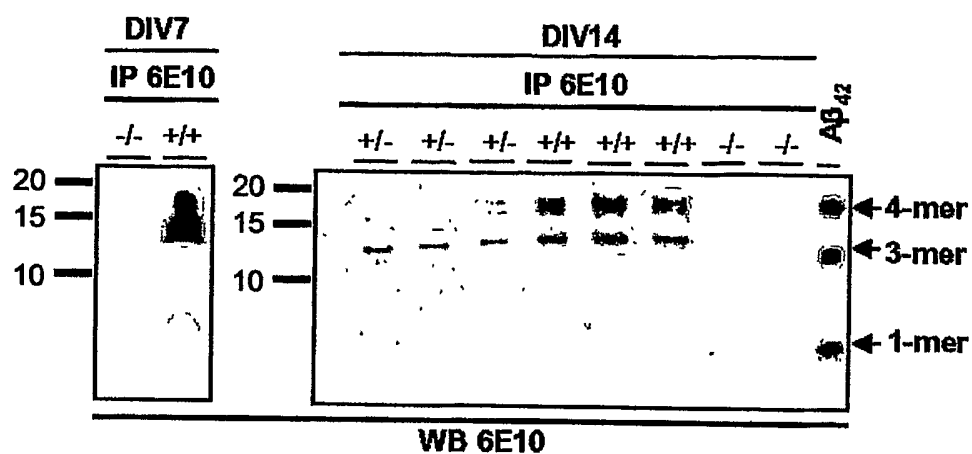
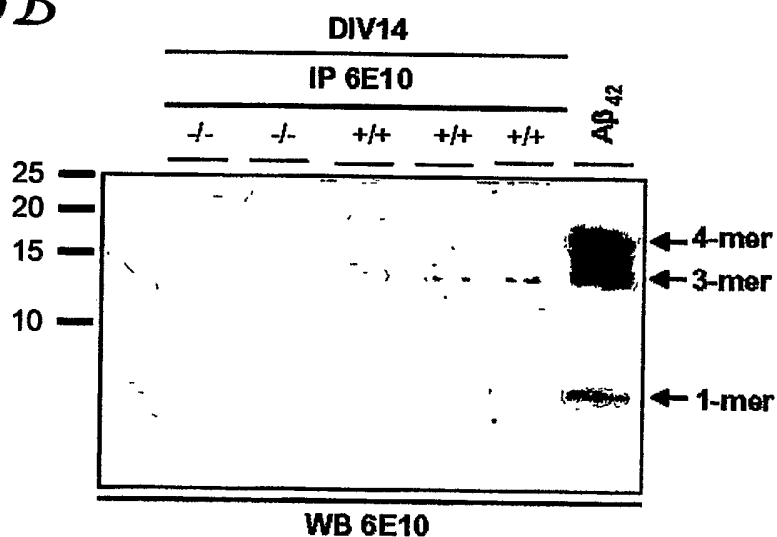
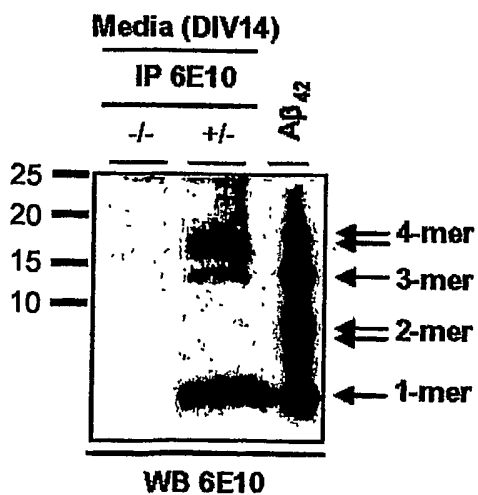
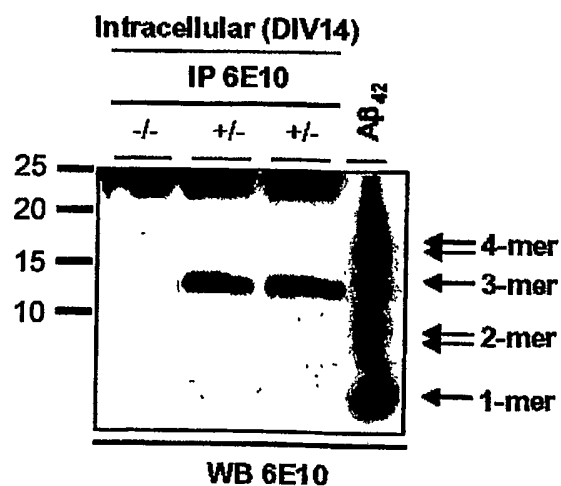


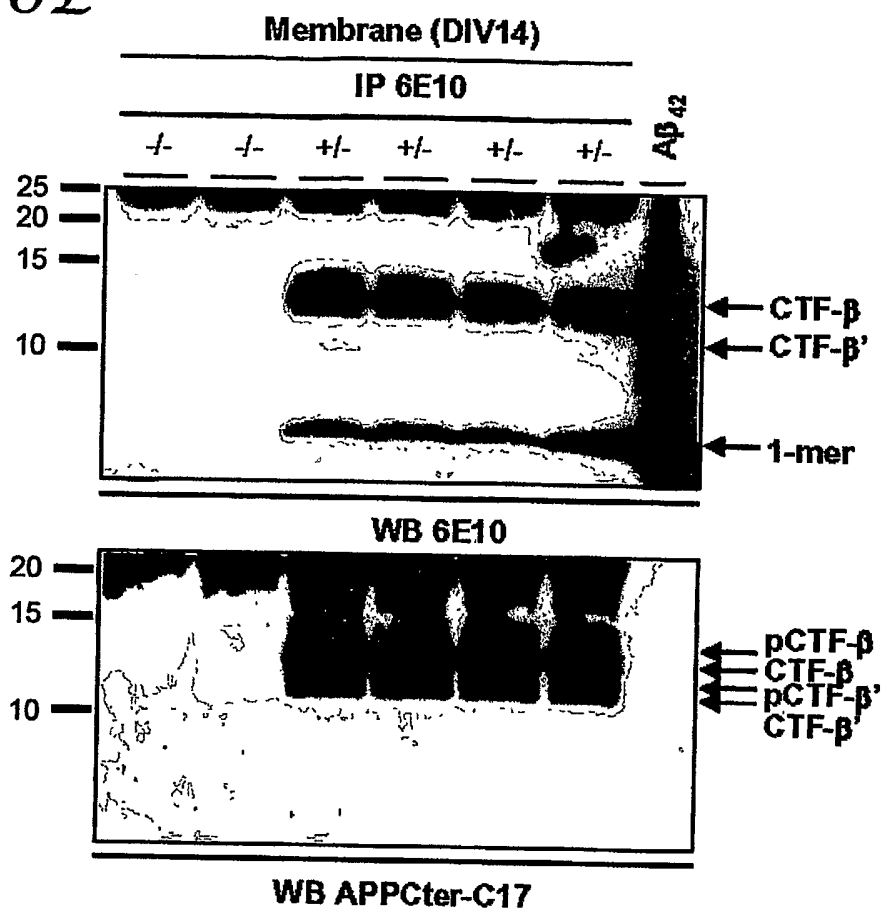
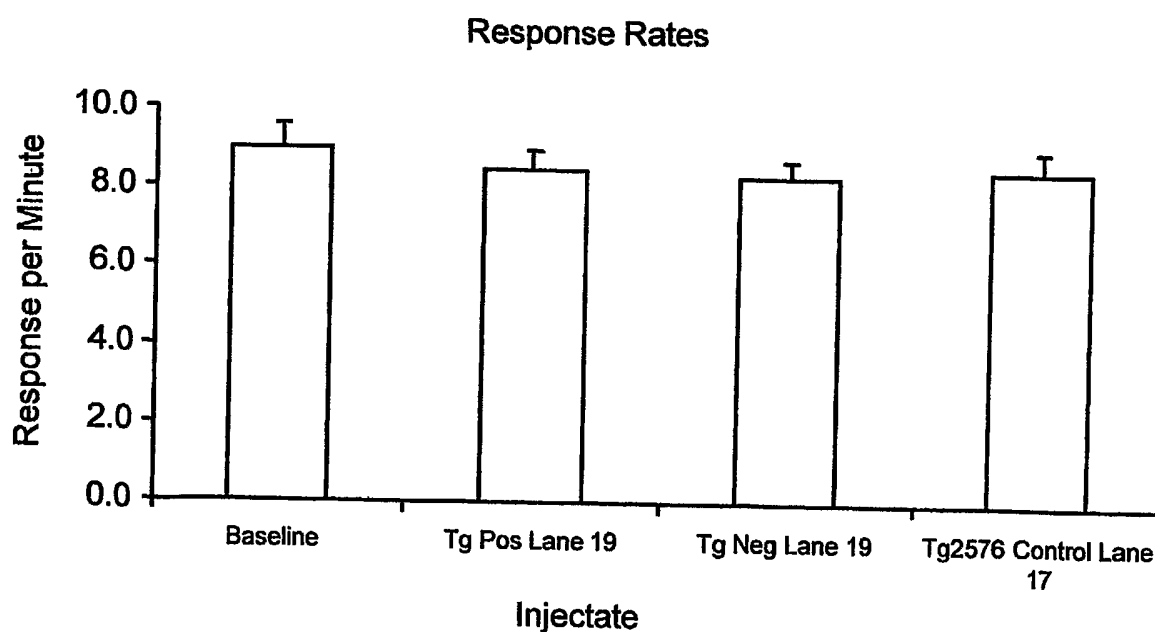
Fig. 9C



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Fig. 10A*Fig. 10B**Fig. 10C**Fig. 10D*

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Fig. 10E*Fig. 11*

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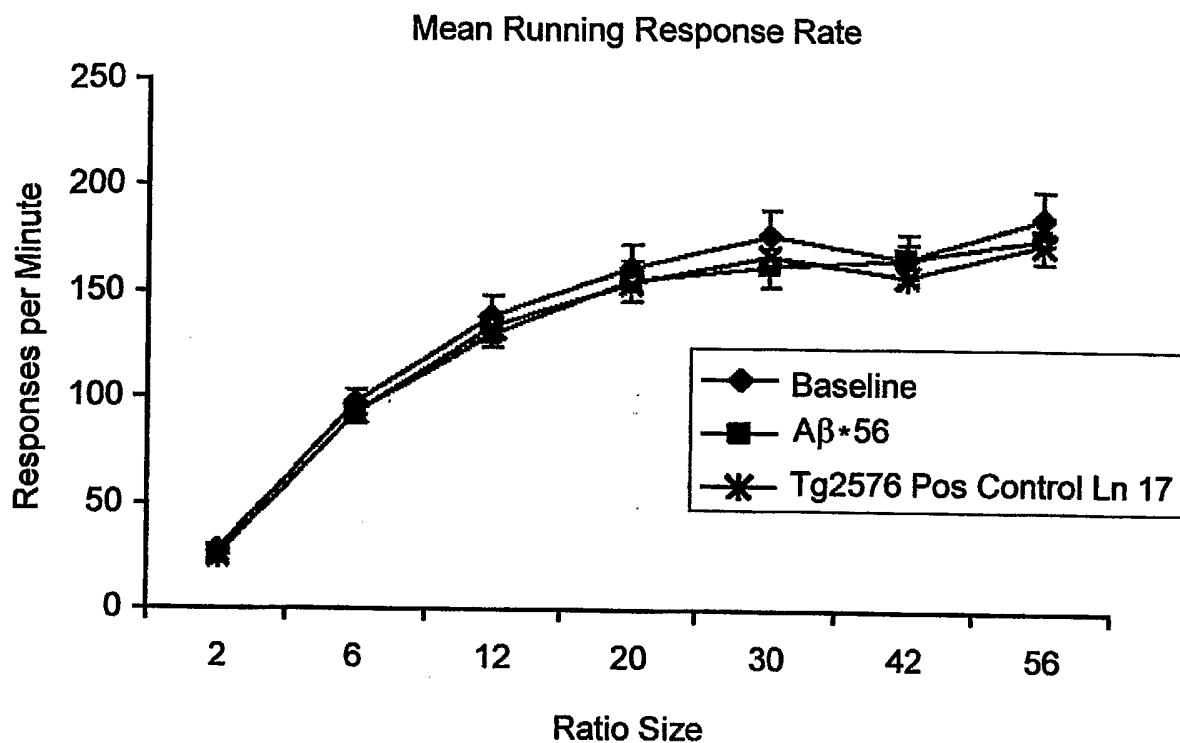
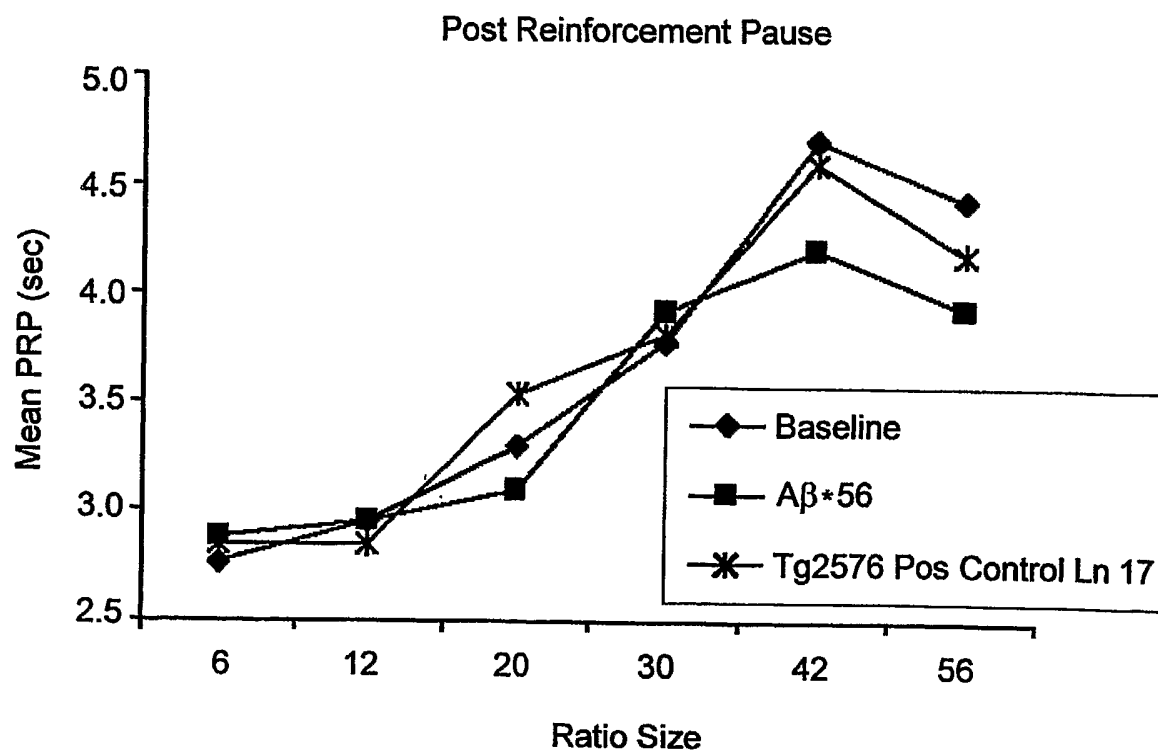
Fig. 12*Fig. 13*

Fig. 14

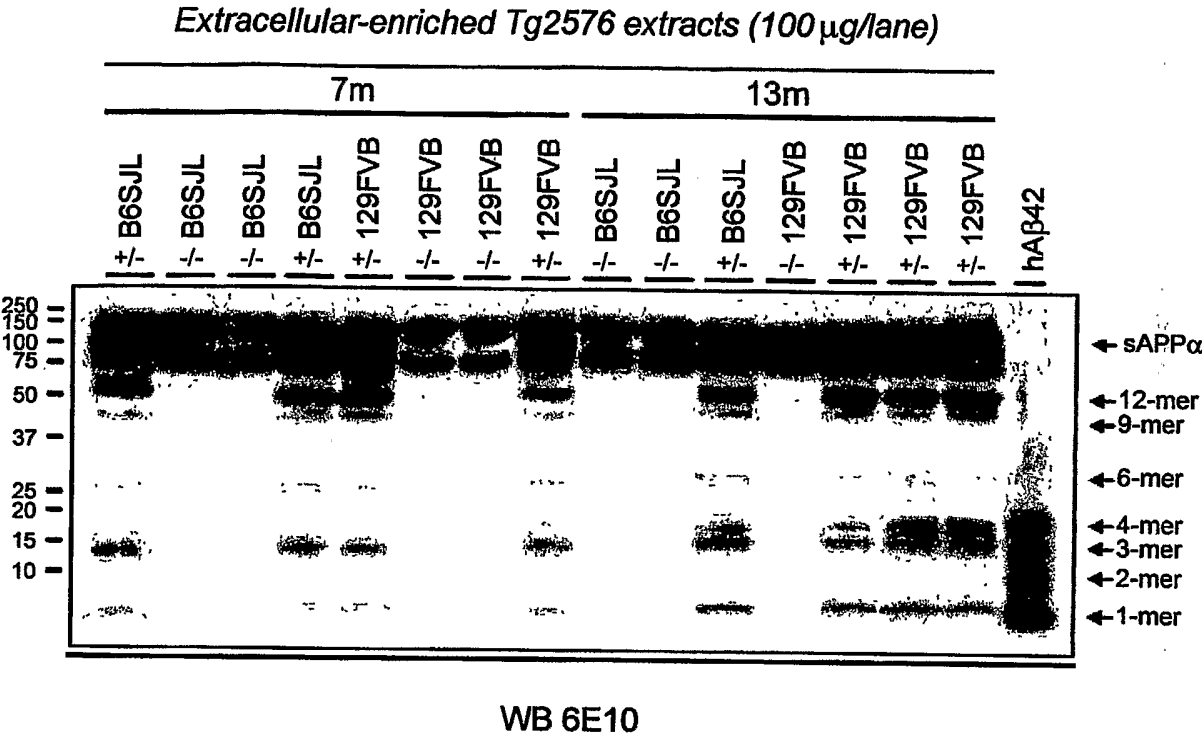
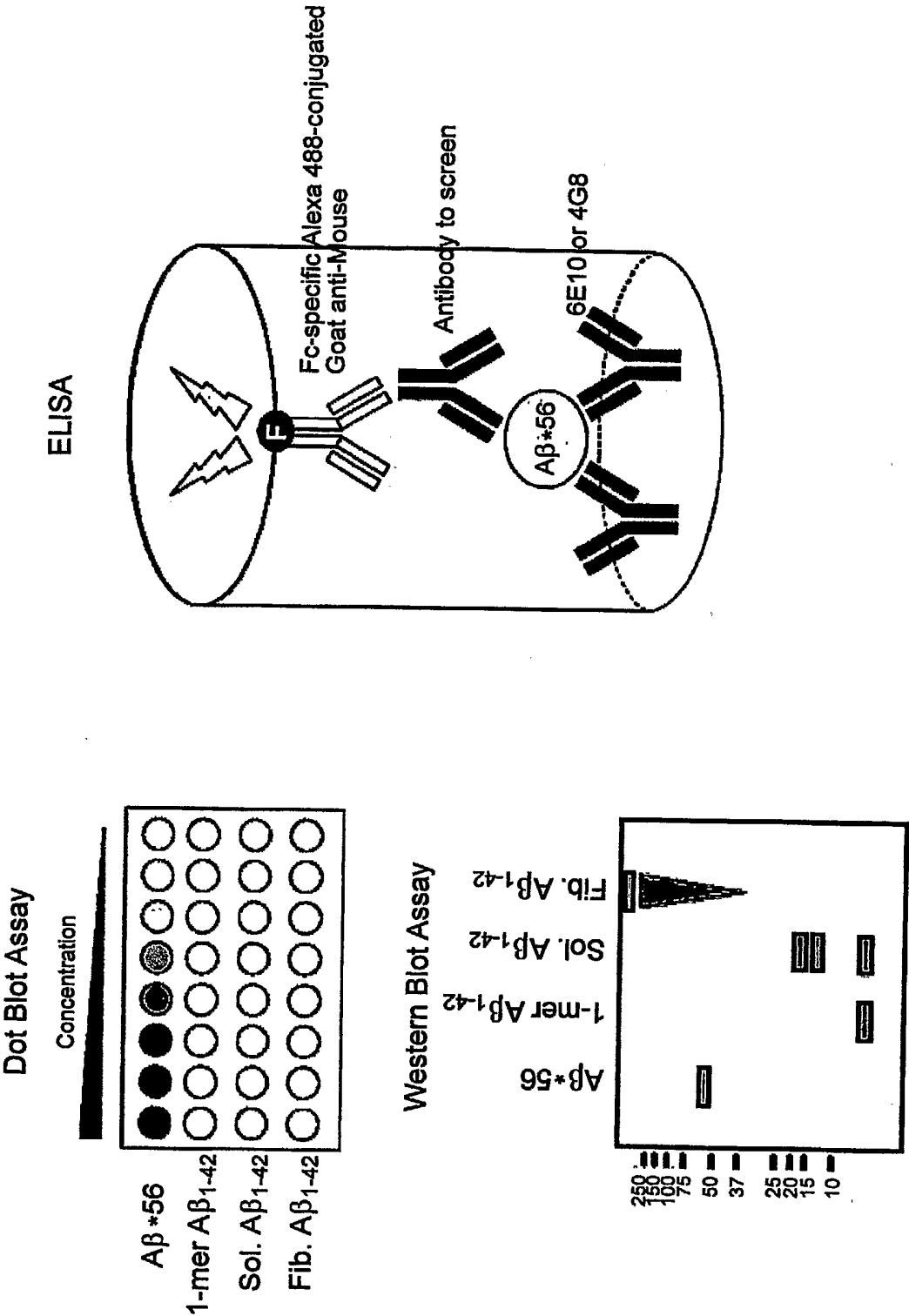


Fig. 15



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Fig. 16A

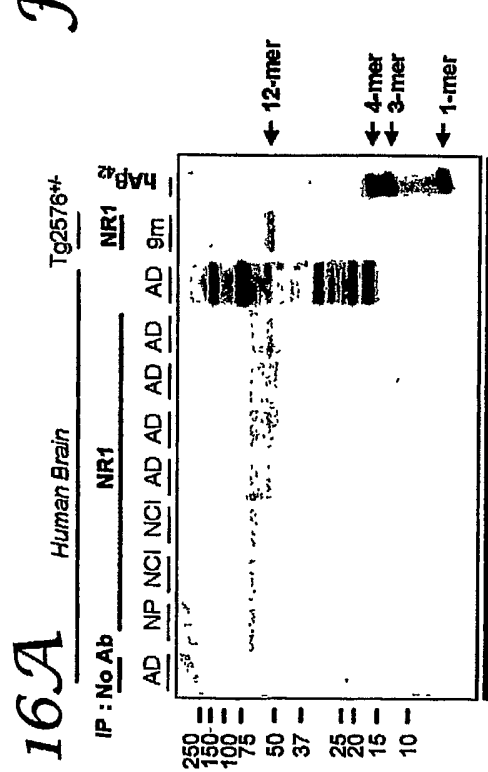


Fig. 16C

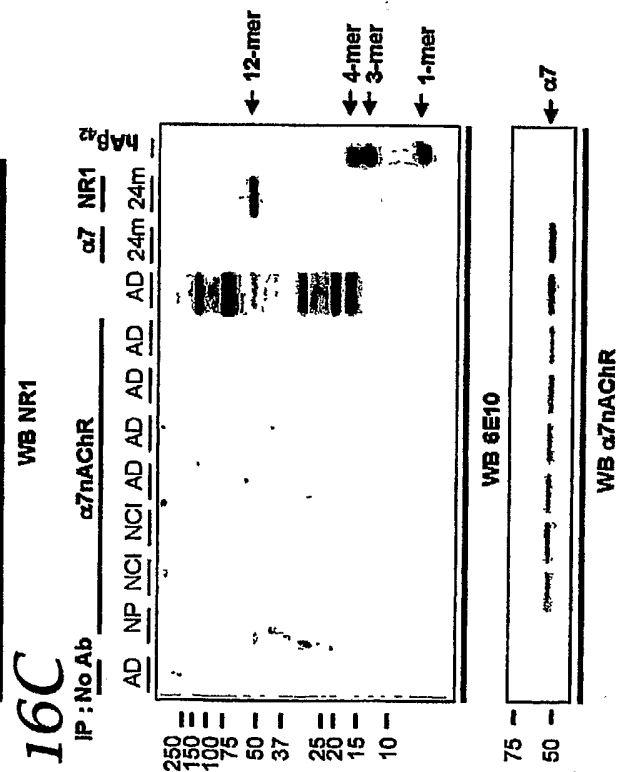
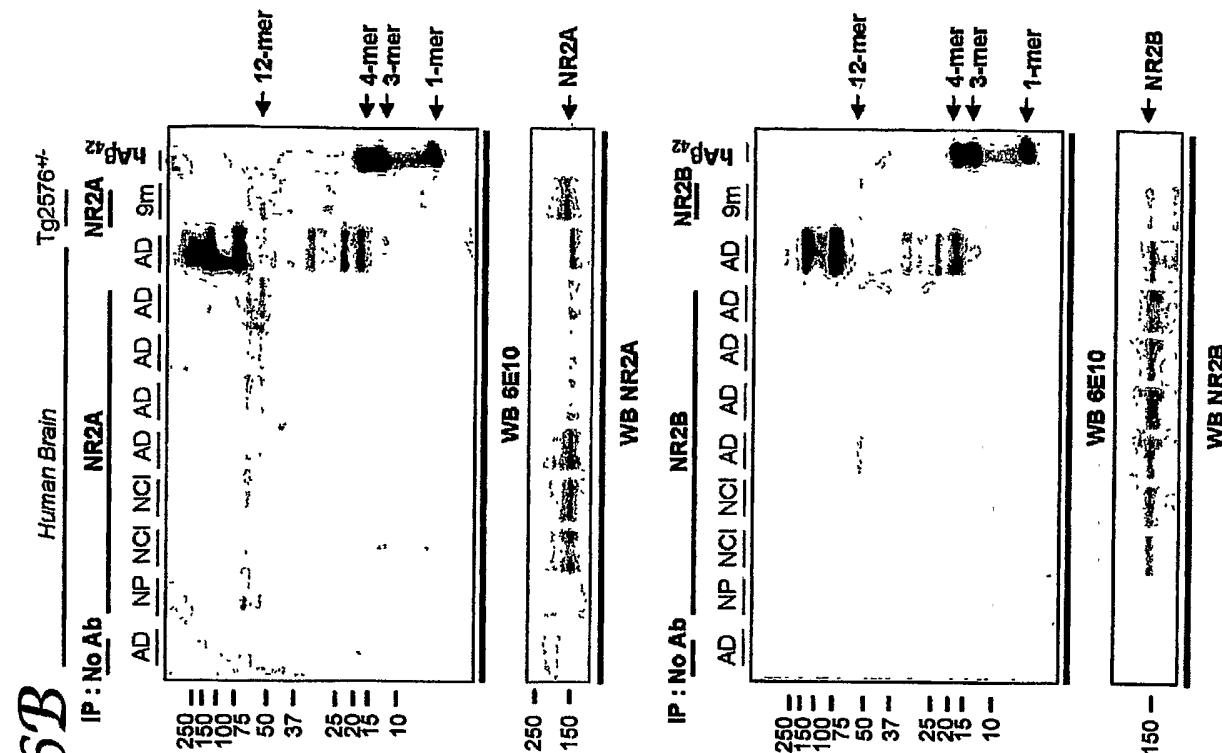


Fig. 16B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/037828

A. CLASSIFICATION OF SUBJECT MATTER C07K14/47 C12N15/12 C07K16/18 G01N33/68 G01N33/53		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) C12N C07K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/031400 A (NORTHWESTERN UNIVERSITY; KLEIN, WILLIAM; KRAFFT, GRANT, A; CHANG, LEI;) 15 April 2004 (2004-04-15) figures 16,18,27; examples 1,3,5,20-22 -----	1-25,27, 28, 33-40, 43-50
X	WALSH D M ET AL: "AMYLOID-BETA OLIGOMERS: THEIR PRODUCTION, TOXICITY AND THERAPEUTIC INHIBITION" BIOCHEMICAL SOCIETY TRANSACTIONS, COLCHESTER, ESSEX, GB, vol. 30, no. 4, 2002, pages 552-557, XP009055250 ISSN: 0300-5127 abstract; figure 1 ----- -/--	1,2, 19-23,50
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family		
Date of the actual completion of the international search 2 March 2006		Date of mailing of the international search report 23/03/2006
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Gurdjian, D

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/037828

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WALSH D M ET AL: "Naturally secreted oligomers of amyloid beta protein potentially inhibit hippocampal long-term potentiation in vivo" NATURE, NATURE PUBLISHING GROUP, LONDON, GB, vol. 416, no. 6880, 4 April 2002 (2002-04-04), pages 535-539, XP002348771 ISSN: 0028-0836 abstract; figures 1-3	1,2, 19-23,50
E	WO 2006/004824 A (REGENTS OF THE UNIVERSITY OF MINNESOTA; ASHE, KAREN, H; CLEARY, JAMES,) 12 January 2006 (2006-01-12) page 8, line 22 - page 11, line 16; claim 26; figure 6 page 15, line 26 - page 16, line 28	1,2, 19-25, 33-38, 45-50
P,X	CLEARY JAMES P ET AL: "Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function." NATURE NEUROSCIENCE. JAN 2005, vol. 8, no. 1, January 2005 (2005-01), pages 79-84, XP002348775 ISSN: 1097-6256 abstract; figures 1,2	1,2, 19-23,50
P,X	WALSH DOMINIC M ET AL: "Certain inhibitors of synthetic amyloid beta-peptide (Abeta) fibrillogenesis block oligomerization of natural Abeta and thereby rescue long-term potentiation." THE JOURNAL OF NEUROSCIENCE : THE OFFICIAL JOURNAL OF THE SOCIETY FOR NEUROSCIENCE. 9 MAR 2005, vol. 25, no. 10, 9 March 2005 (2005-03-09), pages 2455-2462, XP002370352 ISSN: 1529-2401 abstract	1,2, 19-23,50
T	WALSH D M ET AL: "The role of cell-derived oligomers of Abeta in Alzheimer's disease and avenues for therapeutic intervention." BIOCHEMICAL SOCIETY TRANSACTIONS. NOV 2005, vol. 33, no. Pt 5, November 2005 (2005-11), pages 1087-1090, XP002370353 ISSN: 0300-5127 abstract; figure 1	1-25

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 26,29-32,41,42 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

The present claims 39,40 encompass compounds defined only by their desired function, contrary to the requirements of clarity of Article 6 PCT, because the result-to-be-achieved type of definition does not allow the scope of the claim to be ascertained. The fact that any compound could be screened does not overcome this objection, as the skilled person would not have knowledge beforehand as to whether it would fall within the scope claimed, except for the antibody compounds disclosed in the description, see example 3. Undue experimentation would be required to screen compounds randomly. This non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search for claims 39,40.

The search
of claims 39,40 was consequently restricted to antibody compounds .

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

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

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 26,29-32,41,42 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2005/037828

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 2004031400	A	15-04-2004	AU 2003279728 A1 CA 2501091 A1 EP 1545582 A2	23-04-2004 15-04-2004 29-06-2005
WO 2006004824	A	12-01-2006	NONE	