

The amyloid beta peptide: a chemist's perspective. Role in Alzheimer's and fibrillization

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**The Amyloid Beta Peptide: A Chemist's Perspective
Role in Alzheimer's and Fibrillization**

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1. INTRODUCTION

This review is concerned with the role of fibrillization of the amyloid β ($A\beta$) peptide in Alzheimer's disease (AD). The perspective is that of a physical chemist and one aim is to introduce relevant key findings on physico-chemical properties. However, in addition, key aspects of the biology and biochemistry associated with the role of $A\beta$ in AD are also summarized (more detailed reviews of these aspects can be found elsewhere),¹ as are developments in potential therapies and biomarkers.

The aggregation of the amyloid β peptide into oligomers or fibrils is now implicated as a key process associated with progression of AD.² This is the focus of the current review. Whilst the protein tau has an important role in AD progression, its processing occurs downstream of $A\beta$ accumulation.³ A marked decrease or absence of tau expression appears to reduce the neurotoxic effects of $A\beta$.⁴ Proteins including NAC (non-beta-amyloid component) are also co-deposited along with $A\beta$ in plaques.⁵ NAC comprises residues 61-95 of α -synuclein, which is involved in amyloidoses with Lewy bodies such as Parkinson's disease. These topics are not discussed further herein, with the exception of a brief discussion (Section 2.8) of the interaction between $A\beta$ and tau.

Due to the very large number of papers on $A\beta$, this review cannot be exhaustive in the space available. We have attempted to focus on key papers, and work that illustrates the main features of the subjects in the following sections. We have attempted to review work by many groups who have made important contributions. As there are also a large number of previous reviews on the topic of

A β aggregation and its relationship to neurodegenerative disease, we can also only cite a number of the key earlier reviews here.^{1,6}

AD is the most common cause of dementia (representing around 50-80% of all cases⁷) with an estimated 18 million people worldwide currently affected by the condition (according to the World Health Organization).⁸ Its incidence increases dramatically with age, and the number of people with dementia is set to double in the next twenty years.⁷ The annual cost of dementia in the UK is estimated at £23 billion per annum including care and healthcare costs and lost productivity, which equates to £28k per patient.⁹ Alzheimer's disease accounts for about 70% of all late-onset dementia cases.¹⁰ Most cases occur relatively late in life, although around 5% occurs in patients under 60 years old. These cases are termed early onset familial Alzheimer's disease (FAD). Genetic mutations have been linked to these conditions as discussed in section 2.1 below. In AD, neurodegeneration is estimated to start 10-30 years before clinical symptoms are detected.^{2a,11}

Intense research activity is focussed on the development of treatments for Alzheimer's disease (AD) as discussed in Section 3. Several existing treatments can manage the condition but they do not arrest or reverse the progression of AD, i.e. there is no cure. A healthy diet and exercise may contribute to reduced AD risk as might enhanced mental activity and social engagement.¹² Calorie restriction and intermittent fasting also ameliorate age-related behavioural deficits in transgenic mice.¹³ Further discussion of these epidemiological studies is outside the scope of the present review. A number of strategies to treat the condition are actively being pursued by research teams in academia and the pharmaceutical industry.^{12a,14} These

include (i) development of γ -secretase inhibitors (γ -secretase is an enzyme involved in cleavage of amyloid β ($A\beta$) peptides from the Amyloid Precursor Protein), (ii) passive immunization based on $A\beta$ antibodies (iii) inhibition of aggregation of oligomers. These are discussed further in Section 3.

Susceptibility to AD increases with aging, as indicated by large population screening studies and studies using monkeys¹⁵ and transgenic mice.¹⁵ Much research has focussed on early-onset AD for which genetic markers and the role of $A\beta$ are readily identified.^{2c} Table 1 shows characteristics of early-onset AD (EOAD). It is responsible for ~2% of cases and can occur as early as 30 years of age.^{2c} Late-onset AD (LOAD) is the more common variant that causes the majority of the cases of age-dependent dementia. Age is the single biggest known risk factor, with the incidence of the disease increasing from approximately one in ten of those over 65, doubling roughly every five years to affect approximately half of individuals over 85.^{2c,8,16} Susceptibility to LOAD also seems to have a genetic basis, although a single genetic determinant does not exist – several genes associated with susceptibility to the condition are known (as discussed further in Section 2.1) and a combination of genes may also be involved. The progression of AD is similar for EOAD and LOAD and is arbitrarily divided into early/mild, moderate and severe cases.

Oxidative stress may play an important role in the age-dependent susceptibility to AD.¹⁷ Oxidative stress involves the production of free radicals (especially hydroxyl radicals) in the presence of metal ions, which can influence metabolism, and also promote $A\beta$ aggregation, the latter subject being discussed further in section 4.8. The free radicals can cause increased lipid peroxidation, and the formation of associated

byproducts, as well as protein and DNA oxidation in the AD brain. Diminished mitochondrial energy metabolism may play a role in AD pathogenesis, due at least in part to reduced cyclooxygenase (COX) activity (section 2.1).¹⁷ Excitotoxicity is the overstimulation of *N*-methyl-D-aspartate (NMDA) or 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) receptors (NMDARs and AMPARs respectively) by glutamate or aspartate, leading to neuronal hyperexcitability and death. It can also generate excess reactive oxygen species (ROS). The role of oxidative stress is evidenced by the presence of protein glycation end products in A β aggregates, as well as an increase in the number of activated microglial cells (section 2.3).¹⁷ The inflammation that results from oxidative stress as well as A β - (and tau-) induced neurodegeneration has an important role in AD pathology, as reviewed by the neuroinflammation working group.¹⁸

Table 1. Characteristics of Early-Onset AD.^{2c}

Gene	Age of onset, years	Aβ phenotype
APP trisomy 21	50s	Total A β production increased
APP mutations	50s	Total A β production increased A β 42/A β 40 ratio increased
APP triplication of APP gene	50s	Total A β production increased
Presenilin 1	40s and 50s	A β 42/A β 40 ratio increased
Presenilin 2	50s	A β 42/A β 40 ratio increased

Diagnosis of AD is usually through cognitive testing methods detailed elsewhere,^{2c} supported by scanning techniques such as magnetic resonance imaging (MRI).

However, research into biomarkers is a very active and promising field (section 2.4). Prior to development of AD, patients may suffer mild cognitive impairment (MCI), and around 40-60% of patients with this condition develop AD within five years.¹⁹

Factors that can be used to track the risk of progression of AD have recently been reviewed, leading to guidelines for the preclinical assessment of the condition.²⁰ The pathogenic process leading to AD may start many years (a period of approximately one decade has been identified²⁰) before obvious symptoms are noted. The development of biomarkers at an early stage of disease progression would be extremely beneficial.

The insulin/insulin growth factor (IGF) signalling pathway influences ageing and AD progression, as discussed further in section 2.1. Genes undergoing age-related changes in expression have been identified, as have markers of mitochondrial dysfunction in response to oxidative stress.²¹ Another vital regulator of the aging process is autophagy (degradation of intracellular components through lysosomes). Increased autophagy extends lifespan due to reduced insulin-like signalling and it may be stimulated by calorie restriction. Reduced autophagy leads to neurodegeneration, accompanied by the accumulation of ubiquitinated protein aggregates.²¹ This can occur during normal ageing, but reaches pathological levels in neurodegenerative disorders such as AD. Proteasome dysfunction leads to increased levels of ubiquitinated protein and to memory deficits in transgenic mice.²²

The pathology of AD comprises neuritic amyloid plaques and neurofibrillary tangles in the hippocampus, amygdala and association neocortex. Diagnosis with 100% accuracy can only be achieved *post mortem*, however diagnosis with 95% accuracy is possible in living patients using a combination of tools including cognitive testing, brain imaging and analysis of family health history.^{12a}

The A β hypothesis (Fig.1) implicates A β as a key causative agent of AD.

Controversies surrounding the A β hypothesis, including the apparently paradoxical presence of A β deposits in the brains of people not suffering from dementia, and the cause/effect nature of A β deposition, have been discussed.^{3,6e,23} However, these deposits are diffuse and have none of the characteristic surrounding neuritic and glial cytopathology found in mature neuritic plaques.^{6e,24} Biochemical assays such as ELISA and Western blotting indicate that levels of soluble A β correlate better with the presence and extent of cognitive defects than simple plaque counts.²⁵

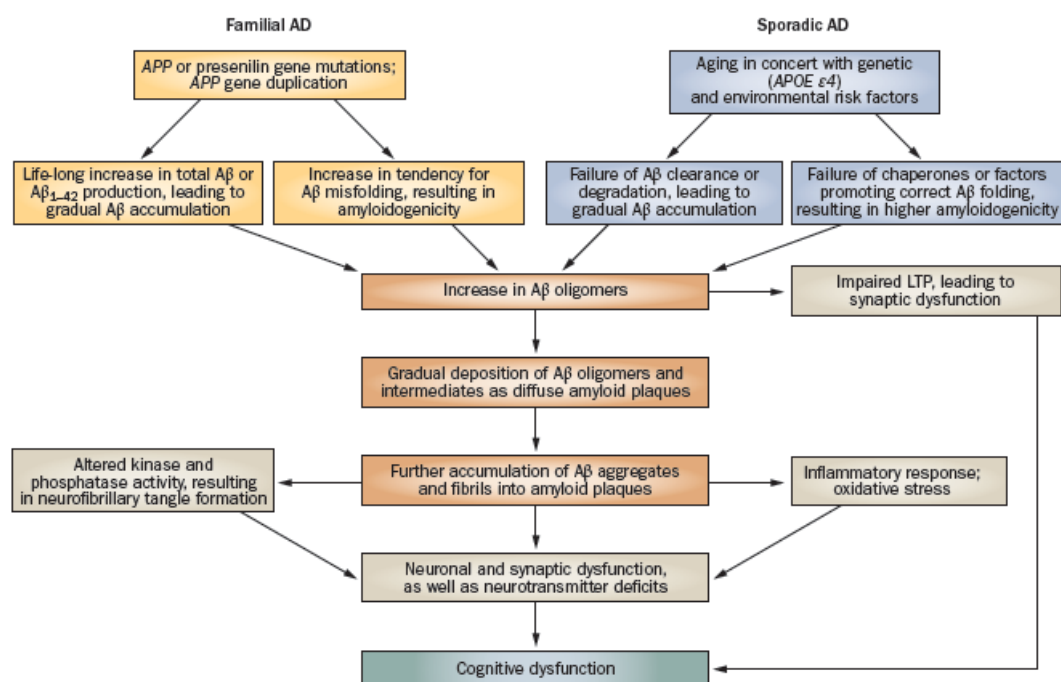


Fig.1. Amyloid cascade hypothesis of AD.²⁶ Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Neurology* 6, 131, copyright 2010.

The term amyloid refers to protein deposits resembling those first observed for starch (amyloid originally meaning starch-like). It is now specifically associated with proteins and peptides adopting fibrils based on the cross- β structure in which the peptide backbone is orthogonal to the fibril axis.²⁷ The β -sheets form fibrils, which have an internal structure such as parallel protofilaments, and the fibrils themselves can further aggregate into larger fibres or bundles (which often comprise twisted fibrils).^{6b,27d} Figure 2 shows representative fibril morphologies for A β peptides, the fibril morphology depends on preparation conditions, and fibril polymorphism is also observed and examples of other fibril structures are shown in section 4.1.

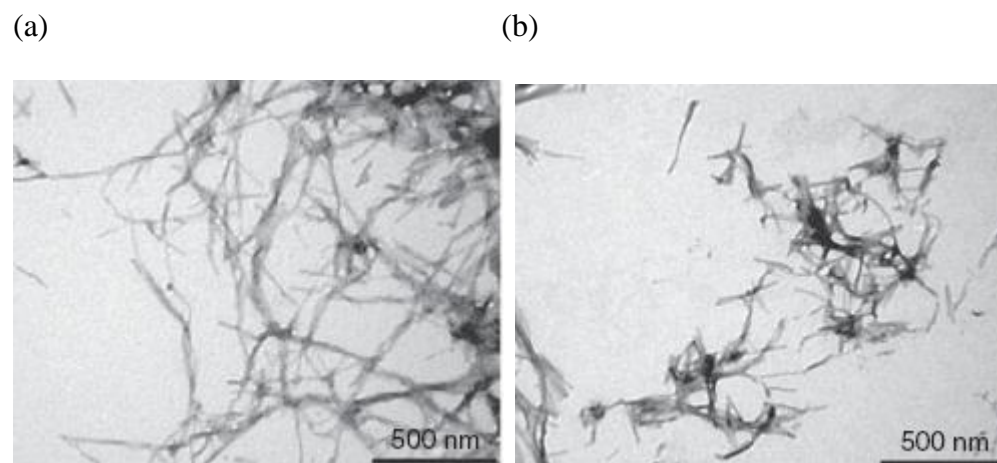


Fig.2. “Typical” A β fibril morphology by TEM, obtained from incubated 50 μ M solutions of (a) A β 40, (b) A β 42.²⁸

Figure 3 shows a timeline of some of the principal discoveries in AD research,^{3,29} also the subject of other historical overviews.^{2a,6e,30} AD is named after Alois Alzheimer who first described the condition now named after him in 1906.^{2a,31} It is generally acknowledged that the first paper to identify A β in association with neuropathology was by Glenner and Wong in 1984,³² who identified a 4 kDa major

component of A β extracted from the blood vessels of a patient with Down's syndrome. By middle age, the brains of Down's syndrome patients inevitably display the neuropathological features of AD, i.e. deposition of A β plaques and AD-type brain lesions, although mental retardation from birth is due to other causes. Glenner and Wong were also able to sequence the first 28 amino acids of A β . In the late 1980's, several different groups were able to use Glenner and Wong's A β sequence to clone the gene encoding amyloid precursor protein (APP) and map it to chromosome 21.^{10,33} Chromosome 21 is duplicated in Down's syndrome, hence the correlation with A β deposition which occurs early in this condition.



Fig.3. Timeline of selected AD discoveries including those associated with the A β hypothesis.³ Reprinted by permission from Macmillan Publishers Ltd: *Nature Medicine* 17, 1060, copyright 2011.

There are two principal variants of the amyloid peptide in humans – A β 40 and A β 42 (this notation will be used consistently for the whole peptide with the number of residues indicated, for fragments the sequence will be indicated).^{1a} The former is more abundant, however A β 42 forms fibrils more rapidly.^{1a,6d,34} A β 43 is also observed³⁵ as are peptides truncated at the C terminus³⁶ such as A β 39.³⁷ N-terminal truncated peptides are also detected.^{36,38} Tryptic degradation of A β from AD patient brains also revealed heterogeneous fragments from the A β (1-5) and A β (6-16) domains.³⁶

It is now thought that oligomers formed in the initial self-assembly process are the toxic agents.^{2a,2d,39} This is discussed in more detail in Section 2.6. Oligomerization of A β occurs intracellularly, as revealed by *in vivo* experiments on human cerebrospinal fluid which yielded SDS-stable dimers of A β .⁴⁰ Incubation did not lead to the production of extracellular oligomers. However, oligomers were detected in neural and non-neural cell lines. The importance of intracellular A β production and its relationship to extracellular production, and re-uptake has been discussed.⁴¹ Whether intra- and inter- cellular pools of A β are distinct or related has been the subject of studies with contradictory conclusions. However, it does appear that extracellular A β may originate from intraneuronal sources, and a dynamic equilibrium may exist between these pools.⁴¹ Since A β is produced via cleavage of APP in membranes (Section 2.2.3) its sites of production include the plasma membrane, but also within the cell in the Golgi and endoplasmic reticulum, as well as endosomes and lysosomes.⁴¹

There is a relationship between the incidence of AD and cerebral ischemia (reduction in blood supply), eg. following a stroke or other cerebrovascular or cardiovascular condition.⁴² Oxidative stress, eg. hypoxia (oxygen deprivation) or ischemia may cause an increase in A β levels in the brain due to an upregulation of APP processing.⁴³ A correlation between serial brain interstitial fluid (ISF) concentration and neurological status (after acute injury) has been noted, A β concentration increasing with improved neurological status.⁴⁴

Amyloid β is produced by proteolytic cleavage of APP, a transmembrane protein discussed further in Section 2.2.3.^{2a} The peptide N terminus is created by cleavage by β -secretase in the extracellular domain of APP, and the C-terminus results from intramembrane cleavage by γ -secretase. A third enzyme, α -secretase cleaves between amino acids 16 and 17 in A β , thus hindering fibrillization. The cleavage by γ -secretase is presenilin-dependent.⁴⁵ As discussed further in Section 2.2.3, γ -secretase is a protein complex involving presenilins, nicastrin, APH-1 and PEN-2, all of which are required for γ -secretase function.⁴⁶ Selkoe thoroughly reviews the historical literature concerning the relationship between the presenilins and γ -secretase.^{1a}

Whilst there have been numerous reviews on the amyloid hypothesis of AD, there are few up-to-date reviews that also discuss the biophysical aspects of A β self-assembly and its influence on AD. The present review aims to provide a unified view of the biological, neurochemical and biophysical aspects of A β aggregation and its relationship to AD. In addition, this review provides a current overview of developments in potential therapeutic strategies.

This review is organized as follows. The first section concerns the features of AD and the properties of A β and APP *in vivo*, including biological and neurochemical characteristics. The development of biomarkers is also considered. This is followed by discussion of therapeutic compounds. The final sections are focussed on different aspects of the biophysical properties of A β .

2. BIOLOGICAL, BIOCHEMICAL AND NEUROCHEMICAL CHARACTERISTICS OF AD, INVOLVING A β AND APP

2.1 Genetic Markers for AD

2.1.1 Genetic Risk Factors

Several genes have been linked to late-onset Alzheimer's disease, most importantly the gene encoding apolipoprotein E (APOE)⁴⁷, especially the ϵ 4 allele.^{47a,47b,47d,48} This is believed to cause the aggressive form of AD with earlier onset.^{3,49} APOE (and related APOC1) diagnostic testing systems have even reached the market although some products have been withdrawn due to IP issues.⁵⁰ The compound rosiglitazone may ameliorate neuronal dendritic spine loss caused by ApoE- ϵ 4, and thus improve cognition in AD patients.⁵¹

Mutations in the genes for APP,⁵² presenilin 1 and 2 (PSEN1 or PS1 and PSEN2 or PS2)^{1a,53} have a role in hereditary forms of AD.⁵⁴ Dominantly inherited forms represent only 1-3% of the total number of cases of AD, most of which are sporadic.^{2a,11,54} Mutations in PS1 and PS2 potentially account for a large fraction of early-onset cases of familial AD.^{1a,55} Mutations in the presenilins cause an increase in A β 42 in AD patients^{1b,55a,56} and also transfected cell lines and transgenic animals

expressing mutant forms of *PS1* or *PS2*.⁵⁷ and this occurs selectively for A β 42/43 over A β 40.⁵⁶⁻⁵⁷ Certain presenilin mutations can disrupt the leakage of calcium (see the discussion of the calcium channel hypothesis in section 2.7) induced by presenilins from the endoplasmic reticulum leading to supranormal release and dyshomeostasis.⁵⁸ This has been investigated in electrophysiology experiments.^{55b,58-59} Missense mutations in PS1 are associated with early and aggressive forms of AD,^{1a} A β 42 plaques being observed as early as 3-4 months.⁶⁰ The G209V, A260V and E280A presenilin A mutations lead to substantial overexpression of A β 42 in the brains of FAD patients.⁶¹ Mutations in *PSEN1* are also associated with *acne inversa* although a correlation between this condition and AD has not been noted.⁶²

The gene encoding ApoE was the first confirmed susceptibility locus for sporadic late onset AD, and its alleles have been widely studied. A recent study using microdialysis in a PDAPP/TRE mouse model (to be discussed shortly) indicates that different isoforms of the gene differentially regulate A β clearance from the brain.⁶³ A genome-wide association study identified three other loci, within *CLU* (which codes for apolipoprotein J, ApoJ or clusterin),^{54,64} within *CRI* (complement receptor 1)⁵⁴ or within *PICALM*.⁶⁴ Single nucleotide polymorphs (SNPs) at these loci (as well as *APOE*) were associated with AD risk. ApoE and CLU are the most abundantly expressed apolipoproteins in the central nervous system.⁵⁴ Earlier work had shown that ApoJ is over-expressed in individuals with AD and is present in CSF⁶⁵ and amyloid plaques.⁶⁶ Clusterin binds soluble A β to form complexes (especially with the more toxic A β 42 peptide) which can cross the blood-brain barrier.⁶⁷ It promotes amyloid plaque formation and is critical for toxicity towards neurons.⁶⁸ Bell *et al.* performed studies on the clearance of radiolabelled (¹²⁵I) A β using a mouse model

and found that A β 42 is cleared more slowly than A β 40 and that A β , ApoE and ApoJ (detected using human-specific ELISAs) are cleared from the brain by different transport mechanisms.^{67b}

A study using a yeast model has identified genetic factors influencing A β toxicity, including *PICALM* but also other previously unidentified genes associated with protein trafficking, stress and metabolism.⁶⁹ This work also confirmed the effect of *PICALM* on A β toxicity using a *C. elegans* model and also using rat cortical neurons.⁶⁹ In terms of mechanisms, the authors suggested that A β affects the endocytic trafficking of a plasma membrane receptor. Another genome-wide survey revealed an association between late-onset AD in carriers of the *APOE*- ϵ 4 allele and SNPs from the *GRB*-associated binding protein 2 (*GAB2*) gene.⁷⁰ ApoD has also been associated with AD, this lipoprotein circulates as a component of serum high density lipoproteins (HDL) and may be involved in cholesterol transport.⁷¹ Apolipoprotein D is involved in lifespan extension in *Drosophila*, conferring resistance to oxidative stress, and its expression is induced in the AD brain.⁷¹

Another gene that was identified as a risk for AD is *CALHM1* (denoting calcium homeostasis modulator 1).⁷² The *CALHM1* protein is localized in the cell membrane, and increased expression leads to enhanced calcium levels within the cytoplasm. Single nucleotide polymorphism of the gene leads to changes in AD susceptibility, specifically a P86L substitution leads to increased A β levels. However, these findings have been challenged – Bertram *et al.* also examined several family-based datasets and number of prior genome-wide association study (GWAS) datasets^{70,73} and found

no correlation between *CALHMI* and AD.⁷⁴ However, the authors of the original study dispute this analysis.⁷⁵

The role of the Orphan G protein-coupled receptor (GPR3) as a modulator of A β production has recently been identified.⁷⁶ The GPR3 gene has been mapped to a candidate AD linkage region in one chromosome as part of a large-scale genome screen using an NIMH (National Institute for Mental Health) sample.⁷⁷ GPR3 expression leads to an increase in production of the γ -secretase complex, and its cell surface localization, in the absence of an effect on Notch processing (discussed further in Section 3.2.1).⁷⁶ Notch proteins are transmembrane proteins involved in development and signaling pathways and a key challenge in the development of effective γ -secretase inhibitors is to avoid side effects caused by interference with these pathways. GPR3 was found to be highly expressed in areas of the normal brain implicated in AD and is elevated in the sporadic AD brain. It thus represents a potential target for therapeutic treatment.

Other proteins associated with A β production or APP processing include the serotonin receptors⁷⁸ and the prostaglandin EP2 receptor.⁷⁹ Prostaglandin E₂ is produced during inflammation due to activity by cytosolic phospholipase A₂ (PLA₂) or cyclooxygenase 2 (COX-2).⁷⁹ The latter enzyme is upregulated in AD brain frontal cortex and synthetic A β peptides induce COX-2 expression in SH-SY5Y neuroblastoma cells *in vitro*.⁸⁰ COX-2 is involved in the inflammatory response and is the target of NSAIDS (non-steroidal anti-inflammatory drugs). A population-based study pointed to the elevation of serum levels of pregnancy zone protein (PZP) in pre-symptomatic AD, compared to controls.⁸¹

Genome-wide studies of genes associated with aging indicate that the insulin/IGF-1 signalling pathway may be involved in Alzheimer's. Reduced signalling causes decreased AD pathology in mice⁸² while paradoxically increased signalling may also be neuroprotective.²¹

2.1.2 Transgenic Mice

Many studies use the PDAPP mouse, also known as PDGF-hAPP (from platelet-derived growth factor) mouse, which overexpresses mutant human APP (V717F mutant) under control of mouse regulatory elements and leads to A β plaque deposition.^{15,83} The PDAPP/TRE model expresses human ApoE. The TgCRND8 murine model of AD expresses a doubly mutant (K670N/M671L and V717F) human APP₆₉₅ transgene.⁸⁴ Tg2576 APP mice expressing the Swedish FAD variant of human APP₆₉₅ (section 2.2.3)⁸⁵ which leads to a selective increase in A β _{42/43} production have also been used in A β immunization experiments.⁸⁶ Tg2576 mice develop memory deficits due to the extracellular accumulation of specific A β oligomeric species, i.e. dodecamers.⁸⁷ A doubly mutant transgenic mouse including the APP(Swe) and mutant PS1 (M146L) has been developed and exhibited a large selective enhancement of A β ₄₂ and plaque deposition.⁶⁰ The 3xTg model in a triply transgenic mouse contains PS1(M146V), APP(Swe), and tau(P301L) transgenes⁸⁸ and this has been used to investigate the interplay of A β and tau (neurofibrillary tangles) pathologies (section 2.8). Contrary to doubly transgenic mice lacking the APP transgene, deposition of plaques and synaptic dysfunction (LTP deficits) are observed with the 3xTg model.⁸⁸ The APP23 mouse overproduces A β ₄₀ and the APPPS1 mouse overexpresses A β ₄₂.⁸⁹

2.2 A β *in vivo*

2.2.1 Characteristics of A β *in vivo*

Evidence that the A β 42 form of A β is the variant preferentially implicated in AD comes from several sources. Studies of the kinetics of aggregation (through turbidity measurements) indicate that A β 42 nucleates more rapidly and is more fibrillogenic than A β 40.^{34,90} The toxicity of A β 42 is much greater than A β 40⁹¹ due to its greater tendency to fibrillise. Some mutations in APP in cultured cells (discussed in section 2.2.2) lead to increased levels of A β 42, whereas wildtype APP predominantly releases A β 40.⁹² It is also found that A β 42 is the principal component of diffuse A β plaques and plaques generated from APP mutants⁹³ and in homogenized brain tissue,⁹⁴ and that early and selective deposition of A β 42 is observed in the brains of AD patients (shorter peptides with different N termini are also found).^{36,38} Despite its lower toxicity, A β 40 is actually produced by a factor of ten times more than A β 42, by γ -secretase cleavage.⁴¹

Whilst A β is generally associated with disease, a functional role for the peptide has also been suggested. Tanzi and coworkers have demonstrated that it is an antimicrobial peptide, i.e. that it is involved in immune reactions.⁹⁵ Antimicrobial activity of A β 40 and A β 42 has been demonstrated against eight common microorganisms including *E. coli* and *S. aureus*. This activity can be blocked by immunodepletion of AD brain homogenates with anti-A β antibodies.⁹⁶ Temporal lobe tissue from AD patients showed higher antimicrobial activity than material from the

brains of age-matched non-AD subjects. Balin's group have suggested that A β may be part of the control mechanism following infection by *C. pneumoniae*,⁹⁷ and that it can mediate infection of cells with this bacterium.⁹⁸ Smith and coworkers argue that A β production is a host response to an underlying condition that develops with age.⁹⁹ However, this is becoming an increasingly contrarian viewpoint in view of the mass of data implicating A β as the causative agent. The ϵ 4 allele of the *APOE* gene, a marker for EOAD (section 2.1.1) may have a beneficial role in enhanced cognitive skills.¹⁰⁰

The fraction of different variants of A β has been investigated. Based on analysis of cell lysates and also tissue from mouse brain, A β 40 has been found to constitute approximately 90% of the secreted A β and A β 42 comprises ~10%,^{57b-d} although somewhat lower^{57c} and much higher^{57e} fractions have also been reported. A β 42 is the most commonly found variant in human CSF.

Since APP is expressed in most peripheral cells, A β is present in plasma in addition to CSF. The level of A β 40 in plasma is generally under 200 pM, and of A β 42(3) is under 60 pM, although both are elevated in patients with *PS1* or *PS2* mutations or in patients with presymptomatic or symptomatic APP patients.⁵⁶ The physiological concentration of A β (in AD patients) in human CSF has been reported by several groups. An A β concentration of less than 500 pg/ml (0.1 nM) is indicative that A β is accumulating in the brain and not circulating in the CSF. Using an ELISA assay, Mehta *et al.* reported for A β 40 $c = 30$ nM.¹⁰¹ and for A β 42 $c = 8$ pM.¹⁰¹ On the other hand, Ida *et al.* using a Western blot assay reported a lower $c = 6$ nM for A β 40 but a

much higher $c = 60$ pM for A β (1-42).³⁷ Similar values for A β 42 for AD patients are reported by Motter *et al.*¹⁰² Values in plasma are also available.^{56,101} The CSF concentration of A β 40 for AD patients is not significantly different to that for nondemented control patients, however the concentration of A β 42 is lower for AD patients.^{37,101-102} This is another evidence for A β 42 as the disease-related species. Peripheral administration of monoclonal antibodies leads to a rapid increase in plasma A β .¹⁰³ The physiological concentration of A β (variant not defined) in normal human CSF is around 1- 2 nM according to ref.¹⁰⁴ whereas a value of 3-8 nM is cited elsewhere.^{1a,102} According to Podlisny *et al.* the physiological concentration of A β 40 is 0.25 – 2.5 nM,¹⁰⁵ in agreement with the value $c = 0.6$ nM reported by other groups.¹⁰⁶ There is no correlation between plasma A β 40 and A β 40 load (in the range 0-40% for 46 nM A β 40 in PDAPP mouse plasma) in the absence of anti-A β antibodies.¹⁰³ The concentration of A β in serial brain interstitial fluid has also been reported.⁴⁴ Ida *et al.* also detected the presence of N-terminally truncated A β species in CSF and plasma.³⁷

The production of A β 40 and A β 42 in the human central nervous system (CNS) does not appear to be different for AD patients compared to control, however the rate of clearance is significantly reduced for AD patients.¹⁰⁷ Production of A β is discussed in the following section. Low-density lipoprotein receptor-related protein (LRP) and the receptor for advanced glycation end products (RAGE) are involved in receptor-mediated flux of A β across the BBB as part of the clearance mechanism.¹⁰⁸ Clearance of A β from the brain to the periphery appears to be mediated by LRP while RAGE is implicated in A β efflux back into the CNS (Fig.4).^{108b}

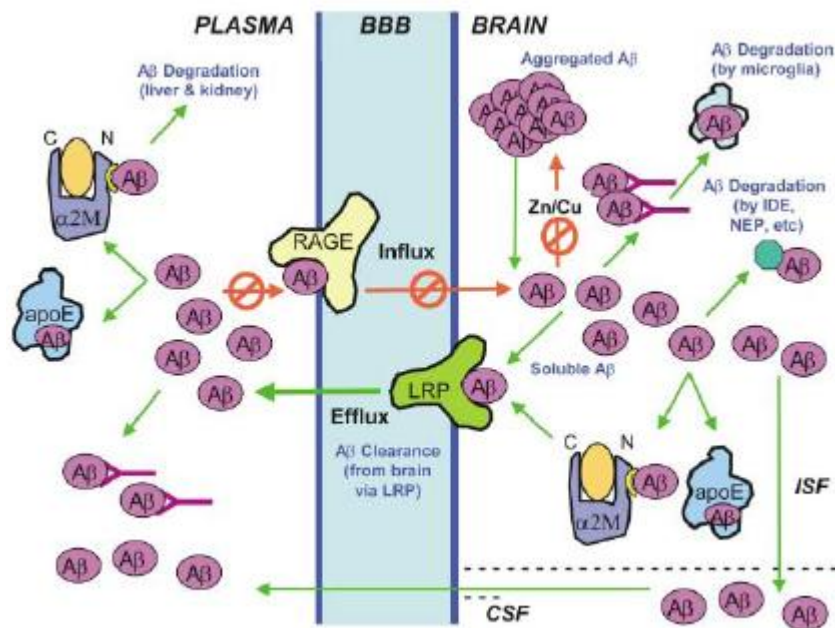


Fig.4. Clearance mechanisms of A β involving LRP and RAGE.^{108b} Blocking interaction of A β with zinc and copper may clear A β . Soluble A β can be removed via enzymatic degradation (via peptidases such as insulin degrading enzyme IDE or neprilysin NEP, and subsequent degradation by activated microglia) or receptor-mediated clearance. LRP receptor-mediated clearance can occur by direct binding or initial binding to the LRP ligands/A β chaperones ApoE and α 2M which can deliver A β to peripheral sites of degradation (liver or kidney). These chaperones can also deliver A β across the BBB into the brain. This process can also occur via the RAGE receptor. Green arrows show pathways that might be pharmacologically relevant. Reprinted from Tanzi, R. E. *et al.*, *Neuron* **2004**, *43*, 605, Copyright 2004, with permission from Elsevier

2.2.2. Modelling AD and A β Deposition in Other Organisms

AD can be modeled using a variety of organisms including fruit flies (*Drosophila melanogaster*),¹⁰⁹ nematode worms (*Caenorhabditis elegans*),^{69,110} potatoes,¹¹¹ and yeast (*Saccharomyces cerevisiae*).^{69,112} *C. elegans* has attracted particular attention as a model to study the function of presenilins, especially in relation to Notch (Section 3.3) since there are great similarities between the *sel-12* gene of *C. elegans* and presenilin genes.^{1a,113} *Drosophila melanogaster* has been engineered to express both wild type human and arctic mutant A β 42.^{109d} Yeast exhibits α -secretase activity on APP.¹¹² The later study showed that yeast can be used to model links between A β , endocytosis and human AD risk factors.⁶⁹ A β (M1-40) and A β (M1-42) i.e. with an N terminal methionine substitution, can be expressed in *E. coli*.^{28,114} The fibrils formed by the recombinant peptides are indistinguishable than those from chemically synthesized peptides.

2.2.3 APP and the Production of A β

APP belongs to the family of type 1 transmembrane glycoproteins.¹¹⁵ It has been hypothesized to act as a vesicular receptor for the motor protein kinesin-I.^{109b} The production of A β in the amyloidogenic pathway involves the sequential cleavage of APP by β -secretase and γ -secretase (Fig.5). The enzyme β -secretase is an integral membrane aspartyl protease encoded by the β -site APP-cleaving enzyme 1 gene (*BACE1*)^{1a,1b,116} while γ -secretase is a membrane-bound protease complex consisting of at least four components including the presenilins (PS1 and PS), nicastrin, and the genes *APH-1* and *PEN-2*.^{110b,117} The γ -secretase complex may also function as an aspartyl protease.^{1a,117b} It has been proposed that A β up-regulates its own production by increasing BACE1 expression,¹¹⁸ possibly involving oxidative stress.¹¹⁹

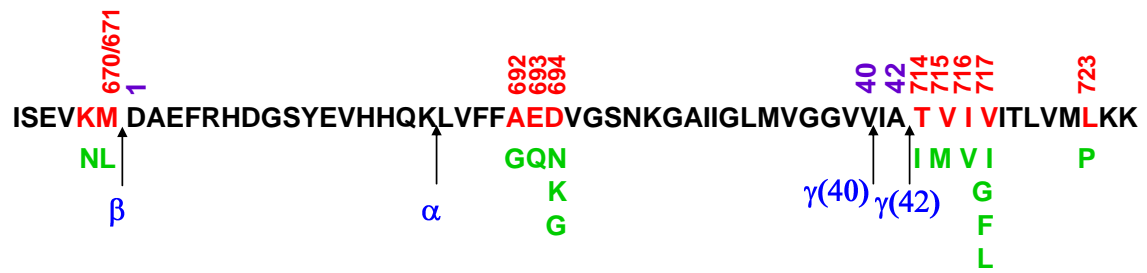


Fig.5. Mutations in APP associated with A β production and expression and AD.

Redrawn from ref. ^{1a} Secretase enzymes are indicated in blue, with cleavage sites arrowed. APP sequence numbers are shown in red, A β in purple. Mutants are indicated with green letters.

In the non-amyloidogenic pathway, a third enzyme, α -secretase cleaves between amino acids 16 and 17 in A β , thus hindering fibrillization of the full peptide. The α -secretases belong to the “A Disintegrin and Metalloprotease” (ADAMs) family of zinc metalloproteinases.¹²⁰

Recent work suggests that in late onset Alzheimer’s disease, A β accumulation occurs intracellularly in late endosomes where enzymes β -secretase and γ -secretase cleave A β , the latter in a presenilin-dependent fashion.¹²¹ The intracellular sites of A β production occur where APP is located, as shown in Fig.6.⁴¹ The gene involved in APP recycling in endosomes has been identified, and is termed *SORL1* and the associated protein is SORLA or LR11. Normally the protein product of the gene directs APP into recycling endosomes (retromer recycling endosomes, Fig.6), however mutations produce a decrease in protein product which leads to the pathway

where A β production via fragment C99 is increased by enzymes in the late endosomes.¹²¹ It has been reported that the proteolytic processing of A β is regulated by glycogen synthase kinase-3 isozymes,⁴⁶ however this has been disputed.¹²²

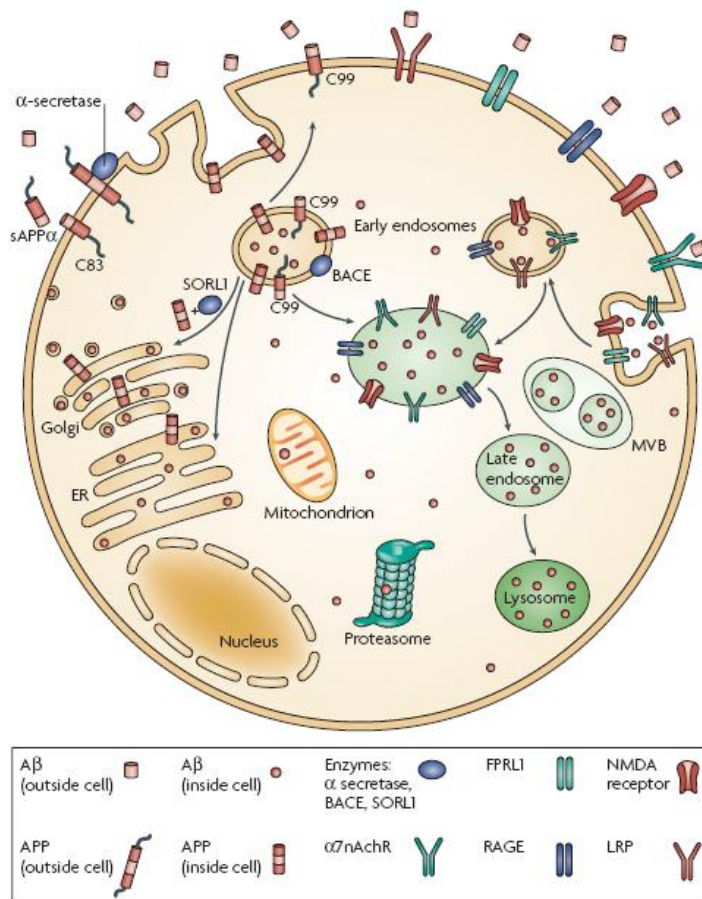


Fig.6. Sites of intracellular A β production.⁴¹ A β is produced within the endoplasmic reticulum (ER) and Golgi system and secreted. APP is localized in the plasma membrane, where it is cleaved by α -secretase, releasing soluble APPs into the extracellular space and leaving an 83-amino acid fragment known as C83 within the membrane. Unprocessed APP can be internalized into early endosomes. In the presence of SORL1, APP is recycled back to the Golgi in retromer endosomes. Early endosomes contain BACE1 which cleaves APP to produce a 99 residue fragment C99, retained within the membrane. C99 can be shuttled back to the ER to be

processed into A β by γ -secretase in the ER, shuttled back to the plasma membrane where the γ -secretase complex is also found, or processed to A β within the endosome/lysosome system. Extracellular A β (i.e. previously secreted A β) can bind to cell surface receptors (including RAGE, LRP, FPRL1, NMDA receptors and α 7-nAChR) and the receptor-A β complex can be internalized into early endosomes [FPRL1 denotes FMLP-receptor-like protein, NMDA denotes *N*-methyl-D-aspartate and α 7-nAChR the α 7 nicotinic acetylcholine receptor]. Intracellular accumulation of A β mainly occurs in the multivesicular body (MVB) and lysosome, but also in the mitochondria, ER, Golgi and cytosol, where it can influence proteasome function. Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Neuroscience* 8, 499, copyright 2010.

In addition to the known isoforms of A β , i.e. A β 42, A β 40 and A β 38, shorter fragments have been identified in CSF.^{120b} This suggested a different APP processing pathway involving concerted cleavage of APP by α - and β -secretases.

It has recently been proposed that rather than A β , another APP fragment may be involved in AD (possibly along with A β).¹²³ The N-APP extracellular N-terminal fragment is adjacent to A β and is also cleaved by BACE. It triggers the cell death cascade by binding to a neuronal receptor called DR6 (death receptor 6), which is highly expressed in regions of the human brain most affected by AD, in the presence of caspase 6.¹²³

Fig.5 summarizes the mutations in APP linked to AD. It is notable that these mutations are located just outside the cleavage sites of β - and γ -secretase beyond the N- and C-termini of A β respectively, as well as close to the α -secretase cleavage site within A β . Mutations within A β are expected to enhance the aggregation properties, and this has been shown for the E693Q mutation (Dutch-type) (Table 2, section 2.2.4). The Flemish A692G mutation leads to a mixture of A β plaque and tangle formation as well as microvascular β -amyloidosis and cerebral hemorrhage due to cerebral amyloid angiopathy.¹²⁴ This mutation also enhances the proportion of APP cleaved by the β -secretase homologue BACE-2.^{1a,125}

APP comprises a group of ubiquitously expressed polypeptides migrating between 110 and 135 kDa on electrophoretic gels.^{1a,126} The heterogeneity arises since there are three main isoforms of human APP with 695, 751 and 770 residues, and additionally due to post-translational modifications including *N*- and *O*-glycosylation, phosphorylation and sulfation. APP forms containing 751 and 770 residues are widely expressed both in neuronal and non-neuronal cells throughout the body whereas the 695-residue form is expressed more highly in neurons, and occurs at very low abundance in other cells.^{1a} The 751 and 770 isoforms contain a KPI (Kunitz-type Protease Inhibitor) domain (Fig.7) and are thus able to inhibit serine proteases such as trypsin and α -chymotrypsin.¹²⁷

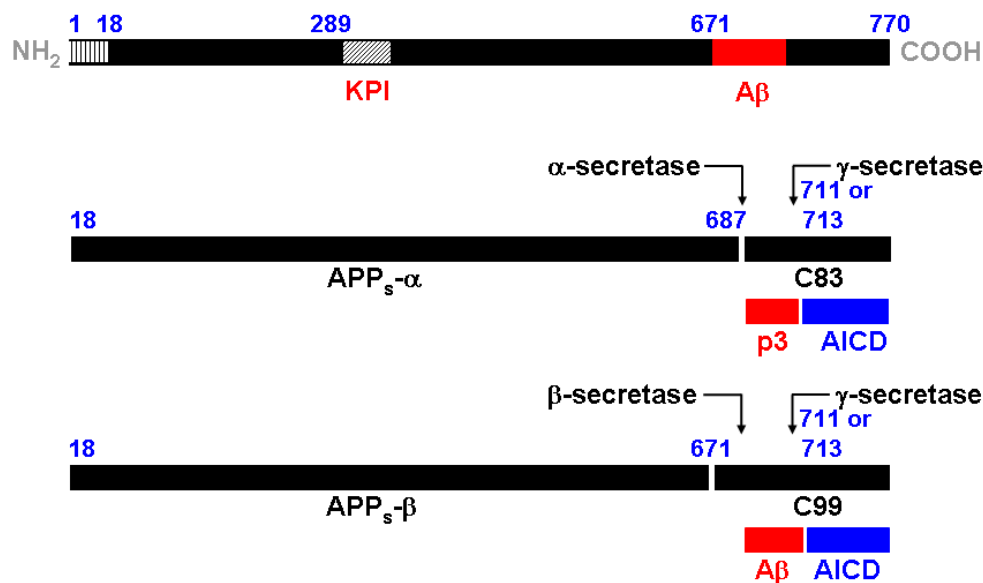


Fig.7. Processing routes of APP to produce A β and other peptide fragments (C83, C99 and p3) as well as the soluble APP_s ectodomain fragments. Redrawn, based on schematics by Selkoe.^{1a,1b} TM denotes transmembrane domain, KPI denotes Kunitz-type inhibitor domain which is a spliced exon of 56 amino acids inserted at residue 289. Cleavage of both C83 and C99 C-terminal fragments by γ -secretase releases the β -amyloid precursor protein intracellular domain (AICD) into the cytoplasm. A 17-residue single peptide is indicated at the N terminus.

Table 2 presents common mutations in APP. The E693Q mutation was the first to be associated with disease, hereditary cerebral haemorrhage with amyloidosis, Dutch type (HCHWA-D).¹²⁸ The A692G Flemish mutation is associated with cerebral haemorrhage with amyloidosis (CHWA).¹²⁸ A mutation in APP K670M671 \rightarrow N670L671 has been associated with the so-called Swedish FAD.¹²⁹ The London

variant involves V717I.^{52,128,130} Detailed information on mutations in presenilins is provided elsewhere.^{10,58a}

Table 2. Effect on A β of APP mutations. Adapted from refs.^{10,131}

Name/FAD variant	Mutation	Effect on APP	Effect on A β
APP-717 (London)	V717F/G/I	Differential γ -secretase cut	Aβ42:Aβ40 ratio increased
APP-670/671 (Swedish)	K670N and M671L	Increased β -secretase cut	Increased Aβ40 and Aβ42 in plasma
APP-692 (Flemish)	A692G	Decreased α -secretase cut?	Decreased Aβ40 and Aβ42 in media, decreased Aβ aggregation, Aβ42:Aβ40 ratio increased
APP-693 (Dutch)	E693Q	Unclear	Decreased Aβ42 in media, increased Aβ aggregation, Aβ42:Aβ40 ratio decreased
APP-693 (Arctic)	E693G ¹³¹	Unclear	Decreased Aβ40 and Aβ42 in plasma, Aβ42:Aβ40 ratio decreased
APP-693 (Italian)	E693K ¹³¹	Unclear	Decreased Aβ42 in media, Aβ42:Aβ40 ratio decreased
APP-694 (Iowa)	D694N	Unclear	Enhanced fibrillization of Aβ40¹³²
PS1-FAD mutations	M139I, H163A, and others ^{55a}	Differential γ -secretase cut	Aβ42:Aβ40 ratio increased
PS2-FAD mutations		Differential γ -secretase cut	Aβ42:Aβ40 ratio increased
Trisomy 21 (Down's syndrome)		Increased APP production	Aβ40 and Aβ42 increased
Apolipoprotein E4		Competes for LDL receptor-related protein (LRP)	Increased Aβ aggregation

Overexpression of the mutant V717F leads to neuronal cell death, and damage to synapse termini, well before the development of amyloid plaques.¹⁵ A double mutant transgenic mouse model also expressing the Swedish FAD mutant led to synapse transmission deficits even in young mice without amyloid plaques. These observations suggest that neurotoxicity of A β is independent of plaque formation, consistent with the neurotoxic agent being other species such as oligomers as discussed in section 2.6.1.¹⁵ A mutation M67I is found to eliminate production of A β 42.¹³³ High levels of A β 42 result in age-dependent formation of amyloid plaque in FAD-mutant hAPP mice, but not in wild-type hAPP mice.¹³³ There is no correlation between synapse damage and hAPP levels or plaque load, although there is an inverse correlation with A β levels. This points to the neurotoxicity of A β even in the absence of plaques.¹³³

A β is produced from APP via cleavage by the secretase enzymes. APP is a transmembrane protein that is postrationally modified through the secretory pathway. The first proteolytic cleavage identified is that by α -secretase, occurring 12 amino acids towards the N terminus from the transmembrane domain (Fig.7).^{1a} This cleavage produces the large soluble α -APPs peptide into the extracellular space, along with release of the 83-residue C-terminal fragment (CTF) in the membrane. Some APP molecules not subjected to α -secretase cleavage can be cleaved by β -secretase releasing the slightly smaller β -APPs ectodomain derivative and retaining a 99-residue CTF (C99) in the residue of the membrane. The β -secretase cleavage can be followed by γ -secretase activity to produce A β , or alternatively sequential action of α - and γ -secretases (the latter acting on C83) produces the p3 peptide fragment (Fig.7).^{1a} The presenilin/ γ -secretase complex can cleave at other sites ϵ and ζ in the

transmembrane domain followed by the final cut at the γ -cleavage C-terminal site to produce A β 38, A β 40 or A β 42.^{6e}

2.2.4 A β Mutations

Strong evidence for the A β hypothesis comes from genetic analyses of FAD (Section 2.2.3), since most mutations in the genes for APP, and *PS1* and *PS2* genes appear to cause accumulation of the A β 42 form.

The following mutations of A β are derived from those for APP (section 2.2):^{91a,134}

A21G Flemish, E22K Italian, E22Q Dutch, E22G arctic, D23N Iowa.

Fig.5 illustrates the location of these mutants, along with the correspondence to the APP sequence. Most mutations occur close to the β -secretase cleavage site, increasing cellular production of A β 40 and A β 42, or just after the γ -secretase cleavage which selectively increases production of the more toxic A β 42.

A systematic investigation of the aggregation tendency of all 798 single-point mutations of A β 42 was carried out using the Zyggregator algorithm (Section 4.5) to quantify aggregation propensity.^{109g} Seventeen mutants were then expressed in *Drosophila melanogaster* and properties including *in vivo* toxicity (survival time) and relative locomotor ability were correlated to the aggregation propensity. Mutants involving E22G (alone, or with one other residue substitution, except I31E/E22G) are found to be most highly pathogenic.^{109g} This is consistent with the observed higher rate of oligomerization and fibrillization of the arctic E22G variant compared to the wild-type peptide.¹³⁵

2.3 Neuronal Toxicity of A β

At least for human neurons, intracellular A β 42 is neurotoxic.^{91b} There is still some controversy about the precise location of A β aggregation *in vivo*,^{1a} although the endoplasmic reticulum and Golgi have been identified (see Fig.6).⁴¹ A review discusses the intra-cellular production of A β .⁴¹ A novel super-resolution fluorescence imaging technique has been used to probe A β 42 fibrillization within HeLa cells (differences in fibril morphology *in vivo* and *in vitro* were also noted).¹³⁶

A β 42 is selectively intracellularly cytotoxic to human neurons, and not to other cell lines.¹³⁷ A β 42 but not A β 40, A β (42-1) or A β (40-1) is toxic to human neurons.¹³⁷ The proapoptotic proteins Bax and p53 are implicated in this intracellular toxicity.¹³⁷⁻¹³⁸ Disturbances in the cell division cycle may influence apoptosis in AD and this has been related to processing of APP and cyclin-dependent phosphorylation of tau.¹³⁸⁻¹³⁹

Long-term potentiation (LTP) is a long-lasting enhancement of synaptic efficacy after brief high frequency stimulation. It has been widely used as a model of synaptic plasticity. A β 42 and A β 40 are known to disrupt LTP in neurons.¹⁴⁰ Neurons from transgenic mice expressing genes encoding mutant APP^{15,88,133,141} or presenilin linked to FAD^{1a,1b,55a,56} exhibit damage to synapses and dendritic spine loss. A β is implicated in these defects because γ -secretase inhibition ameliorates some indicators of synapse damage.^{141f,142} Synthetic A β 42 mediates long-term depression (LTD) in an NMDAR-dependent manner *in vivo*¹⁴³ as does A β secreted by neurons that overexpress APP.¹⁴⁴ Deposition of amyloid plaques and deficits in LTP are observed together, although

spine loss and decrease in pre-synaptic terminal density is observed before plaque deposition probably pointing to the influence of oligomeric or pre-fibrillar A β on neurotoxicity.^{15,141d} This is also supported by the fact that cognitive loss precedes the observation of amyloid plaques.^{88,141a-c,145} Later work specifically implicated soluble oligomeric A β in synaptotoxicity and inhibition of LTP.^{140b,146} (This is also discussed in section 2.6.1).

Loss of glutamate receptors such as those for AMPA and NMDA caused by A β is implicated in synaptic depression and dendritic spine loss.^{142,146d,147} A β is known to inhibit LTP,¹⁴⁸ as do A β fragments such as A β (25-35).¹⁴⁹ The blockage is mediated by stimulation of certain kinases.¹⁵⁰ A β -induced pathology may progress in a neurotransmitter-specific manner with different susceptibility for cholinergic, glutaminergic and GABAergic transmission (GABA = γ -amino butyric acid).¹⁵¹ A β (25-35) was used to investigate the effect of NMDA and GABA receptor antagonists.¹⁵²

A β binds to the α 7 nicotinic acetylcholine receptor, nAChR, in neurons.¹⁵³ This can result in receptor internalization and hence re-uptake of extracellular A β .⁴¹ Since α 7 nAChR is co-localized with A β in plaques and A β disrupts calcium activation and acetylcholine release at the receptor, this interaction may be important in AD pathophysiology.^{153a} A β blocks the response of these nicotinic receptors, at least at high concentration¹⁵⁴ (at low concentration A β 42 seems to activate the α 7 nAChR, although there is still controversy concerning this).¹⁵⁵ Thus, stimulating nicotinic receptors (eg. with nicotine) protects neurons against A β toxicity.^{154a,156}

Neuroprotection can also be achieved using $\alpha 7$ -receptor agonists.^{154a,155a} However, long-term use of nicotinic agonists may induce desensitization of nicotinic receptors.¹⁵⁷ This led to the proposed use of allosteric modulators which bind to a site on nAChR distinct from that of the natural acetylcholine binding site.¹⁵⁷ The specific sequence A β (12–28) was implicated in the inhibition of nicotinic currents.¹⁵⁸

GABA receptors are also potential targets to treat AD. Activation of GABA receptors increases neuronal vulnerability to toxic damage by A β .¹⁵⁹ This can be prevented by taurine (2-aminoethanesulfonic acid, related to tramiprosate, Fig.13) or GABA itself, or GABA_A receptor agonists.^{152,159}

The role of microglia in the deposition of A β plaques (Fig.8) has been examined. Microglia are support cells involved in inflammation that surround senile plaques. Their role is not completely clear,¹⁶⁰ since it has been proposed that they can clear amyloid deposits or alternatively may contribute towards their deposition (and particularly associated inflammation). Reactive microglia associated with A β plaques are involved in inflammation in AD. Fibrillar A β initiates a tyrosine kinase-based response in mouse microglia (and a human cell line) resulting in production of neurotoxic secretory species, proinflammatory cytokines and reactive oxygen species.^{160b} The cytokine TNF- α generated by monocytes and microglia is responsible for most of the A β -induced neurotoxicity.^{160b} Cytokine TGF- β 1 is also involved in the response to injury and has been found in the CNS of AD patients. It has been shown that TGF- β 1 induces A β deposition using a mouse model expressing this cytokine from astrocytes.¹⁶¹

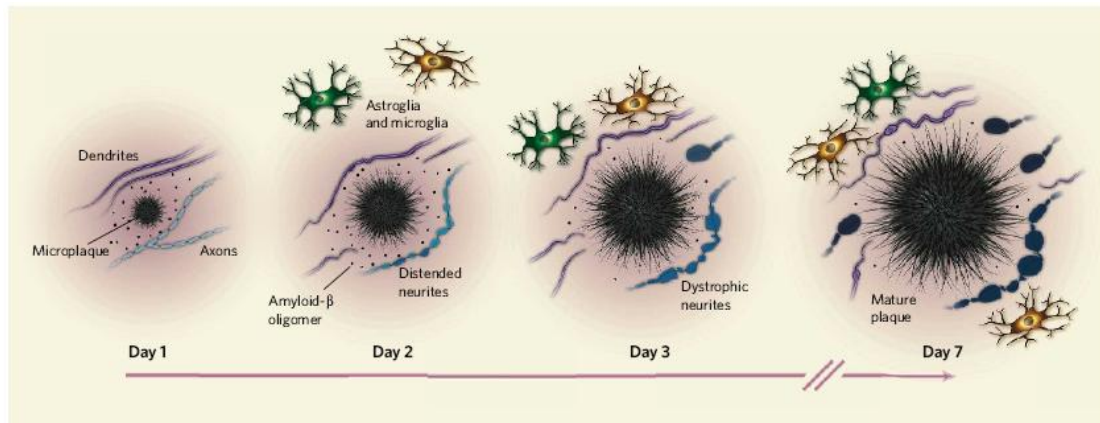


Fig.8. Mechanism of plaque formation and associated migration of glial cells, based on work by Meyer-Luehmann *et al.*¹⁶² Reprinted by permission from Macmillan Publishers Ltd: *Nature* 451, 499, copyright 2008.

Microinjection of fibrillar, but not soluble A β , in the brains of aged rhesus monkeys leads to microglial proliferation as well as loss of neurons and tau phosphorylation.^{160a} Fibrillar A β at plaque-equivalent concentration was found not to be toxic in the brain of young rhesus monkeys, pointing to the role of aging in promoting susceptibility towards A β neurotoxicity.^{160a} Microglia may enhance the toxicity of A β by releasing glutamate through the cysteine-glutamate transporter system x_c⁻, and the neurotoxicity can be eliminated via inhibition of NMDA receptors or system x_c⁻.^{160c} Microglial secretion of ApoE was found to exert a neuroprotective effect.^{160c} On the other hand, Nagele *et al.* investigated the role of microglia in A β plaque formation and found that they can facilitate the conversion of soluble and oligomeric A β into fibrillar form, and that microglia do not remove A β from plaques.¹⁶³ These authors also highlight the role of astrocytes in accumulating A β -positive material as part of their role in debris clearance in response to localized neurodegeneration. It is also suggested that A β fibrillization can occur within the surface plasma membrane of microglia.¹⁶³ In

contrast to the findings of Nagele *et al.*, Simard *et al.* showed using a transgenic mouse model that bone-marrow derived cells (which can cross the BBB) that differentiate into microglia are able to eliminate amyloid deposits by cell-specific phagocytosis.¹⁶⁴ Using multiphoton laser confocal microscopy, Meyer-Luehmann *et al.* showed that microglia are activated within 1-2 days of the appearance of a new plaque and that micro-plaques are rapidly formed that eventually develop into mature plaques (Fig.8).^{160d} Accumulation of microglia during inflammation can be mediated via Chemokine (C-C motif) receptor 2 (CCR2)-signaling and thus CCR2 and its main ligand CCL2 (MCP-1) might also be involved in the altered metabolism of A β underlying Alzheimer's disease (AD).¹⁶⁵ Cannabinoids may also have a role in neuroprotection by blocking microglial activation.¹⁶⁶ Senile plaques express cannabinoid receptors CB₁ and CB₂ together with markers of microglial activation and a synthetic cannabinoid was shown to prevent A β -induced microglial activation.¹⁶⁶

The zinc metalloprotease insulin-degrading enzyme (IDE, insulysin) is central to the turnover of insulin and degrades A β in the mammalian brain.^{108,167} IDE forms a stable complex with A β 40 and with A β (17-27).¹⁶⁸ IDE actually forms a complex with A β monomer and not oligomers and so is not able to inhibit oligomer-induced loss of LTP.^{146a} Monomeric but not aggregated A β was able to associate irreversibly with IDE via the substrate binding site of the protease.¹⁶⁸ The phosphorylation of A β at serine residue S8 reduces its clearance via IDE and angiotensin-converting enzyme (ACE).¹⁶⁹ The other major endopeptidase involved in A β clearance is the zinc metalloprotease neprilysin (NEP),¹⁷⁰ although other proteases capable of degrading A β have been investigated.^{108b,167} Aggregation-mediated A β 42 toxicity is decreased

when aging is slowed (in a *C. elegans* model) by decreasing insulin growth factor-1-like signaling (IIS), pointing to a link between the aging process and aggregation-induced neurodegeneration^{110c} On the other hand, the transcription factors DAF-16 and HSF-1 which express numerous chaperones (Section 3.2.2) regulate A β aggregation and disaggregation activities respectively to promote cellular survival in response to toxic aggregation events (Fig. 9),^{110c} and may be the target for therapeutics. ACE plays an important role in blood pressure and body fluid regulation and sodium homeostasis. It is associated with AD in the Japanese population.¹⁷¹ ACE is found to inhibit A β aggregation and can degrade it by cleavage at N7-S8.^{171b}

Figure 9 shows schematically proposed pathways for *in vivo* aggregation of A β 42, relevant to age-related proteotoxicity.^{110c} The IIS pathway is regulated by the receptor DAF-2 (inhibition of DAF-2 expression extends the lifespan of *C. elegans* worms). The transcription factors heat shock factor (HSF-1) and DAF-16 regulate opposing disaggregation and aggregation processes. The preferred mechanism whereby toxic aggregates are rapidly degraded (5-II) is positively regulated by HSF-1 (stage 5-A) and negatively regulated by DAF-2 (stage 5-C). When the HSF-1-regulated disaggregation mechanism is overloaded, a second comes into play (5-III). This produces less toxic higher M_w aggregates. This is positively regulated by DAF-16 (stage 5-B) and negatively by DAF-2 (stage 5-D). The high M_w aggregates can be eliminated by several methods indicated in the scheme.

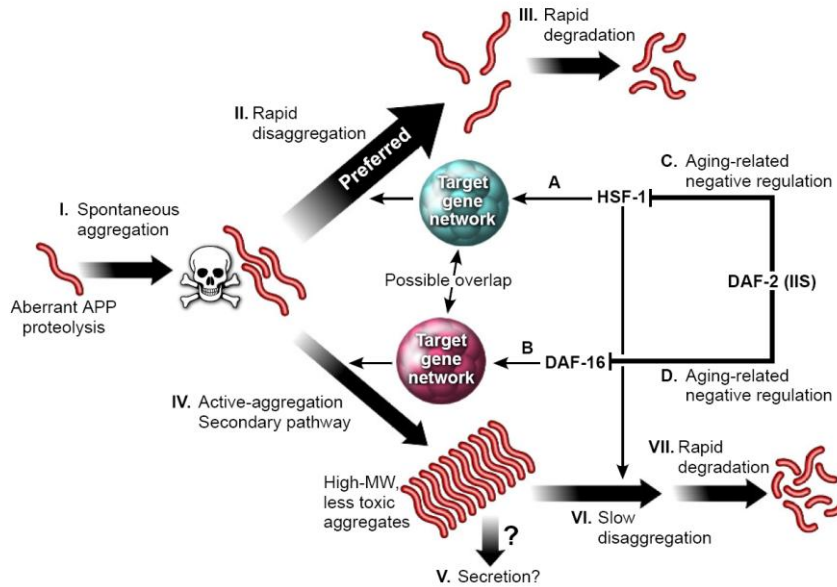


Fig.9. Pathways of regulation of fibrillization in age-onset A β proteolysis.^{110c}

Adapted from Cohen, E. *et al.*, *Science* **2006**, *313*, 1604. Courtesy of Ehud Cohen.

2.4 Biomarkers for AD

Biomarkers for AD are expected to improve the accuracy of diagnosis and to assist in differentiation of cases involving changes in A β metabolism. Biomarkers can also be used to investigate the influence of drugs on A β production (theranostics) along with safety monitoring, eg. of inflammatory responses in the case of adverse effects.²⁶ A major public-private partnership initiative funded by the NIH, non-profit AD research organizations and major international pharma companies is the Alzheimer's Disease Neuroimaging Initiative (ADNI), which aims to identify biomarkers in volunteer patients.¹⁷²

Different biomarkers may be appropriate during the progression of neurodegeneration towards AD leading to a dynamic model for applicable biomarkers as shown in Fig.

10.¹⁷³

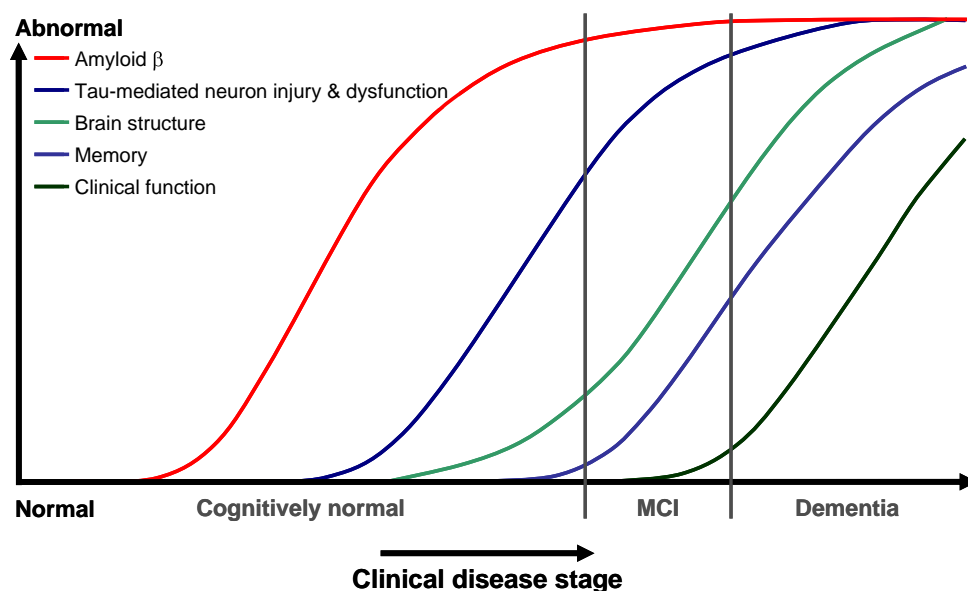


Fig.10. Dynamic events as a basis for use of biomarkers.¹⁷³ A β is identified by CSF A β 42 or PET amyloid imaging. Tau-mediated neuronal injury and dysfunction is identified by CSF tau among other indicators. Brain structure is probed using structural MRI. MCI = mild cognitive impairment.¹⁷³ Reprinted from Jack, C. R. *et al.*, *The Lancet Neurology*, **2010**, 9, 119. Copyright 2010, with permission from Elsevier.

Research on biomarkers in CSF and plasma has recently been reviewed.^{26,173-174} The only established biomarkers are three that can be assayed in CSF: A β 42, total tau (t-tau) and p-tau (phosphorylated tau, at position threonine 181 or threonine 231).^{19,175} A combined analysis of two or more these biomarkers accurately diagnoses AD more accurately than a single one.²⁶ The combination of these three analytes has high

predictive value for prodromal (early symptomatic) AD.²⁶ Reduced CSF A β 42 (and A β 40¹⁷⁶) levels in cognitively normal elderly people who later developed AD have been observed from population-based studies¹⁷⁶⁻¹⁷⁷ and clinical studies.¹⁷⁸ No changes were observed in CSF t-tau or p-tau.¹⁷⁶⁻¹⁷⁷ Biomarkers for tau phosphorylation appear specific to AD in contrast to changes in total tau and A β 42 which are found in patients with other neurodegenerative diseases.²⁶ An analysis of multiple study populations to examine potential CSF biomarkers for AD revealed that these three biomarkers can be used as diagnostics to predict incipient AD in patients with mild cognitive impairment.^{19,175,179} A total of eighteen signalling proteins have been identified in plasma that can predict for AD, the study being based on plasma samples from individuals with presymptomatic to late-stage Alzheimer's disease and from controls.¹⁸⁰

Due to the need for invasive treatment (lumbar puncture) associated with measurements using CSF, reliable biomarkers in blood are also sought. It has been suggested that A β (1-42) plasma levels are not a sensitive and specific indicator for early diagnosis.^{26,174b} This was ascribed to (i) the fact that plasma A β is derived from peripheral tissues and not the brain, (ii) variations in A β levels due to time-dependent fluctuations, (iii) binding of A β to other proteins, (iv) the influence of medications and (v) the involvement of APP and A β 40 in platelet aggregation.^{26,174b} However, it has been reported that a reduction in plasma A β 42/A β 40 ratio is associated with cognitive decline over 9-10 years.¹⁸¹ Tau-related enzymes have been studied as potential blood biomarkers, including kinases involved in tau hyperphosphorylation.^{174b} However, it has been concluded that currently tau-related

biomarkers are not reliable diagnostics.^{174b} Several other potential blood biomarkers are discussed by Blennow *et al.*²⁶

Antibodies associated raised against A β (discussed further in Section 2.5) may be useful biomarkers.^{172a} A recent study reports the development of immunoglobulin G biomarkers for AD via a screening study using synthetic oligomeric peptoids to capture antibodies.¹⁸²

Biomarkers associated with inflammation have also been investigated, in particular proinflammatory cytokines.^{174b} Other disease-related biomarkers include ubiquitin and biomarkers related to cellular senescence such as p53 conformational state or telomere shortening.^{174b} Finally, there are biomarkers associated with cerebrovascular damage.^{174b} Other candidate CSF biomarkers include BACE, APP isoforms, truncated A β isoforms, A β oligomers, endogenous A β antibodies and neuronal and synaptic markers.^{26,174a} In particular, A β oligomers are promising biomarkers, however low CSF concentrations make sensitive detection a challenge.^{174a} Recently, the (small cytokine) chemokine (C-C motif) receptor 2 (CCR2) has been proposed as a CSF biomarker.¹⁶⁵

Methods to identify biomarkers for AD diagnosis based on different ELISA assays, mass spectrometry, DNA and gene chips etc are also discussed elsewhere.^{174b}

Imaging methods such as MRI (magnetic resonance imaging), fMRI (functional MRI) and PET (positron emission tomography) to diagnose AD are reviewed elsewhere.¹⁷³⁻

¹⁷⁴ The use of A β ligands for PET imaging has attracted attention, notably Pittsburgh

compound B (PIB, Fig.11b). This benzothiazole is derived from the well-known amyloid binding compound thioflavin T (Fig.11a) and it enables direct visualization of fibrillar A β load in the brain of living patients.¹⁸³ Another widely used PET reagent is FDG, [¹⁸F]-2-deoxy-D-glucose, which is sensitive to neuronal glucose metabolism.^{174a,183d} Investigation of changes in the retina related to neurodegeneration, i.e. the monitoring of nerve cell death using *in vivo* cell marker methods, has been proposed as a method to screen for AD.¹⁸⁴ Indeed, A β is deposited also in the retina.

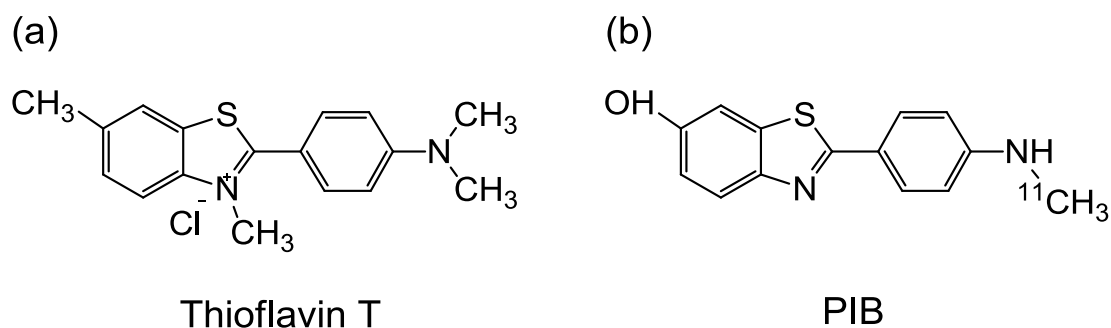


Fig.11. (a) Thioflavin T, (b) Pittsburgh-compound B (PIB) used as a tracer in PET imaging.

The SERPIN (serine protease inhibitor) α_1 -antichymotrypsin has been shown to be a biomarker for Alzheimer's, and is present in CSF.¹⁸⁵ This is probably due to the role of oxidative stress and inflammation in Alzheimer's, specifically in the overproduction of secretase,¹¹⁸⁻¹¹⁹ which in turn is correlated with A β load.¹⁸⁶

2.5. Antibodies to A β and Sequences Therein

Table 3 lists antibodies raised against A β and A β sequences. These are widely used in studies to identify particular A β species produced *in vivo*.

Table3. Commonly used monoclonal A β antibodies. Developed from ref.⁴⁰

Name	Epitope	Ref.
2G3	A β (33-40) A β (31-40) Specific for A β 40	161 187 188
3D6	A β (1-5)	161,187
4G8	A β (17-24) A β (17-28)	87,189 24
6C6	A β (1-16)	106a,190
6E10	A β (1-16) A β (1-17) A β (4-9) A β (4-13)	24,189c 189a 191 168,192
8F5	A β 42 globulomers (docadimeric oligomers)	193
10D5	A β (1-28) epitope A β (x-12)	161
14C2	A β (33-40)	190b
14C12	A β (13-28)	190b
21F12	A β (33-42) A β 42 specific	187 188b
266	A β “central domain” A β (13-28)	103,106a,188a,189b 106a,190a

Several polyclonal antibodies are also used, as listed for instance by Walsh *et al.*⁴⁰

An antibody, now known as A11, that recognises an epitope that is displayed specifically by soluble oligomers of many polypeptides has been identified.¹⁹⁴ The antibodies were raised in rabbits to an oligomeric model antigen comprising gold nanoparticles grafted with A β 40. This antibody inhibits the toxicity of A β 40 and A β 42 oligomers.^{194a} This antibody also recognises oligomers from a range of other proteins and peptides.^{194a} Recognition was not observed for low M_w - or fibrillar- A β

species. This indicates that the antibody recognises a common epitope in soluble oligomers. An antibody termed 8F5 is raised specifically against so-called A β 42 globulomers (docadameric oligomers).¹⁹³ An antibody, distinct from A11, that recognizes on-pathway oligomers (i.e. pre-fibrillar oligomers) called OC has also been reported.^{194b,194c} An antibody against A β (4-10) (FRHDSGY) inhibits A β fibrillization and cytotoxicity, without generating an inflammatory response.¹⁹⁵ Similarly, an antibody against A β (1-11) prevents aggregation of A β 42 and causes disaggregation of preformed A β 42 fibrils.¹⁹⁶ The binding of fluorescently labeled antibody 6E10 to amyloid deposits within the TgCRND8 mouse brain has been observed, up to 2.5 mm away from the site of injection.¹⁹⁷

A number of monoclonal antibodies raised against oligomers and fibrils have been identified, which have activity against binding of A β to cells and reactive oxygen species generation.¹⁹⁸ Antibodies that recognize the N or C terminal residues of A β 42 have been used in immunological studies using soluble dimers.¹⁹⁹ A solution NMR structure of the complex formed between A β 40 and an affibody protein Z_{A β 3} indicates that this affinity ligand protein stabilizes the β -sheet structure, and the hairpin observed in A β 40 (section 4.1) is retained.²⁰⁰ Coexpression of Z_{A β 3} with A β 40 and A β 42 can be used to produce both isoforms recombinantly.²⁰¹

2.6 Oligomers

2.6.1 Toxicity of Oligomers

It is now thought that oligomers formed in the initial self-assembly process of A β are the toxic agents.^{2a,2d,6d,39,91,104,145a,194a,202} Evidence for this comes from several

experiments on disease related and non-disease related proteins. *In vivo* and cell culture experiments showed that A β 42 oligomers, formed under conditions that inhibited fibril formation, were neurotoxic.^{91a,145a,202a} Synthetic A β oligomers inhibit long term potentiation of neuronal cells, as discussed in more detail in section 2.3. It is possible to obtain naturally excreted SDS-stable oligomeric forms of A β and extract them from the conditioned medium of 7PA2 Chinese Hamster Ovary cells.^{40,105,146a,146d,189b} The oligomers are produced soon after generation of human A β in intracellular vesicles in CHO cells which express an APP isoform. The intercellular production of oligomers, principally dimers, was inferred from previous studies using primary human neurons.⁴⁰ The secreted oligomers (predominantly dimers and trimers in the conditioned medium, i.e. released from the microsomes) disrupt the LTP of rat hippocampal neurons^{146a,146c,146d} as discussed further in section 2.3. These oligomeric forms of A β were also shown to disrupt the learning behaviour of rats.¹⁰⁴ The soluble oligomers induce tau hyperphosphorylation leading to disruption of the microtubule skeleton and neuritic degeneration.¹⁹⁹

Further evidence in support of the toxic oligomer hypothesis comes from the observation that molecules that stabilize fibrils by accelerating A β fibril formation leads to a loss of inhibition of LTP by A β oligomers.¹⁹¹ An orcein-related small molecule O4 was found to bind to hydrophobic residues in A β and to promote the formation of β -sheet rich fibrils whilst decreasing the concentration of oligomers.¹⁹¹

Anti-A β antibodies isolated from immunoglobulin strongly disrupt fibrillization.²⁰³ Experiments using polyclonal antibodies indicate that they suppress the toxicity of soluble oligomers whereas there is no antibody response to mature fibrils.^{194a} This has

been proposed as a route to vaccination using A β 42 oligomers.²⁰⁴ Experiments on A β , α -synuclein and transthyretin suggest that cytotoxicity shares a common cause not related to the specific sequence.^{39a,39c} Recent *in vivo* studies using a mouse model suggest that specific soluble A β multimeric species are associated with memory loss in Alzheimers, specifically dodecameric 56 kDa species.⁸⁷

Inhibition of γ -secretase can prevent oligomer formation and restore LTP of rat neurons.^{146a} Two γ -secretase inhibitors (flurbiprofen and semagacestat, Fig.13) recently reached, but failed, phase 3 trials. Possible reasons for the failure of these trials that do not necessarily invalidate the A β hypothesis have been discussed.^{3,49} The γ -secretase inhibitor semagacestat developed by Eli Lilly²⁰⁵ also failed phase 3 trials due to low potency and signs of apparent Notch toxicity including gastrointestinal symptoms and skin cancer.^{3,49}

A β oligomers adversely affect synapse function.^{140b,206} This leads to the damage to neuropili believed to underlie AD.²⁰⁶ A β oligomers may inhibit long-term potentiation and facilitate long-term depression,^{147b} depending on the extent of change in the calcium ion concentration, although there is still controversy around this issue. This has been associated with the synaptic removal of AMPA receptors (AMPA receptors).^{140b} Soluble oligomers, whether naturally secreted or prepared from synthetic A β , inhibit hippocampal long-term potentiation,^{146a,207} due to removal of AMPA receptors^{140b} and disruption of neuronal glutamate uptake.^{147b} They also cause a rapid decrease in membrane expression of memory-related receptors such as NMDA and EphB2.²⁰⁶

The importance of “gatekeeper residues” that cap aggregation prone sequences in natural proteins and help to hinder aggregation into fibrils has been highlighted.^{202c}

There is clearly scope for evolutionary pressure to ensure that proteins contain residues that hinder aggregation and/or promote folding into the native state.^{202c,208}

Specific residues that oppose aggregation were analysed using a computer algorithm that analyses sequence aggregation propensity, including those in A β as discussed further in Section 4.5.^{202c}

Several characteristics of the AD phenotype can only be replicated using oligomers, including synaptic loss, hippocampal synaptic plasticity, microgliosis and tau hyperphosphorylation.^{3,49} The presence of oligomers trapped within plaques, points to the dynamic equilibrium that may exist between these species. Oligomers are formed intracellularly in human neurons.⁴⁰ They appear mainly in the form of dimers.⁴⁰ Oligomers influence synaptic plasticity and impair LTP in brain tissue.^{6e,140b,146,188b} The molecular conformation of a highly synaptotoxic A β oligomer structure has recently been elucidated using ssNMR.²⁰⁹ This study revealed that the oligomer formation is controlled by an N-terminal β -strand.

Stable SDS-resistant oligomers have been detected in normal and AD brain.²¹⁰

Oligomers comprising dimers and trimers were detected in tissue extracted from AD brain,^{210b} and AFM revealed that these structures comprise 3-4 nm diameter ellipsoids. A β -Derived Diffusible Ligands (ADDLs) formed by A β 42 have been imaged by liquid state AFM.²¹¹ AFM has also been used to compare the formation of oligomer-like species and protofibrils by A β 40 and A β 42.²¹² AFM has also been used

to image the oligomer-induced formation of membrane pores, as discussed further in section 2.7.

2.6.2 Types of Oligomers

Various types of oligomeric species have been identified, such as protofibrils, paranuclei, globulomers or so-called A β -Derived Diffusible Ligands (ADDLs),^{146d} especially in the early literature which is not reviewed in detail here. Distinctions between these species have been summarized.^{1c,6e,213} ADDLs are larger (4-6 nm diameter) structures than “low n ” (where n is the number of associated monomers) oligomers or globulomers.²¹³ ADDLs are thought to comprise mixtures of monomer and heterogeneous higher n oligomers.^{1c} The observation of these oligomeric structures depends on how synthetic A β is prepared and incubated. More recently, as discussed below, protocols to prepare oligomers from synthetic A β in a controlled and reproducible manner, or in the secreted medium of a rodent cell line (7PA2), have been described.^{146d} The natural A β oligomers are resistant to SDS and insulin-degrading enzyme (IDE) which can only digest monomeric A β .^{6e} All soluble oligomers display a common structure against antibodies raised against them (A11 antibody, section 2.5).^{194a}

Stable *globular oligomers* termed globulomers can be prepared by careful preparation methods starting from A β monomers. Different groups report various protocols to prepare stable oligomers.^{193,214} Using synthetic A β , Kaye and coworkers prepare oligomers by controlled evaporation of HFIP which is used to disperse A β into monomeric form^{194a,215} followed by redispersion in water, or dissolving the peptide in NaOH and diluting this stock solution in a PBS solution containing sodium

azide.^{215b,216} Chromy *et al.* reported a related method to prepare stable oligomers from synthetic peptides.²¹⁴ As mentioned in Section 2.6.1, the 7PA2 CHO cell line expressing mutant V717F APP has been developed to secrete A β in oligomeric form.^{189b,213} The oligomers produced by Chromy *et al.* are neurotoxic²¹⁴ and block LTP.¹⁹³ Electrophoresis in denaturing gels revealed a spectrum of oligomers including dimers, trimers, tetramers, pentamers, and higher order oligomers up to 24-mers.²¹⁷

Globulomers can be prepared by incubation in the presence of SDS or fatty acids.^{193,218} These oligomers appear to be dodecameric species with a mass of 60 kDa. They raise oligomer-specific antibodies 8F5 (cf. Section 2.5).¹⁹³ They could be related to the brain-derived soluble A β *56 dodecamers.^{6e,207} Solution NMR has been used to characterize A β (M1-42) globulomers indicating a mixed parallel and antiparallel configuration²¹⁹ and a dimeric state. To confirm that the globulomers (oligomers) comprise repeats of the dimer, a mutant peptide with L17C, L34C substitution to enable disulfide crosslinking was prepared. This was found to bind to anti-oligomer antibodies with the same affinity as the WT peptide.²¹⁹ The mixed β -sheet configuration is in contrast to fibrils which contain only parallel β -sheets (Section 4.1). A β oligomers can be used to cross-seed tau oligomers.^{215b}

Electrospray mass spectrometry has been used to probe oligomeric states, in particular via analysis of arrival time distributions which can distinguish distinct species with the same charge/mass ratio due to differences in cross-sections of the ions generated.²¹⁷ This technique reveals that unfiltered solutions of A β 42 contain monomers and large oligomers.²¹⁷ Filtration can be used to isolate smaller oligomers – dimers up to dodecamers, the latter being proposed as the species that initiate

fibrillization.²¹⁷ Later, the same ion mobility mass spectrometry method was applied to investigate oligomer formation by A β 42 with a comparative study to A β 40.²²⁰ For A β 42, oligomers up to dodecamers were observed whereas for A β 40 only oligomers up to tetramers were found. The authors proposed different mechanisms of fibril nucleation based on these observations (Fig.12).²²⁰ These distinct aggregation mechanisms were supported by earlier conclusions from experiments using photo-induced cross-linking of unmodified proteins (PICUP)²²¹ to cross-link oligomers which were analysed using a variety of sizing techniques.^{221a} These studies showed that the formation of monomers up to tetramers only are observed for A β 40, whereas pentamer/hexamer paranuclei are formed preferentially by A β 42.

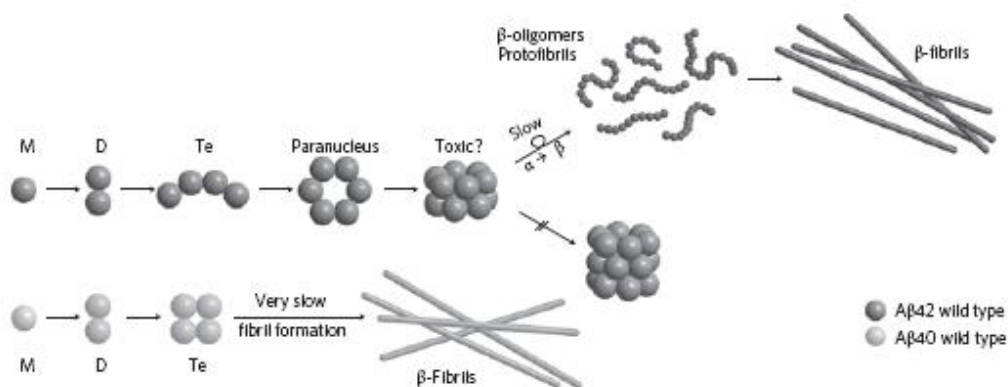


Fig.12. Distinct mechanisms of aggregation of A β 42 and A β 40 proposed by Bernstein *et al.*²²⁰ M denotes monomer, D dimer, Te tetramer and the initial toxic species for A β 42 is proposed to be the dodecamer whilst the planar hexamer serves as a paranucleus. Reprinted by permission from Macmillan Publishers Ltd: *Nature Chemistry* 1, 326, copyright 2009.

Single molecule fluorescence methods indicate that in *vitro* A β 40 forms a heterogeneous distribution of small oligomers (from dimers to 50-mers).²²² The oligomers represent about 1% of the total number of species present, at the concentrations examined (30 nM to 2 μ M). Oligomers have been shown, by SAXS, SDS-page and Western blotting, to bind to APP.²²³ Dimers of A β cause APP to dissociate from the native homodimer conformation into monomers, whereas A β oligomers bind to APP but its homodimer structure is preserved.²²³

2.7 Ion Channel Hypothesis

The mechanism of A β cytotoxicity may be due to its ability to form membrane pores or channels.^{2d,58a,224} This could be due to the exposure of hydrophobic regions in misfolded proteins such as those that form amyloid fibrils.^{224b} Positive charge on a peptide that enables interaction with negatively charged lipid membranes may also be important.^{224b} According to the channel hypothesis, pore formation is responsible for the neurotoxicity of A β . The original work by the group of Arispe *et al.*²²⁵ established that A β is capable of forming membrane channels.

The oligomeric form of A β is implicated in pore formation.^{224g,226} Lambert *et al.* showed that A β 42 oligomers bind to cell membranes and cause cytotoxicity under conditions in which mature fibrils do not form.^{202a} Further support for this is the finding that pore formation is inhibited by Congo red binding,²²⁷ indicating that the A β needs to be aggregated into protofilaments/oligomers for this mechanism to be effective. A β forms pores in lipid membranes that contain multimers of the protein, as

revealed by AFM.^{226a} Evidence for pore formation *in vivo* has also been obtained via TEM on neuronal cell membranes.²²⁸

Uptake of Ca^{2+} across the ion channels leads to neuronal degeneration in a dose- and time- dependent manner and ultimately cell death.^{226a} Changes in the calcium level and the morphology of cultured cells was also found to be sensitive to the aggregation state of $\text{A}\beta_{42}$.²²⁹ $\text{A}\beta$ pore formation leading to an increase in intracellular calcium has been linked to depletion of synaptic vesicles and hence blocked neurotransmission.²³⁰ Transient Ca^{2+} currents are observed near $\text{A}\beta$ plaques in the brain of transgenic mice suggesting the presence of clusters of “hyperactive” neurons.²³¹ A correlation between an increase in calcium ion production and a decrease in $\text{A}\beta$ production has also been noted in studies on SERCA (sarco ER Ca^{2+} ATPase) which is a calcium channel-forming protein in the endoplasmic reticulum (ER) membrane.²³² Down-regulation of SERCA leads to increased Ca^{2+} and reduced $\text{A}\beta$ levels and over-expression leads to increased $\text{A}\beta$ production. SERCA activity was also shown to be decreased in fibroblasts lacking the *PS1* and *PS2* presenilin genes.²³²

2.8 Interaction of $\text{A}\beta$ with tau

The tau protein is involved in microtubule assembly and stabilization within the cytoskeleton (in particular in F-actin fibrils). Mutations can lead to filamentous deposits which have been observed for several neurodegenerative diseases such as Pick’s disease, Parkinsonism-dementia complex of Guam etc.^{2a} Filamentous tau deposits are invariably present even in the absence of $\text{A}\beta$ deposits and it is not clear in the context of fibril deposition precisely how $\text{A}\beta$ and tau interact although there seems to be a synergistic effect which enhances actin bundling and

neurodegeneration.^{2a} Neuronal degeneration induced by tau has been studied *in vivo*, although this is outside the scope of the current review. However, tau can influence A β -induced neuronal dysfunction, as exemplified by a study using transgenic mice that express APP along with tau.^{4a} Synergistic interactions between A β , tau and α -synuclein can accelerate neuropathology and cognitive decline, as indicated by a study using transgenic mice.²³³ These authors note that the aggregation of α -synuclein into Lewy bodies is a pathology associated with up to 50% of AD cases.

A correlation between an increase in CSF tau and ptau-181 and the amount of cortical amyloid has been reported via brain imaging studies using the PIB biomarker (Section 2.4).²³⁴ There is an inverse relationship involving cortical PIB binding, i.e. A β 42 deposition is inversely related to CSF A β 42 levels, but this is not true for plasma species.^{183c,234}

3. THERAPEUTIC TREATMENTS

3.1 Existing treatments

The only drugs currently available do not cure AD but may delay the development of symptoms. The current standard of care for mild to moderate AD includes treatment with acetylcholinesterase inhibitors to improve cognitive function and memantine, an NMDA antagonist. The acetylcholinesterase inhibitors include galantamine, an alkaloid available commercially as Reminyl (Shire), Razadyne (Janssen), rivastigmine (Exelon, Novartis) and donepezil (Aricept, Pfizer).^{8,31,235} The patent on the latter drug expired in 2010,²³⁶ however it has recently been shown that it may also be effective in the treatment of moderate-to-severe AD as well as mild-to-moderate symptoms.²³⁷ The cholinesterase inhibitor tacrine is rarely used due to poor oral bioavailability and

several potential adverse drug reactions.³¹ The NMDA receptor antagonist memantine developed by Eli Lilly is also available under trade names including Ebixa (Lundbeck) and Namenda (Forest).^{8,31} The development of the market for these drugs (sales forecasts) has been assessed – sales were \$4 billion in 2005.⁸

Although developed as an acetylcholinesterase inhibitor, galantamine also acts to inhibit A β aggregation.²³⁸ It is also known as an allosteric modulator of nicotinic receptors.²³⁹ Memantine is thought to function therapeutically as an open-channel blocker of NMDA receptors,²⁴⁰ and also attenuates the ADDL-induced increase in intraneuronal calcium.²⁴¹

3.2 Inhibitors of Fibrillization/Oligomerization

3.2.1 Small Molecules

Since there have been a very large number of papers on small molecule inhibitors of A β fibrillization (also γ -secretase inhibitors) we are not able to review all of them. Reviews on this topic are also available.²⁴² Here, we focus on key classes of compounds and individual compounds that have attracted particular interest, for example moving to advanced stage clinical trials. Other reviews cover many more of the compounds researched to date. The main focus in the following is on A β fibrillization inhibitors, as this has been the main focus of small molecule inhibitor approaches.^{189c,216,242a,242b,242d} However, some compounds have been developed to inhibit β -secretase or γ -secretase.^{242a}

A large number of small molecules have been studied for their ability to influence A β aggregation and toxicity.^{216,242} Possibly the most high profile work has been on

tramiprosate (Alzhemed, 3-amino-1-propanesulfonic acid, Fig.13) which reached phase III trials, which however were not successful.^{3,49} This compound is a glycosaminoglycan (GAG) mimetic (*vide infra*) shown to bind to soluble A β 40 and A β 42 and to maintain them in a non-fibrillar form.^{242d,243} It also decreases A β 42-induced neurotoxicity, is able to cross the blood-brain barrier (BBB) and, using a TgCRND8 mouse model, can reduce amyloid plaque and cerebral levels of A β 40 and A β 42.^{243a,244}

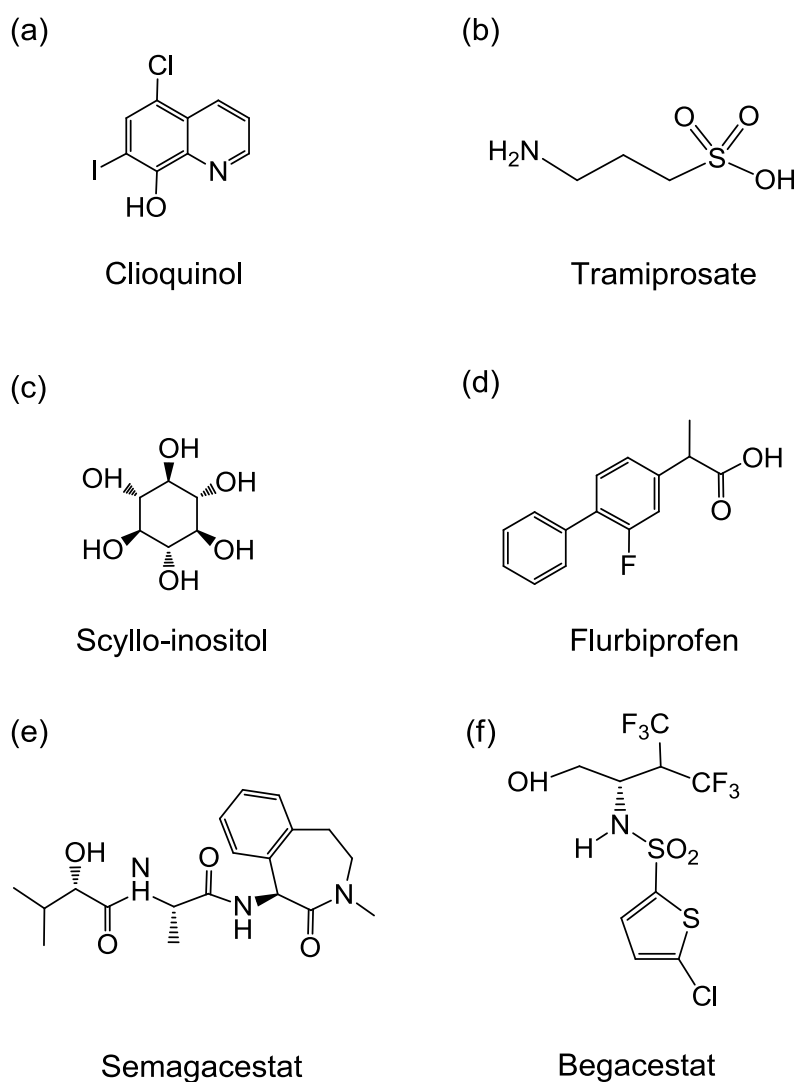


Fig.13. Therapeutic compounds for AD.

A number of dye compounds have been investigated in terms of their effect on A β aggregation and neurotoxicity.¹⁹⁶ Congo red (CR), an amyloid-staining dye, has been the subject of several studies in this regard.²⁴⁵ CR is found to inhibit fibril formation and neurotoxicity towards rat hippocampal cells.^{245b} NMR and light scattering suggest that it binds to A β 40 monomers^{245e} and AFM indicates that this ultimately leads to a distinct aggregation pathway.^{245f} However, CR is potentially toxic due to the metabolic release of benzidine, and has poor BBB permeability.^{245d} CR has been found by Podlisny *et al.* to inhibit the oligomerisation of A β 40 using the A β expressed by 7PA2 cells in conditioned medium (see Section 2.6.1).^{105,189b} In contrast, Knowles and Dobson *et al.* found CR has no effect on A β fibrillization.²⁴⁶ It has been suggested that CR binding arises from a specific conformation of the sulfonate groups in the compound which bind A β via electrostatic interactions.^{242d} Other sulfonated dyes investigated as A β aggregation inhibitors include chrysamine G and thioflavin S.^{242d} Methylene blue inhibits A β oligomerization by stimulating fibrillization.²⁴⁷ It has low toxicity and is able to cross the BBB. Wong and coworkers have studied a series of Brilliant blue derivatives, which are food dyes with blood-brain barrier permeability properties.^{189c} Reduction in A β -induced cytotoxicity due to the formation of off-pathway non-toxic aggregates was noted for some derivatives.

Necula and coworkers investigated a large series of compounds including many dye molecules and classified them according to whether they selectively inhibit the fibrillization or oligomerization of A β 42, or both.²¹⁶ The existence of these different classes of inhibitors might suggest that the pathways of A β oligomerization and fibrillization are independent. However, as discussed in section 2.6, whether

oligomers are on- or off-pathway intermediates is still an open question.²⁴⁸ In a similar analysis of a smaller number of aromatic compounds, including dyes and polyphenols, Ladiwala *et al.*²⁴⁹ classified the molecules according to whether they (i) remodel soluble oligomers into large non-toxic off-pathway aggregates (some also remodel fibrils), (ii) convert soluble A β oligomers into fibrils but are inactive against fibrillar A β or (iii) disaggregate soluble oligomers or fibrils into non-toxic low-molecular weight species (Fig.14).

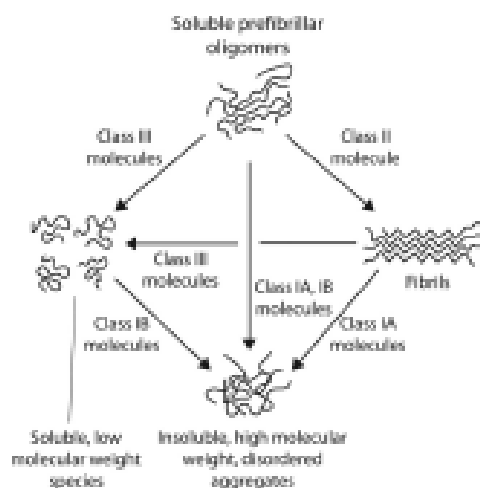


Fig.14. Pathways of aggregation for different classes of small molecule inhibitors of A β oligomerization/fibrillization.²⁴⁹ This research was originally published in Ladiwala, A.R.A. *et al.*, *Journal of Biological Chemistry* **2011**, 286, 3209. © The American Society for Biochemistry and Molecular Biology.

Polyphenols are also able to influence A β aggregation. A review provides details of the many compounds investigated in this context²⁵⁰ and another review describes the associated mechanisms of action with a focus on the antioxidant role of these compounds.²⁵¹ Polyphenols that have attracted particular attention such as tannic acid and epigallocatechin gallate (EGCG) have been shown to reduce A β cytotoxicity in

cell-based assays,^{242a,242d,249,252} and can disassemble mature A β 42 fibrils^{252b} although these compounds are not able to cross the BBB.^{189c} Catechins and related polyphenols have also been shown to inhibit A β fibrillization, as assayed using ThT fluorescence.^{242a,253} It has been noted that competitive binding of the analyte and ThT means that these results need to be treated with caution.²⁵⁴ Other biologically derived polyphenols including NDGA (nordihydroguaiaretic acid), curcumin and rosmarinic acid have also been examined as A β aggregation inhibitors, or in terms of disaggregation of pre-formed fibrils.^{242a,242d} Research has developed to the stage of *in vivo* studies using Tg2576 mice, with a focus on the pathway of A β aggregation, i.e. via oligomers or otherwise.²⁵⁵ Curcumin can cross the BBB and has been shown to reduce plaque burden using a Tg2576 mouse model.²⁵⁶ Resveratrol, a polyphenol with antioxidant properties found in wine has been shown to inhibit A β 42 fibril formation and to reduce cytotoxicity.²⁵⁷

Gazit and coworkers have screened a series of small molecule inhibitors of A β aggregation that contain aromatic recognition elements as well as β -breaker units.²⁵⁸ A lead compound NH₂-D-Trp-Aib-OH was identified. This compound is able to inhibit the formation of toxic oligomers and *in vivo* studies using a model mouse (expressing human APP Swedish and London mutations) also indicated improved cognitive function.²⁵⁸

Metal ion chelators, in particular of Cu²⁺ and Zn²⁺, have also been shown to inhibit or reverse aggregation of A β *in vitro*.^{242a,242d,259} Cherny *et al.* indicate that to efficiently extract A β from brain tissue using metal ion chelators including EGTA and ethylene diamine, Ca²⁺ or Mg²⁺ are also required.^{259a} Metal ion chelators can be specific to

particular ions, for example clioquinol (Fig.13a), an anti-malarial quinone, selectively binds Zn^{2+} and Cu^{2+} with greater affinity than Ca^{2+} and Mg^{2+} and reduces brain A β deposition in an Tg2576 mouse model.²⁶⁰ Studying A β 40, Raman *et al.* found that Zn^{2+} and Cu^{2+} but not Fe^{3+} reduce fibril formation, however pre-formed fibrils are stable in the presence of these metal ions. Clioquinol-induced resumption of fibril growth suppressed by Cu^{2+} but not Zn^{2+} , points both to a chelation effect but also a synergistic effect of a Zn^{2+} -clioquinol complex on A β 40 fibrillization.^{259b} A successor to clioquinol is the 8-hydroxyquinoline analogue PBT2 which reached phase II clinical trials, and shows ability to reduced CSF A β 42 levels as well as cognitive performance.²⁶¹ This compound is believed to perform as a superior ionophore than clioquinol, i.e. to more effectively promote the transport of copper and zinc ions across cell membranes.^{261b} It is also designed to be easier to synthesize, more soluble and to have increased BBB permeability.^{261b} The role of metal ions in the inhibition of A β fibrillization or promotion is discussed further in Section 4.8. Inspired by clioquinol, bifunctional compounds that can interact with both metal ions and A β have been developed based on pyridine/stilbene derivatives²⁶² and related pyridinones.²⁶³ These compounds can disaggregate A β 40. A responsive copper chelator that is released when a pro-chelator is cleaved by β -secretase has been developed and shown to inhibit Cu^{2+} -induced A β aggregation.²⁶⁴ Similarly, a pro-drug compound comprising a glycosylated (glucose-receptor targeting) metal ion chelator (hydroxypridinone) is able to cross the BBB with enzymatic loss of the glucose unit.²⁶⁵ The compound has an antioxidant property.

Compounds that can block A β -induced channel formation in lipid membranes including tromethamine have been investigated via conductance

experiments.^{225a,225b,266} Zinc can also block such channels.^{229,266b} Arispe *et al.* also developed a peptide-based channel blocker, designed specifically to modulate late A β effects on caspase activation and apoptosis.^{266b}

Glycosaminoglycans or proteoglycans have been associated with AD since sulfated GAGs such as heparan or chondroitin sulfate are present in neuritic plaques, neurofibrillary tangles and vascular amyloid deposits.^{242d,267} Binding of some sulfated GAGs can prevent the proteolytic degradation of fibrillar A β . Heparan or heparan sulfate can accelerate the fibrillization of A β *in vitro*,^{267b} probably due to electrostatic binding to a specific domain in the A β (11-28) region.^{245a} Chondroitin sulfate also promotes the aggregation of A β 42 into stable fibrils of reduced toxicity.²⁶⁸ These studies suggested that inhibitors of this interaction might prove useful as therapeutic agents.^{242a} In a related manner, sulfated compounds such as heparin and dextran sulfate can inhibit the interaction of heparan sulfate with A β .²⁶⁹ Some sulfated compounds inhibit A β aggregation. Synthetic glycopolymers bearing sulfated saccharide units are also able to suppress the fibrillization of A β 42, A β 40 and A β (25-35).²⁷⁰ Simple sugars can either accelerate or inhibit fibrillization.²⁷¹ As mentioned above, the GAG-mimetic tramiprosate (Fig.13b) reached phase III clinical trials but was not successful despite reducing CSF A β 42 levels in mild-to-moderate AD patients.^{243b} Reviews that discuss the effect of GAGs on A β aggregation are available^{267b,267c,272} (the former considers also proteins and lipids). The location and distribution of sulfate groups on the GAG chains may define the interaction with A β .^{267c,273} Fibril formation of A β 42 is actually promoted in the presence of GAGs with a suitable spacing of sulfate units, although the cationic polysaccharide chitosan can inhibit such aggregation.²⁷³ Fraser and coworkers showed that in the presence of

low concentrations of sulfate ions, A β (11-28), A β (13-28), and A β (11-25), but not A β (15-28) undergo extensive lateral aggregation into “macrofibers”.^{245a} Ariga *et al.* point to the key role of the A β (13-16) HHQK region in the binding process, especially the histidine residues.²⁷⁴ They also note that low molecular-weight heparins can inhibit fibril formation.

As discussed in the Introduction, oxidative stress plays an important role in AD.¹⁷ The role of antioxidants as novel neuroprotective agents has been reviewed, including polyphenols and other nutraceuticals such as flavonoids and turmeric.^{235,251} The effects of a series of flavonoids, catechins and related compounds on A β 42 fibrillization has been compared and cytotoxicity studies were performed on fibril inhibitors (some fibril promoters were also identified) leading to the identification of promising lead compounds.²⁷⁵ The structural features of derivatives of the flavonoid fisetin involved in A β 42 fibril inhibition have been probed.²⁷⁴ Several studies have suggested that antioxidant vitamins may reduce neurotoxicity, due to their role in alleviating oxidative stress.^{242a}

Lipid-based small molecule inhibitors have also been developed, since the presence of lipid membranes may accelerate A β fibrillization,^{267c} since A β deposition is initiated in a plasma membrane-bound form (Section 4.7). A particular focus has been on the interaction of A β with phosphatidylinositol since this causes a dramatic increase in fibril growth. This can be inhibited using headgroups from other members of the phosphatidylinositol family.²⁷⁶ This led to the identification of *scyllo*-inositol (cyclohexanehexol, Fig.13c) as lead compound.²⁷⁷ This compound stabilizes an oligomeric form of A β .²⁷⁷ Administration to TgCRND8 mice prevented A β plaque formation,

synaptic toxicity and cognitive deficits.^{84b} These effects were seen both in prophylactic and treatment studies.²⁷⁸ The compound has high availability due to the presence of the inositol transporters at the BBB. This compound has reached phase II trials (as AZD-103, Transition Therapeutics).²⁷⁹

The action of other compounds on A β aggregation including nicotine, melatonin, rifampicin and tetracyclines has been reviewed.^{242a}

Inhibition of A β aggregation has been targeted via use of self-recognition elements (SREs). These are molecules based on fragments of the A β peptide, which are capable of binding to the corresponding sequence in the native peptide, but are modified so as to disrupt β -sheet fibrillization.^{242d} Findeis *et al.* proved that compounds based on a core sequence of the A β peptide implicated in fibrillization, A β (16-20) (KLVFF), showed promise as SREs.²⁸⁰ Murphy and co-workers have investigated the effect on A β aggregation of compounds based on KLVFF extended at the C-terminus by cationic or anionic residues to give, for example, KLVFFKKKKKK or KLVFFEEEE.²⁸¹ Molecules containing three or more lysines in the extension were found to be most effective.²⁸¹⁻²⁸² Modified versions of these compounds have been used to examine the role of surface tension on the kinetics of aggregation of A β 40.²⁸³ The compounds contained modifications including charge, branching, D/L-isomer substitution and counterion type (motivated by the Hofmeister series) that influence the stabilisation of the protein structure. Gordon and co-workers studied the *N*-methylated compound A β (16-20)m and showed that it inhibits fibrillization of A β (1-40).²⁸⁴ *N*-methylation of alternate residues disrupts β -sheet self-assembly due to the presentation on one face of the β -strand of residues incapable of adopting the usual

hydrogen bonding pattern.²⁸⁴ Doig and co-workers have followed this up and screened a number of *N*-methylated “meptides” based originally on the same sequence, but with all D-amino acids and with modifications of the termini and incorporation of branched residues in certain positions, that are promising as SREs towards targeted aggregation inhibitors, and which also contain elements to improve their pharmacokinetics.^{242c,285} A lead compound based on a pentapeptide framework has been shown to have favourable cytotoxicity, and to reverse the inhibition of LTP by A β 40.^{242c} Doig *et al.* have also reviewed other promising compounds.^{242b,242c} Austen *et al.* have developed compounds based on KLVFF but with terminal modifications to aid solubility and showed that these were effective inhibitors of toxicity using human neuroblastoma cells.²⁸⁶ The binding of β -alanine and GABA-modified peptide fragments to amyloid fibrils formed by α -synuclein, A β 40 and amylin has recently been examined.²⁸⁷ The binding sequence was based on self-recognition element V⁷⁷AQKTV⁸² of the full length α -synuclein peptide and shorter sequences therefrom. Pentapeptides containing gamma-amino acids and the KLVFF SRE (or its D-amino acid variant) have also been examined, and a promising compound able to reduce A β toxicity, due to inhibition of fibrillization, was identified.²⁸⁸ Watanabe and coworkers have shown by a binding assay using immobilized KLVFF in the presence of fluorescently-labelled KLVFF in solution that the KLVFF motif is a self-recognition element.²⁸⁹ Incorporation of the retro-inverse peptide ffvlk into cross-linked PEG networks (as a monomer, dimer or tetramer, all linked to PEG via a cysteine residue) produces gels that can bind A β 42.²⁹⁰ It was proposed that these gels could serve as sinks or “detoxification depots” to capture A β .

Ligands that stabilize the A β (13-26) domain in an α -helical conformation (as revealed by NMR, section 4.1) have been studied as a means to reduce aggregation of A β 40 and A β 42, and also cytotoxicity.^{109e} The inhibitors were based on self-recognition modelling of the A β (13-23) region and comprise two peptidomimics and *N*-decanoyl-diethylenetriamine. Studies using a *Drosophila melanogaster* model (expressing human A β 42) also suggested reduced neurodegeneration in the presence of the molecules.^{109e} Schrader and coworkers have explored the use of functionalized aminopyrazole derivatives in binding to A β 42.²⁹¹ Ligands bearing multiple lysine residues were found to interact with the ladder of stacked E22 residues and to completely dissolve pre-existing fibrils.^{291b} These ligands interact through electrostatic and hydrophobic interactions with the KLVFF sequence although lipophilic groups on some of the designed ligands can interact with the nonpolar residues between I31 and V36.²⁹¹ This group have also developed lysine-specific molecular tweezers (Fig.15) with aromatic pincers able to inhibit the aggregation and toxicity of A β 40 and A β 42.²⁹² Mihara *et al.* have shown that even dipeptides LF and CF can form mixed fibrils with small amounts of A β 42, hence “capturing” the peptide.²⁹³

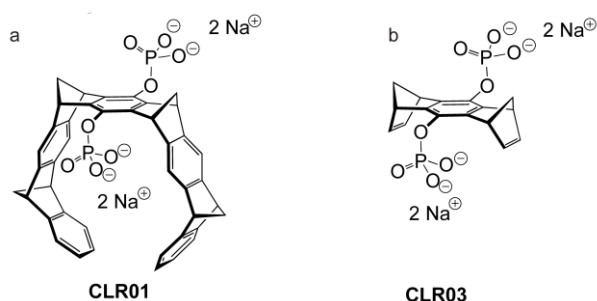


Fig.15. Lysine-specific molecular tweezers CLR01 and CLR03. Reprinted with permission from ref.²⁹². Copyright 2011 American Chemical Society.

Statins reduce cholesterol levels in blood and this in turn correlates to A β production and aggregation as discussed in Section 4.7. Reduction in cholesterol using lovastatin and methyl- β -cyclodextrin inhibits the production of A β in hippocampal neurons without altering APP production.²⁹⁴

In a novel approach related to the “toxic oligomer” hypothesis (Section 2.6.1), the action of selective fibrillization accelerators (with an oligomer-reduction activity) has been probed.¹⁹¹ Compounds related to the dye orcein are shown to accelerate A β 42 fibril growth, while reducing oligomer formation.

3.2.2 Proteins and Particles

Chaperone proteins have been shown to bind to A β and to inhibit fibrillization/oligomerisation. Several proteins that interact with intracellular A β *in vivo* have been identified based on mass spectrometry analysis of human A β expressed in *C. elegans*, including the small heat-shock protein α B-crystallin and related proteins such as members of the HSP70 family.²⁹⁵ α B-crystallin is a chaperone for A β , binding to it, and preventing fibril growth.²⁹⁶ It prevents the spontaneous fibrillization of A β 42²⁹⁶ and the A β 42-seeded growth of A β 40.^{296a} The extracellular chaperone protein clusterin has been shown to sequester oligomers of A β 40 during aggregation and disaggregation.¹⁰¹ As discussed above, clusterin (apolipoprotein J) is an identified risk factor in Alzheimer’s disease.^{54,64} A large-scale screening study has recently identified small molecule proteostasis regulators that induce expression of the chaperone heat shock transcription factor-1 (HSF-1).²⁹⁷ This may form the basis to

treat a number of protein conformational diseases including AD. The inhibition of fibrillization in the presence of clusterin (Section 2.1.1) has also been examined.²⁹⁸

Other proteins and peptides have been shown to bind A β and to influence its aggregation. The secretory protein gelsolin²⁹⁹ and the ganglioside GM1³⁰⁰ are able to bind A β , and it has been proposed that peripheral treatment with these materials can be used to reduce A β levels in brain (mouse model).³⁰¹ Transthyretin, itself an amyloidogenic protein, and variants, can inhibit the aggregation of A β *in vitro* and *in vivo*.³⁰² The cellular prion protein PrP^C is also able to bind A β oligomers at nanomolar affinity, indicating that it may act as a receptor and that it is involved in A β -induced synaptic dysfunction in the mouse brain.³⁰³ However, these findings have not been replicated by two other groups who found that mice suffered memory deficits even in the absence of prion protein, carefully excluding PrP^C.^{146e,146f} Very recent work suggests that cellular PrP^C is essential for oligomeric A β -induced cell death, since PrP^C antibody blocks A β oligomer-induced neurotoxicity and mice expressing PrP^C are resistant to A β toxicity.³⁰⁴ It has been proposed that PrP^C may have a physiological role in modulating NMDAR activity, mediated by copper ions, which is disrupted in the presence of A β 42 (section 4.8).³⁰⁵

Polymeric nanoparticles (uncharged acrylamide-based copolymers) inhibit the fibrillization of A β 40, an observation ascribed to the binding of A β (in monomeric or oligomeric form) to the nanoparticles.¹¹⁴ The binding mainly affects nucleation, and the lag time was found to be strongly influenced by the copolymer composition. The binding is due to a combination of hydrophobicity (controlled via copolymer composition) and hydrogen bonding between polar groups on the polymer and in

A β .¹¹⁴ In the case of cationically (amide) functionalized polystyrene nanoparticles, inhibition of fibrillization is observed for high particle surface area, whereas fibrillization is accelerated for low particle surface areas due to reduction of the lag phase.³⁰⁶

Inorganic nanoparticles can function as A β fibrillization inhibitors,³⁰⁷ although this was demonstrated with cytotoxic CdTe nanoparticles. The precise mechanism is unclear. Polyoxometalates which comprise inorganic early transition metal clusters also inhibit the aggregation of A β .³⁰⁸ Surprisingly, organic nanoparticles based on porous silica have been shown to penetrate the brains of fruit flies (*D. melanogaster*), without exhibiting neurotoxic effects and potentially enabling delivery across the BBB.³⁰⁹ BBB permeability can be modeled using the parallel artificial membrane permeability assay, which measures passive diffusion of small molecule through an artificial lipid membrane.^{262,310}

One example of a study using dendrimer molecules employed maltose-functionalized dendrimers to influence the fibrillization of A β 40.³¹¹ A smaller dendrimer led to fibrillar clumps, sequestering the A β and reducing toxicity, whereas a larger one produced amorphous aggregates, toxic to cells.

3.3 Inhibitors of the secretase enzymes

It has proved difficult to identify small molecule inhibitors of β -secretase (BACE1) with favorable pharmacokinetic characteristics.^{242a} The crystal structure of β -secretase complexed to an 8-residue peptide inhibitor EVNLADEF has been reported – the enzyme has the usual binding cleft of an aspartyl protease.³¹² A Japanese group have

developed several peptide-based compounds based on phenylnorstatine.³¹³ A lead compound KMI-429^{313b} was explored further *in vivo* using a mouse model.³¹⁴ Since the enzyme is membrane bound, an inhibitor has recently been developed by linking a peptide β -secretase inhibitor to a sterol moiety designed to insert in the membrane.³¹⁵ Statins may be used to inhibit cholesterol biosynthesis and the expression of BACE and ultimately A β production.³¹⁶ Statins can inhibit the dimerization of BACE by inhibiting lipidation.³¹⁷ This may provide a therapeutic target to reduce A β production. The role of protein-protein interactions in the correct assembly of BACE, which is required for A β production has been discussed.³¹⁷ BACE associates with APP in cholesterol-rich rafts.³¹⁸ Knocking out the β -secretase gene *BACE1* causes no adverse phenotype in mice,³¹⁹ indicating that β -secretase inhibitors may still provide an attractive target for treatments.

A greater number of compounds have been developed as potential inhibitors of γ -secretase. A complicating factor here is that NOTCH1 and other ligands are also γ -secretase substrates (as well as APP)^{242a} and therefore Notch-related side effects may be problematic.³²⁰ However, certain non-steroidal anti-inflammatory drugs (NSAIDs) can modulate γ -secretase cleavage without blocking Notch cleavage.³²¹ Some NSAIDs can reduce A β levels independent of cyclooxygenase (COX enzyme, associated with inflammation) activity.^{321a-c} Application of NSAIDs including ibuprofen, flurbiprofen and indomethacin inhibits the release of amyloidogenic A β 42 from cultured cells.^{321a} Flurbiprofen (Fig.13d, in the *R* enantiomeric form, shown to be particularly effective³²²) reached phase III clinical trials, but these were unsuccessful. The target of γ -secretase modulators, such as flurbiprofen and related

compounds, has been located to A β (28-36) based on investigation of the localization of fluorescently labeled and biotinylated variants of these NSAIDs.^{321e} Some γ -secretase modulators were shown to alter the production of cell-derived A β oligomers^{321e} while compounds that interact with this region of A β act as γ -secretase inhibitors, highlighting the interplay between γ -secretase-influenced A β production and A β aggregation.^{321e} Furthermore, since some NSAIDs bind to to an APP substrate rather than γ -secretase, Notch toxicity may be avoided in this way.^{321e}

The γ -secretase inhibitor Semagacestat (Fig.13e) was shown to reduce formation of A β in cell assays and also *in vivo* studies.^{205,323} Lanz *et al.* show that this compound leads to lowering of A β in plasma (in guinea pigs) at low doses, but an elevation of A β levels at low concentrations.³²³ However, Semagacestat was not successful (due to inferior performance relative to placebo) in phase III clinical trials.

Other strategies have been employed to avoid side effects from Notch impairment.

Other than NSAIDs, γ -secretase inhibitors that block A β production without influencing Notch activity such as the thiophene sulfonamide Begacestat (Fig.13f) have reached clinical trials, although the mechanisms are unclear.³²⁴ Serneels *et al.* have targeted specific components of the γ -secretase complex (Section 2.2.3), specifically produced by two *APH1* (*APH1A* and *APH1B*) genes.³²⁵ Inactivation of the *APH1B* complex decreases A β plaque deposition and improves behavioural deficits. The different *APH1* complexes also produce A β of different length.³²⁵ As an alternative to targeting γ -secretase, the reduction of γ -secretase-activating protein (GSAP) has been shown to decrease A β production in cells and *in vivo* using a mouse

model.³²⁶ The anticancer drug imatinib (Gleevec, Novartis) is able to inhibit A β production without affecting Notch cleavage since it prevents interaction of GSAP with the γ -secretase substrate. Thus, GSAP is a potential selective therapeutic target avoiding side effects associated with γ -secretase inhibition.³²⁶ Phiel *et al.* showed that therapeutically relevant doses of lithium chloride, a GSK-3 inhibitor, block the production of A β peptides by interfering with APP cleavage at the γ -secretase step, but do not inhibit Notch processing.⁴⁶

3.4. Immunization

In the development of A β -based immunotherapies, several strategies have been pursued including passive immunization with monoclonal anti-A β antibodies, active immunization with synthetic A β 42 and active immunization with modified A β fragments (Fig.16).³²⁷ Progress with active immunization has been dogged by the failure of phase IIa clinical trials due to the development of meningoencephalitis in several patients (*vide infra*).

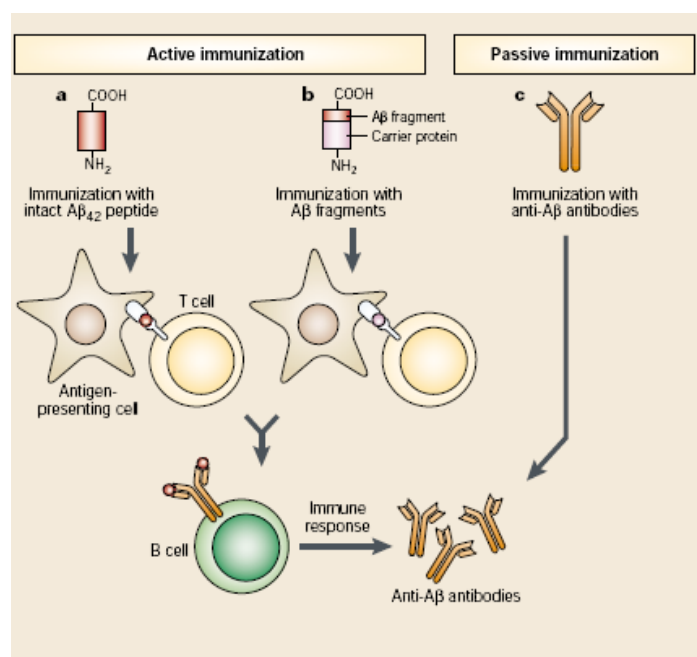


Fig.16. Antibody strategies.^{327a} Three different approaches are illustrated. The first (panel **a**) involves immunization with full-length A β 42. After injection, the peptide is taken up by antigen-presenting cells, and fragments of the peptide are presented to T cells. Subsequently, various B cells that recognize epitopes on A β 42 are engaged and proliferate. These eventually produce anti-A β antibodies. The second active immunization method (panel **b**) involves administration of small fragments of A β conjugated to an unrelated carrier protein. This approach is similar to the first with the exception that the T cells are stimulated by the carrier protein rather than the A β fragment (which lacks T cell epitopes). This approach yields a strong antibody response to part of the A β peptide. The third strategy (panel **c**) is to administer anti-A β antibodies directly. This does not require any immunological response from the host and might be useful in individuals in which an immune response is not otherwise produced. Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Neuroscience* 3, 824, copyright 2002.

Active immunization of transgenic mice with fibrillar A β 42 leads to a reduction in A β deposits in the brain (mouse model) and prevents development of plaque pathology when administered before its formation.^{83c,328} The A β antibodies generated were reported to reduced A β plaque deposition without reducing overall A β levels.^{84a,329} An improvement in the cognitive performance of mice was also noted.^{84a,329} Phase IIa trials pursued by Elan pharmaceuticals, based on this approach (using aggregated A β 42 termed AN1792 with an immunogenic adjuvant QS-21) were halted when 6% of patients developed meningoencephalitis.^{327a,328,330} However, after one year patients producing antibodies that targeted plaques had a lower rate of cognitive decline.³²⁸ In

further follow-up studies it was found that there was a reduction in CSF tau levels following A β 42 immunization.³³¹ and a decrease in brain volume.³³² Although clearance of A β plaques occurred, this did not prevent neurodegeneration.³³³ Using a triply transgenic mouse (3xTg) model it has been shown that A β immunotherapy can not only reduce A β plaques, but also early hyperphosphorylated tau pathology.³³⁴ Vaccination with soluble oligomers of A β 42 has also been proposed as a method to produce toxicity-reducing antibodies.²⁰⁴ Intraperitoneal injection of A β -laden brain extracts into the brains of transgenic mice leads to the deposition of amyloid plaques after prolonged incubation time.³³⁵ These observations point to the possible prion-like behaviour of A β and the need for great caution (at the very least) in the development of immunization using A β directly.

Sigurdsson *et al.*³³⁶ investigated immunization using the modified peptide K₆A β (1-30)-NH₂, with an N-terminal hexa-lysine extension to A β (1-30), the oligo(lysine) enhancing immunogenicity, this extension following work by Palitto *et al.*²⁸² (section 3.2.1). Using a transgenic APP mouse model, this peptide was found to reduce the burden of hippocampal and cortical A β , and more particularly the amount of soluble A β 42 in brain tissue.³³⁶

Passive immunization using antibodies prevents amyloid formation by brain extract from AD patients or APP23 transgenic mice (which over-express A β 40 which deposits in diffuse and filamentous form), pointing to the suppression of seeding activity.⁸⁹ It has been suggested that A β disrupts the integrity of the BBB and that this is restored after immunization as the immune system clears A β from the brain.³³⁷ The

ability of A β 40 to cross the BBB and increase its permeability had been observed earlier, using a cell culture model.³³⁸

Passive immunization using the humanized monoclonal antibody bapineuzumab against A β got as far as phase II clinical trials.³³⁹ However, no significant benefit was observed in cognitive performance tests, and in addition some patients exhibited vasogenic edema.^{339a} However, this compound continues to be investigated in ongoing phase III trials.^{14b,279a,340} as is the related compound solaneuzumab.^{14b,279a,341}

Passive immunization using monoclonal antibodies (mAbs) has been investigated by Solomon *et al.*^{190b,342} They found via *in vitro* studies that selected mAbs prevent the aggregation of A β , antibodies recognizing epitopes within A β (1-28) and A β (8-17).³⁴²

Antibodies raised against A β (1-28) can disaggregate A β fibrils *in vitro* and reduce the neurotoxic effects of A β *in vivo* (MTT cytotoxicity assays using PC12 cells).^{190b}

Antibodies selectively directed against residues 4–10 of A β 42, inhibit both A β fibrillogenesis and cytotoxicity without eliciting an inflammatory response, in addition they can disaggregate preformed A β 42 fibrils.¹⁹⁵ De Mattos *et al.* showed that an antibody (m266, Table 3) against A β 40 is able to bind and completely sequester plasma A β .^{188a} Peripheral administration leads to a large and rapid increase in plasma A β due to a change in A β equilibrium in plasma and in CNS. The m266 antibody does not bind to A β deposits in the brain.^{188a} However, using mouse anti-A β IgG₁ antibodies (recognizing A β (1-16)) some clearance of compact amyloid deposits is observed after several days, along with microglial activation.⁸⁶ The increase in plasma A β levels was correlated to A β load in the hippocampus and cortex (of PDAPP mice) after immunization.¹⁰³ Administration of m266 to PDAPP can improve

cognitive learning, despite no alteration in brain A β burden.³⁴³ A complex of m266 and A β was found in the plasma and CSF of treated mice. Bard *et al.*³⁴⁴ noted the presence of anti-A β antibodies in the central nervous system after peripheral administration, which were able to bind A β plaques and to reduce the pre-existing A β burden. The mechanisms of clearance of A β *in vivo* by immunotherapy have been investigated, this showed that direct disruption of plaques as well as Fc-dependent phagocytosis was involved.³⁴⁵

Immunization using antibodies to human serum amyloid P component leads to the elimination of visceral amyloid deposits, in mice.³⁴⁶ The plasma glycoprotein human serum amyloid P (SAP) component non-selectively binds all kinds of amyloid deposits.³⁴⁷ Clinically, it is possible to reduce circulating human SAP using a known SAP-depleting compound, thereby stimulating antibody production. Inhibitors of SAP binding to A β fibrils have also been developed, and the lead compound (CPHPC) also reduced levels of circulating human SAP.³⁴⁶⁻³⁴⁸ This technology is proceeding to human clinical trials.³⁴⁹

3.5 Other Approaches

Other treatment strategies are being pursued, these are discussed elsewhere^{8,12a,31} and are not considered further here as they do not involve A β . Summaries of compounds in phase I, II and III clinical trials are available.^{14,235,279}

Due to a correlation between insulin resistance (section 2.1) and AD, the peroxisome proliferator-activated receptor- γ (PPAR- γ) agonist rosiglitazone has been investigated,⁵¹ and has reached phase III trials.³¹ This compound is an insulin sensitizer and

mitochondrial activator, and it activates the PPAR- γ pathway. This increases dendritic spin density and rescues spine loss caused by APOE- $\epsilon 4$.⁵¹ Nerve Growth Factor (NGF) mimics have also reached phase III trials,³¹ however since this does not involve A β it is outside the scope of the present review. An antihistamine drug Dimebon³⁵⁰ reached phase III clinical trials for AD which failed due to the absence of a significant effect.³⁵¹ The clearance of A β is facilitated by ApoE (section 2.1.1). This process is impaired in AD. The compound bexarotene that influences ApoE expression has been shown to enhance clearance of soluble A β in an ApoE-dependent manner, also improving cognitive performance in mice.³⁵²

4. BIOPHYSICAL CHEMISTRY STUDIES OF A β AND FRAGMENT PEPTIDES– STRUCTURE AND AGGREGATION

4.1 A β Conformation and Structure of Fibrils

Amyloid fibrils contain bundles of β -sheets with backbones orthogonal to the fibre axis, in the so-called “cross- β ” structure (Fig.17), and this is observed in fibre X-ray diffraction (XRD) patterns obtained from stalks of A β and fragments.^{27a,27b,27d,27e,353}

The prominent equatorial reflections in Fig.17a (a typical amyloid fibre XRD pattern) arise from the spacing of stacked β -sheets (10-12 Å, the range of values arising from different side chain packing modes) and the meridional reflections arise from the β -strand spacing (4.7-4.8 Å).

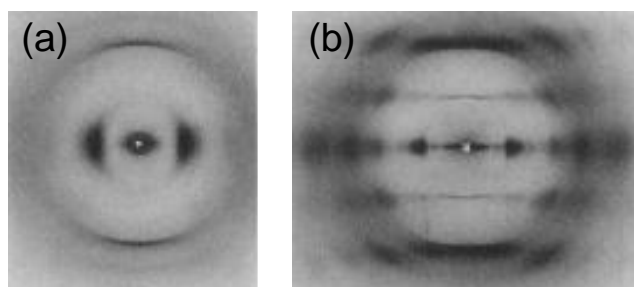


Fig.17. Fibre X-ray diffraction patterns from (a) A β 40, (b) A β (11-28).^{353a} Reprinted from Inouye, H. *et al. Biophysical Journal* **1993**, 64, 502, Copyright 1993, with permission from Elsevier

Many studies have analysed the conformational tendencies of A β and associated fragments. Early work is reviewed by Serpell^{27d} and others.³⁵⁴ A study using CD and NMR showed that A β 40 in aqueous buffer solution adopts a predominantly polyproline II conformation at 0 °C and a random coil state at 60 °C.³⁵⁵ The residues in the central hydrophobic region A β (12-28) tend to adopt β -strand-like conformations at temperatures below 20 °C. The thermal PPII – random coil transition was found to be weakly cooperative. The conformation of the N terminal A β (1-9) domain was also analysed.³⁵⁵ CD has also been exploited to examine the secondary structure formation of A β (1-28), A β (1-39), A β 42 and A β (29-42) in water and TFE or HFIP.³⁵⁶ In aqueous TFE, A β 42, A β (1-28) and A β (1-39) adopt an α -helical structure at low and high pH, but a β -sheet structure at intermediate pH.^{356a} A β 42 and A β 40 have a net charge of +3 at pH 7.4 and β -sheet formation is possible under these conditions. The solubility of A β 42 and shorter peptides in aqueous solution was studied as a function of pH and concentration and aggregation was found to be enhanced at low pH.^{90a} Murphy and coworkers investigated the aggregation of A β (1-28), including the kinetics of aggregation via light scattering.³⁵⁷

Much effort has focussed on analysing the conformation of the core hydrophobic domain of A β . NMR provides the most detailed conformational information. Benzinger *et al.* applied ^{13}C cross-polarization magic angle spinning solid state NMR to probe the conformation of A β (10-35).³⁵⁸ Multiple quantum analysis using the DRAWS pulse sequence enables the registry of specific labelled residues to be determined. This revealed a parallel β -sheet structure with residues in register. The parallel in-register structure persists despite pH dependent variation in fibril morphology as revealed by electron microscopy.³⁵⁹ Similar solid state NMR techniques (with constraints from X-ray diffraction and TEM measurements) have indicated a parallel configuration of β -strands of A β 40, each molecule of which has a β -strand/turn/ β -strand arrangement (Fig.18).³⁶⁰ A similar parallel in-register arrangement was deduced from solid-state NMR data for A β 42.³⁵⁹ A turn structure was located in the A β (26-29) domain based on proteolysis of disulfide-bridged A β (10-43) analogues.³⁶¹ A turn was also predicted around residue 26, based on a primary sequence conformation index.³⁶² This feature was also anticipated based on MD simulations of A β (16-35) and A β (10-35) that showed turns in the A β (24-27) region due to intramolecular D23-K28 salt bridging.³⁶³ In contrast to A β 40 and A β 42 which show in-register parallel β -sheets, NMR studies reveal that Iowa mutant D23N A β 40 can form antiparallel β -sheets.³⁶⁴

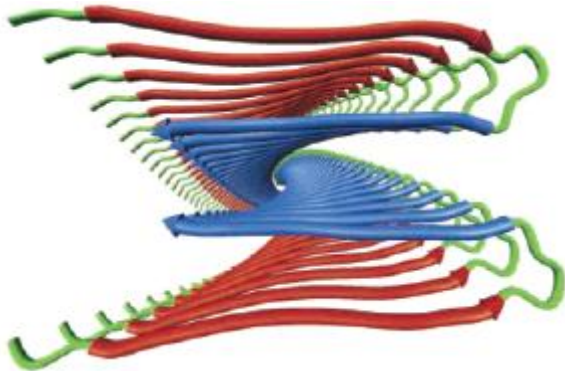


Fig.18. Stacking of A β 40 into parallel β -sheets according to modelling with constraints from solid state NMR. Reprinted with permission from ref. ^{360b,360c}. Copyright 2011 American Chemical Society.

Detailed conformations of the β -strand/turn/ β -strand U-shaped arrangement have been compared for the Ma-Nussinov³⁶³ models and the NMR derived conformations of Lühns³⁶⁵ and Tycko.^{360b,360c,366} Replica-exchange molecular dynamics (REMD) and discrete molecular dynamics (DMD) simulations³⁶⁷ have also confirmed these features, as discussed in Section 4.2. Later MD simulations confirmed a turn in the A β (23-28) domain.³⁶⁸ Benzinger *et al.* did not find evidence for a turn structure in the A β (25-29) domain in their study of A β (10-35),^{358b} highlighting the importance of the E22 and V24 residues in salt bridging and hydrophobic interactions respectively. An analysis of fibre X-ray diffraction data from A β (11-25) fragments was also consistent with a hairpin turn structure, but in the L17-F20 domain.^{353b} However, in a later report from the same group, the unit cell was modelled based on an extended conformation of A β (11-25).³⁶⁹ The β -sheet structure of this peptide has been imaged by cryo-TEM which indicates in-register β -strands.³⁷⁰

In contrast to work focussed on the core hydrophobic domain, there have been fewer studies on the conformation of the C terminal region. The hydrophobic C-terminal A β (29-42) segment forms a β -sheet structure independent of pH, solvent or temperature³⁵⁶ pointing to the high aggregation propensity of this domain, in agreement with computer simulations discussed above. The important role of the A β (33-35) domain was also shown by Pike *et al.* who performed a thorough study on the secondary structure of variants (amino acid deletions and substitutions) of A β (25-35) via CD and on the neurotoxicity of these peptides.³⁷¹ These authors also imaged fibril morphology using electron microscopy.³⁷¹ Aggregation of β -sheets is found to be maximal at pH 5.4.^{356b} A low resolution NMR study indicated a pleated antiparallel β -sheet structure for A β (34-42).³⁷² Hoyer and coworkers reported, on the basis of NMR and computer modelling, a β -hairpin structure for A β 40 in a complex with a phage-display selected affibody protein.²⁰⁰ The hairpin comprises residues A β (17-36). On the other hand, SDS at pH 7.2 stabilizes an A β 42 conformation comprising an extended chain (D1-G9), two α -helices (Y10-V24 and K28- A42), and a looped region (G25-S26-N27) as revealed by solution NMR.³⁷³ Helical content of A β (12-28) in the K16-V24 domain in presence of SDS is also confirmed by CD.³⁷⁴ Figure 19 shows the β -strand/turn/ β -strand conformation of A β (18-42) (residues 1-17 are disordered) within the cross- β fibril structure.

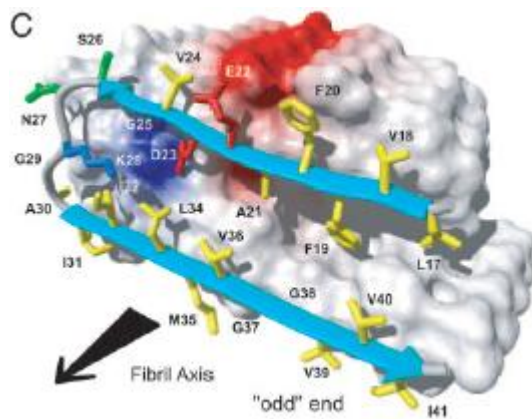


Fig.19. Conformation of A β (17-42) as revealed by H/D-exchange NMR.³⁶⁵ PDB: 2BEG. The hydrophobic, polar, negatively charged and positively charged amino acids are shown in yellow, green, red and blue respectively. Positively and negatively charged surface patches are shown in blue and red, others in white. Copyright 2005 National Academy of Sciences, USA.

Distinct from longer A β fragments (except the Iowa mutant, discussed above), the A β (16-22) peptide appears to adopt an antiparallel β -sheet arrangement as indicated by solid state NMR,³⁷⁵ and isotope-edited FTIR on labelled peptides (combined with ssNMR)³⁷⁶ and computer simulation.³⁶³

The crystal structure of several A β fragments has recently been reported, specifically A β (16-21) (for which three polymorphs were observed), A β (27-32), A β (29-34), A β (30-35), A β (35-42) (in two forms), A β (35-40) (in two forms) and A β (37-42).³⁷⁷ These peptides all form steric zipper structures, i.e. based on self-complementary pairs of β -sheets. Sequences prone to form steric zipper structures were screened using a 3D-profile self-association energy calculation, which indicated a cluster of aggregation-prone sequences in the C terminal A β (30-42) domain.³⁷⁷ In the first

report by Eisenberg's group on crystal structures of amyloid steric zipper structures A β peptides including A β (37-42) GGVVIA and A β (35-40) MVGGVV were among the peptides studied.³⁷⁸ GGVVIA belongs to the class of parallel up-down face-to-back β -sheet structures while MVGGVV β -sheets adopt an antiparallel up-down face-to-back arrangement.

Based on cryo-TEM images, A β 40 fibrils are reported to comprise two protofibrils whatever the overall fibril morphology (considerable polymorphism was noted, see also section 4.4).³⁷⁹ An initial report based on cryo-TEM suggests that A β 42 forms hollow fibrils in which the hairpin A β (17-42) regions fit within the reconstructed density map of the shell.³⁸⁰ However, a later report from the same group based also on cryo-TEM along with additional mass-per-length measurements from scanning TEM, indicates that A β 42 forms a single filament structure without a hollow core (Fig.20).³⁸¹ The cryo-TEM images published for A β (11-25) also do not show a hollow interior.³⁷⁰ Malinchik *et al.* had earlier proposed a hollow filament structure for A β 40 on the basis of TEM cross-section images on plastic-embedded samples, as well as analysis of XRD data.³⁸² Although hollow fibrils were proposed as a common structure for amyloid fibrils,³⁸³ there is in now consensus that this is not the case, and A β fibrils are not hollow nanotubes, although these structures can be observed for fragments such as A β (16-22) under appropriate conditions (section 4.5). On the other hand, MD simulations constrained by the cryo-TEM density maps for A β 42, and NMR coordinates based on data from A β (17-42) suggest that a hollow core structure might be relevant under physiological and acidic pH conditions.³⁸⁴

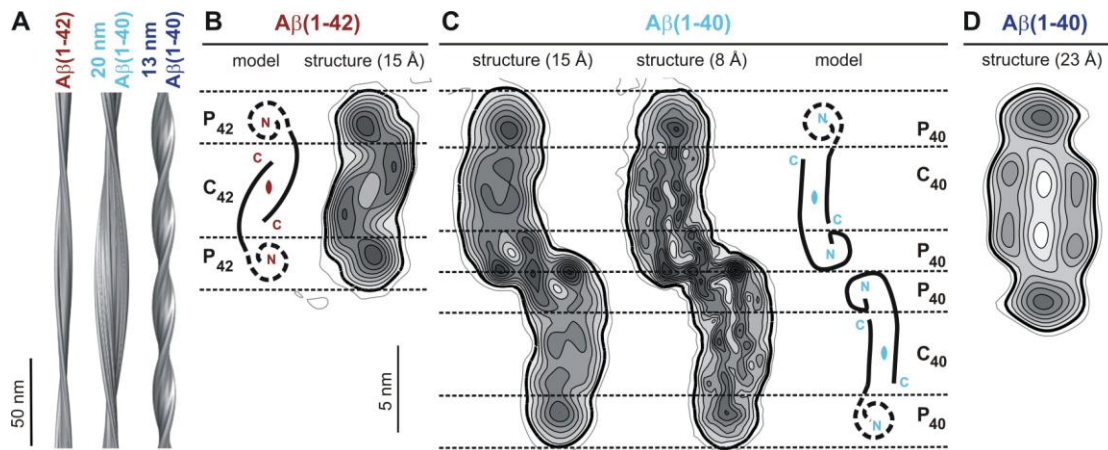


Fig.20. Electron density maps for A β 40 and A β 42, reconstructed from cryo-TEM images.³⁸¹ Copyright 2009 National Academy of Sciences, USA.

The extent of unfolding of A β and ultimately the fibril morphology seem to depend on the level of denaturation.³⁸⁵ By studying A β 40 and A β 42 in which the methionine (residue 35) side chain was in oxidised or reduced state, Hou *et al.* found on the basis of solution NMR, residue-specific interactions in the early stages of aggregation.³⁸⁶ These authors suggest that both hydrophobic and turn-like structures are required in the first self-assembly steps. The solution structure of the methionine-oxidised form of A β 40 has also been studied by NMR and CD by Craik and coworkers – random coil structures were observed at pH 4 in water.³⁸⁷ In SDS solutions, a helical region in the A β (16-24) is noted for this peptide.³⁸⁷ The specific region of A β 40 involved in contacts between fibrils has been identified by solution-state NMR, and corresponds to A β (15-24).³⁸⁸ The NMR data also show that there is an exchange between a monomeric, soluble state and an oligomeric aggregated state under appropriate (physiological) conditions of salt concentration. The equilibrium can be shifted by varying anionic strength.³⁸⁸

4.2 Computer Simulations of A β Conformation and Aggregation

This subject has recently been discussed in several reviews, and some aspects are mentioned in the preceding section.^{366,389} Discrete molecular dynamics simulations using a four bead model (corresponding to different peptide sequences) with hydrogen bonding interactions have been used to model the oligomerization of A β 40 and A β 42.³⁹⁰ These simulations uncovered that the most common species are dimers for A β 40 and pentamers for A β 42, although oligomers up to 9-mers were found for each. These results may be compared with the experimental data on oligomer size distribution for these two A β peptides discussed in Section 2.6.2. Intramolecular contacts were analysed and a turn structure was proposed to stabilize the pentamer structure favoured by A β 42.³⁹⁰ REMD has elucidated the conformation of A β 42 in which loops and turns predominate, although helical regions are found near the C terminus.³⁹¹ It was proposed that these helices are involved in the formation of the oligomeric paranucleus revealed by ion mobility mass spectrometry (Section 2.6.2).³⁹¹

The structure of A β (17-42) protofilaments in solution has been modeled via MD simulations, along with the influence of mutations at E22 and M35.³⁹² Steric zipper formation is observed due to favorable C- and N- terminal interface interactions. Double layered models of oligomers were constructed with association via CC or NN interfaces (Fig.21), these being stabilized by salt bridges. The U-shaped β -strand/turn/ β -strand conformation (section 4.1) leads to a hydrophobic cavity within the stacked β -sheets.³⁹² All-atom MD simulations have been performed to elucidate the structural stability and conformational dynamics of A β (9-40) for wild type and

mutated sequences.³⁶⁸ The peptides adopt in-register parallel β -sheets. Salt bridges are formed between N23 and K28, solvated by water molecules, leading to a hydrated channel along the fibril axis.³⁶⁸ The results were compared to models based on solid state NMR data (section 4.1).^{360b,360c}

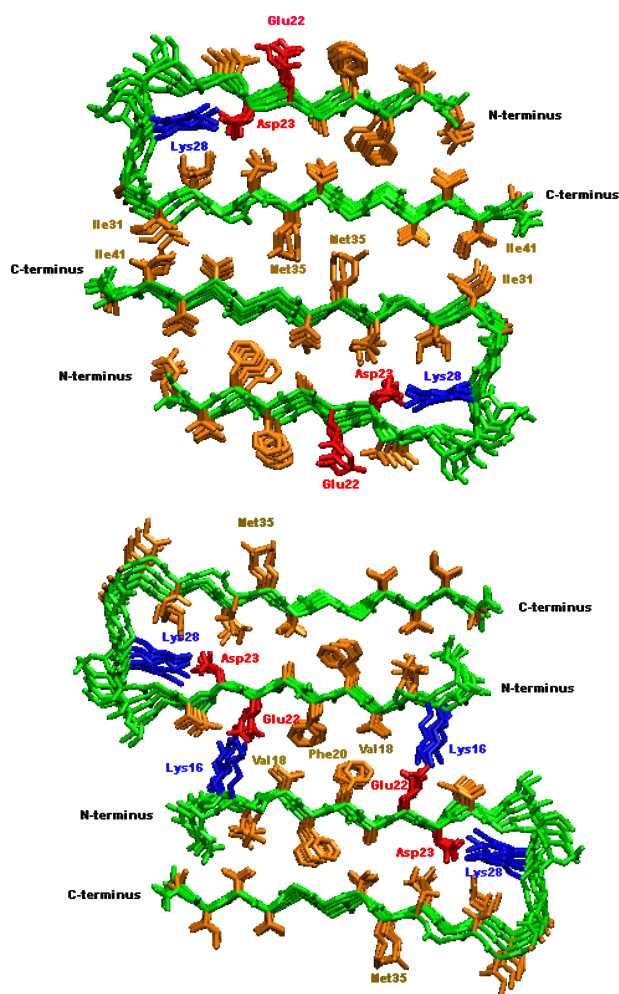


Fig.21. Double layer models used for A β (17-42) oligomers.³⁹² Top- association via C-terminal interfaces; Bottom- association via N-terminal interfaces. Charged residues are shown in red and blue. Reprinted from Zheng, J. *et al. Biophysical Journal* **2007**, 93, 3046, Copyright 2007, with permission from Elsevier.

The oligomerization of A β peptide fragments A β (16-22) and A β (25-35) has been studied by all-atom Monte Carlo simulations.³⁹³ The aggregation of the weakly hydrophobic A β (25-35) peptide is driven by the tendency to form hydrogen bonds that stabilize β -sheets, and this peptide aggregates in a single step. The formation of a critical nucleus involving four peptides was noted for this peptide. In contrast, for the more hydrophobic A β (16-22) peptide, initial collapse into disordered oligomers was observed. In these oligomers, hydrophobic residues are sequestered from the solvent. The subsequent reorganization of oligomers into β -sheet aggregates involves inter-chain H-bonding interactions and exposure of certain residues to solvent.³⁹³ MD simulations have elucidated the structure of dimers formed by A β (25-35) with high β -sheet content aggregates coexisting with less structured dimers.³⁹⁴ The effect of the self-recognition KLVFF domain and the β -sheet breaker peptide LPFFD on the oligomerization of A β (16-22) was examined by all atom MD simulations.³⁹⁵ The binding domain near the N-terminal involving H13 was identified, and binding energies were calculated. LPFFD as expected has a greater inhibitory effect on aggregation.

DMD simulations using a “united atom” model (specifying all atoms except hydrogens) have been performed to examine the conformation of A β (21-30).^{367b} A hairpin conformation driven by hydrophobic interactions between V24 and the butyl units of K28, and stabilized by transient salt bridging between E22/N23 and K28, was identified.^{367b} REMD simulations have been carried out for the same fragment with similar conclusions.^{367c} The significance of the mutations at the E22 residue (Section 2.2.4) in influencing the stability of turn structures, and hence of aggregation into toxic assemblies, was noted.^{367c} These observations are consistent with ssNMR

experiments as mentioned above and in the preceding section. The results of REMD simulations on A β (21-30) have been compared to ion mobility mass spectrometry data, in particular the collision cross-section can be modelled, providing information on conformation.^{367e} A particular focus was the turn structure and the influence of the E22G, E22Q, E22K and D23N mutations.^{367e} The same group also performed all-atom MD simulations in explicit water of the folding of A β (21-30) and the Dutch mutant with the E22Q substitution.^{367a} Similar conclusions were drawn concerning the presence of loops in the V24-K28 region in the wild-type peptide, although this is not observed for the Dutch mutant sequence. The influence of salt ions on the salt bridging interaction was examined.^{367a}

MD simulations have been used to investigate the adsorption of A β (17-42) oligomers (from dimers to hexamers) onto self-assembled monolayers (SAMS) with different end-functional groups.³⁹⁶ The SAMS are proposed to serve as models for cell membranes. The simulations complement experimental studies (CD, AFM, SPR) on A β 42 by the same group,³⁹⁷ which shows that fibrillization is accelerated in the presence of SAMs, to the greatest extent for the hydrophobic -CH₃ capped SAMS or the cationic -NH₂ functionalized SAMS. This observation points to the role of both hydrophobic and electrostatic interactions. The simulations suggest that trimers constitute the smallest nucleus that can seed A β polymerization.

4.3 Kinetics and Mechanisms of Fibrillization

4.3.1 Mechanisms

As mentioned in Section 2.2.1, A β 42 is more aggregation prone and exhibits faster fibril growth than A β 40. A detailed model for the aggregation into initial aggregates (oligomers, termed “micelles” in the original work) and subsequently fibrils has been developed.³⁹⁸ This permits nucleation and elongation rate constants to be calculated.

Fibrillization of A β appears to occur via a proto- or pre- fibrillar stage.^{6d,212,221a,399}

These oligomeric species are consumed as fibrillization proceeds.^{212,399a} The pre-fibrillar stage has been proposed to correspond to micelle formation. Fibrillization of A β 40 occurs above a critical concentration which has been described in analogy with a critical micelle concentration (cmc) as shown in Fig.22.^{6a,90b,400} Fibrillization can be described using the corresponding one-dimensional model of self-assembly.⁴⁰¹ The initially formed protofibrillar species for several proteins including A β 40 and A β 42 are spherical annular-shaped species^{212,221a,399b} which may be linked into chains.^{221a,399b} The proto-fibril formation process may occur before or during the lag phase.

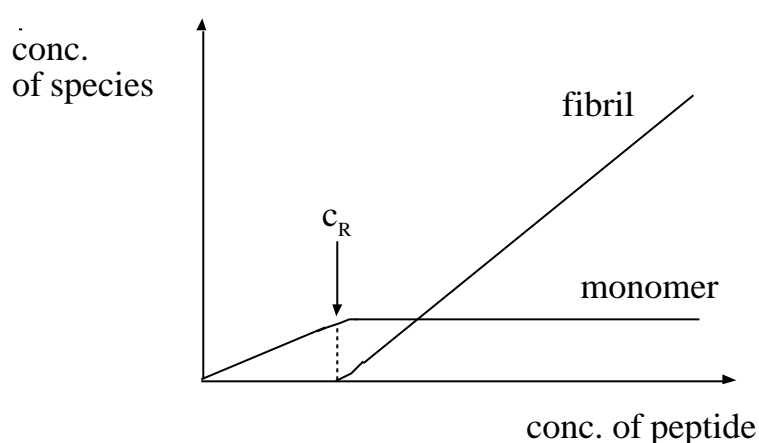


Fig.22. Amount of fibril and monomer as a function of added protein. Fibrils are formed above a critical concentration, c_R . From ref.⁴⁰², redrawn from ref.^{6a}

Models that extend beyond the Oosawa-Asakura model⁴⁰³ for one-dimensional self-assembly have been developed to describe more realistically amyloid aggregation kinetics.⁴⁰⁴ In particular, the influence of fragmentation, i.e. the generation of secondary nuclei, has been considered. It will be interesting to see this model applied to further analyse the kinetics of A β fibril growth.

It is commonly observed that fibrillization occurs after a lag phase, suggesting a nucleation and growth process.^{6a,90b,208,385a,400,405} The lag phase can be eliminated by addition of pre-formed aggregates, i.e. by seeding (Fig.23).^{6a,90b} The influence of A β 40 seeds on A β 42 aggregation and *vice versa* has been examined using immobilized seeds.⁴⁰⁶ Oligomeric forms of the peptides were found to be more effective seeds than either monomers or fibrils and A β 42 monomers aggregated onto A β 42 fibrils more rapidly than other combinations.

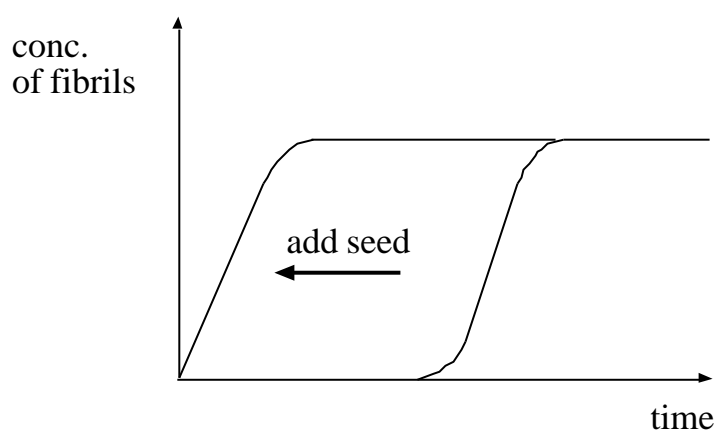


Fig.23. Addition of seed can eliminate the lag time in fibrillization. From ref. ⁴⁰², redrawn from ref. ^{6a}

AFM on A β 40 and A β 42 provide a picture of the initial formation of protofilaments followed by their replacement with fibrils,²¹² this technique being complemented with light scattering, and TEM and SEC analysis of the molecular weight of fractions obtained at different stages of the polymerization process.^{399a}

The aggregation of A β on planar substrates, hydrophilic mica and hydrophobic graphite, has been investigated. On mica, pseudo-micellar aggregates were noted at low concentration and fibrils at higher concentration. In contrast, on graphite, sheets were observed with a thickness equal to the extended peptide length, oriented along the graphite lattice directions.²¹² A later AFM study examined A β 42 fibrillization and plaque formation, and the interaction between A β 40 and A β 42.⁴⁰⁶ Deposition was studied on a synthetic template comprising an *N*-hydroxysuccinimide ester-activated solid surface. A β 42 oligomers were found to be more effective seeds for fibril growth than monomers or mature fibrils.

Lin and coworkers investigated the kinetics and mechanisms of A β 40 fibrillization under different conditions including variable temperature, ionic strength and pH.⁴⁰⁷ The rate of fibrillization was enhanced with increasing temperature or ionic strength. A two-state mechanism of growth was proposed, nucleation being the rate-determining step. The aggregation mechanism was dependent on ionic strength since addition of monomers to fibrils can occur either at the ends (at low ionic strength), or depending on screening of electrostatic interactions at high ionic strength, by lateral aggregation.⁴⁰⁷ Linse *et al.* suggest that A β 42 (D1M mutant) fibrillization occurs via a two-phase state involving soluble A β (liquid phase) and aggregated A β (solid phase).⁴⁰⁸ They used ThT fluorescence and ELISA to monitor fibril growth, and free

A β concentration respectively. The free A β concentration varied linearly with total A β concentration up to 0.2 μ M, followed by a decrease to an asymptotic value. This suggests a *cmc* of 0.2 μ M in the buffer conditions examined.⁴⁰⁸ These authors also point to the need to be extremely careful with the preparation conditions to obtain reproducible fibrillization data on A β 42 due to the known complications associated with avoiding initial A β aggregation (for example, aggregation of A β 40 even on resin during solid phase synthesis has been observed by SSNMR⁴⁰⁹) as well as nucleation at interfaces (air/water interface of bubbles, surface of vessel).⁴⁰⁸ It is already known that very careful protocols have to be followed in studying fibrillization of A β 42 for example, for example starting from a well-defined state of unaggregated peptide (achieved by initial dissolution in a hydrophobic solvent) and then carefully controlling the addition of water or buffer to a dried film, as discussed in Section 2.6.2.²¹⁴ Agitation, e.g. via sonication, is known to have a particularly marked effect on fibril morphology and indeed can be used to create specific polymorphs.^{364,410}

Exchange dynamics between monomers and NMR-invisible (dark state) protofibrils of A β 40 and A β 42 have been investigated using single residue ¹⁵N dark-state exchange saturation transfer NMR.⁴¹¹ This revealed that the first 8 residues at the N terminus exist in a mobile tethered state while the hydrophobic central residues are either tethered to, or in contact with, the protofibril surface. The C terminal residues display lower affinity for the protofibril surface. The ¹⁵N relaxation rates of the C terminus residues are larger for A β 42 than A β 40 and this was proposed as an explanation for the higher fibrillization rate of the former peptide.⁴¹¹

4.3.2 Kinetics

In the growth state of fibrillization, early work on A β 40 suggested that the kinetics are first order, i.e. the rate of fibril elongation is proportional to the concentration of monomers.^{400,412} This was confirmed via light scattering on A β 40 in 0.1 M HCl⁴⁰⁰ (aggregation kinetics are pH dependent), *in vitro* studies of deposition onto plaques in unfixed Alzheimers disease brain tissue^{412a} and ThT fluorescence studies.^{412b} However, in the absence of seeds, A β aggregation exhibits faster than first order kinetics and an optimal pH of 5 instead of 7 as for templated aggregation.^{412a} The temperature dependence of fibril extension for A β 40 in 0.1 M HCl (determined from size measurements via dynamic light scattering) follows the Arrhenius equation,⁴¹³ with an activation energy 96 kJ mol⁻¹, comparable to the value for unfolding of several other peptides.⁴¹³ The concentration dependence of fibrillization kinetics was also analysed. Light scattering has also been used to monitor the aggregation of A β 40 in PBS at different concentrations, and the size and shape of (proto-)fibrils was analysed,⁴¹⁴ and previously, in a similar fashion, this method was applied for A β (1-28)³⁵⁷ and A β (1-39).⁴¹⁵

The rate of fibrillization is strongly influenced by seeding.^{90b} Fibril morphology is also influenced by seeding, as revealed by TEM and solid state NMR studies on A β 40 (see also section 4.4).⁴¹⁰ The kinetics of A β fibril growth by covalent attachment of seeds to the surface can be monitored using using quartz crystal microbalance (QCM),^{246,416} surface plasmon resonance (SPR)⁴¹⁷ or AFM⁴¹⁸ techniques. Peptide can be immobilized on the QCM crystal via, for example, attachment of the peptide *N*-terminus to a tethered 16-mercaptohexadecanoic acid monolayer (via carbodiimide/*N*-hydroxysuccinimide activation) or biotinylated peptide attached via avidin.^{416b} The rate of elongation is observed as a change in resonant frequency of the crystal which

correlates to an increase in surface-bound mass. In this way, monomer addition to A β 40 fibrils was studied, these were found to elongate in a reversible fashion with a rate that varies with monomer concentration and immobilized seed density.^{416b} The growth was consistent with a first-order kinetic model for the single growth phase observed.

Rate constants have been determined for A β fibril growth using several methods.

Direct comparison between results from these measurements is often not possible, due to differences in the precise definition of the measured quantity. Based on 2D studies using immobilized A β 40, the following values were reported for the rates of association and dissociation: $k_{\text{ass}} = 3.6 \times 10^{-4} \mu\text{M}^{-1} \text{min}^{-1}$, $k_{\text{dis}} = 7.6 \times 10^{-5} \text{min}^{-1}$ and the association constant $K_d = k_{\text{dis}}/k_{\text{ass}} = 210 \text{nM}$.^{416b} On the other hand, based on isotope exchange ESI-MS experiments on the dissociation of molecules from fibrils, the following values were determined for A β 42: $k_{\text{dis}} = 0.01 \text{min}^{-1}$ for A β 40 and $k_{\text{dis}} = 1.67 \times 10^{-4} \text{s}^{-1}$ for A β 42.⁴¹⁹ Linse *et al.* studied fibrillization of A β (M1-40) on polymeric nanoparticles and reported $k_{\text{ass}} = 0.13\text{-}0.28 \text{min}^{-1}$, with a lag time 37-250 min.¹¹⁴

Wetzel gives rate constants as shown in Table 4.^{417,420} These measurements were based on surface plasmon resonance measurements using immobilized seeds and may not represent bulk values.

Table 4. Rate constants for dissociation and association determined by Wetzel and coworkers^{417,420} in the rapid reversible binding of monomer to the fibril in step 1 is followed by two successive relatively slow processes (steps 2 and 3).

Mechanistic Step	k_{dis} (s^{-1})	k_{ass}
1	8.1×10^{-1}	$6.6 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$
2	4.4×10^{-3}	$6.4 \times 10^{-2} \text{ s}^{-1}$
3	4.3×10^{-4}	$4.6 \times 10^{-3} \text{ s}^{-1}$

Lomakin *et al.*³⁹⁸ obtained for A β 40 at pH 2 (0.1M HCl) a fiber nucleation rate $k_n = 2.4 \times 10^{-6} \text{ s}^{-1}$ and elongation rate $k_e = 90 \text{ M}^{-1} \text{ s}^{-1}$. These authors propose mechanisms of fibrillization of A β 40 under these conditions depending on whether the concentration exceeds the *cmc*, c^* (Section 4.6) or not, as illustrated in Fig.24.⁴⁰⁰

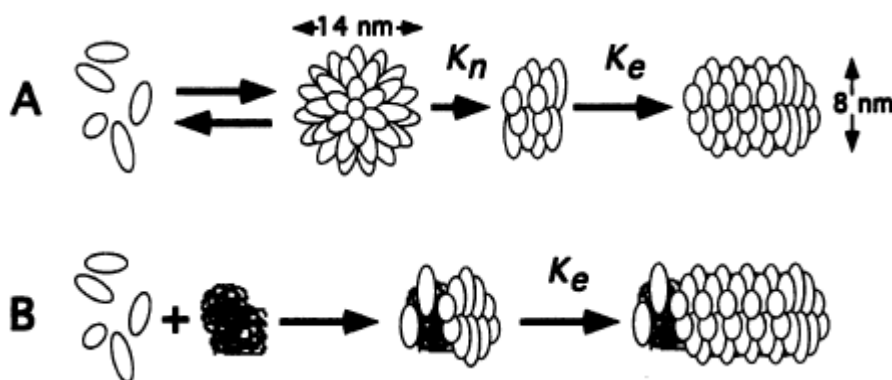


Fig.24. A) Homogeneous nucleation for $c > cmc$ via formation of initial nuclei (rate constant k_n) followed by extension, rate constant k_e . Initially monomers associate into micelles with $R_n = 7 \text{ nm}$, B) Heterogeneous nucleation for $c < cmc$, nucleation occurs mainly on seeds not comprising A β . Figure from ref.⁴⁰⁰ Copyright 1996 National Academy of Sciences, USA.

Inouye and Kirschner⁴²¹ analysed the kinetics of Congo red binding to A β 40 at pH 5.8, based on the measurements by Wood *et al.*⁴²² Using the Oosawa-Asakura model for one-dimensional self-assembly, they obtained values for the dissociation constant for spontaneous assembly of $K_d = 55.1 \mu\text{M}$ and $k_{\text{dis}} = 2 \text{ h}^{-1}$ ($K_d = 53.7 \mu\text{M}$ and $k_{\text{dis}} = 5 \text{ h}^{-1}$ for seeded assembly) as well as the rate coefficient and proton dissociation constant.⁴²¹ The authors note that the kinetics of seeded fibril formation at pH 5.8 are faster than observed by Lomakin *et al.*⁴⁰⁰ at pH 2.

4.3.3 Thermodynamics

Wetzel *et al.* have analysed the thermodynamics of amyloid fibrillization.⁴²⁰ The free energy of fibril elongation of wild type A β 40 was found to be approximately $-37.7 \text{ kJ mol}^{-1}$.⁴²⁰ This group note that seeded fibril growth can be described as a dissociation/elongation equilibrium. The equilibrium is achieved with a remaining pool of monomer with a concentration of $0.7 - 1 \mu\text{M}$ (corresponding to the *cmc*).⁴²⁰ The equilibrium constant K_d was found to be around $0.8 - 1 \mu\text{M}$ (the value being higher for mutants, and for fibril growth in the presence of ThT). A three step mechanism for elongation was proposed with three different sets of dissociation/association constants as described in the preceding section.⁴²⁰ The change in Gibbs energy for a series of mutants of A β 40 was analysed in a similar fashion.⁴²³ The exchange of A β molecules due to fibril association and dissociation has been monitored via hydrogen/deuterium exchange electrospray ionization mass spectrometry.⁴¹⁹ Molecular recycling was found to be much more prevalent for A β 40 than for A β 42. The free energy of fibril growth for A β 40 can be determined from

the critical concentration (Section 4.6) and this leads to $\Delta G_{el}^0 = -46.1 \text{ kJ mol}^{-1}$,⁴²⁴ in reasonable agreement with the value from Wetzel's study quoted above. The Wetzel group also studied the effect of A β 40 alanine mutations on the free energy of elongation.⁴²⁴ By measuring the amount of soluble monomer, and total protein concentrations at steady state for a series of amyloid forming proteins including A β and others, the free energy of elongation was determined and these values may be compared with those for A β 40.⁴²⁵ A correlation with the number of residues in the peptide was noted.

4.3.4. Lack of Sequence Specificity in A β Aggregation

The cross- β structure (Section 4.1) is a common feature for amyloids formed by many different proteins and peptides, including A β .^{2d,39b,426} Evidence that formation of amyloid fibrils is a common state for many if not all proteins comes from several types of experiments. First, fibrils can be induced to form by partial denaturing of proteins not involved with any disease⁴²⁷ or using *de novo* designed peptide fragments (see Section 4.5). Secondly, amyloids can be induced to form by seeding with fibrils of the same, related or unrelated protein,^{6a,89,385a,405b,428} a process that may be implicated in the transmission of prion diseases,^{6a} although the transmission of spongiform encephalopathies may involve cofactors in addition to prions, the full mechanism being unclear as yet.⁴²⁹

A study of mutants of A β 40 (including Dutch type, Section 2.2.4, but also variants for rodents compared to primates) revealed that N-terminal substitutions that distinguish primate A β 40 from rodent A β 40 do not have a significant effect on fibril

morphology.⁴³⁰ The fibrils formed by the Dutch mutant were found to have enhanced stability at high pH compared to the other variants.

Using fusion constructs with green fluorescent protein (GFP), libraries of mutants of wild-type A β 42 have been prepared in which hydrophobic residues at the C terminus⁴³¹ or 8-12 other residues in the C terminal domain⁴³² have been substituted with random nonpolar residues. The folding and fluorescence of GFP is prevented by A β 42, and mutations in A β 42 that disrupt aggregation then lead to increased fluorescence.⁴³³ It was shown that fibrillization is promoted with hydrophobic residues at positions 41 and 42.⁴³¹ and all the hydrophobic mutations (8-12 residues).⁴³² This implies that generic hydrophobic sequences may be sufficient to promote A β 42 fibrillization.⁴³² In related work, A β 42-GFP fusion constructs with A β 42 mutations across the whole sequence were expressed and A β 42 aggregation was probed. This also identified key hydrophobic sequences involved in fibrillization.⁴³³

4.4 Polymorphism

The morphology of A β fibrils can be controlled through the growth conditions, and seeding, leading to distinct polymorphism. This in turn is related to the selection of specific conformations.^{389b} An important contribution on the subject of polymorphism was the study by Petkova *et al.*⁴¹⁰ on A β 40 polymorphism, controlled through application of sonication or not, and imaged by TEM (Fig.25). Solid state NMR was used to identify cross-correlations between different residues, i.e. to probe

conformations in different polymorphs. The cytotoxicity towards rat neurons was also shown to depend on A β 40 polymorphism. *In vivo*, the phenotype of seeded amyloidosis depends on both the source and the host.⁸⁹ This was examined using APP23 and APPPS1 transgenic mice, which overexpress A β 40 and A β 42 respectively. It was suggested that the dependence of amyloid morphology on the seed indicates the existence of A β polymorphism with associated distinct polymorph biological activity, reminiscent of prion strains.⁸⁹

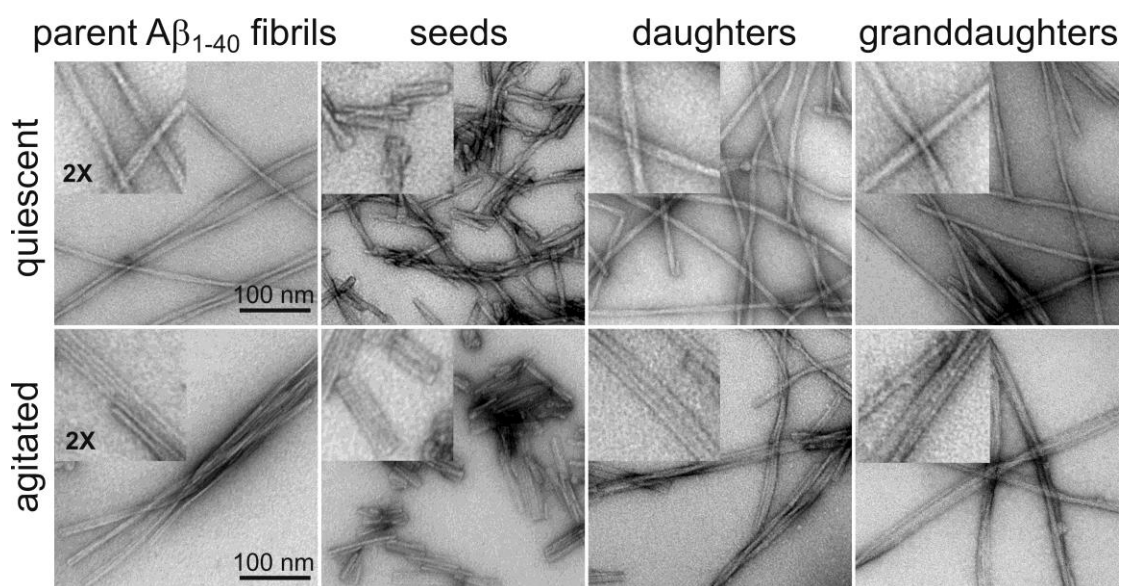


Fig.25. Polymorphism of A β 40 fibrils.⁴¹⁰ From Petkova, A. T. *et al.*, *Science* **2005**, 307, 262. Reprinted with permission from AAAS.

Tycko's group have recently shown that the polymorphism of A β 40 (Iowa mutant) can be reduced by repeated seeding (up to nine generations were studied), which can be used to amplify a particular polymorph, even when it is initially a minor component.³⁶⁴

Seeds comprising nanotubes (self-assembled in methanol) of the modified peptide AAKLVFF (containing A β (16-20), KLVFF) can seed nanotube formation in aqueous solution (conditions that favour non-nanotube fibrillization of unseeded peptide).⁴³⁴ TEM was complemented by solid-state NMR which probed differences in the strand registry and residue packing in the nanotubes and fibrils. These observations point to the non-equilibrium nature of the seeded polymorphism.

The factors that can influence amyloid fibril polymorphism are complex, and apart from factors such as agitation during sample preparation, the presence of hydrophobic interfaces or the air-water interface can influence fibrillization.⁴³⁵

4.5 Fragments

The fibrillization of fragments of A β 40 and A β 42 has been extensively investigated. Several early studies are summarized by Teplow^{6b} and Serpell.^{27d}

Aggregation-prone sequences in A β have been identified by computer modelling of measures of β -sheet forming propensity. The TANGO algorithm, based on a statistical mechanical model of protein conformation (based on measures of propensity to form defined secondary structures), predicts a strong tendency for aggregation of A β (17-21) and A β (31-36), with enhanced aggregation of A β 42 compared to A β 40.⁴³⁶ It can also account for the aggregation propensity of some of the mutant forms of A β (Section 2.2.4). The Zyggregator algorithm is based on measures such as hydrophobicity and patterning of hydrophobic residues that lead to a parameter set obtained by screening of sequences in protein databases.⁴³⁷ It predicts a strong tendency for aggregation of A β (15-23) and A β (30-42), i.e. similar domains as

indicated by TANGO.^{202c,437} These predictions have been compared to results from solid state NMR experiments for both protofibrils and mature fibrils and protofibrils are found generally to have shorter aggregation domains.⁴³⁸

Several key regions are associated with aggregation propensity, including the transmembrane hydrophobic domain and the C-terminal hydrophobic domain. In the following, we focus on fibrillization by the shortest core fragment critical to fibril formation which is believed to lie in the A β (16-22), KLVFFAE, region. Sequences in this central region of A β 42 are of great interest because cleavage by the enzyme α -secretase occurs between K and L.^{2a} Hilbich *et al.* showed that a region in the hydrophobic core around residues 17 to 20, i.e. LVFF, is crucial for β -sheet formation.⁴³⁹ They prepared variants of A β 43, with substitution of various residues 17 to 20, and investigated fibrillization by CD, FTIR and TEM. Substitution with hydrophilic amino acids led to a significant reduction in amyloid formation. Tjernberg *et al.* studied the binding of fragments and variant fragments of A β 40 to the full peptide.⁴⁴⁰ Binding of ¹²⁵I-labelled A β 40 was studied by autoradiography. A series of fragments of A β 40 ranging from 3 to 10 residues was prepared. Only pentapeptides or longer showed significant binding to A β 40, and fragment A β (16-20), i.e. KLVFF, is contained in all strongly binding sequences.^{440b} By preparing pentapeptide variants of KLVFF with substituted amino acids, it was found that residues 1, 2 and 5 (K, L, F) are most important for binding of this fragment to A β 40.⁴⁴⁰ A model for the binding of KLVFF to A β (13-23) confirmed the importance of these residues in forming an anti-parallel β -sheet. The binding capacity of pentapeptides containing D-amino acids instead of L-amino acids was also studied, since the latter are resistant to proteolysis. Residues 2 and 3 were found to be most critical for binding, D-Lys, D-Phe enhancing

binding.^{440b} Findeis *et al.* presented a very thorough study on A β -based inhibitors of A β fibrillization.²⁸⁰ This revealed again the importance of the A β (16-21) region. A derivative of A β (17-21), cholyl-LVFFA-OH was found to be a particularly potent inhibitor of fibrillization, although with limited biochemical stability. The D-amino acid version however was found to be stable in monkey cerebrospinal fluid. Other researchers have explored the role of molecular architecture on the inhibition of fibrillization. Dendritic tetramers containing terminal KLVFF motifs inhibit the aggregation of low molecular weight and protofibrillar A β (1-42) into fibrils, substantially more than the linear KLVFF peptide.⁴⁴¹ Recently, bifunctional molecules with terminal KLVFF pentapeptides linked by aminohexanoic acid spacers, have been shown to recognise early A β oligomers, mainly trimers and tetramers, in mixtures.⁴⁴² The molecules may act as “tweezers”, binding small oligomers if the central spacer has the correct length.

The dependence of fibrillization on fragment size was investigated for A β fragments containing the A β (16-20) sequence.⁴⁴³ Electron microscopy suggested that the shortest fibril-forming sequence was A β (14-23), i.e. the decapeptide HQKLVFFAED. The KLVFF sequence was found not to form fibrils itself. However, more recent work shows that this peptide does form β -sheet fibrils under appropriate conditions in aqueous solution.⁴⁴⁴ Meredith and coworkers later studied variants of KLVFF²⁸⁴ and KLVFFAE⁴⁴⁵ in which amide protons in alternate residues were replaced by *N*-methyl groups.^{284,445} Ac-K(Me)LV(Me)FF-NH₂ was shown to form extended β -strands.²⁸⁴ It is also more water soluble than KLVFF and can permeate phospholipid vesicles and cell membranes. This variant is also resistant to denaturation by addition of solvent, increase of temperature or pH. It is also a potent inhibitor of A β 40 fibrillization, and

can break up preformed A β 40 fibrils, being more effective than KLVFF in both regards²⁸⁴ (as is heptapeptide NH₂-KLV(Me)F(Me)F(Me)A(Me)E-CONH₂⁴⁴⁵). These fragments are believed to form β -strands with distinct faces – one with unmodified groups capable of forming hydrogen bonds and the other containing non-polar methyl groups. This can disrupt the hydrogen bonded β -sheet structure of the A β peptide itself.

Other fragments have been designed to inhibit A β 40 and A β 42 fibrillization. Rational design principles based on the knowledge of the pentapeptide binding sequence led to a study on LPFFD,⁴⁴⁶ now known as the β -sheet breaker peptide. This peptide incorporates proline, known to be a β -sheet blocker and was found to reduce amyloid deposition *in vivo* (rat model) and to disassemble pre-formed fibrils *in vitro*.⁴⁴⁶ Prior investigations of A β (12-26) with proline substitutions had shown that Pro replacement of any residue in the A β (17-23) LVFFAED sequence leads to a loss of fibril formation⁴⁴⁷ and this was confirmed via F19P substitution in A β 42, although oligomeric species were still detected.²¹⁷ This observation also points to the role of Pro residues in forming the core of β -sheets. The capped version of the β -breaker peptide, CH₃CO-LPFFD-CONH₂, has been shown to improve spatial learning in a rat model.⁴⁴⁸

The retro-inverse peptide ffv_Dlk (lower case indicates D-amino acids) binds A β 40 fibrils with moderate affinity but this binding can be significantly enhanced by attaching multiple copies of this peptide to an eight-arm branched PEG.⁴⁴⁹ Tandem dimers of ffv_Dlk linked by a k(β A) (k denotes D-lysine) spacer or a difunctional PEG

chain also showed some enhancement of binding. All of these conjugates are effective in inhibiting fibrillization of the full A β 40 peptide.⁴⁴⁹

TEM indicated that KLVFF itself forms fibrils in aqueous PBS solutions (pH 7.4),²⁸⁴ contrary to the reports by Tjernberg *et al.*⁴⁴³ There existed some controversy as to whether this fragment itself fibrillizes. This was resolved in a study which used cryogenic-TEM (cryo-TEM) among other techniques to confirm that KLVFF does form amyloid fibrils in aqueous solution, at sufficiently high concentration.⁴⁴⁴ Cryo-TEM importantly avoids artifacts when drying to prepare samples for conventional negative stain TEM, this can be problematic in the case of weakly fibrillizing peptides. Fibril formation has been reported for the heptapeptide A β (16-22), CH₃CO-KLVFFAE-NH₂.³⁷⁵ It has been suggested that “fibrils” of this peptide actually comprise nanotubes, based on electron microscopy, atomic force microscopy and small-angle scattering data, and a detailed model for the lamination of the β -sheets in the nanotube wall has been proposed.⁴⁵⁰ The related capped peptide A β (16-22) E22L self-assembles into nanotubes in water/acetonitrile solution and the grooved surfaces of these were used to probe the binding of Congo red, which was found to bind parallel to the long axis.⁴⁵¹ Analysis of the 3D structure of A β 42 from NMR (discussed in detail in section 4.1) indicates that residues A β (18-26) form a β -sheet as do residues 31-42 within the overall β -strand/turn/ β -strand structure of residues 18-42 as shown in Fig.19 (residues 1-17 are disordered).³⁶⁵ The sequence A β (17-23) which seems to be vital in amyloid self-assembly has also been shown to be important in forming the correct β -pleated sheet structure of the A β peptide.^{439,447} As discussed in section 4.2, computer modelling predicts that A β (17-21) should be prone to β -sheet aggregation.^{202c,452} Using algorithms based on the aggregation properties of the

constituent amino acids, Kallberg *et al.*⁴⁵³ suggest that A β (16-23) is a so-called discordant sequence of amino acids, in the sense that this sequence is predicted to adopt a β -strand conformation, whereas the full protein structure in the protein data base (ref 1ba6) indicates an α -helix for this region of A β 40. The protein database structure 1ba6³⁸⁷ is for A β 40 with oxidised methionine (residue 35) in aqueous SDS solution, a solvent which is known to favour α -helices, observed between residues 16 and 24 (section 4.1). NMR data on A β 40 in aqueous solution also indicate an α -helix for residues 15-24 in a water/TFE solution⁴⁵⁴ and for residues 1-36 in SDS solution⁴⁵⁵ (the data from Sticht *et al.*⁴⁵⁴ gives pdb structure 1AML). As mentioned in Section 4.1, NMR in aqueous solution³⁶⁵ indicates a β -sheet in this region of A β 42. Different methods to predict secondary structure indeed lead to different predictions for the conformation of KLVFF. The method of Garnier predicts α -helices for KLVFF, whereas the Chou-Fasman method predicts residues KLV are in β -strand and FF in α -helix structures.^{202c} A β (9-11), i.e. NH₂-GYE-OH, forms amyloid-like fibrils in aqueous solution.⁴⁵⁶ MTT assays indicate that the peptide is toxic to neurons.

N-terminal variants of A β may also play an important role in AD pathophysiology.^{192,457} Hilbich *et al.* used CD and EM to investigate the fibril-forming properties of A β 43 and N-terminal truncated variants along with variants of A β (10-23) with substitution of hydrophobic residues.³⁶¹ Peptides in which phenylalanine residues are substituted for less hydrophobic residues show an enhanced solubility in salt solution compared to the native sequence. These authors also investigated variants in which pairs of residues (D23K28, V24G29, G25A30) in the A β (10-43) peptide were replaced by pairs of cysteines, in order to examine the influence of disulfide bridging,

i.e. to fix an artificial turn structure. As discussed in section 4.1, the native peptide is believed to adopt a turn structure in the A β (23-30) domain.³⁶¹

The fibrillization of A β variants with N-terminal glutamines replaced with pyroglutamyl residues has been investigated. Peptides with this form of post-transcriptional modification are observed *in vivo*, in the brains of AD and Down's syndrome patients⁴⁵⁷⁻⁴⁵⁸ due to presenilin 1 mutations.^{55a} In particular, the aggregation of pGlu-A β (3-42)⁴⁵⁸ and pGlu-A β (11-42) has been examined.⁴⁵⁹ These peptides exhibit accelerated aggregation compared to the unmodified A β 40 and A β 42 and their possible role in seeding aggregation *in vivo* was noted.⁴⁵⁹

The important role of the C-terminal hydrophobic domain, in particular A β (34-42) in driving fibril formation has been examined.^{34,372,460} The length of the C terminus critically influences the rate of amyloid formation but only has a minor effect on the solubility.³⁴ Peptides containing the C terminal sequence A β (36-42/43) can seed fibrillization by peptides lacking the C terminal residues (A β 40-42).³⁴

4.6 Micelles

A β has surfactant-like properties, for example it is able to reduce surface tension in a concentration-dependent manner and both A β 40 and A β 42 and shorter variants (C-terminal truncations) exhibit an apparent critical micelle concentration (*cmc*).⁴⁶¹ The "micelles" may in fact correspond to proto-fibrillar species believed to be involved in the nucleation of fibril formation (section 4.3.1). The formation of SDS-stable aggregates was also confirmed by SDS-PAGE. For a series of C-truncated peptides, the *cmc* was found to be 25 μ M in aqueous solution, and measurements on the

partitioning of a fluorescent dye DPH (1,6-diphenyl 1,3,5-hexatriene) suggested that residues 29-42 form the interior hydrophobic domain.⁴⁶¹ The *cmc* of A β 40 has also been deduced from the concentration dependence of fibril growth kinetics and was found to be $c^* = 0.1$ mM in acidic aqueous solution.⁴⁰⁰ Surface-pressure area experiments and pyrene fluorescence measurements indicate $c^* = 17.5$ - 17.6 μ M in aqueous Tris buffer solution.⁴⁶² Wetzel and coworkers report a value $c^* = 0.7 - 1$ μ M in aqueous PBS solution, measured by SPR and also deduced from fibril dissociation equilibrium constants, obtained from kinetic measurements of ThT fluorescence.⁴²⁰ Linse *et al.* obtained $c^* = 0.2$ μ M for A β (M1-42) in the buffer system they studied, and noted that this is lower than the value observed by Wetzel and coworkers for A β 40 due to differences in ionic strength of buffer as well as the peptide length.⁴⁰⁸ The aggregation number of the micelles ($N = 25$) was estimated using fluorescence quenching techniques. The *cmc* of A β (11-25) determined from FRET experiments is 3 μ M at pH 5 and 70 μ M at pH 7.4.⁴⁶³

Above the *cmc*, Lomakin *et al.* found that the initial rate of elongation and the final size of fibrils were independent of A β concentration due to the monomer-micelle equilibrium.⁴⁰⁰ The surfactant *n*-dodecylhexaoxyethylene glycol monoether (C₁₂E₆) slowed nucleation and elongation of A β 40 fibrils in a concentration-dependent manner.⁴⁰⁰ The hydrodynamic radius of the micelles was approximately 7 nm. Detailed SANS experiments later provided a model for the shape and dimensions of “spherocylindrical” micelles.⁴⁶⁴ The authors identified these species as aggregates comprising 30-50 monomers, therefore they seem to correspond to proto-fibrils rather than oligomers.

SDS micelles hinder the formation of β -sheet fibrils by A β 40 and A β 42, and instead stabilize helical conformations.³⁷³ However, at lower concentration (below the *cmc*), SDS promotes the formation of oligomers by A β 42 (not A β 40).⁴⁶⁵ The interaction of A β 40 with SDS has been investigated by small-angle X-ray and neutron scattering (SAXS and SANS).⁴⁶⁶ It was shown by time-resolved SAXS that A β 40 rapidly forms a complex with pre-existing SDS micelles due to interaction between the sulfate groups and the hydrophilic headgroup units of the surfactant.⁴⁶⁶ Below the critical micelle concentration of SDS, globular core-shell aggregates were also formed but lagging behind the CD-observed transition in secondary structure of A β 40 from random coil to (predominantly) α -helical. These measurements also showed that aggregation of A β 40 in the presence of HCl can be monitored by SAXS. Aggregates of A β 42 with SDS (sub-micellar concentrations) have been observed via AFM, and the effect of SDS on secondary structure was probed by CD and FTIR.⁴⁶⁷ The cationic surfactant hexadecyl-*N*-methylpiperidinium bromide is able to inhibit A β aggregation well below its *cmc*, pointing to a mechanism not involving micellar solubilisation.⁴⁶⁸ A similar phenomenon is reported for a tetrameric quaternary ammonium cationic surfactant which can also disassemble existing A β 40 fibrils.⁴⁶⁹ Instead, it was suggested that a specific binding surface on A β 40 (absent for other amyloid forming proteins) is able to bind such amphiphilic molecules.⁴⁶⁸ A cationic surfactant containing an azobenzene moiety influences A β 40 fibrillization differently depending on its conformation, which can be photo-switched.⁴⁷⁰ The interaction of A β (12-28) with SDS has been investigated by CD and NMR, an α -helical conformation for residues 16-24 was noted (see also section 4.1).³⁷⁴

The interaction of A β 40 with the bio-derived surfactant surfactin is also dependent on the aggregation state of the surfactant.⁴⁷¹ Below the *cmc*, surfactin causes A β 40 to unfold and to fibrillize. Well above the *cmc*, β -sheet fibril formation is inhibited.

4.7 Interactions with Lipid Membranes

The importance of lipid interactions with A β is highlighted by the fact that ApoE, (especially the ϵ 4 allele) a key genetic risk factor for AD (section 2.1), is involved in lipid metabolism.⁴⁷² Lipid membranes have a number of important roles in modulating amyloid fibrillization. These include: (partially) unfolding the peptide, increasing the local concentration of peptide bound to the membrane, orienting the bound protein in an aggregation-prone manner and variation of penetration depth into the membrane affecting the nucleation propensity.⁴⁷³ Lipid rafts are implicated in A β dimer and oligomer formation.⁴⁷⁴ and may provide platforms for selective deposition of different A β aggregates (this also depends on the ordering of the lipids within the membranes which may be different in the rafts⁴⁷⁵).⁴⁷⁶ Ganglioside-rich lipid rafts induce A β oligomerization, for which cholesterol appears not to be essential.^{474b} The involvement of different types of membrane structure (vesicles formed by different phospholipids and gangliosides) in A β fibril growth has been discussed in reviews of membrane-amyloid interactions.^{224g,473,477} The role of membranes in the formation of annular structures that may comprise arrays of oligomers has been revealed by AFM.^{224g,226} and the role of oligomers in creating pores/ion channels has been revealed by membrane conductance measurements (see also section 2.7).^{215a}

A β is generated via regulated intramembrane proteolysis (RIP),^{6e} which involves the shedding of the ectodomain of APP through membrane-anchored secretases (section 2.2.3). The membrane-bound stubs can then be cleaved within their transmembrane domains to release small peptides (A β in the case of APP) into the extracellular space and intracellular domains into the cytoplasm.^{6e}

A β , which is a cationic peptide at neutral pH, (residue-specific pKa values are available^{374,478}) interacts with anionic lipid membranes through electrostatic interactions, depending on pH.^{224g,477a} On the other hand, it has been reported that A β can interact with cationic or zwitterionic lipids as readily as anionic lipids.⁴⁷⁹ This suggests that association of A β with lipid membranes is driven to a substantial extent by hydrophobic interactions with hydrophobic regions in the peptide. Preferential incorporation of A β into anionic lipid membranes is however noted.⁴⁸⁰ The interaction of A β with negatively charged lipids is driven by electrostatic interactions whereas insertion into the membrane is driven by the hydrophobic tail of A β . It has been demonstrated that A β 40 spontaneously inserts into anionic DPPG (1,2-dipalmitoyl phosphatidylglycerol) membranes but not zwitterionic DPPC (1,2-dipalmitoyl phosphatidylcholine) membranes.⁴⁸¹ Furthermore, the DPPG membrane induces β -sheet “crystallization” of A β ,⁴⁸¹ although this interaction was eliminated above pH 7.4 where A β becomes anionic. Enhanced binding of A β 40 (tryptophan labeled Y10W for fluorescence experiments) oligomers to vesicles has been observed when DPPG is incorporated as compared to pure DPPC vesicles.⁴⁸² Terzi *et al.* studied A β (25-35) [and A β (25-35Nle)] on anionic lipid membranes and noted an increase in β -sheet formation (in solution both random coil and β -sheet structures coexist for this peptide)

as probed by CD spectroscopy, in the presence of the negatively charged vesicles.⁴⁸³ These authors were also able to measure binding enthalpies.⁴⁸³ The C terminus capped version of this peptide however adopts a random coil structure on binding to the lipid vesicles, pointing to the role of electrostatics in the binding process, also confirmed by salt screening experiments.⁴⁸³ This group found that A β 40 can insert into anionic monolayers at sufficiently low packing density.⁴⁸⁰ These authors also noted transitions in secondary structure random coil – β -sheet – α -helix depending on the lipid-to-peptide ratio.⁴⁸⁰ A shift to an initial α -helical conformation has been noted upon binding of A β peptides to membranes,³⁷⁴ however the enrichment of peptide concentration close to the membrane may subsequently favour β -sheet formation.^{224g,477a} Thus, membranes can inhibit fibrillization at high lipid-to-peptide ratio, but accelerate it at low relative lipid concentration.^{224g} Biological lipids (in liposomes) can resolubilize A β 42 fibrils, and convert them into more toxic oligomeric forms, and the potential biological relevance of this was noted.⁴⁸⁴

A β can form cation-selective channels when incorporated in lipid bilayers as revealed by conductance measurements.^{225a-c,485} A β peptides disrupt membranes comprised of negatively charged phospholipids, in a pH-dependent manner (which is important in the context of different pH levels in endosomes vs. the extracellular matrix, for example).⁴⁸⁶ AFM shows the formation of channel structures by A β 42 reconstituted in planar lipid bilayers.^{226a} On the other hand, Kaye *et al.* report that A β oligomers increase permeability (quantified via conductance measurements) without any evidence for pore formation or ion selectivity.^{215a} Serpell and coworkers have observed that the A β aggregation state influences its ability to permeate vesicles (probed via calcein fluorescence measurements), and that oligomers have the highest

membrane disruption activity.⁴⁸⁷ The less fibrillogenic A β 40 has a reduced tendency to permeabilize membranes than A β 42. The role of GM1 ganglioside receptors was also highlighted.⁴⁸⁷

Nussinov and coworkers have performed MD simulations of A β in lipid bilayers, focussing on A β (17-42) protofibrils, and A β pore structures were examined.^{366,488} The formation of subunit structures within the channels was observed. The selectivity of the channels for Ca²⁺ observed experimentally was also confirmed from the models.⁴⁸⁸ Consistent with AFM images of A β 40 in a DOPC (dioleoyl phosphatidylcholine) bilayer,^{226b} break-up of the channels into subunits was observed (Fig.26).⁴⁸⁸ Strodel *et al.* have also performed MD simulations on A β pore structures, for A β 42, modeling oligomers.⁴⁸⁹ They found that membrane-spanning β -sheets adopt ordered configurations for dimers to hexamers, however separation into subunits was only observed for octameric oligomers which separated into distinct subunits (*cf.* Fig.24).⁴⁸⁹ A molecular dynamics simulation of the interaction between A β 42 and zwitterionic and anionic lipids indicated that peptide-peptide interactions are favoured in the vicinity of the membrane, driving oligomerization in the case of the anionic lipid membranes.⁴⁹⁰ The dependence on pH was also modelled.

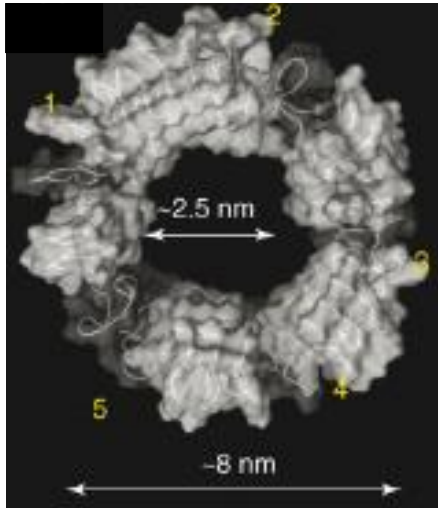


Fig.26. MD simulation of a 24-mer channel formed by A β (17-42) in a DOPC lipid bilayer.^{366,488} The yellow numbers label sub-units. Reprinted from Jang, H. B. *et al. Trends in Biochemical Science* **2008**, 33, 91, Copyright 2008, with permission from Elsevier.

The binding of A β and gangliosides [ganglioside = glycosphingolipid] has been the subject of several studies, although with conflicting conclusions.^{477a,491} Ganglioside membranes have been reported to accelerate A β aggregation^{300,474c} or to inhibit it and stabilize α -helical structures⁴⁹² or to induce β -sheet structure.⁴⁹³ Another report claims that gangliosides induce A β to adopt a mixed α/β conformation at neutral pH.⁴⁸⁶ Actually, a transition from random coil to α -helix might be favoured at low peptide-ganglioside lipid ratios, whereas higher ratios promote the adoption of a β -sheet conformation.^{224g,493b} It has been proposed that ganglioside clusters may form sites that seed A β fibril formation.^{224g,477a} The interaction of A β 40 and A β 42 with phosphatidylinositol (PI) with different inositol headgroups was examined.²⁷⁶ The

formation of β -sheet structures was found to be pH-dependent in the presence of PI vesicles – both A β 40 and A β 42 aggregate at pH 6 (close to the pI = 5.5)^{491,494} but only A β 42 aggregates at pH 7. The work with different PI vesicles formed part of the basis for the development of an inositol-based inhibitor of A β aggregation,²⁷⁶ as discussed further in Section 3.2.1. formation of A β 40 fibrils is accelerated in the presence of ganglioside-containing POPC (1-palmitoyl-2-oleoyl phosphatidylcholine) vesicles.^{300,495} The binding of the peptide to the membrane was analysed quantitatively. The binding was found to be ganglioside-specific.^{300,495} AFM and TEM imaging show aggregation and fibril formation of A β 40 and A β 42 on total brain extract lipids.⁴⁹⁶

Bokvist *et al.* used MAS-NMR (MAS: magic angle spinning) and CD to probe the interaction of A β 40 in different lipid membranes.⁴⁹⁷ Charged membranes can act as templates for aggregation of surface-associated A β 40 in the case that the peptide is released in soluble form. However, membrane inserted A β 40 is anchored by the K28 residue by electrostatic interactions with negatively charged lipids.⁴⁹⁷⁻⁴⁹⁸ By studying a series of full length peptides and fragments, Chauhan *et al.* were able to highlight the role of aliphatic residues at the C terminus of A β interacting with the fatty acid chains as well as the electrostatic interaction involving K28.⁴⁹⁸ Several groups have noted that the interaction of A β with membranes depends on the ordering of the lipid membranes.^{479b,499} Murphy and coworkers used fluorescence anisotropy of an inserted probe to examine interaction of A β 40 with lipid membranes.^{479b,491} They observe that A β aggregates only affect lipid membrane fluidity above, and not below, the lipid chain melting temperature.^{479b} Aggregated A β decreases membrane fluidity, but A β

monomer does not. Aggregation rate and surface hydrophobicity were greater for A β 40 prepared at pH 6 compared to pH 7.⁴⁹¹ However, Wood *et al.* observe that A β 40 does not form amyloid fibrils at pH 5.8 (approximate pH of endosomes and conditions for proteolytic cleavage), but rather forms larger aggregates which lead to turbidity of the solution.⁴²² Inhibition of A β fibril formation was observed to depend on the state of the lipid membrane in studies of the interaction of A β (M1-40) and A β (M1-42) (recombinantly expressed in *E. Coli*, Section 2.2.2) with liposomes.⁴⁹⁹ The largest retardation is observed when DPPC bilayers are in the solid gel phase. The mobility of A β 42 prefibrillar and fibrillar oligomers on the membrane of living cells or lipid membranes has been investigated via single particle tracking techniques (using quantum dots as labels).⁵⁰⁰ The dynamic behaviour is distinct depending on the aggregation state (and conformation) of the peptide, although motion for oligomers is largely confined. Diffusion coefficients were obtained and differ significantly for fibrillar and prefibrillar oligomers recognized by the A11 antibody.⁵⁰⁰

The insertion of the transmembrane fragment A β (25-35) into phospholipid bilayers has been examined by neutron diffraction using deuterium labeling techniques.⁵⁰¹ The location of the peptide C terminus was studied in two different lipid compositions and was found to be dependent on the surface charge of the membrane (zwitterionic or anionic). The adsorption of A β 40 on phospholipid monolayers has been probed using grazing incidence x-ray diffraction and IRRAS.⁵⁰² On negatively charged monolayers, the peptide adsorbs at the air-water interface and inserts into the monolayer, although it is squeezed out at high surface pressure. A β 40 can also penetrate into disordered anionic monolayers in buffer due to salt screening of electrostatic interactions.⁵⁰² The influence of A β (25-35) and A β (22-40) on the picosecond dynamics of lipid

membranes has been examined by quasi-elastic neutron scattering.⁵⁰³ The main influence was on long-range translational diffusion, although localized diffusion was also considered. By selection of the A β (11-22) fragment, the role of charge, and the influence of pH, in governing the interaction of A β with lipids was examined.⁵⁰⁴ Only at endosomal pH (approximately 6) does the peptide insert into negatively charged membranes, with a conformation change (increase in α -helix content in the presence of lipids) detected by CD. Specifically, the protonation state of H13 and H14 was found to be important (as in the case of interactions with metal ions, section 4.8).⁵⁰⁴

Aggregation of A β 42 proceeds with distinct aggregate morphology (and kinetics) when adsorbed on hydrophilic mica or hydrophobic graphite.⁵⁰⁵ and this was related to fibrillization at interfaces such as membranes.

The role of cholesterol in AD has been examined due to the mutual interaction of A β and cholesterol (the metabolism of which is modulated by ApoE).^{472a,474c,477a,506}

Cholesterol mediates A β aggregation and reciprocally A β influences cholesterol dynamics in neurons, leading to tauopathy.^{472a} Whether cholesterol inhibits or promotes A β fibrillization may depend on its content within the membrane,^{224g,507} and the presence of metal ions.⁵⁰⁸ Serum and CSF levels of cholesterol may provide a biomarker for AD (others are discussed in Section 2.4) although the relationship between HDL (high density lipoprotein) level and AD is not clearly established at present.^{472a} A correlation between cholesterol (uncharged) content in the model membranes and A β 42 deposition was noted, due to the effect of cholesterol on membrane rigidity (addition of A β 40 increased vesicle rigidity).^{496a} The structure of lipid membranes extracted from AD patient brain tissue has been compared to control,

using SAXS to obtain electron density profiles and differences were ascribed to the increase in cholesterol level in the AD brain.⁵⁰⁹ The changes in membrane rigidity in turn influence Ca^{2+} ion transport across neuronal cell membranes.⁴⁸⁵ An inverse correlation between membrane cholesterol level and $\text{A}\beta$ -cell surface binding and cytotoxicity was observed.^{496a} The role of cholesterol in amyloid aggregation and tau phosphorylation has been reviewed.^{472,510} Statins may have a role in alleviating AD pathologies associated with cholesterol since they can influence cholesterol metabolism in the human brain.^{472a,506,511} Several cholesterol derivatives have been found to enhance $\text{A}\beta_{42}$ fibrillization, and aspirin can inhibit this cholesterol-mediated fibrillization.⁵¹² The formation of the GM1 ganglioside-bound $\text{A}\beta$ (GM1/ $\text{A}\beta$) complex (found in the brains of AD patients and proposed to seed $\text{A}\beta$ aggregation) is cholesterol-dependent.⁵¹³ Cholesterol byproducts from antibody-induced ozonolysis during inflammation have been detected in human brains.⁵¹⁴ These compounds which contain aldehydes dramatically accelerate $\text{A}\beta$ aggregation *in vitro*. The authors note that these observations relate to some common features of AD and atherosclerosis, in particular in terms of inflammation.⁵¹⁴ The mechanism of interaction of these cholesterol metabolites with $\text{A}\beta_{40}$ was subsequently analysed in detail.⁵¹⁵

4.8 Effect of Metal Ions

Aggregation of $\text{A}\beta$ may be promoted by metal ions.^{16d,516} High concentrations of metal ions (Cu^{2+} , Fe^{3+} , Zn^{2+} , Al^{3+} ...) are found to be co-localized at abnormally high concentration with senile plaques in AD brains.⁵¹⁷ $\text{A}\beta$ rapidly aggregates in the presence of physiological concentrations of Zn^{2+} at pH 7.4.^{106b,518} Enhanced Cu^{2+} -induced aggregation is noted when the pH is lowered to 6.8.^{518b} Mildly acidic

conditions are often associated with inflammation. APP binds copper in the N terminal domain within the APP(135-175) sequence.^{16d} The copper binding domain contains a His-X-His motif.^{16d} Metal ions are involved in processes associated with inflammation in AD patients (*vide infra*). Treatment with metal ion chelators can reduce the deposition of A β in brains, as discussed further in section 3.2.1.^{242a,242d,259} The focus of most studies has been Cu²⁺, Zn²⁺, Al³⁺ and Fe³⁺, which enhance fibril formation.

Transition metal ions may interfere with transport across ion channels, eg. Al³⁺ ions^{225a} and Zn²⁺ ions^{225d,226a,229,266b} have been shown to block these channels. In the latter case, this can be reversed using a Zn²⁺ chelator.^{225d} Arispe *et al.* also point to the formation of channels in bilayer membranes as a possible tool to screen for possible therapeutic compounds.^{225a} Zn²⁺ and Cu²⁺ ions induce A β 42 and A β 40 insertion into vesicles in a suitable pH range with an accompanying formation of α -helical structures.⁵⁰⁸

Surface plasmon resonance (SPR) biosensing has been used to investigate the aggregation of A β 40 in the presence of Cu²⁺, Ca²⁺, Fe²⁺ and Fe³⁺.⁵¹⁹ All ions promoted A β aggregation, but with different rate constants, that for Cu²⁺ being highest, although the aggregates were unstable. The chelator EDTA (ethylenediamine tetraacetic acid) can dissociate metal-ion induced A β aggregates.⁵¹⁹ Rottkamp *et al.* showed that if A β is pretreated with the iron chelator deferoxamine, neuronal toxicity is significantly reduced while conversely, incubation of A β with excess free iron restores toxicity to original levels.⁵²⁰

The aggregation of human A β is stimulated by the presence of zinc ions at sufficiently high concentration due to binding mediated by histidine.^{106b} The importance of the H13 residue in A β in binding Cu²⁺ and Zn²⁺ in a pH-dependent manner was highlighted (the other N terminal histidines H6 and H14 also play a role).^{259b,518b,521} The Cu²⁺ binding site can also involve D1 or E11.⁵²² A solid state NMR study has recently elucidated the Cu²⁺ binding site of A β 40.⁵²³ It was reported that under physiological conditions, aluminium, iron, and zinc strongly promote A β aggregation (rate enhancement of 100-1,000-fold) whereas the other metal ions (including calcium, copper and sodium) studied do not.⁵²⁴ The aggregation of A β induced by iron or aluminium ions is distinguished from that of Zn²⁺ by its rate, extent and pH- and temperature-dependence as probed via sedimentation experiments using ¹²⁵I-labelled A β (1-40).⁵²⁴ It has been proposed that rapid formation of a pre-oligomeric peptide/metal/peptide complex follows binding of Cu²⁺ to A β , leading to inhibition of oligomer formation,⁵²⁵ as observed at low Cu²⁺ concentration.⁵²⁶ Ascorbate-dependent hydroxyl radical generation, is inhibited by A β (1-16) or A β 42 for Cu²⁺ or Fe³⁺.^{521e}

AFM imaging directly illustrates the influence of copper and zinc ions on the aggregation of A β 42, preventing fibrillization even in trace amounts.⁵²⁷ Using immobilized A β seeds (Section 4.3.1), Cu²⁺ and Zn²⁺ at neutral pH are found to accelerate the deposition of A β 40 and A β 42 but produce amorphous aggregates whereas Fe³⁺ induces the formation of fibrils.^{418b} The effects of mixtures of Cu²⁺, Zn²⁺ and Fe³⁺ ions on A β 42 aggregation has also been examined via AFM using the same technique.⁵²⁸

The role of aluminium in the etiology of AD is controversial.^{516d,529} It was originally found to be associated with plaques in AD patients, in the form of aluminosilicates⁵³⁰ specifically associated with neurofibrillary tangles.^{529b,531} Aluminium has significantly higher cytotoxicity in complexes with A β 42 than Cu²⁺, Zn²⁺ and Fe³⁺ and the aggregation properties of A β in the presence of Al³⁺ are also substantially different.⁵³²

The effect of metal ions on A β aggregation has been correlated to oxidative stress.^{517b,520} Both iron and copper have high affinity for A β and are reduced by it, with the subsequent production of hydrogen peroxide and oxidised A β .⁵³³ Iron has been implicated as a key species in oxidative stress, due to its involvement in the creation of free radicals (in particular hydroxyl radicals^{517b}) from H₂O₂ via the Fenton reaction.⁵³⁴ It has been suggested that iron enhances the toxicity of A β by delaying the deposition of the peptide into well-defined fibrils.⁵³⁵ The cytotoxic effects of A β can be attenuated by antioxidants and free radical scavengers such as vitamin E.

The zinc binding motif, strongly conserved among members of the APP family has been identified.⁵³⁶ APP can catalyze the reduction of H₂O₂ and ensuing oxidation of Cu⁺ to Cu²⁺ in a peroxidative reaction *in vitro*, leading via a Fenton-type reaction to free radical formation.^{536c}

4.9 Conjugates of A β with Polymers and Lipid Chains

Conjugation of peptides to synthetic polymers such as PEG may lead to improved solubility, enhanced stability against dilution, reduced toxicity and

immunogenicity.⁵³⁷ The solution self-assembly of peptide-containing copolymers has been reviewed.⁵³⁸

In a pioneering series of papers, Meredith and coworkers have confirmed the formation of fibrils in aqueous solutions of PEG-peptide diblocks where the peptide block was based on the central hydrophobic domain A β (10-35) of the β -amyloid peptide and the PEG block had a molar mass of 3000 g mol⁻¹.⁵³⁹ They found from SANS and TEM that the PEG forms a coating around the fibril, thus acting as a “steric stabilization” layer. The self-assembly in aqueous solution of PEG-peptides, with peptide sequences based on KLVFF A β (16-20) motif has been investigated, with PEG molar mass in the range 1000 – 3000 g mol⁻¹. Conjugates FFKLVFF-PEG⁵⁴⁰ and YYKLVFF-PEG⁵⁴¹ form core-shell fibrils and aggregation into nematic and hexagonal columnar liquid crystal phases is observed at high concentration. Conjugate β A β AKLVFF-PEG was used in studies of enzymatic cleavage (using α -chymotrypsin to cleave between the two phenylalanine residues).⁵⁴² The conjugate forms spherical micelles which are degraded by the enzyme, releasing peptide β A β AKLVF (which does not aggregate into amyloid).

The formation of amyloid-like structures at the air/water interface was reported for peptide amphiphiles (PAs) with a peptide sequence based on A β (31-35), i.e. IIGLM, attached to a C₁₈ chain.⁵⁴³ Epifluorescence microscopy showed the formation of threadlike and needle-like aggregates. Yilin Wang's group have shown that PA C₁₂-A β (11-17) forms fibrils.⁵⁴⁴ The double tail analogue PAs 2C₁₂-Lys-A β (12-17) and C₁₂-A β (11-17)-C₁₂ self-assemble into fibrils or twisted ribbons/tapes respectively, the latter dependent on pH.⁵⁴⁵

5. SUMMARY AND OUTLOOK

This review has focussed on the biological and biophysical properties of the Amyloid β ($A\beta$) peptide and its role in Alzheimer's disease. The biological, biochemical and neurochemical characteristics of AD, involving $A\beta$ and APP have been discussed. Therapeutic treatments including those on the market, or those that have been or continue to be the subject of clinical trials have been described. Finally, aspects of the biophysical chemistry of $A\beta$ and $A\beta$ fragment peptides have been outlined, including various aspects of structure at the molecular and supra-molecular level, and aggregation mechanisms and kinetics.

It is now more than a century since Alzheimer identified the disease now named after him, and more than a quarter century since aggregation of amyloid beta peptide was first associated with the condition. As a major global healthcare challenge, there has been intense research activity in this period. This has led to deep insights into the causative agents, the current consensus being that $A\beta$ is the primary suspect.

However, the progression of AD is not straightforward, and other factors are almost certainly involved, including other proteins such as tau, but also influences such as oxidative stress. Further large-scale genetic and proteomic screening studies will almost certainly reveal other risk factors.

A number of compounds are available to ameliorate the early stage symptoms of AD, however there is still no effective treatment that can halt or reverse progression into the debilitating late stage of the disease. Several approaches have led to compounds

that have reached phase III clinical trials, some of which failed. However, given the complexity associated with treatment of brain disease and the fact that it involves a subtle target, i.e. a protein misfolding process, some setbacks along the road are probably inevitable. The problem of finding a selective therapeutic agent is challenging given the diversity of biochemical pathways involved in brain signalling and other neuronal growth and differentiation processes. Nonetheless, there is reason for optimism as clinical trials of a number of therapeutic agents continue. There has been some refocusing by big pharma in the brain (and central nervous system) disease field, but some significant players are still very active in R&D related to AD therapies. Furthermore, governments internationally are increasingly recognising the magnitude of the problem of diseases of aging, especially AD, and there is currently substantial investment in further research through various funding agencies and networks. Probably closer to realisation are effective diagnostic systems based on blood or plasma analysis, or brain scanning methods.

In terms of biophysical measurements, it has to be noted that A β is a difficult peptide to work with, in the sense that its aggregation properties are highly sensitive to sequence, purity and preparation conditions. The latter include initial dispersal solvent, nature and concentration of the aqueous or buffer solutions, but also the effect of shear during mixing and potentially the nature of the surface of the vessel. Small differences in preparation conditions can lead to distinct polymorphs, which can propagate, as discussed in section 4.4.

Despite these caveats, there is now a great wealth of data on the fibrillization properties of A β , variants and fragments under defined conditions. These

measurements provide a strong framework to underpin the ongoing biological research activity.

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References

- (1) (a) Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741. (b) Selkoe, D. J. *Ann. Intern. Med.* **2004**, *140*, 627. (c) Shankar, G. M.; Walsh, D. M. *Mol. Neurodegener.* **2009**, *4*.
- (2) (a) Goedert, M.; Spillantini, M. G. *Science* **2006**, *314*, 777. (b) Hamley, I. W. *Angew. Chem., Int. Ed. Engl.* **2007**, *46*, 8128. (c) Irvine, G. B.; El-Agnaf, O. M. A.; Shankar, G. M.; Walsh, D. M. *Molecular Medicine* **2008**, *14*, 451. (d) Glabe, C. G. *Neurobiology of Aging* **2006**, *27*, 570.
- (3) Selkoe, D. J. *Nature Medicine* **2011**, *17*, 1060.
- (4) (a) Roberson, E. D.; Scarce-Levie, K.; Palop, J. J.; Yan, F. R.; Cheng, I. H.; Wu, T.; Gerstein, H.; Yu, G. Q.; Mucke, L. *Science* **2007**, *316*, 750. (b) Vossel, K. A.; Zhang, K.; Brodbeck, J.; Daub, A. C.; Sharma, P.; Finkbeiner, S.; Cui, B. X.; Mucke, L. *Science* **2010**, *330*, 198.
- (5) (a) Ueda, K.; Fukushima, H.; Masliah, E.; Xia, Y.; Iwai, A.; Yoshimoto, M.; Otero, D. A. C.; Kondo, J.; Ihara, Y.; Saitoh, T. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 11282. (b) Bodles, A. M.; Guthrie, D. J. S.; Greer, B.; Irvine, G. B. *J. Neurochem.* **2001**, *78*, 384.
- (6) (a) Harper, J. D.; Lansbury, P. T. *Annu. Rev. Biochem.* **1997**, *66*, 385. (b) Teplow, D. B. *Amyloid: Int. J. Exp. Clin. Invest.* **1998**, *5*, 121. (c) Howlett, D. R. In *Bioimaging in Neuroscience*; Broderick, P. A., Rahni, D. N., Kolodny, E. H., Eds.; Humana: Totowa, NJ, 2005, p 61. (d) Lansbury, P. T.; Lashuel, H. A. *Nature* **2006**, *443*, 774. (e) Haass, C.; Selkoe, D. J. *Nature Reviews Molecular Cell Biology* **2007**, *8*, 101. (f) DeToma, A. S.; Salamekh, S.; Ramamoorthy, A.; Lim, M. H. *Chem. Soc. Rev.* **2012**, *41*, 608.
- (7) Abbott, A. *Nature* **2011**, *475*, S2.

- (8) Mount, C.; Downton, C. *Nature Medicine* **2006**, *12*, 780.
- (9) Luengo-Fernandez, R.; Leal, J.; Gray, A. † Dementia 2010, ‡ Health Economics Research Centre, 2010.
- (10) Tanzi, R. E. In *Scientific American Molecular Neurology*; Martin, J. B., Ed.; Scientific American: New York, 1998.
- (11) Gandy, S. *Nature* **2011**, *475*, S15.
- (12) (a) Mucke, L. *Nature (London)* **2009**, *461*, 895. (b) Deweerdt, S. *Nature* **2011**, *475*, S16.
- (13) Halagappa, V. K. M.; Guo, Z. H.; Pearson, M.; Matsuoka, Y.; Cutler, R. G.; LaFerla, F. M.; Mattson, M. P. *Neurobiol. Dis.* **2007**, *26*, 212.
- (14) (a) Melnikova, I. *Nature Rev. Drug Disc.* **2007**, *6*, 341. (b) Sabbagh, M. N. *American Journal of Geriatric Pharmacotherapy* **2009**, *7*, 167.
- (15) Hsia, A. Y.; Masliah, E.; McConlogue, L.; Yu, G. Q.; Tatsuno, G.; Hu, K.; Kholodenko, D.; Malenka, R. C.; Nicoll, R. A.; Mucke, L. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 3228.
- (16) (a) Evans, D. A.; Funkenstein, H.; Albert, M. S.; Scherr, P. A.; Cook, N. R.; Chown, M. J.; Hebert, L. E.; Hennekens, C. H.; Taylor, J. O. *J. Am. Med. Assoc.* **1989**, *262*, 2551. (b) Kukull, W. A.; Bowen, J. D. *Med. Clin. N. Am.* **2002**, *86*, 573. (c) Kukull, W. A.; Higdon, R.; Bowen, J. D.; McCormick, W. C.; Teri, L.; Schellenberg, G. D.; van Belle, G.; Jolley, L.; Larson, E. B. *Arch. Neurol.* **2002**, *59*, 1737. (d) Brown, D. R. *Dalton Trans.* **2009**, *21*, 4069.
- (17) Markesbery, W. R. *Free Radical Biology and Medicine* **1997**, *23*, 134.
- (18) Akiyama, H.; Barger, S.; Barnum, S.; Bradt, B.; Bauer, J.; Cole, G. M.; Cooper, N. R.; Eikelenboom, P.; Emmerling, M.; Fiebich, B. L.; Finch, C. E.; Frautschy, S.; Griffin, W. S. T.; Hampel, H.; Hull, M.; Landreth, G.; Lue, L.

- F.; Mrak, R.; Mackenzie, I. R.; McGeer, P. L.; O'Banion, M. K.; Pachter, J.; Pasinetti, G.; Plata-Salaman, C.; Rogers, J.; Rydel, R.; Shen, Y.; Streit, W.; Strohmeyer, R.; Tooyoma, I.; Van Muiswinkel, F. L.; Veerhuis, R.; Walker, D.; Webster, S.; Wegrzyniak, B.; Wenk, G.; Wyss-Coray, T. *Neurobiology of Aging* **2000**, *21*, 383.
- (19) Hansson, O.; Zetterberg, H.; Buchhave, P.; Londos, E.; Blennow, K.; Minthon, L. *Lancet Neurol.* **2006**, *5*, 228.
- (20) Sperling, R. A.; Aisen, P. S.; Beckett, L. A.; Bennett, D. A.; Craft, S.; Fagan, A. M.; Iwatsubo, T.; Jack, C. R.; Kaye, J.; Montine, T. J.; Park, D. C.; Reiman, E. M.; Rowe, C. C.; Siemers, E.; Stern, Y.; Yaffe, K.; Carrillo, M. C.; Thies, B.; Morrison-Bogorad, M.; Wagster, M. V.; Phelps, C. H. *Alzheimers. Dement.* **2011**, *7*, 280.
- (21) Bishop, N. A.; Lu, T.; Yankner, B. A. *Nature* **2010**, *464*, 529.
- (22) Fischer, D. F.; van Dijk, R.; van Tijn, P.; Hobo, B.; Verhage, M. C.; van der Schors, R. C.; Li, K. W.; van Minnen, J.; Hol, E. M.; van Leeuwen, F. W. *Neurobiology of Aging* **2009**, *30*, 847.
- (23) Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
- (24) Dickson, D. W. *J. Neuropath. Exp. Neurol.* **1997**, *56*, 321.
- (25) (a) Roher, A. E.; Palmer, K. C.; Yurewicz, E. C.; Ball, M. J.; Greenberg, B. D. *J. Neurochem.* **1993**, *61*, 1916. (b) McLean, C. A.; Cherny, R. A.; Fraser, F. W.; Fuller, S. J.; Smith, M. J.; Beyreuther, K.; Bush, A. I.; Masters, C. L. *Ann. Neurol.* **1999**, *46*, 860. (c) Lue, L. F.; Kuo, Y. M.; Roher, A. E.; Brachova, L.; Shen, Y.; Sue, L.; Beach, T.; Kurth, J. H.; Rydel, R. E.; Rogers, J. *Am. J. Pathol.* **1999**, *155*, 853. (d) Näslund, J.; Haroutunian, V.; Mohs, R.; Davis, K.

- L.; Davies, P.; Greengard, P.; Buxbaum, J. D. *J. Am. Med. Assoc.* **2000**, 283, 1571.
- (26) Blennow, K.; Hampel, H.; Weiner, M.; Zetterberg, H. *Nat. Rev. Neurol.* **2010**, 6, 131.
- (27) (a) Eanes, E. D.; Glenner, G. G. *J. Histochem. Cytochem.* **1968**, 16, 673. (b) Kirschner, D. A.; Abraham, C.; Selkoe, D. J. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, 83, 503. (c) Sunde, M.; Blake, C. C. F. *Adv. Protein Chem.* **1997**, 50, 123. (d) Serpell, L. C. *Biochim. Biophys. Acta* **2000**, 1502, 16. (e) Makin, O. S.; Serpell, L. C. *FEBS J* **2005**, 272, 5950.
- (28) Walsh, D. M.; Thulin, E.; Minogue, A. M.; Gustavsson, N.; Pang, E.; Teplow, D. B.; Linse, S. *FEBS J* **2009**, 276, 1266.
- (29) Selkoe, D. J. *Nature Medicine* **2011**, 17, 1521.
- (30) Schnabel, J. *Nature* **2011**, 475, S12.
- (31) Roberson, E. D.; Mucke, L. *Science* **2006**, 314, 781.
- (32) Glenner, G. G.; Wong, C. W. *Biochem. Biophys. Res. Commun.* **1984**, 120, 885.
- (33) (a) Masters, C. L.; Simms, G.; Weinman, N. A.; Multhaup, G.; McDonald, B. L.; Beyreuther, K. *Proc. Natl. Acad. Sci. U. S. A.* **1985**, 82, 4245. (b) Tanzi, R. E.; Gusella, J. F.; Watkins, P. C.; Bruns, G. A. P.; StGeorgehyslop, P.; Vankeuren, M. L.; Patterson, D.; Pagan, S.; Kurnit, D. M.; Neve, R. L. *Science* **1987**, 235, 880. (c) Kang, J.; Lemaire, H. G.; Unterbeck, A.; Salbaum, J. M.; Masters, C. L.; Grzeschik, K. H.; Multhaup, G.; Beyreuther, K.; Mullerhill, B. *Nature* **1987**, 325, 733. (d) Goldgaber, D.; Lerman, M. I.; McBride, O. W.; Saffiotti, U.; Gajdusek, D. C. *Science* **1987**, 235, 877. (e) Robakis, N. K.; Ramakrishna, N.; Wolfe, G.; Wisniewski, H. M. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, 84, 4190.

- (34) Jarrett, J. T.; Berger, E. P.; Lansbury, P. T. *Biochemistry* **1993**, *32*, 4693.
- (35) (a) Qi-Takahara, Y.; Morishima-Kawashima, M.; Tanimura, Y.; Dolios, G.; Hirotsu, N.; Horikoshi, Y.; Kametani, F.; Maeda, M.; Saido, T. C.; Wang, R.; Ihara, Y. *J. Neurosci.* **2005**, *25*, 436. (b) Welander, H.; Franberg, J.; Graff, C.; Sundstrom, E.; Winblad, B.; Tjernberg, L. O. *J. Neurochem.* **2009**, *110*, 697.
- (36) Roher, A. E.; Lowenson, J. D.; Clarke, S.; Wolkow, C.; Wang, R.; Cotter, R. J.; Reardon, I. M.; Zurchernecky, H. A.; Heinrikson, R. L.; Ball, M. J.; Greenberg, B. D. *J. Biol. Chem.* **1993**, *268*, 3072.
- (37) Ida, N.; Hartmann, T.; Pantel, J.; Schroder, J.; Zerfass, R.; Forstl, H.; Sandbrink, R.; Masters, C. L.; Beyreuther, K. *J. Biol. Chem.* **1996**, *271*, 22908.
- (38) Roher, A. E.; Lowenson, J. D.; Clarke, S.; Woods, A. S.; Cotter, R. J.; Gowing, E.; Ball, M. J. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 10836.
- (39) (a) Bucciantini, M.; Giannoni, E.; Chiti, F.; Baroni, F.; Formigli, L.; Zurdo, J.; Taddei, N.; Ramponi, G.; Dobson, C. M.; Stefani, M. *Nature* **2002**, *416*, 507. (b) Dobson, C. M. *Trends Biochem. Sci.* **1999**, *24*, 329. (c) Bucciantini, M.; Calloni, G.; Chiti, F.; Formigli, L.; Nosi, D.; Dobson, C. M.; Stefani, M. *J. Biol. Chem.* **2004**, *279*, 31374.
- (40) Walsh, D. M.; Tseng, B. P.; Rydel, R. E.; Podlisny, M. B.; Selkoe, D. J. *Biochemistry* **2000**, *39*, 10831.
- (41) LaFerla, F. M.; Green, K. N.; Oddo, S. *Nat. Rev. Neurosci.* **2007**, *8*, 499.
- (42) (a) Kokmen, E.; Whisnant, J. P.; Ofallon, W. M.; Chu, C. P.; Beard, C. M. *Neurology* **1996**, *46*, 154. (b) Moroney, J. T.; Bagiella, E.; Desmond, D. W.; Paik, M. C.; Stern, Y.; Tatemichi, T. K. *Stroke* **1996**, *27*, 1283. (c) Tatemichi, T. K.; Paik, M.; Bagiella, E.; Desmond, D. W.; Stern, Y.; Sano, M.; Hauser,

- W. A.; Mayeux, R. *Neurology* **1994**, *44*, 1885. (d) Kalaria, R. N. *Neurobiology of Aging* **2000**, *21*, 321.
- (43) (a) Abe, K.; Tanzi, R. E.; Kogure, K. *Neurosci. Lett.* **1991**, *125*, 172. (b) Kalaria, R. N.; Bhatti, S. U.; Palatinsky, E. A.; Pennington, D. H.; Shelton, E. R.; Chan, H. W.; Perry, G.; Lust, W. D. *Neuroreport* **1993**, *4*, 211. (c) Kogure, K.; Kato, H. *Stroke* **1993**, *24*, 2121. (d) Koistinaho, J.; Pyykonen, I.; Keinanen, R.; Hokfelt, T. *Neuroreport* **1996**, *7*, 2727. (e) Yokota, M.; Saido, T. C.; Tani, E.; Yamaura, I.; Minami, N. *J. Cereb. Blood Flow Metab.* **1996**, *16*, 1219. (f) Jendroska, K.; Hoffmann, O. M.; Patt, S. *Ann. N. Y. Acad. Sci.* **1997**, *826*, 401. (g) Marshall, A. J.; Rattray, M.; Vaughan, P. F. T. *Brain Res.* **2006**, *1099*, 18.
- (44) Brody, D. L.; Magnoni, S.; Schwetye, K. E.; Spinner, M. L.; Esparza, T. J.; Stocchetti, N.; Zipfel, G. J.; Holtzman, D. M. *Science* **2008**, *321*, 1221.
- (45) De Strooper, B.; Saftig, P.; Craessaerts, K.; Vanderstichele, H.; Guhde, G.; Annaert, W.; Von Figura, K.; Van Leuven, F. *Nature* **1998**, *391*, 387.
- (46) Phiel, C. J.; Wilson, C. A.; Lee, V. M. Y.; Klein, P. S. *Nature* **2003**, *423*, 435.
- (47) (a) Schmechel, D. E.; Saunders, A. M.; Strittmatter, W. J.; Crain, B. J.; Hulette, C. M.; Joo, S. H.; Pericakvance, M. A.; Goldgaber, D.; Roses, A. D. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 9649. (b) Saunders, A. M.; Strittmatter, W. J.; Schmechel, D.; Georgehyslop, P. H. S.; Pericakvance, M. A.; Joo, S. H.; Rosi, B. L.; Gusella, J. F.; Crappermaclachlan, D. R.; Alberts, M. J.; Hulette, C.; Crain, B.; Goldgaber, D.; Roses, A. D. *Neurology* **1993**, *43*, 1467. (c) Corder, E. H.; Saunders, A. M.; Strittmatter, W. J.; Schmechel, D. E.; Gaskell, P. C.; Small, G. W.; Roses, A. D.; Haines, J. L.; Pericakvance, M. A. *Science* **1993**, *261*, 921. (d) Bales, K. R.; Verina, T.; Dodel, R. C.; Du, Y. S.; Altstiel,

- L.; Bender, M.; Hyslop, P.; Johnstone, E. M.; Little, S. P.; Cummins, D. J.; Piccardo, P.; Ghetti, B.; Paul, S. M. *Nature Genetics* **1997**, *17*, 263.
- (48) (a) Strittmatter, W. J.; Weisgraber, K. H.; Huang, D. Y.; Dong, L. M.; Salvesen, G. S.; Pericakvance, M.; Schmechel, D.; Saunders, A. M.; Goldgaber, D.; Roses, A. D. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 8098. (b) Bales, K. R.; Liu, F.; Wu, S.; Lin, S. Z.; Koger, D.; DeLong, C.; Hansen, J. C.; Sullivan, P. M.; Paul, S. M. *J. Neurosci.* **2009**, *29*, 6771.
- (49) Selkoe, D. J. *Nature Medicine* **2011**, *17*, 1693.
- (50) Hayden, E. C. *Nature* **2008**, *455*, 1155.
- (51) Brodbeck, J.; Balestra, M. E.; Saunders, A. M.; Roses, A. D.; Mahley, R. W.; Huang, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 1343.
- (52) Goate, A.; Chartierharlin, M. C.; Mullan, M.; Brown, J.; Crawford, F.; Fidani, L.; Giuffra, L.; Haynes, A.; Irving, N.; James, L.; Mant, R.; Newton, P.; Rooke, K.; Roques, P.; Talbot, C.; Pericakvance, M.; Roses, A.; Williamson, R.; Rossor, M.; Owen, M.; Hardy, J. *Nature* **1991**, *349*, 704.
- (53) (a) Sherrington, R.; Rogaev, E. I.; Liang, Y.; Rogaeva, E. A.; Levesque, G.; Ikeda, M.; Chi, H.; Lin, C.; Li, G.; Holman, K.; Tsuda, T.; Mar, L.; Foncin, J. F.; Bruni, A. C.; Montesi, M. P.; Sorbi, S.; Rainero, I.; Pinessi, L.; Nee, L.; Chumakov, I.; Pollen, D.; Brookes, A.; Sanseau, P.; Polinsky, R. J.; Wasco, W.; Dasilva, H. A. R.; Haines, J. L.; Pericakvance, M. A.; Tanzi, R. E.; Roses, A. D.; Fraser, P. E.; Rommens, J. M.; St George-Hyslop, P. H. *Nature* **1995**, *375*, 754. (b) Rogaev, E. I.; Sherrington, R.; Rogaeva, E. A.; Levesque, G.; Ikeda, M.; Liang, Y.; Chi, H.; Lin, C.; Holman, K.; Tsuda, T.; Mar, L.; Sorbi, S.; Nacmias, B.; Piacentini, S.; Amaducci, L.; Chumakov, I.; Cohen, D.;

- Lannfelt, L.; Fraser, P. E.; Rommens, J. M.; St George-Hyslop, P. H. *Nature* **1995**, *376*, 775.
- (54) Lambert, J. C.; Heath, S.; Even, G.; Campion, D.; Sleegers, K.; Hiltunen, M.; Combarros, O.; Zelenika, D.; Bullido, M. J.; Tavernier, B.; Letenneur, L.; Bettens, K.; Berr, C.; Pasquier, F.; Fievet, N.; Barberger-Gateau, P.; Engelborghs, S.; De Deyn, P.; Mateo, I.; Franck, A.; Helisalmi, S.; Porcellini, E.; Hanon, O.; de Pancorbo, M. M.; Lendon, C.; Dufouil, C.; Jaillard, C.; Leveillard, T.; Alvarez, V.; Bosco, P.; Mancuso, M.; Panza, F.; Nacmias, B.; Bossu, P.; Piccardi, P.; Annoni, G.; Seripa, D.; Galimberti, D.; Hannequin, D.; Licastro, F.; Soininen, H.; Ritchie, K.; Blanche, H.; Dartigues, J. F.; Tzourio, C.; Gut, I.; Van Broeckhoven, C.; Alperovitch, A.; Lathrop, M.; Amouyel, P. *Nature Genetics* **2009**, *41*, 1094.
- (55) (a) Russo, C.; Schettini, G.; Saido, T. C.; Hulette, C.; Lippall, C.; Lannfelt, L.; Ghetti, B.; Gambetti, P.; Tabaton, M.; Teller, J. K. *Nature* **2000**, *405*, 531. (b) Yoo, A. S.; Cheng, I.; Chung, S.; Grenfell, T. Z.; Lee, H.; Pack-Chung, E.; Handler, M.; Shen, J.; Xia, W. M.; Tesco, G.; Saunders, A. J.; Ding, K.; Frosch, M. P.; Tanzi, R. E.; Kim, T. W. *Neuron* **2000**, *27*, 561.
- (56) Scheuner, D.; Eckman, C.; Jensen, M.; Song, X.; Citron, M.; Suzuki, N.; Bird, T. D.; Hardy, J.; Hutton, M.; Kukull, W.; Larson, E.; LevyLahad, E.; Viitanen, M.; Peskind, E.; Poorkaj, P.; Schellenberg, G.; Tanzi, R.; Wasco, W.; Lannfelt, L.; Selkoe, D.; Younkin, S. *Nature Medicine* **1996**, *2*, 864.
- (57) (a) Borchelt, D. R.; Thinakaran, G.; Eckman, C. B.; Lee, M. K.; Davenport, F.; Ratovitsky, T.; Prada, C. M.; Kim, G.; Seekins, S.; Yager, D.; Slunt, H. H.; Wang, R.; Seeger, M.; Levey, A. I.; Gandy, S. E.; Copeland, N. G.; Jenkins, N. A.; Price, D. L.; Younkin, S. G. *Neuron* **1996**, *17*, 1005. (b) Duff, K.;

- Eckman, C.; Zehr, C.; Yu, X.; Prada, C. M.; Pereztur, J.; Hutton, M.; Buee, L.; Harigaya, Y.; Yager, D.; Morgan, D.; Gordon, M. N.; Holcomb, L.; Refolo, L.; Zenk, B.; Hardy, J.; Younkin, S. *Nature* **1996**, *383*, 710. (c) Citron, M.; Westaway, D.; Xia, W. M.; Carlson, G.; Diehl, T.; Levesque, G.; JohnsonWood, K.; Lee, M.; Seubert, P.; Davis, A.; Kholodenko, D.; Motter, R.; Sherrington, R.; Perry, B.; Yao, H.; Strome, R.; Lieberburg, I.; Rommens, J.; Kim, S.; Schenk, D.; Fraser, P.; Hyslop, P. S.; Selkoe, D. J. *Nature Medicine* **1997**, *3*, 67. (d) Tomita, T.; Maruyama, K.; Saido, T. C.; Kume, H.; Shinozaki, K.; Tokuhiro, S.; Capell, A.; Walter, J.; Grunberg, J.; Haass, C.; Iwatsubo, T.; Obata, K. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 2025. (e) Oyama, F.; Sawamura, N.; Kobayashi, K.; Morishima-Kawashima, M.; Kuramochi, T.; Ito, M.; Tomita, T.; Maruyama, K.; Saido, T. C.; Iwatsubo, T.; Capell, A.; Walter, J.; Grunberg, L.; Ueyama, Y.; Haass, C.; Ihara, Y. *J. Neurochem.* **1998**, *71*, 313.
- (58) (a) Smith, I. F.; Green, K. N.; LaFerla, F. M. *Cell Calcium* **2005**, *38*, 427. (b) Tu, H. P.; Nelson, O.; Bezprozvanny, A.; Wang, Z. N.; Lee, S. F.; Hao, Y. H.; Serneels, L.; De Strooper, B.; Yu, G.; Bezprozvanny, I. *Cell* **2006**, *126*, 981. (c) Zhang, H.; Sun, S. Y.; Herreman, A.; De Strooper, B.; Bezprozvanny, I. *J. Neurosci.* **2010**, *30*, 8566.
- (59) Cheung, K. H.; Shineman, D.; Muller, M.; Cardenas, C.; Mei, L. J.; Yang, J.; Tomita, T.; Iwatsubo, T.; Lee, V. M. Y.; Foskett, J. K. *Neuron* **2008**, *58*, 871.
- (60) Holcomb, L.; Gordon, M. N.; McGowan, E.; Yu, X.; Benkovic, S.; Jantzen, P.; Wright, K.; Saad, I.; Mueller, R.; Morgan, D.; Sanders, S.; Zehr, C.; O'Campo, K.; Hardy, J.; Prada, C. M.; Eckman, C.; Younkin, S.; Hsiao, K.; Duff, K. *Nature Medicine* **1998**, *4*, 97.

- (61) (a) Lemere, C. A.; Lopera, F.; Kosik, K. S.; Lendon, C. L.; Ossa, J.; Saido, T. C.; Yamaguchi, H.; Ruiz, A.; Martinez, A.; Madrigal, L.; Hincapie, L.; Arango, J. C. L.; Anthony, D. C.; Koo, E. H.; Goate, A. M.; Selkoe, D. J.; Arango, J. C. V. *Nature Medicine* **1996**, *2*, 1146. (b) Mann, D. M. A.; Iwatsubo, T.; Cairns, N. J.; Lantos, P. L.; Nochlin, D.; Sumi, S. M.; Bird, T. D.; Poorkaj, P.; Hardy, J.; Hutton, M.; Prihar, G.; Crook, R.; Rossor, M. N.; Haltia, M. *Ann. Neurol.* **1996**, *40*, 149.
- (62) Wang, B. X.; Yang, W.; Wen, W.; Sun, J.; Su, B.; Liu, B.; Ma, D. L.; Lv, D.; Wen, Y. R.; Qu, T.; Chen, M.; Sun, M. A.; Shen, Y.; Zhang, X. *Science* **2010**, *330*, 1065.
- (63) Castellano, J. M.; Kim, J.; Stewart, F. R.; Jiang, H.; DeMattos, R. B.; Patterson, B. W.; Fagan, A. M.; Morris, J. C.; Mawuenyega, K. G.; Cruchaga, C.; Goate, A. M.; Bales, K. R.; Paul, S. M.; Bateman, R. J.; Holtzman, D. M. *Sci. Transl. Med.* **2011**, *3*, 89ra57.
- (64) Harold, D.; Abraham, R.; Hollingworth, P.; Sims, R.; Gerrish, A.; Hamshere, M. L.; Pahwa, J. S.; Moskvin, V.; Dowzell, K.; Williams, A.; Jones, N.; Thomas, C.; Stretton, A.; Morgan, A. R.; Lovestone, S.; Powell, J.; Proitsi, P.; Lupton, M. K.; Brayne, C.; Rubinsztein, D. C.; Gill, M.; Lawlor, B.; Lynch, A.; Morgan, K.; Brown, K. S.; Passmore, P. A.; Craig, D.; McGuinness, B.; Todd, S.; Holmes, C.; Mann, D.; Smith, A. D.; Love, S.; Kehoe, P. G.; Hardy, J.; Mead, S.; Fox, N.; Rossor, M.; Collinge, J.; Maier, W.; Jessen, F.; Schurmann, B.; van den Bussche, H.; Heuser, I.; Kornhuber, J.; Wiltfang, J.; Dichgans, M.; Frolich, L.; Hampel, H.; Hull, M.; Rujescu, D.; Goate, A. M.; Kauwe, J. S. K.; Cruchaga, C.; Nowotny, P.; Morris, J. C.; Mayo, K.; Sleegers, K.; Bettens, K.; Engelborghs, S.; De Deyn, P. P.; Van Broeckhoven, C.; Livingston, G.; Bass,

- N. J.; Gurling, H.; McQuillin, A.; Gwilliam, R.; Deloukas, P.; Al-Chalabi, A.; Shaw, C. E.; Tsolaki, M.; Singleton, A. B.; Guerreiro, R.; Muhleisen, T. W.; Nothen, M. M.; Moebus, S.; Jockel, K. H.; Klopp, N.; Wichmann, H. E.; Carrasquillo, M. M.; Pankratz, V. S.; Younkin, S. G.; Holmans, P. A.; O'Donovan, M.; Owen, M. J.; Williams, J. *Nature Genetics* **2009**, *41*, 1088.
- (65) (a) Ghiso, J.; Matsubara, E.; Koudinov, A.; Choimiura, N. H.; Tomita, M.; Wisniewski, T.; Frangione, B. *Biochem. J.* **1993**, *293*, 27. (b) Matsubara, E.; Frangione, B.; Ghiso, J. *J. Biol. Chem.* **1995**, *270*, 7563.
- (66) Calero, M.; Rostagno, A.; Matsubara, E.; Zlokovic, B.; Frangione, B.; Ghiso, J. *Microsc. Res. Tech.* **2000**, *50*, 305.
- (67) (a) DeMattos, R. B.; Cirrito, J. R.; Parsadanian, M.; May, P. C.; O'Dell, M. A.; Taylor, J. W.; Harmony, J. A. K.; Aronow, B. J.; Bales, K. R.; Paul, S. M.; Holtzman, D. M. *Neuron* **2004**, *41*, 193. (b) Bell, R. D.; Sagare, A. P.; Friedman, A. E.; Bedi, G. S.; Holtzman, D. M.; Deane, R.; Zlokovic, B. V. *J. Cereb. Blood Flow Metab.* **2007**, *27*, 909.
- (68) DeMattos, R. B.; O'Dell, M. A.; Parsadanian, M.; Taylor, J. W.; Harmony, J. A. K.; Bales, K. R.; Paul, S. M.; Aronow, B. J.; Holtzman, D. M. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 10843.
- (69) Treusch, S.; Hamamichi, S.; Goodman, J. L.; Matlack, K. E. S.; Chung, C. Y.; Baru, V.; Shulman, J. M.; Parrado, A.; Bevis, B. J.; Valastyan, J. S.; Han, H.; Lindhagen-Persson, M.; Reiman, E. M.; Evans, D. A.; Bennett, D. A.; Olofsson, A.; DeJager, P. L.; Tanzi, R. E.; Caldwell, K. A.; Caldwell, G. A.; Lindquist, S. *Science* **2011**, *334*, 1241.
- (70) Reiman, E. M.; Webster, J. A.; Myers, A. J.; Hardy, J.; Dunckley, T.; Zismann, V. L.; Joshipura, K. D.; Pearson, J. V.; Hu-Lince, D.; Huentelman, M. J.;

- Craig, D. W.; Coon, K. D.; Liang, W. S.; Herbert, R. H.; Beach, T.; Rohrer, K. C.; Zhao, A. S.; Leung, D.; Bryden, L.; Marlowe, L.; Kaleem, M.; Mastroeni, D.; Grover, A.; Heward, C. B.; Ravid, R.; Rogers, J.; Hutton, M. L.; Melquist, S.; Petersen, R. C.; Alexander, G. E.; Caselli, R. J.; Kukull, W.; Papassotiropoulos, A.; Stephan, D. A. *Neuron* **2007**, *54*, 713.
- (71) Kalman, J.; McConathy, W.; Araoz, C.; Kasa, P.; Lacko, A. G. *Neurol. Res.* **2000**, *22*, 330.
- (72) Dreses-Werringloer, U.; Lambert, J. C.; Vingdeux, V.; Zhao, H. T.; Vais, H.; Siebert, A.; Jain, A.; Koppel, J.; Rovelet-Lecrux, A.; Hannequin, D.; Pasquier, F.; Galimberti, D.; Scarpini, E.; Mann, D.; Lendon, C.; Campion, D.; Amouyel, P.; Davies, P.; Foskett, J. K.; Campagne, F.; Marambaud, P. *Cell* **2008**, *133*, 1149.
- (73) Li, H.; Wetten, S.; Li, L.; Jean, P. L. S.; Upmanyu, R.; Surh, L.; Hosford, D.; Barnes, M. R.; Briley, J. D.; Borrie, M.; Coletta, N.; Delisle, R.; Dhalla, D.; Ehm, M. G.; Feldman, H. H.; Fornazzari, L.; Gauthier, S.; Goodgame, N.; Guzman, D.; Hammond, S.; Hollingworth, P.; Hsiung, G. Y.; Johnson, J.; Kelly, D. D.; Keren, R.; Kertesz, A.; King, K. S.; Lovestone, S.; Loy-English, I.; Matthews, P. M.; Owen, M. J.; Plumpton, M.; Pryse-Phillips, W.; Prinjha, R. K.; Richardson, J. C.; Saunders, A.; Slater, A. J.; George-Hyslop, P. H. S.; Stinnett, S. W.; Swartz, J. E.; Taylor, R. L.; Wherrett, J.; Williams, J.; Yarnall, D. P.; Gibson, R. A.; Irizarry, M. C.; Middleton, L. T.; Roses, A. D. *Arch. Neurol.* **2008**, *65*, 45.
- (74) Bertram, L.; Schjeide, B. M. M.; Hooli, B.; Mullin, K.; Hiltunen, M.; Soininen, H.; Ingelsson, M.; Lannfelt, L.; Blacker, D.; Tanzi, R. E. *Cell* **2008**, *135*, 993.

- (75) Campagne, F.; Lambert, J. C.; Dreses-Werringloer, U.; Vingtheux, V.; Lendon, C.; Campion, D.; Amouyel, P.; Lee, A. T.; Gregersen, P. K.; Davies, P.; Marambaud, P. *Cell* **2008**, *135*, 994.
- (76) Thathiah, A.; Spittaels, K.; Hoffmann, M.; Staes, M.; Cohen, A.; Horre, K.; Vanbrabant, M.; Coun, F.; Baekelandt, V.; Delacourte, A.; Fischer, D. F.; Pollet, D.; De Strooper, B.; Merchiers, P. *Science* **2009**, *323*, 946.
- (77) Blacker, D.; Bertram, L.; Saunders, A. J.; Moscarillo, T. J.; Albert, M. S.; Wiener, H.; Perry, R. T.; Collins, J. S.; Harrell, L. E.; Go, R. C. P.; Mahoney, A.; Beaty, T.; Fallin, M. D.; Avramopoulos, D.; Chase, G. A.; Folstein, M. F.; McInnis, M. G.; Bassett, S. S.; Doheny, K. J.; Pugh, E. W.; Tanzi, R. E. *Hum. Mol. Genet.* **2003**, *12*, 23.
- (78) Nitsch, R. M.; Deng, M. H.; Growdon, J. H.; Wurtman, R. J. *J. Biol. Chem.* **1996**, *271*, 4188.
- (79) Pooler, A. M.; Arjona, A. A.; Lee, R. K.; Wurtman, R. J. *Neurosci. Lett.* **2004**, *362*, 127.
- (80) Pasinetti, G. M.; Aisen, P. S. *Neuroscience* **1998**, *87*, 319.
- (81) Ijsselstijn, L.; Dekker, L. J. M.; Stingl, C.; van der Weiden, M. M.; Hofman, A.; Kros, J. M.; Koudstaal, P. J.; Smitt, P.; Ikram, M. A.; Breteler, M. M. B.; Luijckx, T. M. *J. Proteome Res.* **2011**, *10*, 4902.
- (82) (a) Cohen, E.; Paulsson, J. F.; Blinder, P.; Burstyn-Cohen, T.; Du, D. G.; Estepa, G.; Adame, A.; Pham, H. M.; Holzenberger, M.; Kelly, J. W.; Masliah, E.; Dillin, A. *Cell* **2009**, *139*, 1157. (b) Freude, S.; Hettich, M. M.; Schumann, C.; Stohr, O.; Koch, L.; Kohler, C.; Udelhoven, M.; Leeser, U.; Muller, M.; Kubota, N.; Kadowaki, T.; Krone, W.; Schroder, H.; Bruning, J. C.; Schubert,

- M. *FASEB J.* **2009**, *23*, 3315. (c) Moloney, A. M.; Griffin, R. J.; Timmons, S.; O'Connor, R.; Ravid, R.; O'Neill, C. *Neurobiology of Aging* **2010**, *31*, 224.
- (83) (a) Games, D.; Adams, D.; Alessandrini, R.; Barbour, R.; Berthelette, P.; Blackwell, C.; Carr, T.; Clemens, J.; Donaldson, T.; Gillespie, F.; Guido, T.; Hagopian, S.; Johnsonwood, K.; Khan, K.; Lee, M.; Leibowitz, P.; Lieberburg, I.; Little, S.; Masliah, E.; McConlogue, L.; Montoyazavala, M.; Mucke, L.; Paganini, L.; Penniman, E.; Power, M.; Schenk, D.; Seubert, P.; Snyder, B.; Soriano, F.; Tan, H.; Vitale, J.; Wadsworth, S.; Wolozin, B.; Zhao, J. *Nature* **1995**, *373*, 523. (b) Rockenstein, E. M.; McConlogue, L.; Tan, H.; Power, M.; Masliah, E.; Mucke, L. *J. Biol. Chem.* **1995**, *270*, 28257. (c) Schenk, D.; Barbour, R.; Dunn, W.; Gordon, G.; Grajeda, H.; Guido, T.; Hu, K.; Huang, J. P.; Johnson-Wood, K.; Khan, K.; Kholodenko, D.; Lee, M.; Liao, Z. M.; Lieberburg, I.; Motter, R.; Mutter, L.; Soriano, F.; Shopp, G.; Vasquez, N.; Vandeventer, C.; Walker, S.; Wogulis, M.; Yednock, T.; Games, D.; Seubert, P. *Nature* **1999**, *400*, 173.
- (84) (a) Janus, C.; Pearson, J.; McLaurin, J.; Mathews, P. M.; Jiang, Y.; Schmidt, S. D.; Chishti, M. A.; Horne, P.; Heslin, D.; French, J.; Mount, H. T. J.; Nixon, R. A.; Mercken, M.; Bergeron, C.; Fraser, P. E.; St George-Hyslop, P.; Westaway, D. *Nature* **2000**, *408*, 979. (b) McLaurin, J.; Kierstead, M. E.; Brown, M. E.; Hawkes, C. A.; Lambermon, M. H. L.; Phinney, A. L.; Darabie, A. A.; Cousins, J. E.; French, J. E.; Lan, M. F.; Chen, F. S.; Wong, S. S. N.; Mount, H. T. J.; Fraser, P. E.; Westaway, D.; St George-Hyslop, P. *Nature Medicine* **2006**, *12*, 801.
- (85) Hsiao, K.; Chapman, P.; Nilsen, S.; Eckman, C.; Harigaya, Y.; Younkin, S.; Yang, F. S.; Cole, G. *Science* **1996**, *274*, 99.

- (86) Wilcock, D. M.; DiCarlo, G.; Henderson, D.; Jackson, J.; Clarke, K.; Ugen, K. E.; Gordon, M. N.; Morgan, D. *J. Neurosci.* **2003**, *23*, 3745.
- (87) Lesné, S.; Koh, M. T.; Kotilinek, L.; Kaye, R.; Glabe, C. G.; Yang, A.; Gallagher, M.; Ashe, K. H. *Nature* **2006**, *440*, 352.
- (88) Oddo, S.; Caccamo, A.; Shepherd, J. D.; Murphy, M. P.; Golde, T. E.; Kaye, R.; Metherate, R.; Mattson, M. P.; Akbari, Y.; LaFerla, F. M. *Neuron* **2003**, *39*, 409.
- (89) Meyer-Luehmann, M.; Coomaraswamy, J.; Bolmont, T.; Kaeser, S.; Schaefer, C.; Kilger, E.; Neuenschwander, A.; Abramowski, D.; Frey, P.; Jaton, A. L.; Vigouret, J. M.; Paganetti, P.; Walsh, D. M.; Mathews, P. M.; Ghiso, J.; Staufenbiel, M.; Walker, L. C.; Jucker, M. *Science* **2006**, *313*, 1781.
- (90) (a) Burdick, D.; Soreghan, B.; Kwon, M.; Kosmoski, J.; Knauer, M.; Henschen, A.; Yates, J.; Cotman, C.; Glabe, C. *J. Biol. Chem.* **1992**, *267*, 546. (b) Jarrett, J. T.; Lansbury, P. T. *Cell (Cambridge, Mass.)* **1993**, *73*, 1055.
- (91) (a) Dahlgren, K. N.; Manelli, A. M.; Stine, W. B.; Baker, L. K.; Krafft, G. A.; LaDu, M. J. *J. Biol. Chem.* **2002**, *277*, 32046. (b) Zhang, Y.; McLaughlin, R.; Goodyer, C.; LeBlanc, A. *J. Cell Biol.* **2002**, *156*, 519.
- (92) (a) Citron, M.; Oltersdorf, T.; Haass, C.; McConlogue, L.; Hung, A. Y.; Seubert, P.; Vigopelfrey, C.; Lieberburg, I.; Selkoe, D. J. *Nature* **1992**, *360*, 672. (b) Cai, X. D.; Golde, T. E.; Younkin, S. G. *Science* **1993**, *259*, 514. (c) Suzuki, N.; Cheung, T. T.; Cai, X. D.; Odaka, A.; Otvos, L.; Eckman, C.; Golde, T. E.; Younkin, S. G. *Science* **1994**, *264*, 1336.
- (93) Iwatsubo, T.; Odaka, A.; Suzuki, N.; Mizusawa, H.; Nukina, N.; Ihara, Y. *Neuron* **1994**, *13*, 45.

- (94) Gravina, S. A.; Ho, L. B.; Eckman, C. B.; Long, K. E.; Otvos, L.; Younkin, L. H.; Suzuki, N.; Younkin, S. G. *J. Biol. Chem.* **1995**, *270*, 7013.
- (95) Soscia, S. J.; Kirby, J. E.; Washicosky, K. J.; Tucker, S. M.; Ingelsson, M.; Hyman, B.; Burton, M. A.; Goldstein, L. E.; Duong, S.; Tanzi, R. E.; Moir, R. D. *Plos One* **2010**, *5*.
- (96) Soscia, S. J.; Kirby, J. E.; Washicosky, K. J.; Tucker, S. M.; Ingelsson, M.; Hyman, B.; Burton, M. A.; Goldstein, L. E.; Duong, S.; Tanzi, R. E.; Moir, R. D. *Plos One* **2010**, *5*, e9505.
- (97) Balin, B. J.; Little, C. S.; Hammond, C. J.; Appelt, D. M.; Whittum-Hudson, J. A.; Gerard, H. C.; Hudson, A. P. *J. Alzheimers Dis.* **2008**, *13*, 371.
- (98) Appelt, D. M.; Roupas, M. R.; Way, D. S.; Bell, M. G.; Albert, E. V.; Hammond, C. J.; Balin, B. J. *BMC Neuroscience* **2008**, *9*, 13.
- (99) Castellani, R. J.; Lee, H. G.; Siedlak, S. L.; Nunomura, A.; Hayashi, T.; Nakamura, M.; Zhu, X. W.; Perry, G.; Smith, M. A. *J. Alzheimers Dis.* **2009**, *18*, 447.
- (100) (a) Yu, Y. W. Y.; Lin, C.-H.; Chen, S.-P.; Hong, C.-J.; Tsai, S.-J. *Neurosci. Lett.* **2000**, *294*, 179. (b) Han, S. D.; Drake, A. I.; Cessante, L. M.; Jak, A. J.; Houston, W. S.; Delis, D. C.; Filoteo, J. V.; Bondi, M. W. *J. Neurol. Neurosurg. Psychiatry* **2007**, *78*, 1103. (c) Marchant, N. L.; King, S. L.; Tabet, N.; Rusted, J. M. *Neuropsychopharmacology* **2010**, *35*, 1090.
- (101) Mehta, P. D.; Pirttila, T.; Mehta, S. P.; Sersen, E. A.; Aisen, P. S.; Wisniewski, H. M. *Arch. Neurol.* **2000**, *57*, 100.
- (102) Motter, R.; Vigopelfrey, C.; Kholodenko, D.; Barbour, R.; Johnsonwood, K.; Galasko, D.; Chang, L.; Miller, B.; Clark, C.; Green, R.; Olson, D.;

- Southwick, P.; Wolfert, R.; Munroe, B.; Lieberburg, I.; Seubert, P.; Schenk, D. *Ann. Neurol.* **1995**, *38*, 643.
- (103) Demattos, R. B.; Bales, K. R.; Cummins, D. J.; Paul, S. M.; Holtzman, D. M. *Science* **2002**, *295*, 2264.
- (104) Cleary, J. P.; Walsh, D. M.; Hofmeister, J. J.; Shankar, G. M.; Kuskowski, M. A.; Selkoe, D. J.; Ashe, K. H. *Nature Neuroscience* **2005**, *8*, 79.
- (105) Podlisny, M. B.; Walsh, D. M.; Amarante, P.; Ostaszewski, B. L.; Stimson, E. R.; Maggio, J. E.; Teplow, D. B.; Selkoe, D. J. *Biochemistry* **1998**, *37*, 3602.
- (106) (a) Seubert, P.; Vigopelfrey, C.; Esch, F.; Lee, M.; Dovey, H.; Davis, D.; Sinha, S.; Schlossmacher, M.; Whaley, J.; Swindlehurst, C.; McCormack, R.; Wolfert, R.; Selkoe, D.; Lieberburg, I.; Schenk, D. *Nature* **1992**, *359*, 325. (b) Bush, A. I.; Pettingell, W. H.; Multhaup, G.; Paradis, M. D.; Vonsattel, J. P.; Gusella, J. F.; Beyreuther, K.; Masters, C. L.; Tanzi, R. E. *Science* **1994**, *265*, 1464.
- (107) Mawuenyega, K. G.; Sigurdson, W.; Ovod, V.; Munsell, L.; Kasten, T.; Morris, J. C.; Yarasheski, K. E.; Bateman, R. J. *Science* **2010**, *330*, 1774.
- (108) (a) Zlokovic, B. V. *J. Neurochem.* **2004**, *89*, 807. (b) Tanzi, R. E.; Moir, R. D.; Wagner, S. L. *Neuron* **2004**, *43*, 605.
- (109) (a) Rosen, D. R.; Martinmorris, L.; Luo, L. Q.; White, K. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 2478. (b) Gunawardena, S.; Goldstein, L. S. B. *Neuron* **2001**, *32*, 389. (c) Iijima, K.; Liu, H. P.; Chiang, A. S.; Hearn, S. A.; Konsolaki, M.; Zhong, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 6623. (d) Crowther, D. C.; Kinghorn, K. J.; Miranda, E.; Page, R.; Curry, J. A.; Duthie, F. A. I.; Gubb, D. C.; Lomas, D. A. *Neuroscience* **2005**, *132*, 123. (e) Nerelius, C.; Sandegren, A.; Sargsyan, H.; Raunak, R.; Leijonmarck, H.;

- Chatterjee, U.; Fisahn, A.; Imarisio, S.; Lomas, D. A.; Crowther, D. C.; Stromberg, R.; Johansson, J. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 9191.
- (f) Chakraborty, R.; Vepuri, V.; Mhatre, S. D.; Paddock, B. E.; Miller, S.; Michelson, S. J.; Delvadia, R.; Desai, A.; Vinokur, M.; Melicharek, D. J.; Utreja, S.; Khandelwal, P.; Ansaloni, S.; Goldstein, L. E.; Moir, R. D.; Lee, J. C.; Tabb, L. P.; Saunders, A. J.; Marena, D. R. *Plos One* **2011**, *6*. (g) Luheshi, L. M.; Tartaglia, G. G.; Brorsson, A. C.; Pawar, A. P.; Watson, I. E.; Chiti, F.; Vendruscolo, M.; Lomas, D. A.; Dobson, C. M.; Crowther, D. C. *Plos Biology* **2007**, *5*, 2493.
- (110) (a) Link, C. D. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 9368. (b) Yu, G.; Nishimura, M.; Arawaka, S.; Levitan, D.; Zhang, L. L.; Tandon, A.; Song, Y. Q.; Rogaeva, E.; Chen, F. S.; Kawaral, T.; Supala, A.; Levesque, L.; Yu, H.; Yang, D. S.; Holmes, E.; Millman, P.; Liang, Y.; Zhang, D. M.; Xu, D. H.; Sato, C.; Rogaev, E.; Smith, M.; Janus, C.; Zhang, Y. N.; Aebersold, R.; Farrer, L.; Sorbi, S.; Bruni, A.; Fraser, P.; St George-Hyslop, P. *Nature* **2000**, *407*, 48. (c) Cohen, E.; Bieschke, J.; Perciavalle, R. M.; Kelly, J. W.; Dillin, A. *Science* **2006**, *313*, 1604.
- (111) Youm, J. W.; Kim, H.; Lo Han, J. H.; Jang, C. H.; Ha, H. J.; Mook-Jung, I.; Jeon, J. H.; Choi, C. Y.; Kim, Y. H.; Kim, H. S.; Joung, H. Y. *FEBS Lett.* **2005**, *579*, 6737.
- (112) (a) Zhang, H. Y.; Komano, H.; Fuller, R. S.; Gandy, S. E.; Frail, D. E. *J. Biol. Chem.* **1994**, *269*, 27799. (b) Komano, H.; Seeger, M.; Gandy, S.; Wang, G. T.; Krafft, G. A.; Fuller, R. S. *J. Biol. Chem.* **1998**, *273*, 31648.
- (113) (a) Baumeister, R.; Leimer, U.; Zweckbronner, I.; Jakubek, C.; Grunberg, J.; Haass, C. *Genes and function* **1997**, *1*, 149. (b) Levitan, D.; Doyle, T. G.;

- Brousseau, D.; Lee, M. K.; Thinakaran, G.; Slunt, H. H.; Sisodia, S. S.; Greenwald, I. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 14940.
- (114) Cabaleiro-Lago, C.; Quinlan-Pluck, F.; Lynch, I.; Lindman, S.; Minogue, A. M.; Thulin, E.; Walsh, D. M.; Dawson, K. A.; Linse, S. *J. Am. Chem. Soc.* **2008**, *130*, 15437.
- (115) Halim, A.; Brinkmalm, G.; Ruetschi, U.; Westman-Brinkmalm, A.; Portelius, E.; Zetterberg, H.; Blennow, K.; Larson, G.; Nilsson, J. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 11848.
- (116) Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. F.; Frigon, N.; Hong, J.; Jacobson-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensari, S. M.; Wang, S. W.; Walker, D.; Zhao, J.; McConlogue, L.; John, V. *Nature* **1999**, *402*, 537.
- (117) (a) Francis, R.; McGrath, G.; Zhang, J. H.; Ruddy, D. A.; Sym, M.; Apfeld, J.; Nicoll, M.; Maxwell, M.; Hai, B.; Ellis, M. C.; Parks, A. L.; Xu, W.; Li, J. H.; Gurney, M.; Myers, R. L.; Himes, C. S.; Hiebsch, R.; Ruble, C.; Nye, J. S.; Curtis, D. *Dev. Cell* **2002**, *3*, 85. (b) De Strooper, B. *Neuron* **2003**, *38*, 9. (c) St George-Hyslop, P.; Schmitt-Ulms, G. *Nature* **2010**, *467*, 36.
- (118) Tabaton, M.; Zhu, X. W.; Perry, G.; Smith, M. A.; Giliberto, L. *Exp. Neurol.* **2010**, *221*, 18.
- (119) Guglielmotto, M.; Aragno, M.; Autelli, R.; Giliberto, L.; Novo, E.; Colombatto, S.; Danni, O.; Parola, M.; Smith, M. A.; Perry, G.; Tamagno, E.; Tabaton, M. *J. Neurochem.* **2009**, *108*, 1045.
- (120) (a) Allinson, T. M. J.; Parkin, E. T.; Turner, A. J.; Hooper, N. M. *J. Neurosci. Res.* **2003**, *74*, 342. (b) Portelius, E.; Price, E.; Brinkmalm, G.; Stiteler, M.;

- Olsson, M.; Persson, R.; Westman-Brinkmalm, A.; Zetterberg, H.; Simon, A. J.; Blennow, K. *Neurobiology of Aging* **2011**, *32*, 1090.
- (121) Rogaeva, E.; Meng, Y.; Lee, J. H.; Gu, Y.; Kawarai, T.; Zou, F.; Katayama, T.; Baldwin, C. T.; Cheng, R.; Hasegawa, H.; Chen, F.; Shibata, N.; Lunetta, K. L.; Pardossi-Piquard, R.; Bohm, C.; Wakutani, Y.; Cupples, L. A.; Cuenco, K. T.; Green, R. C.; Pinessi, L.; Rainero, I.; Sorbi, S.; Bruni, A.; Duara, R.; Friedland, R. P.; Inzelberg, R.; Hampe, W.; Bujo, H.; Song, Y. Q.; Andersen, O. M.; Willnow, T. E.; Graff-Radford, N.; Petersen, R. C.; Dickson, D.; Der, S. D.; Fraser, P. E.; Schmitt-Ulms, G.; Younkin, S.; Mayeux, R.; Farrer, L. A.; St George-Hyslop, P. *Nature Genetics* **2007**, *39*, 168.
- (122) Jaworski, T.; Dewachter, I.; Lechat, B.; Gees, M.; Kremer, A.; Demedts, D.; Borghgraef, P.; Devijver, H.; Kugler, S.; Patel, S.; Woodgett, J. R.; Van Leuven, F. *Nature* **2011**, *480*, E4.
- (123) Nikolaev, A.; McLaughlin, T.; O'Leary, D. D. M.; Tessier-Lavigne, M. *Nature* **2009**, *457*, 981.
- (124) Hendriks, L.; Vanduijn, C. M.; Cras, P.; Cruts, M.; Vanhul, W.; Vanharskamp, F.; Warren, A.; McInnis, M. G.; Antonarakis, S. E.; Martin, J. J.; Hofman, A.; Vanbroeckhoven, C. *Nature Genetics* **1992**, *1*, 218.
- (125) Farzan, M.; Schnitzler, C. E.; Vasilieva, N.; Leung, D.; Choe, H. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 9712.
- (126) Selkoe, D. J.; Podlisny, M. B.; Joachim, C. L.; Vickers, E. A.; Lee, G.; Fritz, L. C.; Oltersdorf, T. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 7341.
- (127) Sinha, S.; Dovey, H. F.; Seubert, P.; Ward, P. J.; Blacher, R. W.; Blaber, M.; Bradshaw, R. A.; Arici, M.; Mobley, W. C.; Lieberburg, I. *J. Biol. Chem.* **1990**, *265*, 8983.

- (128) Goate, A. M. *Cell. Mol. Life Sci.* **1998**, *54*, 897.
- (129) Mullan, M.; Crawford, F.; Axelman, K.; Houlden, H.; Lilius, L.; Winblad, B.; Lannfelt, L. *Nature Genetics* **1992**, *1*, 345.
- (130) (a) Chartierharlin, M. C.; Crawford, F.; Houlden, H.; Warren, A.; Hughes, D.; Fidani, L.; Goate, A.; Rossor, M.; Roques, P.; Hardy, J.; Mullan, M. *Nature* **1991**, *353*, 844. (b) Sinha, S.; Lieberburg, I. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 11049.
- (131) Nilsberth, C.; Westlind-Danielsson, A.; Eckman, C. B.; Condrón, M. M.; Axelman, K.; Forsell, C.; Sten, C.; Luthman, J.; Teplow, D. B.; Younkin, S. G.; Naslund, J.; Lannfelt, L. *Nature Neuroscience* **2001**, *4*, 887.
- (132) (a) Grabowski, T. J.; Cho, H. S.; Vonsattel, J. P. G.; Rebeck, G. W.; Greenberg, S. M. *Ann. Neurol.* **2001**, *49*, 697. (b) Van Nostrand, W. E.; Melchor, J. P.; Cho, H. S.; Greenberg, S. M.; Rebeck, G. W. *J. Biol. Chem.* **2001**, *276*, 32860.
- (133) Mucke, L.; Masliah, E.; Yu, G. Q.; Mallory, M.; Rockenstein, E. M.; Tatsuno, G.; Hu, K.; Kholodenko, D.; Johnson-Wood, K.; McConlogue, L. *J. Neurosci.* **2000**, *20*, 4050.
- (134) Takahashi, T.; Mihara, H. *Chem. Comm.* **2012**, *48*, 1568.
- (135) Sian, A. K.; Frears, E. R.; El-Agnaf, O. M. A.; Patel, B. P.; Manca, M. F.; Siligardi, G.; Hussain, R.; Austen, B. M. *Biochem. J.* **2000**, *349*, 299.
- (136) Schierle, G. S. K.; van de Linde, S.; Erdelyi, M.; Esbjorner, E. K.; Klein, T.; Rees, E.; Bertocini, C. W.; Dobson, C. M.; Sauer, M.; Kaminski, C. F. *J. Am. Chem. Soc.* **2011**, *133*, 12902.
- (137) Zhang, Y.; McLaughlin, R.; Goodyer, C.; LeBlanc, A. *J. Cell Biol.* **2002**, *156*, 519.
- (138) Nagy, Z.; Esiri, M. M. *Neurobiology of Aging* **1997**, *18*, 565.

- (139) (a) Nagy, Z.; Esiri, M. M.; Cato, A. M.; Smith, A. D. *Acta Neuropathol.* **1997**, *94*, 6. (b) Nagy, Z.; Esiri, M. M.; Smith, A. D. *Neuroscience* **1998**, *87*, 731.
- (140) (a) Chen, Q. S.; Kagan, B. L.; Hirakura, Y.; Xie, C. W. *J. Neurosci. Res.* **2000**, *60*, 65. (b) Miñano-Molina, A. J.; España, J.; Martin, E.; Barneda-Zahonero, B.; Fadó, R.; Solé, M.; Trullás, R.; Saura, C. A.; Rodríguez-Alvarez, J. *J. Biol. Chem.* **2011**, *286*, 27311.
- (141) (a) Chapman, P. F.; White, G. L.; Jones, M. W.; Cooper-Blacketer, D.; Marshall, V. J.; Irizarry, M.; Younkin, L.; Good, M. A.; Bliss, T. V. P.; Hyman, B. T.; Younkin, S. G.; Hsiao, K. K. *Nature Neuroscience* **1999**, *2*, 271. (b) Larson, J.; Lynch, G.; Games, D.; Seubert, P. *Brain Res.* **1999**, *840*, 23. (c) Giacchino, J.; Criado, J. R.; Games, D.; Henriksen, S. *Brain Res.* **2000**, *876*, 185. (d) Lanz, T. A.; Carter, D. B.; Merchant, K. M. *Neurobiol. Dis.* **2003**, *13*, 246. (e) Spires, T. L.; Meyer-Luehmann, M.; Stern, E. A.; McLean, P. J.; Skoch, J.; Nguyen, P. T.; Bacskai, B. J.; Hyman, B. T. *J. Neurosci.* **2005**, *25*, 7278. (f) Almeida, C. G.; Tampellini, D.; Takahashi, R. H.; Greengard, P.; Lin, M. T.; Snyder, E. M.; Gouras, G. K. *Neurobiol. Dis.* **2005**, *20*, 187.
- (142) Snyder, E. M.; Nong, Y.; Almeida, C. G.; Paul, S.; Moran, T.; Choi, E. Y.; Nairn, A. C.; Salter, M. W.; Lombroso, P. J.; Gouras, G. K.; Greengard, P. *Nature Neuroscience* **2005**, *8*, 1051.
- (143) Kim, J. H.; Anwyl, R.; Suh, Y. H.; Djamgoz, M. B. A.; Rowan, M. J. *J. Neurosci.* **2001**, *21*, 1327.
- (144) Kamenetz, F.; Tomita, T.; Hsieh, H.; Seabrook, G.; Borchelt, D.; Iwatsubo, T.; Sisodia, S.; Malinow, R. *Neuron* **2003**, *37*, 925.
- (145) (a) Hartley, D. M.; Walsh, D. M.; Ye, C. P. P.; Diehl, T.; Vasquez, S.; Vassilev, P. M.; Teplow, D. B.; Selkoe, D. J. *J. Neurosci.* **1999**, *19*, 8876. (b) Moechars,

- D.; Dewachter, I.; Lorent, K.; Reverse, D.; Baekelandt, V.; Naidu, A.; Tesseur, I.; Spittaels, K.; Van Den Haute, C.; Checler, F.; Godaux, E.; Cordell, B.; Van Leuven, F. *J. Biol. Chem.* **1999**, *274*, 6483. (c) Billings, L. M.; Oddo, S.; Green, K. N.; McGaugh, J. L.; LaFerla, F. M. *Neuron* **2005**, *45*, 675.
- (146) (a) Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Cullen, W. K.; Anwyl, R.; Wolfe, M. S.; Rowan, M. J.; Selkoe, D. J. *Nature* **2002**, *416*, 535. (b) Wang, H. W.; Pasternak, J. F.; Kuo, H.; Ristic, H.; Lambert, M. P.; Chromy, B.; Viola, K. L.; Klein, W. L.; Stine, W. B.; Krafft, G. A.; Trommer, B. L. *Brain Res.* **2002**, *924*, 133. (c) Townsend, M.; Shankar, G. M.; Mehta, T.; Walsh, D. M.; Selkoe, D. J. *J. Physiol.-London* **2006**, *572*, 477. (d) Selkoe, D. J. *Behavioral Brain Research* **2008**, *192*, 106. (e) Kessels, H. W.; Nguyen, L. N.; Nabavi, S.; Malinow, R. *Nature* **2010**, *466*, E3. (f) Balducci, C.; Beeg, M.; Stravalaci, M.; Bastone, A.; Scip, A.; Biasini, E.; Tapella, L.; Colombo, L.; Manzoni, C.; Borsello, T.; Chiesa, R.; Gobbi, M.; Salmona, M.; Forloni, G. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 2295.
- (147) (a) Hsieh, H.; Boehm, J.; Sato, C.; Iwatsubo, T.; Tomita, T.; Sisodia, S.; Malinow, R. *Neuron* **2006**, *52*, 831. (b) Li, S. M.; Hong, S. Y.; Shepardson, N. E.; Walsh, D. M.; Shankar, G. M.; Selkoe, D. *Neuron* **2009**, *62*, 788.
- (148) Cullen, W. K.; Suh, Y. H.; Anwyl, R.; Rowan, M. J. *Neuroreport* **1997**, *8*, 3213.
- (149) Freir, D. B.; Holscher, C.; Herron, C. E. *J. Neurophysiol.* **2001**, *85*, 708.
- (150) Wang, Q. W.; Walsh, D. M.; Rowan, M. J.; Selkoe, D. J.; Anwyl, R. *J. Neurosci.* **2004**, *24*, 3370.
- (151) Bell, K. F. S.; Ducatenzeiler, A.; Ribeiro-da-Silva, A.; Duff, K.; Bennett, D. A.; Cuello, A. C. *Neurobiology of Aging* **2006**, *27*, 1644.
- (152) Lee, B. Y.; Ban, J. Y.; Seong, Y. H. *Neurosci. Res.* **2005**, *52*, 347.

- (153) (a) Wang, H. Y.; Lee, D. H. S.; D'Andrea, M. R.; Peterson, P. A.; Shank, R. P.; Reitz, A. B. *J. Biol. Chem.* **2000**, *275*, 5626. (b) Wang, H. Y.; Lee, D. H. S.; Davis, C. B.; Shank, R. P. *J. Neurochem.* **2000**, *75*, 1155.
- (154) (a) Kihara, T.; Shimohama, S.; Sawada, H.; Kimura, J.; Kume, T.; Kochiyama, H.; Maeda, T.; Akaike, A. *Ann. Neurol.* **1997**, *42*, 159. (b) Liu, Q. S.; Kawai, H.; Berg, D. K. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 4734.
- (155) (a) Dineley, K. T.; Bell, K. A.; Bui, D.; Sweatt, J. D. *J. Biol. Chem.* **2002**, *277*, 25056. (b) Spencer, J. P.; Weil, A.; Hill, K.; Hussain, I.; Richardson, J. C.; Cusdin, F. S.; Chen, Y. H.; Randall, A. D. *Neuroscience* **2006**, *137*, 795. (c) Abbott, J. J.; Howlett, D. R.; Francis, P. T.; Williams, R. J. *Neurobiology of Aging* **2008**, *29*, 992.
- (156) Kihara, T.; Shimohama, S.; Urushitani, M.; Sawada, H.; Kimura, J.; Kume, T.; Maeda, T.; Akaike, A. *Brain Res.* **1998**, *792*, 331.
- (157) Maelicke, A. *Dement. Geriatr. Cogn. Disord.* **2000**, *11*, 11.
- (158) Pettit, D. L.; Shao, Z.; Yakel, J. L. *J. Neurosci.* **2001**, *21*.
- (159) Louzada, P. R.; Lima, A. C. P.; Mendonca-Silva, D. L.; Noel, F.; De Mello, F. G.; Ferreira, S. T. *FASEB J.* **2004**, *18*, 511.
- (160) (a) Geula, C.; Wu, C. K.; Saroff, D.; Lorenzo, A.; Yuan, M. L.; Yankner, B. A. *Nature Medicine* **1998**, *4*, 827. (b) Combs, C. K.; Karlo, J. C.; Kao, S. C.; Landreth, G. E. *J. Neurosci.* **2001**, *21*, 1179. (c) Qin, S.; Colin, C.; Hinnert, I.; Gervais, A.; Cheret, C.; Mallat, M. *J. Neurosci.* **2006**, *26*, 3345. (d) Meyer-Luehmann, M.; Spiess-Jones, T. L.; Prada, C.; Garcia-Alloza, M.; de Calignon, A.; Rozkalne, A.; Koenigsknecht-Talboo, J.; Holtzman, D. M.; Bacskai, B. J.; Hyman, B. T. *Nature* **2008**, *451*, 720.

- (161) Wyss-Coray, T.; Masliah, E.; Mallory, M.; McConlogue, L.; Johnson-Wood, K.; Lin, C.; Mucke, L. *Nature* **1997**, *389*, 603.
- (162) Masliah, E. *Nature* **2008**, *451*, 638.
- (163) Nagele, R. G.; Wegiel, J.; Venkataraman, V.; Imaki, H.; Wang, K. C.; Wegiel, J. *Neurobiology of Aging* **2004**, *25*, 663.
- (164) Simard, A. R.; Soulet, D.; Gowing, G.; Julien, J. P.; Rivest, S. *Neuron* **2006**, *49*, 489.
- (165) Westin, K.; Buchhave, P.; Nielsen, H. M.; Minthon, L.; Janciauskiene, S. M.; Hansson, O. *Plos One* **2012**, *7*, e30525.
- (166) Ramirez, B. G.; Blazquez, C.; del Pulgar, T. G.; Guzman, N.; de Ceballos, M. A. L. *J. Neurosci.* **2005**, *25*, 1904.
- (167) Selkoe, D. J. *Neuron* **2001**, *32*, 177.
- (168) Llovera, R. E.; de Tullio, M.; Alonso, L. G.; Leissring, M. A.; Kaufman, S. B.; Roher, A. E.; Gay, G. D.; Morelli, L.; Castano, E. M. *J. Biol. Chem.* **2008**, *283*, 17039.
- (169) Kumar, S.; Singh, S.; Hinze, D.; Josten, M.; Sahl, H. G.; Siepmann, M.; Walter, J. *J. Biol. Chem.* **2012**, *287*, 8641.
- (170) Hellstrom-Lindahl, E.; Ravid, R.; Nordberg, A. *Neurobiology of Aging* **2008**, *29*, 210.
- (171) (a) Hu, J.; Miyatake, F.; Aizu, Y.; Nakagawa, H.; Nakamura, S.; Tamaoka, A.; Takahash, R.; Urakami, K.; Shoji, M. *Neurosci. Lett.* **1999**, *277*, 65. (b) Hu, J. G.; Igarashi, A.; Kamata, M.; Nakagawa, H. *J. Biol. Chem.* **2001**, *276*, 47863.
- (172) (a) Williams, R. *Nature* **2011**, *475*, S5. (b) Buckholtz, N. S. *Nature* **2011**, *475*, S8.

- (173) Jack, C. R.; Knopman, D. S.; Jagust, W. J.; Shaw, L. M.; Aisen, P. S.; Weiner, M. W.; Petersen, R. C.; Trojanowski, J. Q. *Lancet Neurol.* **2010**, *9*, 119.
- (174) (a) Hampel, H.; Frank, R.; Broich, K.; Teipel, S. J.; Katz, R. G.; Hardy, J.; Herholz, K.; Bokde, A. L. W.; Jessen, F.; Hoessler, Y. C.; Sanhai, W. R.; Zetterberg, H.; Woodcock, J.; Blennow, K. *Nature Rev. Drug Disc.* **2010**, *9*, 560. (b) Humpel, C. *Trends in Biotechnology* **2011**, *29*, 26.
- (175) Blennow, K.; Hampel, H. *Lancet Neurol.* **2003**, *2*, 605.
- (176) Skoog, I.; Davidsson, P.; Aevarsson, O.; Vanderstichele, H.; Vanmechelen, E.; Blennow, K. *Dement. Geriatr. Cogn. Disord.* **2003**, *15*, 169.
- (177) Gustafson, D. R.; Skoog, I.; Rosengren, L.; Zetterberg, H.; Blennow, K. *J. Neurol. Neurosurg. Psychiatry* **2007**, *78*, 461.
- (178) Stomrud, E.; Hansson, O.; Blennow, K.; Minthon, L.; Londos, E. *Dement. Geriatr. Cogn. Disord.* **2007**, *24*, 118.
- (179) Mattsson, N.; Zetterberg, H.; Hansson, O.; Andreasen, N.; Parnetti, L.; Jonsson, M.; Herukka, S. K.; van der Flier, W. M.; Blankenstein, M. A.; Ewers, M.; Rich, K.; Kaiser, E.; Verbeek, M.; Tsolaki, M.; Mulugeta, E.; Rosen, E.; Aarsland, D.; Visser, P. J.; Schroder, J.; Marcusson, J.; de Leon, M.; Hampel, H.; Scheltens, P.; Pirttila, T.; Wallin, A.; Jonhagen, M. E.; Minthon, L.; Winblad, B.; Blennow, K. *J. Am. Med. Assoc.* **2009**, *302*, 385.
- (180) Ray, S.; Britschgi, M.; Herbert, C.; Takeda-Uchimura, Y.; Boxer, A.; Blennow, K.; Friedman, L. F.; Galasko, D. R.; Jutel, M.; Karydas, A.; Kaye, J. A.; Leszek, J.; Miller, B. L.; Minthon, L.; Quinn, J. F.; Rabinovici, G. D.; Robinson, W. H.; Sabbagh, M. N.; So, Y. T.; Sparks, D. L.; Tabaton, M.; Tinklenberg, J.; Yesavage, J. A.; Tibshirani, R.; Wyss-Coray, T. *Nature Medicine* **2007**, *13*, 1359.

- (181) (a) Okereke, O. I.; Xia, W. M.; Selkoe, D. J.; Grodstein, F. *Arch. Neurol.* **2009**, *66*, 1247. (b) Yaffe, K.; Weston, A.; Graff-Radford, N. R.; Satterfield, S.; Simonsick, E. M.; Younkin, S. G.; Younkin, L. H.; Kuller, L.; Ayonayon, H. N.; Ding, J. Z.; Harris, T. B. *J. Am. Med. Assoc.* **2011**, *305*, 261. (c) Yaffe, K.; Weston, A. *J. Am. Med. Assoc.* **2011**, *305*, 1655.
- (182) Reddy, M. M.; Wilson, R.; Wilson, J.; Connell, S.; Gocke, A.; Hynan, L.; German, D.; Kodadek, T. *Cell* **2011**, *144*, 132.
- (183) (a) Mathis, C. A.; Wang, Y. M.; Holt, D. P.; Huang, G. F.; Debnath, M. L.; Klunk, W. E. *J. Med. Chem.* **2003**, *46*, 2740. (b) Klunk, W. E.; Engler, H.; Nordberg, A.; Wang, Y. M.; Blomqvist, G.; Holt, D. P.; Bergstrom, M.; Savitcheva, I.; Huang, G. F.; Estrada, S.; Ausen, B.; Debnath, M. L.; Barletta, J.; Price, J. C.; Sandell, J.; Lopresti, B. J.; Wall, A.; Koivisto, P.; Antoni, G.; Mathis, C. A.; Langstrom, B. *Ann. Neurol.* **2004**, *55*, 306. (c) Fagan, A. M.; Mintun, M. A.; Mach, R. H.; Lee, S. Y.; Dence, C. S.; Shah, A. R.; LaRossa, G. N.; Spinner, M. L.; Klunk, W. E.; Mathis, C. A.; DeKosky, S. T.; Morris, J. C.; Holtzman, D. M. *Ann. Neurol.* **2006**, *59*, 512. (d) Forsberg, A.; Engler, H.; Almkvist, O.; Blomqvist, G.; Hagman, G.; Wall, A.; Ringheim, A.; Langstrom, B.; Nordberg, A. *Neurobiology of Aging* **2008**, *29*, 1456. (e) Tapiola, T.; Alafuzoff, I.; Herukka, S. K.; Parkkinen, L.; Hartikainen, P.; Soininen, H.; Pirttila, T. *Arch. Neurol.* **2009**, *66*, 382.
- (184) (a) Cordeiro, M. F.; Guo, L.; Coxon, K. M.; Duggan, J.; Nizari, S.; Normando, E. M.; Sensi, S. L.; Sillito, A. M.; Fitzke, F. W.; Salt, T. E.; Moss, S. E. *Cell Death Dis.* **2010**, *1*. (b) Guo, L.; Duggan, J.; Cordeiro, M. F. *Current Alzheimer Research* **2010**, *7*, 3.

- (185) (a) Nielsen, H. M.; Minthon, L.; Londos, E.; Blennow, K.; Miranda, E.; Perez, J.; Crowther, D. C.; Lomas, D. A.; Janciauskiene, S. M. *Neurology* **2007**, *69*, 1569. (b) Schneider, P.; Hampel, H.; Buerger, K. *CNS Neurosci. Ther.* **2009**, *15*, 358.
- (186) Li, R.; Lindholm, K.; Yang, L. B.; Yue, X.; Citron, M.; Yao, R. Q.; Beach, T.; Sue, L.; Sabbagh, M.; Cai, H. B.; Wong, P.; Price, D.; Shen, Y. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 3632.
- (187) Johnson-Wood, K.; Lee, M.; Motter, R.; Hu, K.; Gordon, G.; Barbour, R.; Khan, K.; Gordon, M.; Tan, H.; Games, D.; Lieberburg, I.; Schenk, D.; Seubert, P.; McConlogue, L. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 1550.
- (188) (a) DeMattos, R. B.; Bales, K. R.; Cummins, D. J.; Dodart, J. C.; Paul, S. M.; Holtzman, D. M. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 8850. (b) Shankar, G. M.; Bloodgood, B. L.; Townsend, M.; Walsh, D. M.; Selkoe, D. J.; Sabatini, B. L. *J. Neurosci.* **2007**, *27*, 2866.
- (189) (a) Kim, K. S.; Wen, G. Y.; Bancher, C.; Chen, C. M. J.; Sapienza, V. J.; Hong, H.; Wisniewski, H. M. *Neurosci. Res. Commun.* **1990**, *7*, 113. (b) Podlisny, M. B.; Ostaszewski, B. L.; Squazzo, S. L.; Koo, E. H.; Rydell, R. E.; Teplow, D. B.; Selkoe, D. J. *J. Biol. Chem.* **1995**, *270*, 9564. (c) Wong, H. E.; Qi, W.; Choi, H. M.; Fernandez, E.; Kwon, I. *ACS Chem. Neurosci.* **2011**, *2*, 645.
- (190) (a) Haass, C.; Schlossmacher, M. G.; Hung, A. Y.; Vigopelfrey, C.; Mellon, A.; Ostaszewski, B. L.; Lieberburg, I.; Koo, E. H.; Schenk, D.; Teplow, D. B.; Selkoe, D. J. *Nature* **1992**, *359*, 322. (b) Solomon, B.; Koppel, R.; Frankel, D.; HananAharon, E. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 4109.
- (191) Bieschke, J.; Herbst, M.; Wiglenda, T.; Friedrich, R. P.; Boeddrich, A.; Schiele, F.; Kleckers, D.; del Amo, J. M. L.; Gruning, B. A.; Wang, Q. W.; Schmidt,

- M. R.; Lurz, R.; Anwyl, R.; Schnoegl, S.; Fandrich, M.; Frank, R. F.; Reif, B.; Gunther, S.; Walsh, D. M.; Wanker, E. E. *Nature Chemical Biology* **2012**, *8*, 93.
- (192) Sergeant, N.; Bombois, S.; Ghestem, A.; Drobecq, H.; Kostanjevecki, V.; Missiaen, C.; Watzet, A.; David, J. P.; Vanmechelen, E.; Sergheraert, C.; Delacourte, A. *J. Neurochem.* **2003**, *85*, 1581.
- (193) Barghorn, S.; Nimmrich, V.; Striebinger, A.; Krantz, C.; Keller, P.; Janson, B.; Bahr, M.; Schmidt, M.; Bitner, R. S.; Harlan, J.; Barlow, E.; Ebert, U.; Hillen, H. *J. Neurochem.* **2005**, *95*, 834.
- (194) (a) Kaye, R.; Head, E.; Thompson, J. L.; McIntire, T. M.; Milton, S. C.; Cotman, C. W.; Glabe, C. G. *Science* **2003**, *300*, 486. (b) Kaye, R.; Head, E.; Sarsoza, F.; Saing, T.; Cotman, C. W.; Necula, M.; Margol, L.; Wu, J.; Breydo, L.; Thompson, J. L.; Rasool, S.; Gurlo, T.; Butler, P.; Glabe, C. G. *Mol. Neurodegener.* **2007**, *2*. (c) Glabe, C. G. *J. Biol. Chem.* **2008**, *283*, 29639.
- (195) McLaurin, J.; Cecal, R.; Kierstead, M. E.; Tian, X.; Phinney, A. L.; Manea, M.; French, J. E.; Lambermon, M. H. L.; Darabie, A. A.; Brown, M. E.; Janus, C.; Chishti, M. A.; Horne, P.; Westaway, D.; Fraser, P. E.; Mount, H. T. J.; Przybylski, M.; St George-Hyslop, P. *Nature Medicine* **2002**, *8*, 1263.
- (196) Mamikonyan, G.; Necula, M.; Mkrtychyan, M.; Ghochikyan, A.; Petrushina, I.; Movsesyan, N.; Mina, E.; Kiyatkin, A.; Glabe, C. G.; Cribbs, D. H.; Agadjanyan, M. G. *J. Biol. Chem.* **2007**, *282*, 22376.
- (197) McLean, D.; Cooke, M. J.; Wang, Y.; Fraser, P.; St George-Hyslop, P.; Shoichet, M. S. *J. Controlled Release* **2012**, *in press*.

- (198) Lambert, M. P.; Velasco, P. T.; Chang, L.; Viola, K. L.; Fernandez, S.; Lacor, P. N.; Khuon, D.; Gong, Y. S.; Bigio, E. H.; Shaw, P.; De Felice, F. G.; Krafft, G. A.; Klein, W. L. *J. Neurochem.* **2007**, *100*, 23.
- (199) Jin, M.; Shepardson, N.; Yang, T.; Chen, G.; Walsh, D.; Selkoe, D. J. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 5819.
- (200) Hoyer, W.; Gronwall, C.; Jonsson, A.; Stahl, S.; Hard, T. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 5099.
- (201) Macao, B.; Hoyer, W.; Sandberg, A.; Brorsson, A. C.; Dobson, C. M.; Hard, T. *BMC Biotechnol.* **2008**, *8*.
- (202) (a) Lambert, M. P.; Barlow, A. K.; Chromy, B. A.; Edwards, C.; Freed, R.; Liosatos, M.; Morgan, T. E.; Rozovsky, I.; Trommer, B.; Viola, K. L.; Wals, P.; Zhang, C.; Finch, C. E.; Krafft, G. A.; Klein, W. L. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 6448. (b) Andersson, K.; Olofsson, A.; Nielsen, E. H.; Svehag, S. E.; Lundgren, E. *Biochem. Biophys. Res. Commun.* **2002**, *294*, 309. (c) Rousseau, F.; Schmykovitz, J.; Serrano, L. *Curr. Opin. Struct. Biol.* **2006**, *16*, 118.
- (203) Du, Y. S.; Wei, X.; Dodel, R.; Sommer, N.; Hampel, H.; Gao, F.; Ma, Z. Z.; Zhao, L. M.; Oertel, W. H.; Farlow, M. *Brain* **2003**, *126*, 1935.
- (204) Lambert, M. P.; Viola, K. L.; Chromy, B. A.; Chang, L.; Morgan, T. E.; Yu, J. X.; Venton, D. L.; Krafft, G. A.; Finch, C. E.; Klein, W. L. *J. Neurochem.* **2001**, *79*, 595.
- (205) Yi, P.; Hadden, C.; Kulanthaivel, P.; Calvert, N.; Annes, W.; Brown, T.; Barbuch, R. J.; Chaudhary, A.; Ayan-Oshodi, M. A.; Ring, B. J. *Drug Metab. Dispos.* **2010**, *38*, 554.

- (206) Lacor, P. N.; Buniel, M. C.; Furlow, P. W.; Clemente, A. S.; Velasco, P. T.; Wood, M.; Viola, K. L.; Klein, W. L. *J. Neurosci.* **2007**, *27*, 796.
- (207) Lesne, S.; Koh, M. T.; Kotilinek, L.; Kaye, R.; Glabe, C. G.; Yang, A.; Gallagher, M.; Ashe, K. H. *Nature* **2006**, *440*, 352.
- (208) Dobson, C. M. *Nature* **2003**, *426*, 884.
- (209) Haupt, C.; Leppert, J.; Röncke, R.; Meinhardt, J.; Yadav, J. K.; Ramachandran, R.; Ohlenschläger, O.; Reymann, K. G.; Görlach, M.; Fändrich, M. *Angew. Chem., Int. Ed. Engl.* **2012**, *51*, 1576.
- (210) (a) Kuo, Y. M.; Emmerling, M. R.; Vigo-Pelfrey, C.; Kasunic, T. C.; Kirkpatrick, J. B.; Murdoch, G. H.; Ball, M. J.; Roher, A. E. *J. Biol. Chem.* **1996**, *271*, 4077. (b) Roher, A. E.; Chaney, M. O.; Kuo, Y. M.; Webster, S. D.; Stine, W. B.; Haverkamp, L. J.; Woods, A. S.; Cotter, R. J.; Tuohy, J. M.; Krafft, G. A.; Bonnell, B. S.; Emmerling, M. R. *J. Biol. Chem.* **1996**, *271*, 20631. (c) Enya, M.; Morishima-Kawashima, M.; Yoshimura, M.; Shinkai, Y.; Kusui, K.; Khan, K.; Games, D.; Schenk, D.; Sugihara, S.; Yamaguchi, H.; Ihara, Y. *Am. J. Pathol.* **1999**, *154*, 271. (d) Funato, H.; Enya, M.; Yoshimura, M.; Morishima-Kawashima, M.; Ihara, Y. *Am. J. Pathol.* **1999**, *155*, 23.
- (211) Shekhawat, G. S.; Chand, A.; Sharma, S.; Verawati; Dravid, V. P. *Appl. Phys. Lett.* **2009**, *95*, 233114.
- (212) Harper, J. D.; Wong, S. S.; Lieber, C. M.; Lansbury, P. T. *Chemistry and Biology* **1997**, *4*, 119.
- (213) Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Rowan, M. J.; Selkoe, D. J. *Biochem. Soc. Trans.* **2002**, *30*, 552.

- (214) Chromy, B. A.; Nowak, R. J.; Lambert, M. P.; Viola, K. L.; Chang, L.; Velasco, P. T.; Jones, B. W.; Fernandez, S. J.; Lacor, P. N.; Horowitz, P.; Finch, C. E.; Krafft, G. A.; Klein, W. L. *Biochemistry* **2003**, *42*, 12749.
- (215) (a) Kaye, R.; Sokolov, Y.; Edmonds, B.; McIntire, T. M.; Milton, S. C.; Hall, J. E.; Glabe, C. G. *J. Biol. Chem.* **2004**, *279*, 46363. (b) Lasagna-Reeves, C. A.; Castillo-Carranza, D. L.; Guerrero-Munoz, M. J.; Jackson, G. R.; Kaye, R. *Biochemistry* **2010**, *49*, 10039.
- (216) Necula, M.; Kaye, R.; Milton, S.; Glabe, C. G. *J. Biol. Chem.* **2007**, *282*, 10311.
- (217) Bernstein, S. L.; Wytttenbach, T.; Baumketner, A.; Shea, J. E.; Bitan, G.; Teplow, D. B.; Bowers, M. T. *J. Am. Chem. Soc.* **2005**, *127*, 2075.
- (218) Gellermann, G. P.; Byrnes, H.; Striebinger, A.; Ullrich, K.; Mueller, R.; Hillen, H.; Barghorn, S. *Neurobiol. Dis.* **2008**, *30*, 212.
- (219) Yu, L. P.; Edalji, R.; Harlan, J. E.; Holzman, T. F.; Lopez, A. P.; Labkovsky, B.; Hillen, H.; Barghorn, S.; Ebert, U.; Richardson, P. L.; Miesbauer, L.; Solomon, L.; Bartley, D.; Walter, K.; Johnson, R. W.; Hajduk, P. J.; Olejniczak, E. T. *Biochemistry* **2009**, *48*, 1870.
- (220) Bernstein, S. L.; Dupuis, N. F.; Lazo, N. D.; Wytttenbach, T.; Condrón, M. M.; Bitan, G.; Teplow, D. B.; Shea, J.-E.; Ruotolo, B. T.; Robinson, C. V.; Bowers, M. T. *Nature Chemistry* **2009**, *1*, 326.
- (221) (a) Bitan, G.; Kirkitadze, M. D.; Lomakin, A.; Vollers, S. S.; Benedek, G. B.; Teplow, D. B. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 330. (b) Bitan, G.; Teplow, D. B. *Acc. Chem. Res.* **2004**, *37*, 357.

- (222) Narayan, P.; Orte, A.; Clarke, R. W.; Bolognesi, B.; Hook, S.; Ganzinger, K. A.; Meehan, S.; Wilson, M. R.; Dobson, C. M.; Klenerman, D. *Nature Structural and Molecular Biology* **2012**, *19*, 79.
- (223) Libeu, C. P.; Poksay, K. S.; John, V.; Bredesen, D. E. *J. Alzheimers Dis.* **2011**, *25*, 547.
- (224) (a) Pollard, H. B.; Arispe, N.; Rojas, E. *Cellular and Molecular Neurobiology* **1995**, *15*, 513. (b) Kourie, J. I.; Henry, C. L. *Clin. Exp. Pharmacol. Physiol.* **2002**, *29*, 741. (c) Lashuel, H. A.; Hartley, D.; Petre, B. M.; Walz, T.; Lansbury, P. T. *Nature* **2002**, *418*, 291. (d) Lashuel, H. A.; Lansbury, P. T. *Q. Rev. Biophys.* **2006**, *39*, 167. (e) Marx, J. *Science* **2007**, *318*, 384. (f) Bischofberger, M.; Gonzalez, M. R.; van der Goot, F. G. *Curr. Opin. Cell Biol.* **2009**, *21*, 589. (g) Butterfield, S. M.; Lashuel, H. A. *Angew. Chem.* **2010**, *49*, 5628.
- (225) (a) Arispe, N.; Rojas, E.; Pollard, H. B. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 567. (b) Arispe, N.; Pollard, H. B.; Rojas, E. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 10573. (c) Arispe, N.; Pollard, H. B.; Rojas, E. *Ann. N. Y. Acad. Sci.* **1994**, *747*, 256. (d) Arispe, N.; Pollard, H. B.; Rojas, E. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 1710.
- (226) (a) Lin, H.; Bhatia, R.; Lal, R. *FASEB J.* **2001**, *15*, 2433. (b) Quist, A.; Doudevski, I.; Lin, H.; Azimova, R.; Ng, D.; Frangione, B.; Kagan, B.; Ghiso, J.; Lal, R. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 10427.
- (227) Hirakura, Y.; Lin, M. C.; Kagan, B. L. *J. Neurosci. Res.* **1999**, *57*, 458.
- (228) Inoue, S. *Amyloid-Journal of Protein Folding Disorders* **2008**, *15*, 223.
- (229) Bhatia, R.; Lin, H.; Lal, R. *FASEB J.* **2000**, *14*, 1233.

- (230) Parodi, J.; Sepulveda, F. J.; Roa, J.; Opazo, C.; Inestrosa, N. C.; Aguayo, L. G.
J. Biol. Chem. **2010**, *285*, 2506.
- (231) Busche, M. A.; Eichhoff, G.; Adelsberger, H.; Abramowski, D.; Wiederhold, K.
H.; Haass, C.; Staufenbiel, M.; Konnerth, A.; Garaschuk, O. *Science* **2008**,
321, 1686.
- (232) Green, K. N.; Demuro, A.; Akbari, Y.; Hitt, B. D.; Smith, I. F.; Parker, I.;
LaFerla, F. M. *J. Cell Biol.* **2008**, *181*, 1107.
- (233) Clinton, L. K.; Blurton-Jones, M.; Myczek, K.; Trojanowski, J. Q.; LaFerla, F.
M. *J. Neurosci.* **2010**, *30*, 7281.
- (234) Fagan, A. M.; Mintun, M. A.; Shah, A. R.; Aldea, P.; Roe, C. M.; Mach, R. H.;
Marcus, D.; Morris, J. C.; Holtzman, D. M. *EMBO Mol. Med.* **2009**, *1*, 371.
- (235) Aderinwale, O. G.; Ernst, H. W.; Mousa, S. A. *Am. J. Alzheimers Dis. Other
Dement.* **2010**, *25*, 414.
- (236) Miller, G. *Science* **2010**, *329*, 502.
- (237) Howard, R.; McShane, R.; Lindsay, J.; Ritchie, C.; Baldwin, A.; Barber, R.;
Burns, A.; Denning, T.; Findlay, D.; Holmes, C.; Hughes, A.; Jacoby, R.; Jones,
R.; Jones, R.; McKeith, I.; Macharouthu, A.; O'Brien, J.; Passmore, P.;
Sheehan, B.; Juszczak, E.; Katona, C.; Hills, R.; Knapp, M.; Ballard, C.;
Brown, R.; Banerjee, S.; Onions, C.; Griffin, M.; Adams, J.; Gray, R.;
Johnson, T.; Bentham, P.; Phillips, P. *New England Journal of Medicine* **2012**,
366, 893.
- (238) Matharu, B.; Gibson, G.; Parsons, R.; Huckerby, T. N.; Moore, S. A.; Cooper,
L. J.; Millichamp, R.; Allsop, D.; Austen, B. *J. Neurol. Sci.* **2009**, *280*, 49.
- (239) Coyle, J.; Kershaw, P. *Biol. Psychiatry* **2001**, *49*, 289.
- (240) Lipton, S. A. *Nature Rev. Drug Disc.* **2006**, *5*, 160.

- (241) De Felice, F. G.; Velasco, P. T.; Lambert, M. P.; Viola, K.; Fernandez, S. J.; Ferreira, S. T.; Klein, W. L. *J. Biol. Chem.* **2007**, *282*, 11590.
- (242) (a) Hamaguchi, T.; Ono, K.; Yamada, M. *Cell. Mol. Life Sci.* **2006**, *63*, 1538.
(b) Doig, A. J. *Curr. Opin. Drug Discov. Devel.* **2007**, *10*, 533. (c) Amijee, H.; Madine, J.; Middleton, D. A.; Doig, A. J. *Biochem. Soc. Trans.* **2009**, *37*, 692.
(d) Hawkes, C. A.; Ng, V.; McLaurin, J. *Drug Dev. Res.* **2009**, *70*, 111.
- (243) (a) Gervais, F.; Paquette, J.; Morissette, C.; Krzywkowski, P.; Yu, M.; Azzi, M.; Lacombe, D.; Kong, X. Q.; Aman, A.; Laurin, J.; Szarek, W. A.; Tremblay, P. *Neurobiology of Aging* **2007**, *28*, 537. (b) Aisen, P. S.; Gauthier, S.; Vellas, B.; Briand, R.; Saurnier, D.; Laurin, J.; Garceau, D. *Current Alzheimer Research* **2007**, *4*, 473.
- (244) Aisen, P. S.; Saumier, D.; Briand, R.; Laurin, J.; Gervais, F.; Tremblay, P.; Garceau, D. *Neurology* **2006**, *67*, 1757.
- (245) (a) Fraser, P. E.; Nguyen, J. T.; Chin, D. T.; Kirschner, D. A. *J. Neurochem.* **1992**, *59*, 1531. (b) Lorenzo, A.; Yankner, B. A. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 12243. (c) Carter, D. B.; Chou, K. C. *Neurobiology of Aging* **1998**, *19*, 37. (d) Frid, P.; Anisimov, S. V.; Popovic, N. *Brain Res. Rev.* **2007**, *53*, 135. (e) Pedersen, M. O.; Mikkelsen, K.; Behrens, M. A.; Pedersen, J. S.; Enghild, J. J.; Skrydstrup, T.; Malmendal, A.; Nielsen, N. C. *J. Phys. Chem. B* **2010**, *114*, 16003. (f) Bose, P. P.; Chatterjee, U.; Xie, L.; Johansson, J.; Gothelid, E.; Arvidsson, P. I. *ACS Chem. Neurosci.* **2010**, *1*, 315.
- (246) Buell, A. K.; Dobson, C. M.; Knowles, T. P. J.; Welland, M. E. *Biophys. J.* **2010**, *99*, 3492.
- (247) Necula, M.; Breydo, L.; Milton, S.; Kaye, R.; van der Veer, W. E.; Tone, P.; Glabe, C. G. *Biochemistry* **2007**, *46*, 8850.

- (248) (a) Gorman, P. M.; Yip, C. M.; Fraser, P. E.; Chakrabartty, A. *J. Mol. Biol.* **2003**, *325*, 743. (b) Lee, J.; Culyba, E. K.; Powers, E. T.; Kelly, J. W. *Nature Chemical Biology* **2011**, *7*, 602.
- (249) Ladiwala, A. R. A.; Dordick, J. S.; Tessier, P. M. *J. Biol. Chem.* **2011**, *286*, 3209.
- (250) Ramassamy, C. *Eur. J. Pharmacol.* **2006**, *545*, 51.
- (251) Kelsey, N. A.; Wilkins, H. M.; Linseman, D. A. *Molecules* **2010**, *15*, 7792.
- (252) (a) Ehrnhoefer, D. E.; Bieschke, J.; Boeddrich, A.; Herbst, M.; Masino, L.; Lurz, R.; Engemann, S.; Pastore, A.; Wanker, E. E. *Nat. Struct. Mol. Biol.* **2008**, *15*, 558. (b) Bieschke, J.; Russ, J.; Friedrich, R. P.; Ehrnhoefer, D. E.; Wobst, H.; Neugebauer, K.; Wanker, E. E. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 7710.
- (253) Ono, K.; Yoshiike, Y.; Takashima, A.; Hasegawa, K.; Naiki, H.; Yamada, M. *J. Neurochem.* **2003**, *87*, 172.
- (254) Jones, O. G.; Mezzenga, R. *Soft Matter* **2012**, *8*, 876.
- (255) Hamaguchi, T.; Ono, K.; Murase, A.; Yamada, M. *Am. J. Pathol.* **2009**, *175*, 2557.
- (256) Yang, F. S.; Lim, G. P.; Begum, A. N.; Ubeda, O. J.; Simmons, M. R.; Ambegaokar, S. S.; Chen, P. P.; Kaye, R.; Glabe, C. G.; Frautschi, S. A.; Cole, G. M. *J. Biol. Chem.* **2005**, *280*, 5892.
- (257) (a) Feng, Y.; Wang, X. P.; Yang, S. G.; Wang, Y. J.; Zhang, X.; Du, X. T.; Sun, X. X.; Zhao, M.; Huang, L.; Liu, R. T. *Neurotoxicology* **2009**, *30*, 986. (b) Ladiwala, A. R. A.; Lin, J. C.; Bale, S. S.; Marcelino-Cruz, A. M.; Bhattacharya, M.; Dordick, J. S.; Tessier, P. M. *J. Biol. Chem.* **2010**, *285*, 24228.

- (258) Frydman-Marom, A.; Rechter, M.; Shefler, I.; Bram, Y.; Shalev, D. E.; Gazit, E. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 1981.
- (259) (a) Cherny, R. A.; Legg, J. T.; McLean, C. A.; Fairlie, D. P.; Huang, X. D.; Atwood, C. S.; Beyreuther, K.; Tanzi, R. E.; Masters, C. L.; Bush, A. I. *J. Biol. Chem.* **1999**, *274*, 23223. (b) Raman, B.; Ban, T.; Yamaguchi, K.; Sakai, M.; Kawai, T.; Naiki, H.; Goto, Y. *J. Biol. Chem.* **2005**, *280*, 16157.
- (260) Cherny, R. A.; Atwood, C. S.; Xilinas, M. E.; Gray, D. N.; Jones, W. D.; McLean, C. A.; Barnham, K. J.; Volitakis, I.; Fraser, F. W.; Kim, Y. S.; Huang, X. D.; Goldstein, L. E.; Moir, R. D.; Lim, J. T.; Beyreuther, K.; Zheng, H.; Tanzi, R. E.; Masters, C. L.; Bush, A. I. *Neuron* **2001**, *30*, 665.
- (261) (a) Lannfelt, L.; Blennow, K.; Zetterberg, H.; Batsman, S.; Ames, D.; Harrison, J.; Masters, C. L.; Targum, S.; Bush, A. I.; Murdoch, R.; Wilson, J.; Ritchie, C. W. *Lancet Neurol.* **2008**, *7*, 779. (b) Adlard, P. A.; Cherny, R. A.; Finkelstein, D. I.; Gautier, E.; Robb, E.; Cortes, M.; Volitakis, I.; Liu, X.; Smith, J. P.; Perez, K.; Laughton, K.; Li, Q. X.; Charman, S. A.; Nicolazzo, J. A.; Wilkins, S.; Deleva, K.; Lynch, T.; Kok, G.; Ritchie, C. W.; Tanzi, R. E.; Cappai, R.; Masters, C. L.; Barnham, K. J.; Bush, A. I. *Neuron* **2008**, *59*, 43. (c) Faux, N. G.; Ritchie, C. W.; Gunn, A.; Rembach, A.; Tsatsanis, A.; Bedo, J.; Harrison, J.; Lannfelt, L.; Blennow, K.; Zetterberg, H.; Ingelsson, M.; Masters, C. L.; Tanzi, R. E.; Cummings, J. L.; Herd, C. M.; Bush, A. I. *J. Alzheimers Dis.* **2010**, *20*, 509.
- (262) Choi, J. S.; Braymer, J. J.; Nanga, R. P. R.; Ramamoorthy, A.; Lim, M. H. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 21990.
- (263) Scott, L. E.; Telpoukhovskaia, M.; Rodriguez-Rodriguez, C.; Merkel, M.; Bowen, M. L.; Page, B. D. G.; Green, D. E.; Storr, T.; Thomas, F.; Allen, D.

- D.; Lockman, P. R.; Patrick, B. O.; Adam, M. J.; Orvig, C. *Chem. Sci.* **2011**, *2*, 642.
- (264) Folk, D. S.; Franz, K. J. *J. Am. Chem. Soc.* **2010**, *132*, 4994.
- (265) Schugar, H.; Green, D. E.; Bowen, M. L.; Scott, L. E.; Storr, T.; Bohmerle, K.; Thomas, F.; Allen, D. D.; Lockman, P. R.; Merkel, M.; Thompson, K. H.; Orvig, C. *Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 1716.
- (266) (a) Arispe, N.; Pollard, H. B.; Rojas, E. *Mol. Cell. Biochem.* **1994**, *140*, 119. (b) Simakova, O.; Arispe, N. *J. Biochemistry* **2006**, *45*, 5907.
- (267) (a) Snow, A. D.; Mar, H.; Nochlin, D.; Kimata, K.; Kato, M.; Suzuki, S.; Hassell, J.; Wight, T. N. *Am. J. Pathol.* **1988**, *133*, 456. (b) McLaurin, J.; Franklin, T.; Zhang, X. Q.; Deng, J. P.; Fraser, P. E. *Eur. J. Biochem.* **1999**, *266*, 1101. (c) McLaurin, J.; Yang, D. S.; Yip, C. M.; Fraser, P. E. *Journal of Structural Biology* **2000**, *130*, 259.
- (268) Bravo, R.; Arimon, M.; Valle-Delgado, J. J.; Garcia, R.; Durany, N.; Castel, S.; Cruz, M.; Ventura, S.; Fernandez-Busquets, X. *J. Biol. Chem.* **2008**, *283*, 32471.
- (269) Leveugle, B.; Scanameo, A.; Ding, W.; Fillit, H. *Neuroreport* **1994**, *5*, 1389.
- (270) Miura, Y.; Yasuda, K.; Yamamoto, K.; Koike, M.; Nishida, Y.; Kobayashi, K. *Biomacromolecules* **2007**, *8*, 2129.
- (271) Fung, J.; Darabie, A. A.; McLaurin, J. *Biochem. Biophys. Res. Commun.* **2005**, *328*, 1067.
- (272) Ariga, T.; Miyatake, T.; Yu, R. K. *J. Neurosci. Res.* **2010**, *88*, 2303.
- (273) Valle-Delgado, J. J.; Alfonso-Prieto, M.; de Groot, N. S.; Ventura, S.; Samitier, J.; Rovira, C.; Fernandez-Busquets, X. *FASEB J.* **2010**, *24*, 4250.

- (274) Akaishi, T.; Morimoto, T.; Shibao, M.; Watanabe, S.; Sakai-Kato, K.; Utsunomiya-Tate, N.; Abe, K. *Neurosci. Lett.* **2008**, *444*, 280.
- (275) Kim, H.; Park, B. S.; Lee, K. G.; Choi, C. Y.; Jang, S. S.; Kim, Y. H.; Lee, S. E. *J. Agric. Food Chem.* **2005**, *53*, 8537.
- (276) McLaurin, J.; Franklin, T.; Chakrabartty, A.; Fraser, P. E. *J. Mol. Biol.* **1998**, *278*, 183.
- (277) McLaurin, J.; Golomb, R.; Jurewicz, A.; Antel, J. P.; Fraser, P. E. *J. Biol. Chem.* **2000**, *275*, 18495.
- (278) Fenili, D.; Brown, M.; Rappaport, R.; McLaurin, J. *J. Mol. Med.* **2007**, *85*, 603.
- (279) (a) Palmer, A. M. *Trends Pharmacol. Sci.* **2011**, *32*, 141. (b) Gravitz, L. *Nature* **2011**, *475*, S9.
- (280) Findeis, M. A.; Musso, G. M.; Arico-Muendel, C. C.; Benjamin, H. W.; Hundal, A. M.; Lee, J. J.; Chin, J.; Kelley, M.; Wakefield, J.; Hayward, N. J.; Molineaux, S. M. *Biochemistry* **1999**, *38*, 6791.
- (281) Lowe, T. L.; Strzelec, A.; Kiessling, L. L.; Murphy, R. M. *Biochemistry* **2001**, *40*, 7882.
- (282) Pallitto, M. M.; Ghanta, J.; Heinzelman, P.; Kiessling, L. L.; Murphy, R. M. *Biochemistry* **1999**, *38*, 3570.
- (283) Gibson, T. J.; Murphy, R. M. *Biochemistry* **2005**, *44*, 8898.
- (284) Gordon, D. J.; Tappe, R.; Meredith, S. C. *Journal of Peptide Research* **2002**, *60*, 37.
- (285) (a) Hughes, E.; Burke, R. M.; Doig, A. J. *J. Biol. Chem.* **2000**, *275*, 25109. (b) Kokkoni, N.; Stott, K.; Amijee, H.; Mason, J. M.; Doig, A. J. *Biochemistry* **2006**, *45*, 9906.

- (286) Austen, B. M.; Paleologou, K. E.; Ali, S. A. E.; Qureshi, M. M.; Allsop, D.; El-Agnaf, O. M. A. *Biochemistry* **2008**, *47*, 1984.
- (287) Madine, J.; Wang, X.; Brown, D. R.; Middleton, D. A. *ChemBioChem* **2009**, *10*, 1982.
- (288) Castelletto, V.; Cheng, G.; Hamley, I. W. *Chem. Comm.* **2011**, *47*, 12470.
- (289) Watanabe, K.; Segawa, T.; Nakamura, K.; Kodaka, M.; Konakahara, T.; Okuno, H. *Journal of Peptide Research* **2001**, *58*, 342.
- (290) Sundaram, R. K.; Kasinathan, C.; Stein, S.; Sundaram, P. *Current Alzheimer Research* **2008**, *5*, 26.
- (291) (a) Rzepecki, P.; Schrader, T. *J. Am. Chem. Soc.* **2005**, *127*, 3016. (b) Hochdörffer, K.; Marz-Berberich, J.; Nage-Steger, L.; Epple, M.; Meyer-Zaika, W.; Horn, A. H. C.; Stich, H.; Sinha, S.; Bitan, G.; Schrader, T. *J. Am. Chem. Soc.* **2011**, *133*, 4348.
- (292) Sinha, S.; Lopes, D. H. J.; Du, Z. M.; Pang, E. S.; Shanmugam, A.; Lomakin, A.; Talbiersky, P.; Tennstaedt, A.; McDaniel, K.; Bakshi, R.; Kuo, P. Y.; Ehrmann, M.; Benedek, G. B.; Loo, J. A.; Klarner, F. G.; Schrader, T.; Wang, C. Y.; Bitan, G. *J. Am. Chem. Soc.* **2011**, *133*, 16958.
- (293) Sato, J.; Takahashi, T.; Oshima, H.; Matsumura, S.; Mihara, H. *Chem. Eur. J.* **2007**, *13*, 7745.
- (294) Simons, M.; Keller, P.; De Strooper, B.; Beyreuther, K.; Dotti, C. G.; Simons, K. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 6460.
- (295) Fonte, V.; Kapulkin, V.; Taft, A.; Fluet, A.; Friedman, D.; Link, C. D. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 9439.
- (296) (a) Raman, B.; Ban, T.; Sakai, M.; Pasta, S. Y.; Ramakrishna, T.; Naiki, H.; Goto, Y.; Rao, C. M. *Biochem. J.* **2005**, *392*, 573. (b) Shamma, S. L.;

- Waudby, C. A.; Wang, S. Y.; Buell, A. K.; Knowles, T. P. J.; Ecroyd, H.; Welland, M. E.; Carver, J. A.; Dobson, C. M.; Meehan, S. *Biophys. J.* **2011**, *101*, 1681.
- (297) Calamini, B.; Silva, M. C.; Madoux, F.; Hutt, D. M.; Khanna, S.; Chalfant, M. A.; Saldanha, S. A.; Hodder, P.; Tait, B. D.; Garza, D.; Balch, W. E.; Morimoto, R. I. *Nature Chemical Biology* **2012**, *8*, 185.
- (298) Yerbury, J. J.; Poon, S.; Meehan, S.; Thompson, B.; Kumita, J. R.; Dobson, C. M.; Wilson, M. R. *FASEB J.* **2007**, *21*, 2312.
- (299) Chauhan, V. P. S.; Ray, I.; Chauhan, A.; Wisniewski, H. M. *Biochem. Biophys. Res. Commun.* **1999**, *258*, 241.
- (300) Choo-Smith, L. P.; Garzon-Rodriguez, W.; Glabe, C. G.; Surewicz, W. K. *J. Biol. Chem.* **1997**, *272*, 22987.
- (301) Matsuoka, Y.; Saito, M.; LaFrancois, J.; Saito, M.; Gaynor, K.; Olm, V.; Wang, L. L.; Casey, E.; Lu, Y. F.; Shiratori, C.; Lemere, C.; Duff, K. *J. Neurosci.* **2003**, *23*, 29.
- (302) Schwarzman, A. L.; Tsiper, M.; Wente, H.; Wang, A.; Vitek, M. P.; Vasiliev, V.; Goldgaber, D. *Amyloid-Journal of Protein Folding Disorders* **2004**, *11*, 1.
- (303) Lauren, J.; Gimbel, D. A.; Nygaard, H. B.; Gilbert, J. W.; Strittmatter, S. M. *Nature* **2009**, *457*, 1128.
- (304) Kudo, W.; Lee, H. P.; Zou, W. Q.; Wang, X. L.; Perry, G.; Zhu, X. W.; Smith, M. A.; Petersen, R. B.; Lee, H. G. *Hum. Mol. Genet.* **2012**, *21*, 1138.
- (305) You, H. T.; Tsutsui, S.; Hameed, S.; Kannanayakal, T. J.; Chen, L. N.; Xia, P.; Engbers, J. D. T.; Lipton, S. A.; Stys, P. K.; Zamponi, G. W. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 1737.

- (306) Cabaleiro-Lago, C.; Quinlan-Pluck, F.; Lynch, I.; Dawson, K. A.; Linse, S. *ACS Chem. Neurosci.* **2010**, *1*, 279.
- (307) Yoo, S. I.; Yang, M.; Brender, J. R.; Subramanian, V.; Sun, K.; Joo, N. E.; Jeong, S. H.; Ramamoorthy, A.; Kotov, N. A. *Angew. Chem. Int. Ed. Engl.* **2011**, *50*, 5110.
- (308) Geng, J.; Li, M.; Ren, J. S.; Wang, E. B.; Qu, X. G. *Angew. Chem. Int. Ed. Engl.* **2011**, *50*, 4184.
- (309) Barandeh, F.; Nguyen, P.-L.; Kumar, R.; Iacobucci, G. J.; Kuznicki, M. L.; Kosterman, A.; Bergey, E. J.; Prasad, P. N.; Gunawardena, S. *Plos One* **2012**, *7*, e29424.
- (310) Di, L.; Kerns, E. H.; Fan, K.; McConnell, O. J.; Carter, G. T. *Eur. J. Med. Chem.* **2003**, *38*, 223.
- (311) Klementieva, O.; Benseny-Cases, N.; Gella, A.; Appelhans, D.; Voit, B.; Cladera, J. *Biomacromolecules* **2011**, *12*, 3903.
- (312) Hong, L.; Koelsch, G.; Lin, X. L.; Wu, S. L.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. *Science* **2000**, *290*, 150.
- (313) (a) Kimura, T.; Shuto, D.; Kasai, S.; Liu, P.; Hidaka, K.; Hamada, T.; Hayashi, Y.; Hattori, C.; Asai, M.; Kitazume, S.; Saido, T. C.; Ishiura, S.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1527. (b) Kimura, T.; Shuto, D.; Hamada, Y.; Igawa, N.; Kasai, S.; Liu, P.; Hidaka, K.; Hamada, T.; Hayashi, Y.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 211.
- (314) Asai, M.; Hattori, C.; Iwata, N.; Saido, T. C.; Sasagawa, N.; Szabo, B.; Hashimoto, Y.; Maruyama, K.; Tanuma, S.; Kiso, Y.; Ishiura, S. *J. Neurochem.* **2006**, *96*, 533.

- (315) Rajendran, L.; Schneider, A.; Schlechtingen, G.; Weidlich, S.; Ries, J.; Braxmeier, T.; Schwille, P.; Schulz, J. B.; Schroeder, C.; Simons, M.; Jennings, G.; Knolker, H. J.; Simons, K. *Science* **2008**, *320*, 520.
- (316) Parsons, R. B.; Subramaniam, D.; Austen, B. M. *J. Neurochem.* **2007**, *102*, 1276.
- (317) Parsons, R. B.; Farrant, J. K.; Price, G. C.; Subramaniam, D.; Austen, B. M. *Biochem. Soc. Trans.* **2007**, *35*, 577.
- (318) Parsons, R. B.; Austen, B. M. *Biochem. Soc. Trans.* **2007**, *35*, 974.
- (319) Cai, H. B.; Wang, Y. S.; McCarthy, D.; Wen, H. J.; Borchelt, D. R.; Price, D. L.; Wong, P. C. *Nature Neuroscience* **2001**, *4*, 233.
- (320) Geling, A.; Steiner, H.; Willem, M.; Bally-Cuif, L.; Haass, C. *EMBO Rep.* **2002**, *3*, 688.
- (321) (a) Weggen, S.; Eriksen, J. L.; Das, P.; Sagi, S. A.; Wang, R.; Pietrzik, C. U.; Findlay, K. A.; Smith, T. E.; Murphy, M. P.; Butler, T.; Kang, D. E.; Marquez-Sterling, N.; Golde, T. E.; Koo, E. H. *Nature* **2001**, *414*, 212. (b) Weggen, S.; Eriksen, J. L.; Sagi, S. A.; Pietrzik, C. U.; Ozols, V.; Fauq, A.; Golde, T. E.; Koo, E. H. *J. Biol. Chem.* **2003**, *278*, 31831. (c) Sagi, S. A.; Weggen, S.; Eriksen, J.; Golde, T. E.; Koo, E. H. *J. Biol. Chem.* **2003**, *278*, 31825. (d) Désiré, L.; Bourdin, J.; Loiseau, N.; Peillon, H.; Picard, V.; De Oliveira, C.; Bachelot, F.; Leblond, B.; Tavernier, T.; Beausoleil, E.; Lacombe, S.; Drouin, D.; Schweighoffer, F. *J. Biol. Chem.* **2005**, *280*, 37516. (e) Kukar, T. L.; Ladd, T. B.; Bann, M. A.; Fraering, P. C.; Narlawar, R.; Maharvi, G. M.; Healy, B.; Chapman, R.; Welzel, A. T.; Price, R. W.; Moore, B.; Rangachari, V.; Cusack, B.; Eriksen, J.; Jansen-West, K.; Verbeeck, C.; Yager, D.; Eckman, C.; Ye, W. J.; Sagi, S.; Cottrell, B. A.; Torpey, J.;

- Rosenberry, T. L.; Fauq, A.; Wolfe, M. S.; Schmidt, B.; Walsh, D. M.; Koo, E. H.; Golde, T. E. *Nature* **2008**, *453*, 925.
- (322) Morihara, T.; Chu, T.; Ubeda, O.; Beech, W.; Cole, G. M. *J. Neurochem.* **2002**, *83*, 1009.
- (323) Lanz, T. A.; Karmilowicz, M. J.; Wood, K. M.; Pozdnyakov, N.; Du, P.; Piotrowski, M. A.; Brown, T. M.; Nolan, C. E.; Richter, K. E. G.; Finley, J. E.; Fei, Q.; Ebbinghaus, C. F.; Chen, Y. L.; Spracklin, D. K.; Tate, B.; Geoghegan, K. F.; Lau, L. F.; Auperin, D. D.; Schachter, J. B. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 924.
- (324) (a) Mayer, S. C.; Kreft, A. F.; Harrison, B.; Abou-Gharbia, M.; Antane, M.; Aschmies, S.; Atchison, K.; Chlenov, M.; Cole, D. C.; Comery, T.; Diamantidis, G.; Ellingboe, J.; Fan, K.; Galante, R.; Gonzales, C.; Ho, D. M.; Hoke, M. E.; Hu, Y.; Huryn, D.; Jain, U.; Jin, M.; Kremer, K.; Kubrak, D.; Lin, M.; Lu, P. M.; Magolda, R.; Martone, R.; Moore, W.; Oganessian, A.; Pangalos, M. N.; Porte, A.; Reinhart, P.; Resnick, L.; Riddell, D. R.; Sonnenberg-Reines, J.; Stock, J. R.; Sun, S. C.; Wagner, E.; Wang, T.; Woller, K.; Xu, Z.; Zaleska, M. M.; Zeldis, J.; Zhang, M. S.; Zhou, H.; Jacobsen, J. S. *J. Med. Chem.* **2008**, *51*, 7348. (b) Martone, R. L.; Zhou, H.; Atchison, K.; Comery, T.; Xu, J. Z.; Huang, X. Y.; Gong, X. H.; Jin, M.; Kreft, A.; Harrison, B.; Mayer, S. C.; Aschmies, S.; Gonzales, C.; Zaleska, M. M.; Riddell, D. R.; Wagner, E.; Lu, P. M.; Sun, S. C.; Sonnenberg-Reines, J.; Oganessian, A.; Adkins, K.; Leach, M. W.; Clarke, D. W.; Huryn, D.; Abou-Gharbia, M.; Magolda, R.; Bard, J.; Frick, G.; Raje, S.; Forlow, S. B.; Balliet, C.; Burczynski, M. E.; Reinhart, P. H.; Wan, H. I.; Pangalos, M. N.; Jacobsen, J. S. *J. Pharmacol. Exp. Ther.* **2009**, *331*, 598.

- (325) Serneels, L.; Van Biervliet, J.; Craessaerts, K.; Dejaegere, T.; Horre, K.; Van Houtvin, T.; Esselmann, H.; Paul, S.; Schafer, M. K.; Berezovska, O.; Hyman, B. T.; Sprangers, B.; Sciot, R.; Moons, L.; Jucker, M.; Yang, Z. X.; May, P. C.; Karran, E.; Wiltfang, J.; D'Hooge, R.; De Strooper, B. *Science* **2009**, *324*, 639.
- (326) He, G.; Luo, W. J.; Li, P.; Remmers, C.; Netzer, W. J.; Hendrick, J.; Bettayeb, K.; Flajolet, M.; Gorelick, F.; Wennogle, L. P.; Greengard, P. *Nature* **2010**, *467*, 95.
- (327) (a) Schenk, D. *Nat. Rev. Neurosci.* **2002**, *3*, 824. (b) Birmingham, K.; Frantz, S. *Nature Medicine* **2002**, *8*, 199. (c) Schenk, D.; Hagen, M.; Seubert, P. *Curr. Opin. Immunol.* **2004**, *16*, 599.
- (328) Hock, C.; Konietzko, U.; Streffer, J. R.; Tracy, J.; Signorell, A.; Muller-Tillmanns, B.; Lemke, U.; Henke, K.; Moritz, E.; Garcia, E.; Wollmer, M. A.; Umbricht, D.; de Quervain, D. J. F.; Hofmann, M.; Maddalena, A.; Papassotiropoulos, A.; Nitsch, R. M. *Neuron* **2003**, *38*, 547.
- (329) Morgan, D.; Diamond, D. M.; Gottschall, P. E.; Ugen, K. E.; Dickey, C.; Hardy, J.; Duff, K.; Jantzen, P.; DiCarlo, G.; Wilcock, D.; Connor, K.; Hatcher, J.; Hope, C.; Gordon, M.; Arendash, G. W. *Nature* **2000**, *408*, 982.
- (330) Orgogozo, J. M.; Gilman, S.; Dartigues, J. F.; Laurent, B.; Puel, M.; Kirby, L. C.; Jouanny, P.; Dubois, B.; Eisner, L.; Flitman, S.; Michel, B. F.; Boada, M.; Frank, A.; Hock, C. *Neurology* **2003**, *61*, 46.
- (331) Gilman, S.; Koller, M.; Black, R. S.; Jenkins, L.; Griffith, S. G.; Fox, N. C.; Eisner, L.; Kirby, L.; Rovira, M. B.; Forette, F.; Orgogozo, J. M. *Neurology* **2005**, *64*, 1553.

- (332) Fox, N. C.; Black, R. S.; Gilman, S.; Rossor, M. N.; Griffith, S. G.; Jenkins, L.; Koller, M. *Neurology* **2005**, *64*, 1563.
- (333) Holmes, C.; Boche, D.; Wilkinson, D.; Yadegarfar, G.; Hopkins, V.; Bayer, A.; Jones, R. W.; Bullock, R.; Love, S.; Neal, J. W.; Zotova, E.; Nicoll, J. A. R. *Lancet* **2008**, *372*, 216.
- (334) Oddo, S.; Billings, L.; Kesslak, J. P.; Cribbs, D. H.; LaFerla, F. M. *Neuron* **2004**, *43*, 321.
- (335) Eisele, Y. S.; Obermuller, U.; Heilbronner, G.; Baumann, F.; Kaeser, S. A.; Wolburg, H.; Walker, L. C.; Staufenbiel, M.; Heikenwalder, M.; Jucker, M. *Science* **2010**, *330*, 980.
- (336) Sigurdsson, E. M.; Scholtzova, H.; Mehta, P. D.; Frangione, B.; Wisniewski, T. *Am. J. Pathol.* **2001**, *159*, 439.
- (337) (a) Dickstein, D. L.; Biron, K. E.; Ujii, M.; Pfeifer, C. G.; Jeffries, A. R.; Jefferies, W. A. *FASEB J.* **2006**, *20*, 426. (b) Dickstein, D. L.; Biron, K. E.; Ujii, M.; Pfeifer, C. G.; Jeffries, A. R.; Jefferies, W. A. *FASEB J.* **2006**, *20*, 1573.
- (338) Strazielle, N.; Ghersi-Egea, J. F.; Ghiso, J.; Dehouck, M. P.; Frangione, B.; Patlak, C.; Fenstermacher, J.; Gorevic, P. *J. Neuropath. Exp. Neurol.* **2000**, *59*, 29.
- (339) (a) Salloway, S.; Sperling, R.; Gilman, S.; Fox, N. C.; Blennow, K.; Raskind, M.; Sabbagh, M.; Honig, L. S.; Doody, R.; van Dyck, C. H.; Mulnard, R.; Barakos, J.; Gregg, K. M.; Liu, E.; Lieberburg, I.; Schenk, D.; Black, R.; Grundman, M. *Neurology* **2009**, *73*, 2061. (b) Rinne, J. O.; Brooks, D. J.; Rossor, M. N.; Fox, N. C.; Bullock, R.; Klunk, W. E.; Mathis, C. A.; Blennow, K.; Barakos, J.; Okello, A. A.; de Llano, S. R. M.; Liu, E.; Koller,

- M.; Gregg, K. M.; Schenk, D.; Black, R.; Grundman, M. *Lancet Neurol.* **2010**, *9*, 363.
- (340) <http://www.clinicaltrials.gov/ct2/show/NCT00667810>. In 2012.
- (341) <http://www.clinicaltrials.gov/ct2/show/NCT00905372>. In 2012.
- (342) Solomon, B.; Koppel, R.; Hanan, E.; Katzav, T. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 452.
- (343) Dodart, J. C.; Bales, K. R.; Gannon, K. S.; Greene, S. J.; DeMattos, R. B.; Mathis, C.; DeLong, C. A.; Wu, S.; Wu, X.; Holtzman, D. M.; Paul, S. M. *Nature Neuroscience* **2002**, *5*, 452.
- (344) Bard, F.; Cannon, C.; Barbour, R.; Burke, R. L.; Games, D.; Grajeda, H.; Guido, T.; Hu, K.; Huang, J. P.; Johnson-Wood, K.; Khan, K.; Kholodenko, D.; Lee, M.; Lieberburg, I.; Motter, R.; Nguyen, M.; Soriano, F.; Vasquez, N.; Weiss, K.; Welch, B.; Seubert, P.; Schenk, D.; Yednock, T. *Nature Medicine* **2000**, *6*, 916.
- (345) Bacskai, B. J.; Kajdasz, S. T.; McLellan, M. E.; Games, D.; Seubert, P.; Schenk, D.; Hyman, B. T. *J. Neurosci.* **2002**, *22*, 7873.
- (346) Bodin, K.; Ellmerich, S.; Kahan, M. C.; Tennent, G. A.; Loesch, A.; Gilbertson, J. A.; Hutchinson, W. L.; Mangione, P. P.; Gallimore, J. R.; Millar, D. J.; Minogue, S.; Dhillon, A. P.; Taylor, G. W.; Bradwell, A. R.; Petrie, A.; Gillmore, J. D.; Bellotti, V.; Botto, M.; Hawkins, P. N.; Pepys, M. B. *Nature* **2010**, *468*, 93.
- (347) Pepys, M. B.; Herbert, J.; Hutchinson, W. L.; Tennent, G. A.; Lachmann, H. J.; Gallimore, J. R.; Lovat, L. B.; Bartfai, T.; Alanine, A.; Hertel, C.; Hoffmann, T.; Jakob-Roetne, R.; Norcross, R. D.; Kemp, J. A.; Yamamura, K.; Suzuki,

- M.; Taylor, G. W.; Murray, S.; Thompson, D.; Purvis, A.; Kolstoe, S.; Wood, S. P.; Hawkins, P. N. *Nature* **2002**, *417*, 254.
- (348) Kolstoe, S. E.; Ridha, B. H.; Bellotti, V.; Wang, N.; Robinson, C. V.; Crutch, S. J.; Keir, G.; Kukkastenvehmas, R.; Gallimore, J. R.; Hutchinson, W. L.; Hawkins, P. N.; Wood, S. P.; Rossor, M. N.; Pepys, M. B. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 7619.
- (349) Borman, S. *Chemical and Engineering News* **2010**, *Jan 25 issue*, 30.
- (350) (a) Bachurin, S.; Bukatina, E.; Lermontova, N.; Tkachenko, S.; Afanasiev, A.; Grigoriev, V.; Grigorieva, I.; Ivanov, Y.; Sablin, S.; Zefirov, N. In *Annals of the New York Academy of Sciences. Neuroprotective agents: Fifth International Conference*; New York Academy of Sciences {a} , 2 East 63rd Street, New York, NY, 10021, USA: 2001; Vol. 939, p 425. (b) Doody, R. S.; Gavrilova, S. I.; Sano, M.; Thomas, R. G.; Aisen, P. S.; Bachurin, S. O.; Seely, L.; Hung, D. *Lancet* **2008**, *372*, 207.
- (351) Miller, G. *Science* **2010**, *327*, 1309.
- (352) Cramer, P. E.; Cirrito, J. R.; Wesson, D. W.; Lee, C. Y. D.; Karlo, J. C.; Zinn, A. E.; Casali, B. T.; Restivo, J. L.; Goebel, W. D.; James, M. J.; Brunden, K. R.; Wilson, D. A.; Landreth, G. E. *Science* **2012**, *335*, 1503.
- (353) (a) Inouye, H.; Fraser, P. E.; Kirschner, D. A. *Biophys. J.* **1993**, *64*, 502. (b) Serpell, L. C.; Blake, C. C. F.; Fraser, P. E. *Biochemistry* **2000**, *39*, 13269.
- (354) Nelson, R.; Eisenberg, D. *Curr. Opin. Struct. Biol.* **2006**, *16*, 260.
- (355) Danielsson, J.; Jarvet, J.; Damberg, P.; Graslund, A. *FEBS J* **2005**, *272*, 3938.
- (356) (a) Barrow, C. J.; Zagorski, M. G. *Science* **1991**, *253*, 179. (b) Barrow, C. J.; Yasuda, A.; Kenny, P. T. M.; Zagorski, M. G. *J. Mol. Biol.* **1992**, *225*, 1075.

- (357) Shen, C.-L.; Scott, G. L.; Merchant, F.; Murphy, R. M. *Biophys. J.* **1993**, *65*, 2383.
- (358) (a) Benzinger, T. L. S.; Gregory, D. M.; Burkoth, T. S.; Miller-Auer, H.; Lynn, D. G.; Botto, R. E.; Meredith, S. C. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 13407. (b) Benzinger, T. L. S.; Gregory, D. M.; Burkoth, T. S.; Miller-Auer, H.; Lynn, D. G.; Botto, R. E.; Meredith, S. C. *Biochemistry* **2000**, *39*, 3491.
- (359) Antzutkin, O. N.; Leapman, R. D.; Balbach, J. J.; Tycko, R. *Biochemistry* **2002**, *41*, 15436.
- (360) (a) Antzutkin, O. N.; Balbach, J. J.; Leapman, R. D.; Rizzo, N. W.; Reed, J.; Tycko, R. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 13045. (b) Petkova, A. T.; Ishii, Y.; Balbach, J. J.; Antzutkin, O. N.; Leapman, R. D.; Delaglio, F.; Tycko, R. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 16742. (c) Tycko, R. *Biochemistry* **2003**, *42*, 3151.
- (361) Hilbich, C.; Kisterswoike, B.; Reed, J.; Masters, C. L.; Beyreuther, K. *J. Mol. Biol.* **1991**, *218*, 149.
- (362) Kirschner, D. A.; Inouye, H.; Duffy, L. K.; Sinclair, A.; Lind, M.; Selkoe, D. J. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 6953.
- (363) Ma, B. Y.; Nussinov, R. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 14126.
- (364) Qiang, W.; Yau, W. M.; Tycko, R. *J. Am. Chem. Soc.* **2011**, *133*, 4018.
- (365) Lührs, T.; Ritter, C.; Adrian, M.; Riek-Loher, D.; Bohrmann, B.; Döbeli, H.; Schubert, D.; Riek, R. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 17342.
- (366) Jang, H. B.; Zheng, J.; Lal, R.; Nussinov, R. *Trends Biochem. Sci.* **2008**, *33*, 91.
- (367) (a) Cruz, L.; Urbanc, B.; Borreguero, J. M.; Lazo, N. D.; Teplow, D. B.; Stanley, H. E. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 18258. (b) Borreguero, J. M.; Urbanc, B.; Lazo, N. D.; Buldyrev, S. V.; Teplow, D. B.;

- Stanley, H. E. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 6015. (c) Baumketner, A.; Bernstein, S. L.; Wytttenbach, T.; Lazo, N. D.; Teplow, D. B.; Bowers, M. T.; Shea, J. E. *Protein Science* **2006**, *15*, 1239. (d) Teplow, D. B.; Lazo, N. D.; Bitan, G.; Bernstein, S.; Wytttenbach, T.; Bowers, M. T.; Baumketner, A.; Shea, J. E.; Urbanc, B.; Cruz, L.; Borreguero, J.; Stanley, H. E. *Acc. Chem. Res.* **2006**, *39*, 635. (e) Murray, M. M.; Krone, M. G.; Bernstein, S. L.; Baumketner, A.; Condrón, M. M.; Lazo, N. D.; Teplow, D. B.; Wytttenbach, T.; Shea, J. E.; Bowers, M. T. *J. Phys. Chem. B* **2009**, *113*, 6041.
- (368) Buchete, N. V.; Tycko, R.; Hummer, G. *J. Mol. Biol.* **2005**, *353*, 804.
- (369) Sikorski, P.; Atkins, E. D. T.; Serpell, L. C. *Structure* **2003**, *11*, 915.
- (370) Serpell, L. C.; Smith, J. M. *J. Mol. Biol.* **2000**, *299*, 225.
- (371) Pike, C. J.; Walencewiczwasser, A. J.; Kosmoski, J.; Cribbs, D. H.; Glabe, C. G.; Cotman, C. W. *J. Neurochem.* **1995**, *64*, 253.
- (372) Lansbury, P. T.; Costa, P. R.; Griffiths, J. M.; Simon, E. J.; Auger, M.; Halverson, K. J.; Kocisko, D. A. *Nature Struct. Biol.* **1995**, *2*, 990.
- (373) Shao, H. Y.; Jao, S. C.; Ma, K.; Zagorski, M. G. *J. Mol. Biol.* **1999**, *285*, 755.
- (374) Fletcher, T. G.; Keire, D. A. *Protein Science* **1997**, *6*, 666.
- (375) Balbach, J. J.; Ishii, Y.; Antzutkin, O. N.; Leapman, R. D.; Rizzo, N. W.; Dyda, F.; Reed, J.; Tycko, R. *Biochemistry* **2000**, *39*, 13748.
- (376) (a) Liang, Y.; Pingali, S. V.; Jogalekar, A. S.; Snyder, J. P.; Thiyagarajan, P.; Lynn, D. G. *Biochemistry* **2008**, *47*, 10018. (b) Mehta, A. K.; Lu, K.; Childers, W. S.; Liang, S.; Dong, J.; Snyder, J. P.; Pingali, S. V.; Thiyagarajan, P.; Lynn, D. G. *J. Am. Chem. Soc.* **2008**, *130*, 9829.

- (377) Colletier, J. P.; Laganowsky, A.; Landau, M.; Zhao, M. L.; Soriaga, A. B.; Goldschmidt, L.; Flot, D.; Cascio, D.; Sawaya, M. R.; Eisenberg, D. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 16938.
- (378) Sawaya, M. R.; Sambashivan, S.; Nelson, R.; Ivanova, M. I.; Sievers, S. A.; Apostol, M. I.; Thompson, M. J.; Balbirnie, M.; Wiltzius, J. J. W.; McFarlane, H. T.; Madsen, A. O.; Riek, C.; Eisenberg, D. *Nature* **2007**, *447*, 453.
- (379) (a) Sachse, C.; Fandrich, M.; Grigorieff, N. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 7462. (b) Meinhardt, J.; Sachse, C.; Hortschansky, P.; Grigorieff, N.; Fandrich, M. *J. Mol. Biol.* **2009**, *386*, 869.
- (380) Zhang, R.; Hu, X. Y.; Khant, H.; Ludtke, S. J.; Chiu, W.; Schmid, M. F.; Frieden, C.; Lee, J. M. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 4653.
- (381) Schmidt, M.; Sachse, C.; Richter, W.; Xu, C.; Fandrich, M.; Grigorieff, N. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 19813.
- (382) Malinchuk, S. B.; Inoué, H.; Szumowski, K. E.; Kirschner, D. A. *Biophys. J.* **1998**, *74*, 537.
- (383) Perutz, M. F.; Finch, J. T.; Berriman, J.; Lesk, A. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 5591.
- (384) Miller, Y.; Ma, B. Y.; Tsai, C. J.; Nussinov, R. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 14128.
- (385) (a) Kad, N. M.; Thomson, N. H.; Smith, D. P.; Smith, D. A.; Radford, S. E. *J. Mol. Biol.* **2001**, *313*, 559. (b) Arnaudov, L. N.; de Vries, R. *Biophys. J.* **2005**, *88*, 515.
- (386) Hou, L. M.; Shao, H. Y.; Zhang, Y. B.; Li, H.; Menon, N. K.; Neuhaus, E. B.; Brewer, J. M.; Byeon, I. J. L.; Ray, D. G.; Vitek, M. P.; Iwashita, T.; Makula, R. A.; Przybyla, A. B.; Zagorski, M. G. *J. Am. Chem. Soc.* **2004**, *126*, 1992.

- (387) Watson, A. A.; Fairlie, D. P.; Craik, D. J. *Biochemistry* **1998**, *37*, 12700.
- (388) Narayanan, S.; Reif, B. *Biochemistry* **2005**, *44*, 1444.
- (389) (a) Ma, B. Y.; Nussinov, R. *Curr. Opin. Chem. Biol.* **2006**, *10*, 445. (b) Miller, Y.; Ma, B.; Nussinov, R. *Chem. Rev.* **2010**, *110*, 4820.
- (390) Urbanc, B.; Cruz, L.; Yun, S.; Buldyrev, S. V.; Bitan, G.; Teplow, D. B.; Stanley, H. E. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 17345.
- (391) Baumketner, A.; Bernstein, S. L.; Wyttenbach, T.; Bitan, G.; Teplow, D. B.; Bowers, M. T.; Shea, J. E. *Protein Science* **2006**, *15*, 420.
- (392) Zheng, J.; Jang, H.; Ma, B.; Tsai, C. J.; Nussinov, R. *Biophys. J.* **2007**, *93*, 3046.
- (393) (a) Cheon, M.; Chang, I.; Mohanty, S.; Luheshi, L. M.; Dobson, C. M.; Vendruscolo, M.; Favrin, G. *PLoS Computational Biology* **2007**, *3*, 1727. (b) Cheon, M.; Favrin, G.; Chang, I.; Dobson, C. M.; Vendruscolo, M. *Frontiers in Bioscience* **2008**, *13*, 5614.
- (394) Wei, G. H.; Jewett, A. I.; Shea, J. E. *Phys. Chem. Chem. Phys.* **2010**, *12*, 3622.
- (395) Viet, M. H.; Ngo, S. T.; Lam, N. S.; Li, M. S. *J. Phys. Chem. B* **2011**, *115*, 7433.
- (396) (a) Wang, Q.; Zhao, C.; Zhao, J.; Wang, J.; Yang, J.-C.; Yu, X.; Zheng, J. *Langmuir* **2010**, *26*, 3308. (b) Zhao, J.; Wang, Q.; Liang, G.; Zheng, J. *Langmuir* **2011**, *27*, 14876.
- (397) Wang, Q.; Shah, N.; Zhao, J.; Wang, C.; Zhao, C.; Liu, L.; Li, L.; Zhou, F.; Zheng, J. *Phys. Chem., Chem. Phys.* **2011**, *13*, 15200.
- (398) Lomakin, A.; Teplow, D. B.; Kirschner, D. A.; Benedek, G. B. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 7942.

- (399) (a) Walsh, D. M.; Lomakin, A.; Benedek, G. B.; Condrón, M. M.; Teplow, D. B. *J. Biol. Chem.* **1997**, *272*, 22364. (b) Caughey, B.; Lansbury, P. T. *Annual Review of Neuroscience* **2003**, *26*, 267.
- (400) Lomakin, A.; Chung, D. S.; Benedek, G. B.; Kirschner, D. A.; Teplow, D. B. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 1125.
- (401) Israelachvili, J. N. *Intermolecular and Surface Forces*; Academic Press: San Diego, 1991.
- (402) Hamley, I. W. *Angew. Chem.* **2007**, *46*, 8128.
- (403) (a) Asakura, S.; Oosawa, F. *J. Chem. Phys.* **1954**, *22*, 1255. (b) Oosawa, F.; Asakura, S. *Thermodynamics of the polymerization of protein*; Academic Press: New York, 1975.
- (404) (a) Knowles, T. P. J.; Waudby, C. A.; Devlin, G. L.; Cohen, S. I. A.; Aguzzi, A.; Vendruscolo, M.; Terentjev, E. M.; Welland, M. E.; Dobson, C. M. *Science* **2009**, *326*, 1533. (b) Cohen, S. I. A.; Vendruscolo, M.; Welland, M. E.; Dobson, C. M.; Terentjev, E. M.; Knowles, T. P. J. *J. Chem. Phys.* **2011**, *135*. (c) Cohen, S. I. A.; Vendruscolo, M.; Dobson, C. M.; Knowles, T. P. J. *J. Chem. Phys.* **2011**, *135*. (d) Cohen, S. I. A.; Vendruscolo, M.; Dobson, C. M.; Knowles, T. P. J. *J. Chem. Phys.* **2011**, *135*.
- (405) (a) Andreu, J. M.; Timasheff, S. N. *Methods Enzymol.* **1986**, *130*, 47. (b) Johan, K.; Westermark, G.; Engström, U.; Gustavsson, A.; Hultman, P.; Westermark, P. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 2558. (c) Baskakov, I. V.; Legname, G.; Gryczynski, Z.; Prusiner, S. B. *Protein Science* **2004**, *13*, 586.
- (406) Ha, C.; Park, C. B. *Langmuir* **2006**, *22*, 6977.
- (407) Lin, M. S.; Chen, L. Y.; Tsai, H. T.; Wang, S. S. S.; Chang, Y.; Higuchi, A.; Chen, W. Y. *Langmuir* **2008**, *24*, 5802.

- (408) Hellstrand, E.; Boland, B.; Walsh, D. M.; Linse, S. *ACS Chem. Neurosci.* **2010**, *1*, 13.
- (409) Wang, S.; Ishii, Y. *J. Am. Chem. Soc.* **2012**, *134*, 2848.
- (410) Petkova, A. T.; Leapman, R. D.; Guo, Z. H.; Yau, W. M.; Mattson, M. P.; Tycko, R. *Science* **2005**, *307*, 262.
- (411) Fawzi, N. L.; Ying, J. F.; Ghirlando, R.; Torchia, D. A.; Clore, G. M. *Nature* **2011**, *480*, 268.
- (412) (a) Esler, W. P.; Stimson, E. R.; Ghilardi, J. R.; Vinters, H. V.; Lee, J. P.; Mantyh, P. W.; Maggio, J. E. *Biochemistry* **1996**, *35*, 749. (b) Naiki, H.; Nakakuki, K. *Lab. Invest.* **1996**, *74*, 374.
- (413) Kusumoto, Y.; Lomakin, A.; Teplow, D. B.; Benedek, G. B. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 12277.
- (414) Murphy, R. M.; Pallitto, M. R. *Journal of Structural Biology* **2000**, *130*, 109.
- (415) Shen, C.-L.; Fitzgerald, M. C.; Murphy, R. M. *Biophys. J.* **1994**, *67*, 1238.
- (416) (a) Knowles, T. P.; Shu, W.; Devlin, G. L.; Meehan, S.; Auer, S.; Dobson, C. M.; Welland, M. E. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 10016. (b) Kotarek, J. A.; Johnson, K. C.; Moss, M. A. *Anal. Biochem.* **2008**, *378*, 15.
- (417) Cannon, M. J.; Williams, A. D.; Wetzel, R.; Myszka, D. G. *Anal. Biochem.* **2004**, *328*, 67.
- (418) (a) Ha, C.; Park, C. B. *Langmuir* **2006**, *22*, 6977. (b) Ha, C.; Ryu, J.; Park, C. B. *Biochemistry* **2007**, *46*, 6118.
- (419) Sánchez, L.; Madurga, S.; Pukala, T.; Vilaseca, M.; Lopez-Iglesias, C.; Robinson, C. V.; Giralt, E.; Carulla, N. *J. Am. Chem. Soc.* **2011**, *133*, 6505.
- (420) O'Nuallain, B.; Shivaprasad, S.; Kheterpal, I.; Wetzel, R. *Biochemistry* **2005**, *44*, 12709.

- (421) Inouye, H.; Kirschner, D. A. *Journal of Structural Biology* **2000**, *130*, 123.
- (422) Wood, S. J.; Maleef, B.; Hart, T.; Wetzel, R. *J. Mol. Biol.* **1996**, *256*, 870.
- (423) Wetzel, R.; Shivaprasad, S.; Williams, A. D. *Biochemistry* **2007**, *46*, 1.
- (424) Williams, A. D.; Shivaprasad, S.; Wetzel, R. *J. Mol. Biol.* **2006**, *357*, 1283.
- (425) Baldwin, A. J.; Knowles, T. P. J.; Tartaglia, G. G.; Fitzpatrick, A. W.; Devlin, G. L.; Shammass, S. L.; Waudby, C. A.; Mossuto, M. F.; Meehan, S.; Gras, S. L.; Christodoulou, J.; Anthony-Cahill, S. J.; Barker, P. D.; Vendruscolo, M.; Dobson, C. M. *J. Am. Chem. Soc.* **2011**, *133*, 14160.
- (426) Jahn, T. R.; Radford, S. E. *FEBS J* **2005**, *272*, 5962.
- (427) Chiti, F.; Webster, P.; Taddei, N.; Clark, A.; Stefani, M.; Ramponi, G.; Dobson, C. M. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 3590.
- (428) (a) Krebs, M. R. H.; Wilkins, D. K.; Chung, E. W.; Pitkeathly, M. C.; Chamberlain, A. K.; Zurdo, J.; Robinson, C. V.; Dobson, C. M. *J. Mol. Biol.* **2000**, *300*, 541. (b) Balbirnie, M.; Grothe, R.; Eisenberg, D. S. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 2375. (c) Krebs, M. R. H.; Morozova-Roche, L. A.; Daniel, K.; Robinson, C. V.; Dobson, C. M. *Protein Science* **2004**, *13*, 1933.
- (429) Gaughey, B.; Baron, G. S. *Nature (London)* **2006**, *443*, 803.
- (430) Fraser, P. E.; Nguyen, J. T.; Inouye, H.; Surewicz, W. K.; Selkoe, D. J.; Podlisy, M. B.; Kirschner, D. A. *Biochemistry* **1992**, *31*, 10716.
- (431) Kim, W.; Hecht, M. H. *J. Biol. Chem.* **2005**, *280*, 35069.
- (432) Kim, W. K.; Hecht, M. H. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 15824.
- (433) Wurth, C.; Guimard, N. K.; Hecht, M. H. *J. Mol. Biol.* **2002**, *319*, 1279.
- (434) Madine, J.; Davies, H. A.; Shaw, C.; Hamley, I. W.; Middleton, D. A. *Chem. Comm.* **2012**, *48*, 2976.

- (435) Pronchik, J.; He, X.; Giurleo, J. T.; Talaga, D. S. *J. Am. Chem. Soc.* **2010**, *132*, 9797.
- (436) Fernandez-Escamilla, A. M.; Rousseau, F.; Schymkowitz, J.; Serrano, L. *Nature Biotechnology* **2004**, *22*, 1302.
- (437) Pawar, A. P.; DuBay, K. F.; Zurdo, J.; Chiti, F.; Vendruscolo, M.; Dobson, C. *M. J. Mol. Biol.* **2005**, *350*, 379.
- (438) Scheidt, H. A.; Morgado, I.; Rothemund, S.; Huster, D.; Fandrich, M. *Angew. Chem. Int. Ed. Engl.* **2011**, *50*, 2837.
- (439) Hilbich, C.; Kisterswoike, B.; Reed, J.; Masters, C. L.; Beyreuther, K. *J. Mol. Biol.* **1992**, *228*, 460.
- (440) (a) Tjernberg, L. O.; Naslund, J.; Lindqvist, F.; Johansson, J.; Karlstrom, A. R.; Thyberg, J.; Terenius, L.; Nordstedt, C. *J. Biol. Chem.* **1996**, *271*, 8545. (b) Tjernberg, L. O.; Lilliehook, C.; Callaway, D. J. E.; Naslund, J.; Hahne, S.; Thyberg, J.; Terenius, L.; Nordstedt, C. *J. Biol. Chem.* **1997**, *272*, 12601.
- (441) Chafekar, S. M.; Malda, H.; Merckx, M.; Meijer, E. W.; Viertl, D.; Lashuel, H. A.; Baas, F.; Scheper, W. *ChemBioChem* **2007**, *8*, 1857.
- (442) Reinke, A. A.; Ung, P. M. U.; Quintero, J. J.; Carlson, H. A.; Gestwicki, J. E. *J. Am. Chem. Soc.* **2010**, *132*, 17655.
- (443) Tjernberg, L. O.; Callaway, D. J. E.; Tjernberg, A.; Hahne, S.; Lilliehöök, C.; Terenius, L.; Thyberg, J.; Nordstedt, C. *J. Biol. Chem.* **1999**, *274*, 12619.
- (444) Krysmann, M. J.; Castelletto, V.; Kelarakis, A.; Hamley, I. W.; Hule, R. A.; Pochan, D. J. *Biochemistry* **2008**, *47*, 4597.
- (445) Gordon, D. J.; Sciarretta, K. L.; Meredith, S. C. *Biochemistry* **2001**, *40*, 8237.
- (446) Soto, C.; Sigurdsson, E. M.; Morelli, L.; Kumar, R. A.; Castaño, E. M.; Frangione, B. *Nature Medicine* **1998**, *4*, 822.

- (447) Wood, S. J.; Wetzel, R.; Martin, J. D.; Hurle, M. R. *Biochemistry* **1995**, *34*, 724.
- (448) Chacon, M. A.; Barria, M. I.; Soto, C.; Inestrosa, N. C. *Molecular Psychiatry* **2004**, *9*, 953.
- (449) Zhang, G.; Leibowitz, M. J.; Sinko, P. J.; Stein, S. *Bioconjugate Chemistry* **2003**, *14*, 86.
- (450) (a) Lu, K.; Jacob, J.; Thiagarajan, P.; Conticello, V. P.; Lynn, D. G. *J. Am. Chem. Soc.* **2003**, *125*, 6391. (b) Dong, J.; Lu, K.; Lakdawala, A.; Mehta, A. K.; Lynn, D. G. *Amyloid* **2006**, *13*, 206.
- (451) Childers, W. S.; Mehta, A. K.; Lu, K.; Lynn, D. G. *J. Am. Chem. Soc.* **2009**, *131*, 10165.
- (452) Fernandez-Escamilla, A.-M.; Rousseau, F.; Schymkowitz, J.; Serrano, L. *Nature Biotechnology* **2004**, *22*, 1302.
- (453) Kallberg, Y.; Gustafsson, M.; Persson, B.; Thyberg, J.; Johansson, J. *J. Biol. Chem.* **2001**, *276*, 12945.
- (454) Sticht, H.; Bayer, P.; Willbold, D.; Dames, S.; Hilbich, C.; Beyreuther, K.; Frank, R. W.; Rosch, P. *Eur. J. Biochem.* **1995**, *233*, 293.
- (455) Coles, M.; Bicknell, W.; Watson, A. A.; Fairlie, D. P.; Craik, D. J. *Biochemistry* **1998**, *37*, 11064.
- (456) Naskar, J.; Drew, M. G. B.; Deb, I.; Das, S.; Banerjee, A. *Org. Lett.* **2008**, *10*, 2625.
- (457) Tekirian, T. L. *Journal of Alzheimer's Disease* **2001**, *3*, 241.
- (458) Hosoda, R.; Saido, T. C.; Otvos, L.; Arai, T.; Mann, D. M. A.; Lee, V. M. Y.; Trojanowski, J. Q.; Iwatsubo, T. *J. Neuropath. Exp. Neurol.* **1998**, *57*, 1089.

- (459) (a) He, W. L.; Barrow, C. J. *Biochemistry* **1999**, *38*, 10871. (b) Schilling, S.; Lauber, T.; Schaupp, M.; Manhart, S.; Scheel, E.; Bohm, G.; Demuth, H. U. *Biochemistry* **2006**, *45*, 12393.
- (460) Halverson, K.; Fraser, P. E.; Kirschner, D. A.; Lansbury, P. T. *Biochemistry* **1990**, *29*, 2639.
- (461) Soreghan, B.; Kosmoski, J.; Glabe, C. G. *J. Biol. Chem.* **1994**, *269*, 28551.
- (462) Sabate, R.; Estelrich, J. *J. Phys. Chem. B* **2005**, *109*, 11027.
- (463) Kim, J.; Lee, M. *Biochem. Biophys. Res. Commun.* **2004**, *316*, 393.
- (464) Yong, W.; Lomakin, A.; Kirkitadze, M. D.; Teplow, D. B.; Chen, S. H.; Benedek, G. B. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 150.
- (465) Rangachari, V.; Moore, B. D.; Reed, D. K.; Sonoda, L. K.; Bridges, A. W.; Conboy, E.; Hartigan, D.; Rosenberry, T. L. *Biochemistry* **2007**, *46*, 12451.
- (466) Lin, J. M.; Lin, T. L.; Jeng, U. S.; Huang, Z. H.; Huang, Y. S. *Soft Matter* **2009**, *5*, 3913.
- (467) Sureshbabu, N.; Kirubakaran, R.; Jayakumar, R. *Eur. Biophys. J. Biophys. Lett.* **2009**, *38*, 355.
- (468) Wood, S. J.; MacKenzie, L.; Maleeff, B.; Hurle, M. R.; Wetzel, R. *J. Biol. Chem.* **1996**, *271*, 4086.
- (469) He, C. Q.; Hou, Y. B.; Han, Y. C.; Wang, Y. L. *Langmuir* **2011**, *27*, 4551.
- (470) Hamill, A. C.; Lee, C. T. *J. Phys. Chem. B* **2009**, *113*, 6164.
- (471) Han, Y.; Huang, X.; Cao, M.; Wang, Y. *J. Phys. Chem. B* **2008**, *112*, 15195.
- (472) (a) Michikawa, M. *J. Neurosci. Res.* **2003**, *72*, 141. (b) Michikawa, M. *Mol. Neurobiol.* **2003**, *27*, 1.
- (473) Gorbenko, G. P.; Kinnunen, P. K. *J. Chem. Phys. Lipids* **2006**, *141*, 72.

- (474) (a) Kawarabayashi, T.; Shoji, M.; Younkin, L. H.; Lin, W. L.; Dickson, D. W.; Murakami, T.; Matsubara, E.; Abe, K.; Ashe, K. H.; Younkin, S. G. *J. Neurosci.* **2004**, *24*, 3801. (b) Kim, S. I.; Yi, J. S.; Ko, Y. G. *J. Cell. Biochem.* **2006**, *99*, 878. (c) Okada, T.; Ikeda, K.; Wakabayashi, M.; Ogawa, M.; Matsuzaki, K. *J. Mol. Biol.* **2008**, *382*, 1066.
- (475) Mandal, P. K.; Pettegrew, J. W. *Neurochem. Res.* **2004**, *29*, 2267.
- (476) Morita, M.; Hamada, T.; Tendo, Y.; Hata, t.; Vestergaard, M. C.; Takagi, M. *Soft Matter* **2012**, *8*, 2816.
- (477) (a) Matsuzaki, K. *BBA Biomembranes* **2007**, *1768*, 1935. (b) Aisenbrey, C.; Borowik, T.; Bystrom, R.; Bokvist, M.; Lindstrom, F.; Misiak, H.; Sani, M. A.; Grobner, G. *Eur. Biophys. J. Biophys. Lett.* **2008**, *37*, 247.
- (478) Ma, K.; Clancy, E. L.; Zhang, Y. B.; Ray, D. G.; Wollenberg, K.; Zagorski, M. *G. J. Am. Chem. Soc.* **1999**, *121*, 8698.
- (479) (a) Ege, C.; Lee, K. Y. *C. Biophys. J.* **2004**, *87*, 1732. (b) Kremer, J. J.; Sklansky, D. J.; Murphy, R. M. *Biochemistry* **2001**, *40*, 8563.
- (480) Terzi, E.; Holzemann, G.; Seelig, J. *Biochemistry* **1997**, *36*, 14845.
- (481) Chi, E. Y.; Ege, C.; Winans, A.; Majewski, J.; Wu, G. H.; Kjaer, K.; Lee, K. Y. *C. Proteins* **2008**, *72*, 1.
- (482) Wong, P. T.; Schauerte, J. A.; Wissler, K. C.; Ding, H.; Lee, E. L.; Steel, D. G.; Gafni, A. *J. Mol. Biol.* **2009**, *386*, 81.
- (483) Terzi, E.; Holzemann, G.; Seelig, J. *Biochemistry* **1994**, *33*, 7434.
- (484) Martins, I. C.; Kuperstein, I.; Wilkinson, H.; Maes, E.; Vanbrabant, M.; Jonckheere, W.; Van Gelder, P.; Hartmann, D.; D'Hooge, R.; De Strooper, B.; Schymkowitz, J.; Rousseau, F. *EMBO J.* **2008**, *27*, 224.

- (485) Kawahara, M.; Kuroda, Y.; Arispe, N.; Rojas, E. *J. Biol. Chem.* **2000**, *275*, 14077.
- (486) McLaurin, J.; Chakrabartty, A. *J. Biol. Chem.* **1996**, *271*, 26482.
- (487) Williams, T. L.; Day, I. J.; Serpell, L. C. *Langmuir* **2010**, *26*, 17260.
- (488) Jang, H.; Zheng, J.; Nussinov, R. *Biophys. J.* **2007**, *93*, 1938.
- (489) Strodel, B.; Lee, J. W. L.; Whittleston, C. S.; Wales, D. J. *J. Am. Chem. Soc.* **2010**, *132*, 13300.
- (490) Davis, C. H.; Berkowitz, M. L. *Biophys. J.* **2009**, *96*, 785.
- (491) Kremer, J. J.; Pallitto, M. M.; Sklansky, D. J.; Murphy, R. M. *Biochemistry* **2000**, *39*, 10309.
- (492) McLaurin, J.; Franklin, T.; Fraser, P. E.; Chakrabartty, A. *J. Biol. Chem.* **1998**, *273*, 4506.
- (493) (a) Matsuzaki, K.; Horikiri, C. *Biochemistry* **1999**, *38*, 4137. (b) Okada, T.; Wakabayashi, M.; Ikeda, K.; Matsuzaki, K. *J. Mol. Biol.* **2007**, *371*, 481.
- (494) Wood, S. J.; Maleeff, B.; Hart, T.; Wetzel, R. *J. Mol. Biol.* **1996**, *256*, 870.
- (495) Choo-Smith, L. P.; Surewicz, W. K. *FEBS Lett.* **1997**, *402*, 95.
- (496) (a) Yip, C. M.; Elton, E. A.; Darabie, A. A.; Morrison, M. R.; McLaurin, J. *J. Mol. Biol.* **2001**, *311*, 723. (b) Yip, C. M.; Darabie, A. A.; McLaurin, J. *J. Mol. Biol.* **2002**, *318*, 97.
- (497) Bokvist, M.; Lindstrom, F.; Watts, A.; Grobner, G. *J. Mol. Biol.* **2004**, *335*, 1039.
- (498) Chauhan, A.; Ray, I.; Chauhan, V. P. S. *Neurochem. Res.* **2000**, *25*, 423.
- (499) Hellstrand, E.; Sparr, E.; Linse, S. *Biophys. J.* **2010**, *98*, 2206.
- (500) Calamai, M.; Pavone, F. S. *J. Am. Chem. Soc.* **2011**, *133*, 12001.
- (501) Dante, T.; Hauss, T.; Dencher, N. A. *Biophys. J.* **2002**, *83*, 2610.

- (502) Maltseva, E.; Kerth, A.; Blume, A.; Mohwald, H.; Brezesinski, G.
Chembiochem **2005**, *6*, 1817.
- (503) Buchsteiner, A.; Hauss, T.; Dencher, N. A. *Soft Matter* **2011**, *8*, 424.
- (504) Ravault, S.; Flore, C.; Saurel, O.; Milon, A.; Brasseur, R.; Lins, L. *Langmuir*
2009, *25*, 10948.
- (505) Kowalewski, T.; Holtzman, D. M. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*,
3688.
- (506) Austen, B.; Christodoulou, G.; Terry, J. E. *The journal of nutrition, health &*
aging **2002**, *6*, 377.
- (507) Qiu, L. M.; Lewis, A.; Como, J.; Vaughn, M. W.; Huang, J. Y.; Somerharju, P.;
Virtanen, J.; Cheng, K. H. *Biophys. J.* **2009**, *96*, 4299.
- (508) Curtain, C. C.; Ali, F. E.; Smith, D. G.; Bush, A. I.; Masters, C. L.; Barnham,
K. J. *J. Biol. Chem.* **2003**, *278*, 2977.
- (509) Mason, R. P.; Shoemaker, W. J.; Shajenko, L.; Chambers, T. E.; Herbette, L. G.
Neurobiology of Aging **1992**, *13*, 413.
- (510) Fantini, J.; Yahi, N. *Expert Rev. Mol. Med.* **2010**, *12*, e27.
- (511) Locatelli, S.; Lutjohann, D.; Schmidt, H. H. J.; Otto, C.; Beisiegel, U.; von
Bergmann, K. *Arch. Neurol.* **2002**, *59*, 213.
- (512) Harris, J. R. *Micron* **2002**, *33*, 609.
- (513) Kakio, A.; Nishimoto, S.; Yanagisawa, K.; Kozutsumi, Y.; Matsuzaki, K. *J.*
Biol. Chem. **2001**, *276*, 24985.
- (514) Zhang, Q. H.; Powers, E. T.; Nieva, J.; Huff, M. E.; Dendle, M. A.; Bieschke,
J.; Glabe, C. G.; Eschenmoser, A.; Wentworth, P.; Lerner, R. A.; Kelly, J. W.
Proc. Natl. Acad. Sci. U. S. A. **2004**, *101*, 4752.

- (515) Bieschke, J.; Zhang, Q. H.; Powers, E. T.; Lerner, R. A.; Kelly, J. W.
Biochemistry **2005**, *44*, 4977.
- (516) (a) Bush, A. I. *Curr. Opin. Chem. Biol.* **2000**, *4*, 184. (b) *Metal ions and neurodegenerative disorders*; Zatta, P., Ed.; World Scientific: Singapore, 2003. (c) Adlard, P. A.; Bush, A. I. *J. Alzheimers Dis.* **2006**, *10*, 145. (d) Miu, A. C.; Benga, O. *J. Alzheimers Dis.* **2006**, *10*, 179.
- (517) (a) Smith, M. A.; Harris, P. L. R.; Sayre, L. M.; Perry, G. *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 9866. (b) Sayre, L. M.; Perry, G.; Smith, M. A. *Curr. Opin. Chem. Biol.* **1999**, *3*, 220. (c) Sayre, L. M.; Perry, G.; Harris, P. L. R.; Liu, Y. H.; Schubert, K. A.; Smith, M. A. *J. Neurochem.* **2000**, *74*, 270. (d) Lovell, M. A.; Robertson, J. D.; Teesdale, W. J.; Campbell, J. L.; Markesbery, W. R. *J. Neurol. Sci.* **1998**, *158*, 47. (e) Suh, S. W.; Jensen, K. B.; Jensen, M. S.; Silva, D. S.; Kesslak, P. J.; Danscher, G.; Frederickson, C. J. *Brain Res.* **2000**, 852, 274.
- (518) (a) Bush, A. I.; Pettingell, W. H.; Paradis, M. D.; Tanzi, R. E. *J. Biol. Chem.* **1994**, *269*, 12152. (b) Atwood, C. S.; Moir, R. D.; Huang, X. D.; Scarpa, R. C.; Bacarra, N. M. E.; Romano, D. M.; Hartshorn, M. K.; Tanzi, R. E.; Bush, A. I. *J. Biol. Chem.* **1998**, *273*, 12817.
- (519) Hu, W. P.; Chang, G. L.; Chen, S. J.; Kuo, Y. M. *J. Neurosci. Methods* **2006**, *154*, 190.
- (520) Rottkamp, C. A.; Raina, A. K.; Zhu, X. W.; Gaier, E.; Bush, A. I.; Atwood, C. S.; Chevion, M.; Perry, G.; Smith, M. A. *Free Radical Biology and Medicine* **2001**, *30*, 447.
- (521) (a) Liu, S. T.; Howlett, G.; Barrow, C. J. *Biochemistry* **1999**, *38*, 9373. (b) Curtain, C. C.; Ali, F.; Volitakis, I.; Cherny, R. A.; Norton, R. S.; Beyreuther,

- K.; Barrow, C. J.; Masters, C. L.; Bush, A. I.; Barnham, K. J. *J. Biol. Chem.* **2001**, *276*, 20466. (c) Syme, C. D.; Nadal, R. C.; Rigby, S. E. J.; Viles, J. H. *J. Biol. Chem.* **2004**, *279*, 18169. (d) Miura, T.; Suzuki, K.; Kohata, N.; Takeuchi, H. *Biochemistry* **2000**, *39*, 7024. (e) Nakamura, M.; Shishido, N.; Nunomura, A.; Smith, M. A.; Perry, G.; Hayashi, Y.; Nakayama, K.; Hayashi, T. *Biochemistry* **2007**, *46*, 12737. (f) Dong, J. J.; Shokes, J. E.; Scott, R. A.; Lynn, D. G. *J. Am. Chem. Soc.* **2006**, *128*, 3540. (g) Dong, J.; Canfield, J. M.; Mehta, A. K.; Shokes, J. E.; Tian, B.; Childers, W. S.; Simmons, J. A.; Mao, Z.; Scott, R. A.; Warncke, K.; Lynn, D. G. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 13313.
- (522) Streltsov, V. A.; Titmuss, S. J.; Epa, V. C.; Barnham, K. J.; Masters, C. L.; Varghese, J. N. *Biophys. J.* **2008**, *95*, 3447.
- (523) Parthasarathy, S.; Long, F.; Miller, Y.; Xiao, Y. L.; McElheny, D.; Thurber, K.; Ma, B. Y.; Nussinov, R.; Ishii, Y. *J. Am. Chem. Soc.* **2011**, *133*, 3390.
- (524) Mantyh, P. W.; Ghilardi, J. R.; Rogers, S.; Demaster, E.; Allen, C. J.; Stimson, E. R.; Maggio, J. E. *J. Neurochem.* **1993**, *61*, 1171.
- (525) Pedersen, J. T.; Teilum, K.; Heegaard, N. H. H.; Ostergaard, J.; Adolph, H. W.; Hemmingsen, L. *Angew. Chem. Int. Ed. Engl.* **2011**, *50*, 2532.
- (526) Garai, K.; Sengupta, P.; Sahoo, B.; Maiti, S. *Biochem. Biophys. Res. Commun.* **2006**, *345*, 210.
- (527) Innocenti, M.; Salvietti, E.; Guidotti, M.; Casini, A.; Bellandi, S.; Foresti, M. L.; Gabbiani, C.; Pozzi, A.; Zatta, P.; Messori, L. *J. Alzheimers Dis.* **2010**, *19*, 1323.
- (528) Ryu, J.; Girigoswami, K.; Ha, C.; Ku, S. H.; Park, C. B. *Biochemistry* **2008**, *47*, 5328.

- (529) (a) Gupta, V. B.; Anitha, S.; Hegde, M. L.; Zecca, L.; Garruto, R. M.; Ravid, R.; Shankar, S. K.; Stein, R.; Shanmugavelu, P.; Rao, K. S. J. *Cell. Mol. Life Sci.* **2005**, *62*, 143. (b) Walton, J. R. *Neurotoxicology* **2006**, *27*, 385. (c) Zatta, P. *J. Alzheimers Dis.* **2006**, *10*, 33.
- (530) Candy, J. M.; Oakley, A. E.; Klinowski, J.; Carpenter, T. A.; Perry, R. H.; Atack, J. R.; Perry, E. K.; Blessed, G.; Fairbairn, A.; Edwardson, J. A. *Lancet* **1986**, *1*, 354.
- (531) Good, P. F.; Perl, D. P.; Bierer, L. M.; Schmeidler, J. *Ann. Neurol.* **1992**, *31*, 286.
- (532) (a) Ricchelli, F.; Drago, D.; Filippi, B.; Tognon, G.; Zatta, P. *Cell. Mol. Life Sci.* **2005**, *62*, 1724. (b) Drago, D.; Bettella, M.; Bolognin, S.; Cendron, L.; Scancar, J.; Milacic, R.; Ricchelli, F.; Casini, A.; Messori, L.; Tognon, G.; Zatta, P. *Int. J. Biochem. Cell Biol.* **2008**, *40*, 731.
- (533) (a) Bondy, S. C.; Guo-Ross, S. X.; Truong, A. T. *Brain Res.* **1998**, *799*, 91. (b) Huang, X. D.; Atwood, C. S.; Hartshorn, M. A.; Multhaup, G.; Goldstein, L. E.; Scarpa, R. C.; Cuajungco, M. P.; Gray, D. N.; Lim, J.; Moir, R. D.; Tanzi, R. E.; Bush, A. I. *Biochemistry* **1999**, *38*, 7609. (c) Huang, X. D.; Cuajungco, M. P.; Atwood, C. S.; Hartshorn, M. A.; Tyndall, J. D. A.; Hanson, G. R.; Stokes, K. C.; Leopold, M.; Multhaup, G.; Goldstein, L. E.; Scarpa, R. C.; Saunders, A. J.; Lim, J.; Moir, R. D.; Glabe, C.; Bowden, E. F.; Masters, C. L.; Fairlie, D. P.; Tanzi, R. E.; Bush, A. I. *J. Biol. Chem.* **1999**, *274*, 37111.
- (534) Schubert, D.; Chevion, M. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 702.
- (535) Liu, B.; Moloney, A.; Meehan, S.; Morris, K.; Thomas, S. E.; Serpell, L. C.; Hider, R.; Marciniak, S. J.; Lomas, D. A.; Crowther, D. C. *J. Biol. Chem.* **2011**, *286*, 4248.

- (536) (a) Bush, A. I.; Multhaup, G.; Moir, R. D.; Williamson, T. G.; Small, D. H.; Rumble, B.; Pollwein, P.; Beyreuther, K.; Masters, C. L. *J. Biol. Chem.* **1993**, *268*, 16109. (b) Multhaup, G.; Bush, A. I.; Pollwein, P.; Masters, C. L. *FEBS Lett.* **1994**, *355*, 151. (c) Multhaup, G.; Ruppert, T.; Schlicksupp, A.; Hesse, L.; Bill, E.; Pipkorn, R.; Masters, C. L.; Beyreuther, K. *Biochemistry* **1998**, *37*, 7224.
- (537) (a) Vandermeulen, G. W. M.; Tziatzios, C.; Klok, H.-A. *Macromolecules* **2003**, *36*, 4107. (b) Vandermeulen, G. W. M.; Klok, H.-A. *Macromol. Biosci.* **2004**, *4*, 383. (c) Hamley, I. W. *Block Copolymers in Solution*; Wiley: Chichester, 2005.
- (538) Schlaad, H. *Adv. Polym. Sci.* **2006**, *202*, 53.
- (539) (a) Burkoth, T. S.; Benzinger, T. L. S.; Jones, D. N. M.; Hallenga, K.; Meredith, S. C.; Lynn, D. G. *J. Am. Chem. Soc.* **1998**, *120*, 7655. (b) Burkoth, T. S.; Benzinger, T. L. S.; Urban, V.; Lynn, D. G.; Meredith, S. C.; Thiyagarajan, P. *J. Am. Chem. Soc.* **1999**, *121*, 7429. (c) Thiyagarajan, P.; Burkoth, T. S.; Urban, V.; Seifert, S.; Benzinger, T. L. S.; Morgan, D. M.; Gordon, D.; Meredith, S. C.; Lynn, D. G. *J. Appl. Cryst.* **2000**, *33*, 535.
- (540) (a) Hamley, I. W.; Krysmann, M. J.; Castelletto, V.; Kelarakis, A.; Noirez, L.; Hule, R. A.; Pochan, D. *Chem. Eur. J.* **2008**, *14*, 11369. (b) Hamley, I. W.; Krysmann, M. J.; Castelletto, V.; Noirez, L. *Adv. Mater.* **2008**, *20*, 4394. (c) Castelletto, V.; Newby, G. E.; Zhu, Z.; Hamley, I. W.; Noirez, L. *Langmuir* **2010**, *26*, 9986.
- (541) Castelletto, V.; Newby, G. E.; Hermida-Merino, D.; Hamley, I. W.; Liu, D.; Noirez, L. *Polymer Chemistry* **2010**, *1*, 453.

(542) Castelletto, V.; McKendrick, J. M. E.; Hamley, I. W.; Cenker, C.; Olsson, U.

Langmuir **2010**, *26*, 11624.

(543) Li, C.; Orbulescu, J.; Sui, G.; Leblanc, R. M. *Langmuir* **2004**, *20*, 8641.

(544) Deng, M. L.; Yu, D. F.; Hou, Y. B.; Wang, Y. L. *J. Phys. Chem. B* **2009**, *113*,
8539.

(545) He, C.; Han, Y.; Fan, Y.; Deng, M.; Wang, Y. *Langmuir* **2012**, *28*, 3391.