京都大学学術情報リポジトリ
KURENAI 紅
Kyoto University Research Information Repository

京都大学
KYOTO UNIVERSITY

Title	Conformation-specific antibodies to target amyloid β oligomers and their application to immunotherapy for Alzheimer's disease.
Author(s)	Murakami, Kazuma
Citation	Bioscience, biotechnology, and biochemistry (2014), 78(8): 1293-1305
Issue Date	2014-08-18
URL	http://hdl.handle.net/2433/200745
Right	This is an Accepted Manuscript of an article published by Taylor & Francis in 'Bioscience, biotechnology, and biochemistry' on 18 Aug 2014, available online: http://www.tandfonline.com/10.1080/09168451.2014.940275.
Type	Journal Article
Textversion	author

Kyoto University

Award Review

Running Title: Conformation-specific Antibodies of Aβ

Conformation-specific Antibodies to Target Amyloid β Oligomers and Their Application to Immunotherapy for Alzheimer's Disease

Kazuma Murakami

Division of Food Science and Biotechnology, Graduate School of Agriculture,
Kyoto University, Kyoto 606-8502, Japan

Correspondence: Tel.: +81-75-753-6282; Fax: +81-75-753-6284;
E-mail: alzkazu@kais.kyoto-u.ac.jp

Abstract (123<150 words)

Amyloid β-protein (Aβ) oligomers, intermediates of Aβ aggregation, cause cognitive impairment and synaptotoxicity in the pathogenesis of Alzheimer's disease (AD). Immunotherapy using anti-Aβ antibody is one of the most promising approaches for AD treatment. However, most clinical trials using conventional sequence-specific antibodies have proceeded with difficulty. This is probably due to the unintended removal of the non-pathological monomer and fibrils of Aβ as well as the pathological oligomers by these antibodies that recognize Aβ sequence, which is not involved in synaptotoxicity. Several efforts have been made recently to develop conformation-specific antibodies that target the tertiary structure of Aβ oligomers. Here we review the recent findings of Aβ oligomers and anti-Aβ antibodies including our own, and discuss their potential as therapeutic and diagnostic tools.

Key words:

amyloid β; Alzheimer's disease; oligomer; antibody; conformation

Introduction

Accumulation of aggregated proteins is characteristic of many neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease, and Huntington's disease. AD is generally characterized by the aggregation of extracellular amyloid β-protein (Aβ) in senile plaques. Aβ mainly consists of 40- and 42-mer amyloid β peptides (Aβ40, Aβ42), which are predominantly secreted from Aβ protein precursor (APP) by two proteases (β- and γ-secretases).[1,2] β-Secretase is identified as an aspartyl protease of the pepsin family, called β-site APP-cleaving enzyme (BACE-1).[3] It is noted in amyloid theory that Aβ aggregates through β-sheet formation and shows neurotoxicity. On the other hand, neurofibrillary tangles (NFTs) are another feature of AD pathology and are composed of intracellular deposits of tau protein.[4,5] Abnormal aggregation of tau is related to its hyperphosphorylation. Recent clinical reports by the Alzheimer's Disease Neuroimaging Initiative (ADNI) support the amyloid theory; the accumulation of Aβ occurs earliest during the process of AD as a molecular trigger, followed by neuronal injury, deposition of phosphorylated tau, and a shrunken hippocampus, respectively.[6] The pre-symptomatic and mild cognitive impairment (MCI) stages, prior to AD onset, are dependent on progression based on these biomarkers (Fig. 1).

The cleavage of APP by BACE-1 generates a secreted APPβ (sAPPβ) and a membrane-bound C-terminal fragment of APP (CTFβ), which is a precursor of the following cleavage by γ-secretase (amyloidogenic pathway, Fig. 2). Two homologous presenilins, presenilin 1 (PS1) and presenilin 2 (PS2), play an important role in γ-secretase activity, which requires three other cofactors: nicastrin (Nct), anterior pharynx-defective phenotype (APH-1), and presenilin-enhancer (PEN-2).[7] The broad substrate specificity of γ-secretase at the C-terminal region of APP results in the multiple production of other lengths of Aβs (*e.g.* 37-, 38- or 43-mer).[8,9] Additional Aβ heterogeneity is generated by an enzymatic reaction: isomerase (Asp7, Asp23),[10,11] glutaminylcyclase (Glu3, Glu11),[11,12] aminopeptidases (Aβ3-42),[11] and phosphorylation (Ser8).[13] Despite recent rediscovery of the potent amyloidogenicity and pathogenicity of Aβ43[14] in the animal study, the aggregative ability and

neurotoxicity of Aβ43 does not exceed those of Aβ42.[15] These findings suggest that Aβ42 or these modification products of Aβ42 plays the most critical role in the pathogenesis of AD.[16]

On the other hand, APP is cleaved by α-secretase between residues 16 and 17 to produce secreted APPα (sAPPα) and the C-terminal fragment (CTFα), resulting in no production of Aβ40 and Aβ42 from these cleaved precursors (non-amyloidogenic pathway, Fig. 2). Concurrently, smaller fragments, referred to as p3 (Aβ17-40/42) and APP intracellular domain (AICD), are produced. The physiological role of these APP metabolites remains unclear in spite of their ubiquitous expression in almost all human organs. Furthermore, a proportion of Aβ is also modulated by degrading enzymes, such as insulin-degrading enzyme[17] and neprilysin.[18]

Although most of the present clinical drugs in AD target glutamatergic and cholinergic neurotransmission, their benefits are limited in terms of symptomatic treatments. Disease-modifying drugs to prevent the aggregation of Aβ, to hinder the production of Aβ, and to enhance the degrading activity of Aβ are currently being developed. In particular, immunotherapy using anti-Aβ antibody for Aβ clearance and anti-aggregation has been intensively examined in clinical trials.[19] However, some conventional antibodies targeting Aβ sequence are struggling in trials. In recent years, conformation[§]-specific antibodies that target synaptotoxic Aβ oligomers (intermediate aggregates), rather than the physiological Aβ monomer and fibrils, have received a lot of attention. In the following chapters, this review focuses on the features of Aβ oligomers and unique attempts to develop antibodies against Aβ oligomers, and introduces our findings of a monoclonal antibody against a toxic conformer[§§] of Aβ42 together with its application to AD treatment.

I. Amyloid β Oligomer Hypothesis

1. Aβ oligomers

There is increasing evidence that soluble oligomeric assemblies of Aβ can induce cognitive decline and synaptic dysfunction in the pathology of AD,[20] whereas mature plaques composed of insoluble fibrils are not always consistent with neuronal degeneration[21,22] and

serve as a store of the toxic assembly of Aβ.[23] Accumulated studies on the etiology of Aβ assemblies; paranucleus (5-mer),[24] Aβ*56 (56 kDa, 12-mer),[25] protofibrils (24~700-mer),[26,27] globulomer (38/48 kDa, ~12-mer),[28] AβO (~90 kDa, 15~20-mer),[29] Aβ-derived diffusible ligands (ADDLs; ~90 kDa, ~24-mer),[30] annulus (150~250 kDa, ~50-mer),[31] and amylospheroid (ASPD; 158~669 kDa, ~100-mer)[32] have been appreciated (Fig. 3, Table 1). Paranucleus is supposed to be a unit of protofibrils. In particular, the synaptotoxic potentials of ADDLs are well studied, and they are extensively used as an oligomer model. These synaptotoxic high molecular-weight oligomers are composed of a dimer and/or trimer as a minimum unit of Aβ assemblies (2 x *n*-mer, 3 x *n*-mer).[33,34] More correctly, Aβ40 preferably exists as dimer,[35] while Aβ42 likely form trimer or tetramer.[36] Studies using synthetic dimers (S26C-Aβ40)[37] and *in vivo*-derived dimers[37] and trimers[38] support their significance to the synaptotoxicity.

2. Synaptotoxicity

Long-term potentiation (LTP) is a lasting enhancement in signal transmission among neurons, reflecting synaptic health.[39] Synthetic Aβ oligomers (ADDLs),[30] brain-derived oligomers (Aβ*56)[25] from AD transgenic mice (Tg2576 line), and dimers[37] from human AD patients inhibit LTP and induce dendritic spine shrinkage in rat neurons, resulting in synaptotoxicity in the CA1 region of the hippocampus. Because memory loss is closely related to synaptotoxicity, the removal of Aβ oligomers and prevention of oligomer formation would be a promising approach for AD therapeutics. Shankar *et al*. demonstrated that the inhibition of LTP was neutralized by the administration of anti-Aβ antibodies to a rat model of AD.[37]

3. Oxidative stress

Oxidative stress induced from reactive oxygen species (ROS; *e.g*. superoxide radical, hydroxyl radical) is an early event underlying synaptotoxicity and the subsequent neuronal death by Aβ oligomer. Reports using human brain materials show a strong correlation

between oxidative damage levels (total SOD, catalase, glutathione, protein carbonyls, thiobarbituric acid reactive substances, 3-nitrotyrosine, 4-hydroxynonenal, and acrolein) and the dementia status of subjects.[40] Klein and colleagues proposed that ADDLs induce LTP accompanied with oxidative damage *ex vivo*.[41] Barnham and colleagues proposed that Aβ forms dityrosine cross-linked dimers *via* oxidation of the tyrosine residue at position 10 (Tyr10) under oxidative conditions,[42] and that generic dityrosine levels were also elevated in the AD brain.[43]

Superoxide dismutase (SOD) is one of the major antioxidant metallo-enzymes converting toxic superoxide radicals to hydrogen peroxide. In AD brains, the amount of CuZn-SOD (SOD1), which is found in the peroxisomes and nucleus as well as in the cytosol and intermembrane space of mitochondria, was larger than in non-AD cases.[44] On the other hand, no such increase was found in Mn-SOD (SOD2) in the mitochondrial matrix or in extracellular CuZn-SOD (SOD3) in specific cell types, such as vascular smooth muscular cells, lungs, and plasma.[44] Furthermore, to evaluate the contribution of SOD1 to AD progression, our group previously bred *Sod1*-deficient mice (*Sod1*$^{-/-}$), which showed drusen deposition,[45] fatty liver,[46] skin thinning,[47] and osteoporosis,[48] as a senescence model, with an APP transgenic mouse model (Tg2576) as an AD model. In the resultant double transgenic mice (*hAPP*/*Sod1*$^{-/-}$), Aβ oligomerization associated with memory loss and synaptic loss worsened as compared with control AD mice.[44] BACE1 amounts were also augmented in *hAPP*/*Sod1*$^{-/-}$, implying stimulation of the amyloidogenic pathway by cytoplasmic superoxide radicals.[49] The relevance of oxidative stress to oligomer formation of Aβ in the etiology of AD was described in the previous review.[50]

4. Target receptors

It is still unclear how Aβ oligomer interferes with signaling pathways to inhibit LTP activity. Some candidates for oligomer-targeted receptors at the synaptic plasma membrane have been reported. Snyder *et al.* suggested that the application of naturally secreted Aβ oligomers to cortical slices promoted the endocytosis of *N*-methyl-D-aspartate (NMDA)

receptors by binding the oligomers to α7-nicotinic receptors.[51] Subsequently, the disturbance of NMDA function affected calcium influx and the downstream cascades, such as AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole) receptors.[52] Aβ oligomers also interacted with RAGE (receptor for advanced glycation endproducts) receptor[53] and the insulin receptor[54] to induce oxidative stress. Notably, the cellular prion protein (PrP^C) functions as a specific receptor for Aβ oligomers to inhibit LTP activity and to disrupt insulin activity.[55] These interactions could be dependent on the size, polarity, and conformations of Aβ oligomers.

II. Aβ Immunotherapy

1. Active immunization

Solomon *et al*. reported that anti-Aβ antibody prevented the aggregation of Aβ[56] and disaggregated the pre-existed fibril of Aβ using thioflavin T,[57] which is a reagent showing fluorescence by binding the β-sheet structure within amyloid aggregates.[58] In 1999, Schenk *et al*. first demonstrated active immunization using an AD mouse model (PDAPP). In this study, the administration of synthetic Aβ42 to animals led to a reduction of plaque area[59], and recovery of cognitive impairment was also reported in later studies.[60,61] Subsequently, an experiment using 3xTg-AD mice showed that behavioral improvement by immunization was related to the reduction of Aβ oligomer levels,[62] indicating Aβ oligomers as more optimal targets than plaques for AD treatment.

In a clinical trial (AN1792) in which Elan and Wyeth initiated active immunization in 2001, synthetic Aβ42 combined with the surface-active saponin adjuvant QS-21 was vaccinated. Although phase I was safely conducted, phase II was halted because of severe adverse effects (aseptic meningoencephalitis) in ~6% of patients.[63] The subsequent follow-up study indicated that Aβ plaques were reduced in AD patients but not progressive cognitive impairment.[64] This was likely due to the unintended removal of both pathological and non-pathological Aβ42; the role of the latter in physiological function is currently controversial. Soscia *et al*. reported one interesting study on the involvement of Aβ42 in the

immune system as an antimicrobial protein.[65] Alternatively, the involvement of the excessive induction of T-helper (T_H) 1 lymphocytes by QS-21 adjuvant has been noted, which causes the strong response of the cell-mediated immune system in order to enhance antibody responses in the elderly.

2. Passive immunization

Active Aβ immunization is cost-effective and long-lasting with only a few injections, although it is difficult to avoid the risk of undesirable immune responses because of the use of strong adjuvants to boost antibody generation. In contrast, passive immunization by the intravenous administration of antibodies is moderate even in the elderly, whose proinflammatory cytokine levels are normally higher,[66] and it can be halted at any time if adverse events occur. Additionally, the usage of antibodies only directing the target agent of interest, such as toxic Aβ assemblies or conformations, is one of their advantages over active immunization.

So far, over 600 antibodies against Aβ have been deposited in Alzforum (http://www.alzforum.org/), and most of these were dependent on the Aβ sequence. The initial application of passive immunotherapy using AD mice (PDAPP) described that treatment with anti-Aβ N-terminus monoclonal (3D6) antibodies prevented plaque formation, but not anti-Aβ C-terminus monoclonal antibodies.[67] In particular, the binding of antibody to amyloid plaque could induce the microglical phagocytosis of Aβ burden through Fc receptor.[67] Also, the injection of anti-Aβ middle portion antibody (m266), whose epitope lies in Aβ13-28, to young PDAPP mice prevented plaque formation and decreased the levels of soluble Aβ.[68] The complex of Aβ with antibody in the blood was detected in this study, supporting the potent role of anti-Aβ antibody therapy in AD prevention. These therapeutic effects are suggested to be mediated by the following inhibitory mechanisms: (1) the complex formation of Aβ with antibodies could induce binding of the Fc portion to microglia, leading to the phagocytosis of these complexes.[67] (2) The antibodies could directly prevent the aggregation (oligomerization) of Aβ.[69] These mechanisms are based on the assumption that

antibodies can cross the blood–brain barrier (BBB) in order to bind Aβ within the brain. There is an alternative idea that antibodies in the blood might induce a shift in the concentration gradient of Aβ over BBB, followed by increased efflux of Aβ from the brain to the periphery (sink hypothesis).[68)]

However, some animal experiments using other sequence-specific antibodies of Aβ led to the occurrence of microhemorrhages in the regions of cerebral amyloid angiopathy, despite the mitigation of senile plaques and neuritic dystrophy.[70,71)] Recently, the humanized antibody (bapineuzumab) of 3D6 was tested in clinical trials. Although bapinezumab reduced Aβ plaques examined by plaque-detective positron emission tomography (PET) imaging in AD patients in phase III, almost no clinical benefits were observed, thus resulting in the termination of this trial, according to the report by Lemere *et al*.[72)] These problems may have occurred because the treatment was too late to recover from neurodegenerative decline during the disease process.[73,74)] It is therefore indispensable to develop highly sensitive oligomer-specific antibodies for the purpose of early diagnosis and passive immunization in AD therapeutics.

III. Conformation-specific Antibodies to Target Aβ Oligomers

Wirth *et al*. reported no association of Aβ plaques by [^{11}C] Pittsburgh compound B (PiB) PET and neuronal degeneration in older subjects with normal cognition.[75)] These findings imply the need for a novel detection tool for oligomeric Aβ in place of PiB, which is one of the most reliable techniques for amyloid detection in clinical practice. If the involvement of tau hyperphosphorylation and accumulation is considered in AD pathology, these may be stimulated by Aβ oligomers.[76)] However, well-established detection reagents of Aβ oligomers are presently lacking. Considering the difference of conformations between Aβ oligomers and fibrils based on previous NMR analysis,[77)] several endeavors have been made to develop conformation-specific antibodies to target Aβ oligomers (Table 1).

1. A11, OC, αAPF antibody

Glabe and colleagues generated an oligomer-specific antibody (A11), which does not recognize Aβ fibrils and also reacts with other types of amyloid oligomers (α-synuclein in Parkinson's disease, polyglutamine in Huntington's disease, and prion peptide 106-126 in prion disease), using a molecular mimic of the presumed organization of Aβ oligomers.[78) They used Aβ40 octamer as a hapten, which was synthesized by conjugating the C-terminal thioester Aβ40 to colloidal gold nanoparticles. The gold-coupled Aβ40 octamer forms a typical β-sheet structure in the circular dichroism (CD) spectra.[79)] This octamer is also spherical in atomic force microscopy, but weak in thioflavin T fluorescence.[79)] This is the first antibody that binds intermediates of Aβ aggregation, but not fibrillar Aβ.[80)] In fact, immunohistochemistry using human AD brains showed that the localization of A11 staining was different from that of thioflavin staining.[78)]

Subsequently, they produced OC antibody by immunizing with Aβ42 fibrils, and OC recognized only amyloid fibrils, not prefibrillar oligomer detected by A11.[81)] The mechanism of A11-positive prefibrillar oligomer formation is proposed to be distinct from that of OC-positive fibrillar oligomer formation. They also identified the annular protofibrillar oligomer (αAPF), and made an antiserum selective for αAPF as the second generation of A11.[82)]

Regarding the application of these antibodies to the diagnosis, they performed dot blotting using human materials. The levels of soluble fibrillar oligomer detected by OC were larger in AD brain extracts than in age-matched individuals, and these increased levels were associated with cognitive decline. Surprisingly, levels of soluble prefibrillar oligomer by A11 and αAPF were not associated.[83)] Similar results were obtained in the experiment using mouse brain extracts.[84)] These results raise another concern that there are at least two classes of oligomers: oligomers supposed to move into the fibrillar stage (on-pathway) or those supposed to remain as the intermediate (off-pathway) (Fig. 3). These also suggest that fibrillar deposition may not be necessarily as benign as previously considered. Recent research also showed that αAPF levels in the cerebrospinal fluid (CSF) were elevated during the presymptomatic phase in a hereditary (familial) AD patient.[85)] αAPF might be an optimized biomarker for the early

diagnosis of AD.

2. Anti-ADDLs antibody

Klein and colleagues found that Aβ42-derived ADDLs blocked LTP by binding to synaptic terminals.[30] Aβ40 failed to form ADDLs. Anti-ADDLs antibody (NU-1) was developed based on its ability to discriminate an AD brain from a control brain.[86] Although these antibodies were generated by immunization with ADDLs, the epitope of NU-1 likely lay in the Aβ sequence (Aβ1-28) or its assemblies. Neutralization by these antibodies significantly rescued Aβ42-induced LTP inhibition as well as ROS.[86] The amounts of ADDLs were enhanced in CSF and brain extracts of AD.[87,88]

Shughrue *et al*. also produced an antibody against ADDLs according to the method developed by Klein and colleagues, and one clone (ACU-954) significantly inhibited the loss of dendritic spines induced by ADDLs through its binding to hippocampal neurons.[89] ACU-954 also detected naturally-occurring ADDLs in AD brains, which was localized in the hippocampal dendritic spines as well as in the cortex, but not within neuronal cells.[89]

3. Anti-ASPD antibody

Hoshi and colleagues generated monoclonal antibodies (rpASD1, mASD3) against amylospheroid (ASPD), which are considered to be an off-pathway product of Aβ intermediates because ASPD were not included in mature fibrils and were different from ADDLs in morphology and size.[90] They isolated 10~15-nm spherical Aβ oligomer (named as native ASPD) by immunoisolation using anti-ASPD antibodies from AD brains. The amount of native ASPD correlated with the severity of AD. These antibodies also immunostained dense-core plaques in cryosections as well as paraffin sections of AD brains. Based on an experiment using the antibodies, they proposed that ASPD-mediated toxicity has a distinct mechanism from other oligomers, where ASPD binds a presynaptic target in an NMDA-receptor-independent manner.[90]

The subsequent study by the same group using a combination measurement of

fluorescence correlation spectroscopy and transmission electron microscopy showed that the formation of ASPD begins with a trimer, whereas the initial step of fibrillogenesis is dimerization.[91] The oligomeric size of most toxic ASPD was ~32-mer (~128 kDa). These findings raise a future concern how dimers and trimers show such different toxicity profiles.

4. Anti-globulomer antibody

Hillen and colleagues developed an antibody (A-887755) against synthetic oligomer (globulomer), whose conformation is different from that of Aβ monomer or fibril.[92] In this study, Aβ20-42 was used for preparation of globulomer. They originally found a globulomer made from Aβ42, which is supposed to be a stable neurotoxin,[28] and showed that Aβ42 globulomer inhibited spontaneous synaptic function by modulation of the P/Q-type calcium current.[93] They used a truncated peptide (Aβ20-42) to avoid the reactivity of all Aβ species (monomers, oligomers, fibrils) because of the broad immunogenicity of N-terminal regions. Indeed, the antibody (6G1) against Aβ42 globulomer did not discriminate among monomers, oligomers, and fibrils.[28]

In immnoprecipitation experiments, A-887755 did not recognize Aβ monomer in the CSF and plasma of AD patients. More importantly, A-887755 did not immunolable senile plaque in AD brains (*e.g.* brain parenchym and vessel),[92] suggesting little cross-reactivity of oligomer-targeted A-887755 antibody with Aβ monomer and deposits. Aβ20-42 globulomer-induced synaptotoxicity was also neutralized by A-887755. Regarding the therapeutic approach, active immunization with Aβ20-42 globulomer improved the impaired novel object recognition. Furthermore, passive immunization with A-887755 rescued cognitive impairment as well as synaptic spine density in AD mice.[92] Considering adverse effects with the removal of plaques, A-887755 might be a good candidate for an AD therapeutic agent.

5. Grafted amyloid-motif antibody (Gammabody)

Tessier and colleagues proposed a unique "grafting" approach to develop conformation-

and sequence-specific antibodies for Aβ.[94] This approach is based on the concept, originated by Williamson and colleagues,[95] that selectivity against aggregated Aβ conformers can be enhanced by grafting the Aβ sequence responsible for aggregation into the complementarity-determining region (CDR) in the F_V domain of antibodies, which are generally bound to antigens. They focused on the third CDR (CDR3) of an antibody domain (V_H), whose structure has been identified (PDB: 3B9V). The folding of V_H, which is a stable scaffold, is insensitive to point mutations in the CDR3 loop motif.[96] Systematic grafting of the Aβ sequence revealed that the antibody including the central region (Val18-Ala21) bound to Aβ fibrils, and the antibody including the C-terminal region (Leu34-Ala21) reacted with Aβ oligomers as well as fibrils. However, an oligomer-specific antibody was not obtained. Such broad reactivity may be why the selected grafting sequence is shared between the formation of oligomers and fibrils. Immunohistochemistry has not been performed.

In subsequent studies, these antibodies inhibited the aggregation of Aβ42 by forming Aβ-antibody complex, which was detected by size-exclusion chromatography.[97] These approaches were expanded to other amyloid proteins: islet amyloid polypeptide (type 2 diabetes) and α-synuclein (Parkinson's disease).

6. Antibodies generated by phage display

Phage display is a conventional and powerful technique for antibody selection from libraries by inserting a gene encoding a protein of interest into a phage gene. In general, a virus with the ability to infect and replicate within bacteria is used as a bacteriophage. Fändrich and colleagues demonstrated a phage display using a recombinant library of the camelid VHH domain, and selected the conformation-sensitive VHH-domain B10 by repeated panning using Aβ40 fibrils.[98] The B10 antibody recognized only mature fibrils and prevented fibrillization by stabilizing Aβ40 protofibril.

Their next target was Aβ40 oligomer. The reactivity of the obtained antibody (KW1) in a similar approach was dependent on a hydrophobic and aromatic motif including Aβ fragment (Aβ18-20), which was in good agreement with the results from NMR analysis of the

interaction of Aβ40 with KW1.[99] KW1 bound to high molecular-weight oligomers rather than fibrils and detected brain-derived oligomers in AD patients.

Cattaneo and colleagues carried out advanced phage display selection using an anti-Aβ single chain F_V domain by targeting intracellular Aβ oligomers.[100] They expressed a LexA-Aβ42 fusion protein in yeast cells, and several antibodies were obtained against these intracellular antigens. The antibodies immunostained senile deposits in the AD brain, and the intracellular deposits were also confirmed in the cell-based experiment. These antibodies also inhibited ADDLs-induced toxicity in cell cultures by preventing the binding of ADDLs to the synapse. This will help us to understand the processing and trafficking of intracellular Aβ oligomers.

IV. Antibodies against toxic conformer of Aβ42

Despite accumulated structural research using NMR, MS, and X-ray crystallography on Aβ42[101] and Aβ40,[102,103] studies focusing on the relationship between conformer and neurotoxicity are limited. We have previously proposed the toxic conformer of Aβ42 with a turn at positions 22 and 23, and that this conformer could preferably form oligomeric conformation. Our strategy is to develop the oligomer-targeted antibodies based on the theory of the toxic conformer of Aβ42.[104]

1. Toxic conformer of Aβ42

Some investigations clarified that the *S*-oxidized radical cation in Met35 of Aβ42 is closely related to its neurotoxicity. However, it remains fully unanswered how the radical is formed to induce toxic effects. Moreover, Met35 radical is generally too unstable to cause oxidative damage continuously.[105] Our continued research, including systematic proline replacement and electron spin resonance (ESR), demonstrated that the turn structure at positions 22 and 23 could bring a phenoxy radical into Tyr10, which was generated through trace metals, close to Met35, resulting in the generation of the *S*-oxidized radical cation in Met35 (Fig. 4A). Another turn at Gly38 and Val39 as well as the turn at Glu22 and Asp23

was also involved in aggregation and neurotoxicity.[106] Such an additional C-terminal turn could play a role in the stabilization of the *S*-oxidized radical cation by forming an *S-O* bonding with a carboxylate anion at Ala42 at the C-terminal core (Fig. 4A). Collectively, the resultant core facilitated by an intramolecular β-sheet (Met35~Ala42) would contribute to long-lasting oxidative stress, that is, the neurotoxicity,[15] and thus we have proposed the toxic conformer of Aβ42 with a turn at positions 22 and 23. Further research using solid-state NMR clarified the existence of a non-toxic conformer with a turn at positions 25 and 26 in Aβ42 aggregates as well as a toxic conformer with a turn at positions 22 and 23[107-109] (Fig. 4B). In the following study by Masuda *et al.*, the Aβ42-lactam (E22K-D23E), in which the side chains of Lys22 and Glu23 in the toxic conformer are linked with an amide bond, enhanced oligomer (mainly trimer) formation and the radical-generating ability of Aβ42 as well as the aggregative ability (oligomerization) and neurotoxicity. In contrast, the Aβ42-lactam (G25K-S26E), in which the side chains of Lys25 and Glu26 in the non-toxic conformer are similarly linked, did not.[108] Aβ42 mutant (E22P-Aβ42) with a high ability to form the toxic conformer induced the synaptotoxicity on the rat hippocampal slices.[110] These findins strongly suggest that the formation of toxic conformer could be required to facilitate the oligomeric conformation (termed as "toxic oligomer").

2. 11A1 antibody

We next tried to develop a monoclonal antibody against the toxic conformer of Aβ42. The truncated Aβ peptide (E22P-Aβ10-35) including a toxic turn at positions 22 and 23, as a Pro-X corner (X: variable amino acid residue),[111] based on the optimum length (Aβ10-35) for neurotoxicity,[15] was utilized as a hapten (Fig. 4B). To address whether the obtained antibody (termed 11A1)[104] can react with Aβ oligomers or not, a brain soluble fraction was prepared for western blotting. 11A1 bound a low-molecular-weight oligomer (predominantly trimer), whereas 4G8 against Aβ17-24 and 82E1[112] against the N-terminus of Aβ, recognized mainly the monomer. These observation are consistent with the previous data that Aβ42 mutants with a potent propensity to form a turn structure at positions 22 and 23 accelerated Aβ

oligomerization.[108)]

MTT assay is one of the evaluation methods for Aβ-mediated neurotoxicity. The neurotoxicity of Aβ42 on PC12 cells was recovered by 11A1, but not by 4G8.[104)] 11A1 also inhibited the cytotoxicity of E22P-Aβ42, which can more readily form the toxic conformer of Aβ42. Similar results were obtained in the test using rat primary neurons.[113)] The following dot blotting study of Aβ42 demonstrated the gradual increase of 11A1 reactivity in a time-dependent manner, which preceded neurotoxicity.[114)] On the other hand, the immunoreactivity of Aβ42 by other sequence-specific antibodies remained constant. Moreover, 11A1 potently detected the toxic conformer in Aβ42 mutants related to familial Aβ mutations, such as Italian (E22K) and Arctic (E22G),[114)] which augmented neurotoxicity as well as the aggregative ability of Aβ42.[115)] The neurotoxic effects of these mutants were in good agreement with the levels of reactive oxidative stress tested by the 2’,7’-dichlorodihydrofluorescein (DCF) assay,[114)] supporting the critical role of oligomerization induced from toxic Aβ42 conformers in oxidative stress.

3. Intracellular Aβ

Although the accumulation of oligomeric Aβ within neuronal cells has been considered to be one of the early events during AD progression, there is little information on the conformation of intraneuronal Aβ aggregates.[116)] It has been reported that the intracellular Aβ oligomer accumulates in the endoplasmic reticulum (ER), endosomes, lysosomes, and mitochondria.[117)] Intracellular Aβ deposition precedes the accumulation of extracellular Aβ.[118)] Mitochondrial toxicity, proteasome impairment, and synaptic damage due to intracellular Aβ have been identified.[119)] Our immunohistochemical studies using the frontal lobe and hippocampus of AD patients (provided by Dr. Shigeo Murayama of the Brain Bank for Aging Research, Tokyo Metropolitan Institute of Gerontology) showed that 11A1 recognized not only typical amyloid plaques but also potent intracellular staining (Fig. 4B). On the other hand, only extracellular amyloid plaques were stained by other sequence-dependent antibodies.[104)] Interestingly, mild intracellular staining of 11A1 was

1 found even in non-AD individuals, suggesting that 11A1 can detect toxic species of Aβ within
2 cells before the onset of AD. These do not contradict the previous results[118] of the potent
3 immunoreactivity of intracellular Aβ in a patient with MCI.

4 Similar results using 11A1 have been followed by other researchers. Ohyagi and
5 colleagues showed that intraneuronal staining by 11A1 was more closely related to the onset
6 of memory impairment in 3xTg-AD mice than that by 4G8.[120] They also found the
7 co-localization of 11A1-positive deposits with GRP78, an ER stress marker, in AD brain
8 sections, whose expression was associated with cognitive impairment and dysfunction of
9 endsomes and Golgi-ER trafficking.[120] Kulic *et al*. developed APP transgenic mice with
10 double mutations of Swedish (K670N/M671L in APP) and Osaka (E693Δ in APP), and
11 observed the early depositions of intracellular fibrillar oligomers (11A1-positive) coupled
12 with early memory decline.[121] Osaka mutation (E22Δ in Aβ) favoring oligomerization
13 induced the potent synaptotoxicity of Aβ42,[122] but not Aβ40.[110] Inoue and colleagues using
14 11A1 demonstrated intracellular accumulation of Aβ oligomers with toxic conformer in
15 neuronal cells derived from induced pluripotent stem cells (iPSCs), which were obtained from
16 sporadic patients and a familial AD patient with Osaka mutation.[123] Interestingly,
17 anti-ADDLs antibody (NU-1) also immunostained intracellular Aβ similarly to 11A1. 11A1
18 is thus a unique antibody that preferably recognizes intracellular amyloid in the human brain
19 along with senile plaques. These findings highlight that the toxic conformer of Aβ42 could
20 accumulate within neurons at the early stage during AD progression.

21 Regarding the intracellular accumulation of Aβ in AD pathology, key questions of how
22 intracellular Aβ accumulates remain unanswered, that is, whether Aβ is partially secreted into
23 the extracellular space but remains intracellular, or whether secreted Aβ is transported into the
24 intracellular space. Indeed, some transporters involved in the internalization of Aβ have been
25 reported; the scavenger receptor for advanced glycation end products (RAGE)[53] and the
26 formyl peptide receptor-like 1 (FPRL1).[53,124] Notably, extracellular plaques increase, while
27 intracellular depositions of Aβ decrease.[125] Considering the involvement of tau pathology,
28 intraneuronal Aβ co-existed with NFT inside the neurons.[4,126] Intracellular Aβ may trigger

tau hyperphosphorylation and mitochondrial dysfunction to induce synaptotoxicity. Because the deposition of tau protein starts about 10 years later than Aβ accumulation (Fig. 1), a mediator regulating the cross-talk of Aβ with tau may exist. Quite recently, the synergistical interaction between the accumulation of 11A1-positive intracellular Aβ and human tau could accelerate each other's aggregation.[127] These indicate the meditation role of toxic conformer of Aβ42 in AD pathology.

V. Conclusions and Future Directions

Given the growing medical and social burden, the necessity of the early resolution of AD is stronger than ever. To date, anti-Aβ drugs have been developed;[128] these are mainly divided into three strategies: (1) anti-aggregation and clearance, (2) secretase inhibitors, (3) Aβ degradation activator. Since symptomatic drugs (denopezil, memantine, rivastigmine, and galantamine) have been established, these combination strategies based on an early diagnosis will be more effective. Several structure-based designs of aggregation inhibitors have been also recently reported.[129-131] Conformation-specific antibodies to target the characteristic structure of Aβ oligomers will shed new light on the accurate diagnosis by ELISA development and vaccination therapy. Eventually, it may be possible to extend the diagnosis and intervention to asymptomatic people.

In the application of antibodies to ELISA development, the approach of two-site ELISA has received attention, in which the same sequence-specific antibody (82E1[132] or Ban50[133] against Aβ1-16) for capture and detection is used. These approaches revealed a clear correlation of the oligomer levels in the plasma and brain extracts[132] and CSF[133] in various cognitive levels of AD patients. A recent study using brain lysates showed that two-site ELISA of the antibody (HJ3.4) against the N-terminal Aβ discriminated Aβ dimer from monomer, but the result that HJ3.4 did not discriminate oligomers from plaques caused confusion.[134] Such a strategy aiming at ELISA specificity is questioned. Because 2 x n-mer oligomers with high molecular weight as well as the dimer can be theoretically detected in these strategies, oligomer levels in healthy individuals may be overestimated.[135] Two-site

ELISA recently generated by the same group to target ADDLs using a modified ACU-954 conjugated with a bead-based fluorescent platform was improved in this aspect.[136) Prospectively, the application of conformation-specific antibodies such as 11A1 into ELISA is promising.

Indeed, only a few antibodies can cross the BBB (0.1–0.2%).[137) Even if unprecedented antibodies are developed, this concern may limit their therapeutic application, such as in vaccination. Quite recently, protein manipulation by binding anti-Aβ antibody to transferrin receptor, which is involved in receptor-mediated transcytosis, produced a monovalent "Brain Shuttle" module, leading to increased brain penetration.[138) Consequently, continuous investigations to develop oligomer-specific antibodies with high affinity will be required to move closer to the realization of a world without AD.

Acknowledgements

The work described in this review was conducted both in the Laboratory of Organic Chemistry in Life Science, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University and in the Research Team for Molecular Gerontology, Tokyo Metropolitan Institute of Gerontology. I would like to express special thanks to Professor Kazuhiro Irie (Kyoto University) for his continued encouragement and fruitful discussion, which have been indispensable to the progress in this work. I am grateful to Dr. Takahiko Shimizu (Tokyo Metropolitan Institute of Gerontology, currently Chiba University) for his guidance and valuable discussion on the animal experiment. I also heartily thank Dr. Gal Bitan (University of California, Los Angeles) and Dr. Kevin J. Barnham (University of Melbourne) for their kind encouragement during my stay in their laboratories. I am deeply grateful to Dr. Shigeo Murayama (Tokyo Metropolitan Institute of Gerontology) for providing the brain samples from the Brain Bank for Aging Research. I am thankful to Emeritus Professor Hajime Ohigashi (Kyoto University), Professor Nobuhiro Hirai (Kyoto University), and Dr. Akira Murakami (Kyoto University) for helpful suggestions, and Professor Takuji Shirasawa (Tokyo Metropolitan Institute of Gerontology, currently Juntendo University), Ms.

Nakaba Murata (Tokyo Metropolitan Institute of Gerontology), Dr. Noriaki Kinoshita (Immunobiological Laboratories, currently GeneticLab), Dr. Yuko Horikoshi-Sakuraba (Immunobiological Laboratories), Dr. Hideyuki Hara (Bruker Biospin), Dr. Yuichi Masuda (Tohoku University), Dr. Naotaka Izuo (Chiba University), and Mr. Akira Morimoto for their wonderful collaboration.

Finally, this work was supported in part by Grants-in-Aid for Scientific Research, the Program for the Promotion of Basic Research Activities for Innovative Biosciences, and funds for the Promotion of Science for Young Scientists from The Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government.

References

1) Glenner GG and Wong CW, *Biochem. Biophys. Res. Commun.,* **120**, 885-890 (1984).

2) Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, and Beyreuther K, *Proc. Natl. Acad. Sci. USA,* **82**, 4245-4249 (1985).

3) Karran E, Mercken M, and De Strooper B, *Nat. Rev. Drug Discov.,* **10**, 698-712 (2011).

4) Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, and Binder LI, *Proc. Natl. Acad. Sci. USA,* **83**, 4913-4917 (1986).

5) Ihara Y, Nukina N, Miura R, and Ogawara M, *J Biochem,* **99**, 1807-1810 (1986).

6) Aisen PS, Petersen RC, Donohue MC, Gamst A, Raman R, Thomas RG, Walter S, Trojanowski JQ, Shaw LM, Beckett LA, Jack CR, Jr., Jagust W, Toga AW, Saykin AJ, Morris JC, Green RC, and Weiner MW, *Alzheimers Dement.,* **6**, 239-246 (2010).

7) Takasugi N, Tomita T, Hayashi I, Tsuruoka M, Niimura M, Takahashi Y, Thinakaran G, and Iwatsubo T, *Nature,* **422**, 438-441 (2003).

8) Qi-Takahara Y, Morishima-Kawashima M, Tanimura Y, Dolios G, Hirotani N, Horikoshi Y, Kametani F, Maeda M, Saido TC, Wang R, and Ihara Y, *J. Neurosci.,* **25**, 436-445 (2005).

9) Takami M, Nagashima Y, Sano Y, Ishihara S, Morishima-Kawashima M, Funamoto S, and Ihara Y, *J. Neurosci.,* **29**, 13042-13052 (2009).

10) Roher AE, Lowenson JD, Clarke S, Wolkow C, Wang R, Cotter RJ, Reardon IM,

1 Zurcher-Neely HA, Heinrikson RL, Ball MJ, and Greenberg BD, *J. Biol. Chem.,* **268**,
2 3072-3083 (1993).
3 11) Saido TC, Yamao-Harigaya W, Iwatsubo T, and Kawashima S, *Neurosci. Lett.,* **215**,
4 173-176 (1996).
5 12) Liu K, Solano I, Mann D, Lemere C, Mercken M, Trojanowski JQ, and Lee VM, *Acta*
6 *Neuropathol. (Berl).,* **112**, 163-174 (2006).
7 13) Kumar S, Rezaei-Ghaleh N, Terwel D, Thal DR, Richard M, Hoch M, Mc Donald JM,
8 Wullner U, Glebov K, Heneka MT, Walsh DM, Zweckstetter M, and Walter J, *EMBO*
9 *J.,* **30**, 2255-2265 (2011).
10 14) Saito T, Suemoto T, Brouwers N, Sleegers K, Funamoto S, Mihira N, Matsuba Y,
11 Yamada K, Nilsson P, Takano J, Nishimura M, Iwata N, Van Broeckhoven C, Ihara Y,
12 and Saido TC, *Nat. Neurosci.,* **14**, 1023-1032 (2011).
13 15) Murakami K, Irie K, Ohigashi H, Hara H, Nagao M, Shimizu T, and Shirasawa T, *J.*
14 *Am. Chem. Soc.,* **127**, 15168-15174 (2005).
15 16) Davis J and Van Nostrand WE, *Proc. Natl. Acad. Sci. USA,* **93**, 2996-3000 (1996).
16 17) Qiu WQ, Walsh DM, Ye Z, Vekrellis K, Zhang J, Podlisny MB, Rosner MR, Safavi A,
17 Hersh LB, and Selkoe DJ, *J. Biol. Chem.,* **273**, 32730-32738 (1998).
18 18) Iwata N, Tsubuki S, Takaki Y, Shirotani K, Lu B, Gerard NP, Gerard C, Hama E, Lee
19 HJ, and Saido TC, *Science,* **292**, 1550-1552 (2001).
20 19) Mangialasche F, Solomon A, Winblad B, Mecocci P, and Kivipelto M, *Lancet Neurol.,*
21 **9**, 702-716 (2010).
22 20) Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, and
23 Selkoe DJ, *Nature,* **416**, 535-539 (2002).
24 21) Lee HG, Zhu X, Castellani RJ, Nunomura A, Perry G, and Smith MA, *J. Pharmacol.*
25 *Exp. Ther.,* **321**, 823-829 (2007).
26 22) Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, Parisi JE, and
27 Hyman BT, *Ann. Neurol.,* **41**, 17-24 (1997).
28 23) Meyer-Luehmann M, Spires-Jones TL, Prada C, Garcia-Alloza M, de Calignon A,
29 Rozkalne A, Koenigsknecht-Talboo J, Holtzman DM, Bacskai BJ, and Hyman BT,
30 *Nature,* **451**, 720-724 (2008).
31 24) Bitan G, Kirkitadze MD, Lomakin A, Vollers SS, Benedek GB, and Teplow DB, *Proc.*

1 *Natl. Acad. Sci. USA,* **100**, 330-335 (2003).
2 25) Lesnè S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, and Ashe
3 KH, *Nature,* **440**, 352-357 (2006).
4 26) Walsh DM, Lomakin A, Benedek GB, Condron MM, and Teplow DB, *J. Biol. Chem.,*
5 **272**, 22364-22372 (1997).
6 27) Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A, Benedek
7 GB, Selkoe DJ, and Teplow DB, *J. Biol. Chem.,* **274**, 25945-25952 (1999).
8 28) Barghorn S, Nimmrich V, Striebinger A, Krantz C, Keller P, Janson B, Bahr M,
9 Schmidt M, Bitner RS, Harlan J, Barlow E, Ebert U, and Hillen H, *J. Neurochem.,* **95**,
10 834-847 (2005).
11 29) Deshpande A, Mina E, Glabe C, and Busciglio J, *J. Neurosci.,* **26**, 6011-6018 (2006).
12 30) Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE,
13 Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, and Klein
14 WL, *Proc. Natl. Acad. Sci. USA,* **95**, 6448-6453 (1998).
15 31) Caughey B and Lansbury PT, *Annu. Rev. Neurosci.,* **26**, 267-298 (2003).
16 32) Hoshi M, Sato M, Matsumoto S, Noguchi A, Yasutake K, Yoshida N, and Sato K,
17 *Proc. Natl. Acad. Sci. USA,* **100**, 6370-6375 (2003).
18 33) Roychaudhuri R, Yang M, Hoshi MM, and Teplow DB, *J. Biol. Chem.,* **284**,
19 4749-4753 (2009).
20 34) Benilova I, Karran E, and De Strooper B, *Nat. Neurosci.,* **15**, 349-357 (2012).
21 35) Garzon-Rodriguez W, Sepulveda-Becerra M, Milton S, and Glabe CG, *J. Biol. Chem.,*
22 **272**, 21037-21044 (1997).
23 36) Chen YR and Glabe CG, *J. Biol. Chem.,* **281**, 24414-24422 (2006).
24 37) Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM,
25 Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, and Selkoe
26 DJ, *Nat. Med.,* **14**, 837-842 (2008).
27 38) Townsend M, Shankar GM, Mehta T, Walsh DM, and Selkoe DJ, *J. Physiol.,* **572**,
28 477-492 (2006).
29 39) Cooke SF and Bliss TV, *Brain,* **129**, 1659-1673 (2006).
30 40) Ansari MA and Scheff SW, *J. Neuropathol. Exp. Neurol.,* **69**, 155-167 (2010).
31 41) De Felice FG, Velasco PT, Lambert MP, Viola K, Fernandez SJ, Ferreira ST, and

1 Klein WL, *J. Biol. Chem.,* **282**, 11590-11601 (2007).
2 42) Barnham KJ, Haeffner F, Ciccotosto GD, Curtain CC, Tew D, Mavros C, Beyreuther
3 K, Carrington D, Masters CL, Cherny RA, Cappai R, and Bush AI, *FASEB J.,* **18**,
4 1427-1429 (2004).
5 43) Smith DG, Cappai R, and Barnham KJ, *Biochim. Biophys. Acta,* **1768**, 1976-1990
6 (2007).
7 44) Murakami K, Murata N, Noda Y, Tahara S, Kaneko T, Kinoshita N, Hatsuta H,
8 Murayama S, Barnham KJ, Irie K, Shirasawa T, and Shimizu T, *J. Biol. Chem.,* **286**,
9 44557-44568 (2011).
10 45) Imamura Y, Noda S, Hashizume K, Shinoda K, Yamaguchi M, Uchiyama S, Shimizu
11 T, Mizushima Y, Shirasawa T, and Tsubota K, *Proc. Natl. Acad. Sci. USA,* **103**,
12 11282-11287 (2006).
13 46) Uchiyama S, Shimizu T, and Shirasawa T, *J. Biol. Chem.,* **281**, 31713-31719 (2006).
14 47) Murakami K, Inagaki J, Saito M, Ikeda Y, Tsuda C, Noda Y, Kawakami S, Shirasawa
15 T, and Shimizu T, *Biochem. Biophys. Res. Commun.,* **382**, 457-461 (2009).
16 48) Nojiri H, Saita Y, Morikawa D, Kobayashi K, Tsuda C, Miyazaki T, Saito M, Marumo
17 K, Yonezawa I, Kaneko K, Shirasawa T, and Shimizu T, *J. Bone Miner. Res.,* **26**,
18 2682-2694 (2011).
19 49) Murakami K, Murata N, Noda Y, Irie K, Shirasawa T, and Shimizu T, *Biosci.*
20 *Biotechnol. Biochem.,* **76**, 1098-1103 (2012).
21 50) Murakami K, Shimizu T, and Irie K, *J. Amino Acids,* **2011**, ID654207 (2011).
22 51) Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, Nairn AC, Salter MW,
23 Lombroso PJ, Gouras GK, and Greengard P, *Nat. Neurosci.,* **8**, 1051-1058 (2005).
24 52) Yamin G, *J. Neurosci. Res.,* **87**, 1729-1736 (2009).
25 53) Yan SD, Chen X, Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagashima M,
26 Morser J, Migheli A, Nawroth P, Stern D, and Schmidt AM, *Nature,* **382**, 685-691
27 (1996).
28 54) Giuffrida ML, Caraci F, De Bona P, Pappalardo G, Nicoletti F, Rizzarelli E, and
29 Copani A, *Rev. Neurosci.,* **21**, 83-93 (2010).
30 55) Lauren J, Gimbel DA, Nygaard HB, Gilbert JW, and Strittmatter SM, *Nature,* **457**,
31 1128-1132 (2009).

56) Solomon B, Koppel R, Hanan E, and Katzav T, *Proc. Natl. Acad. Sci. USA,* **93**, 452-455 (1996).

57) Solomon B, Koppel R, Frankel D, and Hanan-Aharon E, *Proc. Natl. Acad. Sci. USA,* **94**, 4109-4112 (1997).

58) Naiki H, Higuchi K, Hosokawa M, and Takeda T, *Anal. Biochem.,* **177**, 244-249 (1989).

59) Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandevert C, Walker S, Wogulis M, Yednock T, Games D, and Seubert P, *Nature,* **400**, 173-177 (1999).

60) Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, Chishti MA, Horne P, Heslin D, French J, Mount HT, Nixon RA, Mercken M, Bergeron C, Fraser PE, St George-Hyslop P, and Westaway D, *Nature,* **408**, 979-982 (2000).

61) Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, Duff K, Jantzen P, DiCarlo G, Wilcock D, Connor K, Hatcher J, Hope C, Gordon M, and Arendash GW, *Nature,* **408**, 982-985 (2000).

62) Oddo S, Vasilevko V, Caccamo A, Kitazawa M, Cribbs DH, and LaFerla FM, *J. Biol. Chem.,* **281**, 39413-39423 (2006).

63) Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank A, and Hock C, *Neurology,* **61**, 46-54 (2003).

64) Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, Jones RW, Bullock R, Love S, Neal JW, Zotova E, and Nicoll JA, *Lancet,* **372**, 216-223 (2008).

65) Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, Burton MA, Goldstein LE, Duong S, Tanzi RE, and Moir RD, *PLoS ONE,* **5**, e9505 (2010).

66) Effros RB, Fletcher CV, Gebo K, Halter JB, Hazzard WR, Horne FM, Huebner RE, Janoff EN, Justice AC, Kuritzkes D, Nayfield SG, Plaeger SF, Schmader KE, Ashworth JR, Campanelli C, Clayton CP, Rada B, Woolard NF, and High KP, *Clin. Infect. Dis.,* **47**, 542-553 (2008).

67) Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R,

1 Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, and
2 Yednock T, *Nat. Med.,* **6**, 916-919 (2000).
3 68) DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, and Holtzman DM, *Proc.*
4 *Natl. Acad. Sci. USA,* **98**, 8850-8855 (2001).
5 69) Klyubin I, Walsh DM, Lemere CA, Cullen WK, Shankar GM, Betts V, Spooner ET,
6 Jiang L, Anwyl R, Selkoe DJ, and Rowan MJ, *Nat. Med.,* **11**, 556-561 (2005).
7 70) Racke MM, Boone LI, Hepburn DL, Parsadainian M, Bryan MT, Ness DK, Piroozi
8 KS, Jordan WH, Brown DD, Hoffman WP, Holtzman DM, Bales KR, Gitter BD, May
9 PC, Paul SM, and DeMattos RB, *J. Neurosci.,* **25**, 629-636 (2005).
10 71) Wilcock DM, Rojiani A, Rosenthal A, Subbarao S, Freeman MJ, Gordon MN, and
11 Morgan D, *J. Neuroinflammation,* **1**, 24 (doi:10.1186/1742-2094-1181-1124) (2004).
12 72) Lemere CA, *Mol. Neurodegener.,* **8**, 36 (2013).
13 73) Lemere CA, and Masliah E, *Nat. Rev. Neurol.,* **6**, 108-119 (2010).
14 74) Liu YH, Giunta B, Zhou HD, Tan J, and Wang YJ, *Nat. Rev. Neurol.,* **8**, 465-469
15 (2012).
16 75) Wirth M, Madison CM, Rabinovici GD, Oh H, Landau SM, and Jagust WJ, *J.*
17 *Neurosci.,* **33**, 5553-5563 (2013).
18 76) De Felice FG, Wu D, Lambert MP, Fernandez SJ, Velasco PT, Lacor PN, Bigio EH,
19 Jerecic J, Acton PJ, Shughrue PJ, Chen-Dodson E, Kinney GG, and Klein WL,
20 *Neurobiol. Aging,* **29**, 1334-1347 (2008).
21 77) Chimon S, Shaibat MA, Jones CR, Calero DC, Aizezi B, and Ishii Y, *Nat. Struct. Mol.*
22 *Biol.,* (2007).
23 78) Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, and Glabe
24 CG, *Science,* **300**, 486-489 (2003).
25 79) Kayed R and Glabe CG, *Methods Enzymol.,* **413**, 326-344 (2006).
26 80) Glabe CG, *Trends Biochem. Sci.,* **29**, 542-547 (2004).
27 81) Kayed R, Head E, Sarsoza F, Saing T, Cotman CW, Necula M, Margol L, Wu J,
28 Breydo L, Thompson JL, Rasool S, Gurlo T, Butler P, and Glabe CG, *Mol.*
29 *Neurodegener.,* **2**, 18 (2007).
30 82) Kayed R, Pensalfini A, Margol L, Sokolov Y, Sarsoza F, Head E, Hall J, and Glabe C,
31 *J. Biol. Chem.,* **284**, 4230-4237 (2009).

1 83) Tomic JL, Pensalfini A, Head E, and Glabe CG, *Neurobiol. Dis.,* **35**, 352-358 (2009).
2 84) Sarsoza F, Saing T, Kayed R, Dahlin R, Dick M, Broadwater-Hollifield C, Mobley S,
3 Lott I, Doran E, Gillen D, Anderson-Bergman C, Cribbs DH, Glabe C, and Head E,
4 *Acta Neuropathol. (Berl).* **118**, 505-517 (2009).
5 85) Ringman JM, Tomic JL, Coppola G, Elashoff D, Gylys KH, and Glabe CG, *Dementia*
6 *Geriatr. Cogn. Disord. Extra,* **2**, 652-657 (2012).
7 86) Lambert MP, Velasco PT, Chang L, Viola KL, Fernandez S, Lacor PN, Khuon D,
8 Gong Y, Bigio EH, Shaw P, De Felice FG, Krafft GA, and Klein WL, *J. Neurochem.,*
9 **100**, 23-35 (2007).
10 87) Gong Y, Chang L, Viola KL, Lacor PN, Lambert MP, Finch CE, Krafft GA, and Klein
11 WL, *Proc. Natl. Acad. Sci. USA,* **100**, 10417-10422 (2003).
12 88) Georganopoulou DG, Chang L, Nam JM, Thaxton CS, Mufson EJ, Klein WL, and
13 Mirkin CA, *Proc. Natl. Acad. Sci. USA,* **102**, 2273-2276 (2005).
14 89) Shughrue PJ, Acton PJ, Breese RS, Zhao WQ, Chen-Dodson E, Hepler RW, Wolfe AL,
15 Matthews M, Heidecker GJ, Joyce JG, Villarreal SA, and Kinney GG, *Neurobiol.*
16 *Aging,* **31**, 189-202 (2010).
17 90) Noguchi A, Matsumura S, Dezawa M, Tada M, Yanazawa M, Ito A, Akioka M,
18 Kikuchi S, Sato M, Ideno S, Noda M, Fukunari A, Muramatsu S, Itokazu Y, Sato K,
19 Takahashi H, Teplow DB, Nabeshima Y, Kakita A, Imahori K, and Hoshi M, *J. Biol.*
20 *Chem.,* **284**, 32895-32905 (2009).
21 91) Matsumura S, Shinoda K, Yamada M, Yokojima S, Inoue M, Ohnishi T, Shimada T,
22 Kikuchi K, Masui D, Hashimoto S, Sato M, Ito A, Akioka M, Takagi S, Nakamura Y,
23 Nemoto K, Hasegawa Y, Takamoto H, Inoue H, Nakamura S, Nabeshima Y, Teplow
24 DB, Kinjo M, and Hoshi M, *J. Biol. Chem.,* **286**, 11555-11562 (2011).
25 92) Hillen H, Barghorn S, Striebinger A, Labkovsky B, Muller R, Nimmrich V, Nolte MW,
26 Perez-Cruz C, van der Auwera I, van Leuven F, van Gaalen M, Bespalov AY,
27 Schoemaker H, Sullivan JP, and Ebert U, *J. Neurosci.,* **30**, 10369-10379 (2010).
28 93) Nimmrich V, Grimm C, Draguhn A, Barghorn S, Lehmann A, Schoemaker H, Hillen
29 H, Gross G, Ebert U, and Bruehl C, *J. Neurosci.,* **28**, 788-797 (2008).
30 94) Perchiacca JM, Ladiwala AR, Bhattacharya M, and Tessier PM, *Proc. Natl. Acad. Sci.*
31 *USA,* **109**, 84-89 (2012).

1 95) Moroncini G, Kanu N, Solforosi L, Abalos G, Telling GC, Head M, Ironside J,
2 Brockes JP, Burton DR, and Williamson RA, *Proc. Natl. Acad. Sci. USA,* **101**,
3 10404-10409 (2004).
4 96) Barthelemy PA, Raab H, Appleton BA, Bond CJ, Wu P, Wiesmann C, and Sidhu SS, *J.*
5 *Biol. Chem.,* **283**, 3639-3654 (2008).
6 97) Ladiwala AR, Bhattacharya M, Perchiacca JM, Cao P, Raleigh DP, Abedini A,
7 Schmidt AM, Varkey J, Langen R, and Tessier PM, *Proc. Natl. Acad. Sci. USA,* **109**,
8 19965-19970 (2012).
9 98) Habicht G, Haupt C, Friedrich RP, Hortschansky P, Sachse C, Meinhardt J,
10 Wieligmann K, Gellermann GP, Brodhun M, Gotz J, Halbhuber KJ, Rocken C, Horn
11 U, and Fandrich M, *Proc. Natl. Acad. Sci. USA,* **104**, 19232-19237 (2007).
12 99) Morgado I, Wieligmann K, Bereza M, Ronicke R, Meinhardt K, Annamalai K,
13 Baumann M, Wacker J, Hortschansky P, Malesevic M, Parthier C, Mawrin C,
14 Schiene-Fischer C, Reymann KG, Stubbs MT, Balbach J, Gorlach M, Horn U, and
15 Fandrich M, *Proc. Natl. Acad. Sci. USA,* **109**, 12503-12508 (2012).
16 100) Meli G, Visintin M, Cannistraci I, and Cattaneo A, *J. Mol. Biol.,* **387**, 584-606 (2009).
17 101) Luhrs T, Ritter C, Adrian M, Riek-Loher D, Bohrmann B, Dobeli H, Schubert D, and
18 Riek R, *Proc. Natl. Acad. Sci. USA,* **102**, 17342-17347 (2005).
19 102) Petkova AT, Ishii Y, Balbach JJ, Antzutkin ON, Leapman RD, Delaglio F, and Tycko
20 R, *Proc. Natl. Acad. Sci. USA,* **99**, 16742-16747 (2002).
21 103) Tycko R, *Q. Rev. Biophys.,* **39**, 1-55 (2006).
22 104) Murakami K, Horikoshi-Sakuraba Y, Murata N, Noda Y, Masuda Y, Kinoshita N,
23 Hatsuta H, Murayama S, Shirasawa T, Shimizu T, and Irie K, *ACS Chem. Neurosci.,* **1**,
24 747-756 (2010).
25 105) Varadarajan S, Kanski J, Aksenova M, Lauderback C, and Butterfield DA, *J. Am.*
26 *Chem. Soc.,* **123**, 5625-5631 (2001).
27 106) Morimoto A, Irie K, Murakami K, Masuda Y, Ohigashi H, Nagao M, Fukuda H,
28 Shimizu T, and Shirasawa T, *J. Biol. Chem.,* **279**, 52781-52788 (2004).
29 107) Irie K, Murakami K, Masuda Y, Morimoto A, Ohigashi H, Hara H, Ohashi R,
30 Takegoshi K, Fukuda H, Nagao M, Shimizu T, and Shirasawa T, *Mini Rev. Med.*
31 *Chem.,* **7**, 1001-1008 (2007).

108) Masuda Y, Uemura S, Ohashi R, Nakanishi A, Takegoshi K, Shimizu T, Shirasawa T, and Irie K, *ChemBioChem,* **10**, 287-295 (2009).

109) Murakami K, Masuda Y, Shirasawa T, Shimizu T, and Irie K, *Geriatr Gerontol Int,* **10** (Suppl. 1), S169-179 (2010).

110) Suzuki T, Murakami K, Izuo N, Kume T, Akaike A, Nagata T, Nishizaki T, Tomiyama T, Takuma H, Mori H, and Irie K, *Int. J. Alzheimers Dis.,* **2011**, 431320 (2010).

111) Chou PY, and Fasman GD, *J. Mol. Biol.,* **115**, 135-175 (1977).

112) Horikoshi Y, Sakaguchi G, Becker AG, Gray AJ, Duff K, Aisen PS, Yamaguchi H, Maeda M, Kinoshita N, and Matsuoka Y, *Biochem. Biophys. Res. Commun.,* **319**, 733-737 (2004).

113) Izuo N, Murakami K, Sato M, Iwasaki M, Izumi Y, Shimizu T, Akaike A, Irie K, and Kume T, *Biochem. Biophys. Res. Commun.,* **438**, 1-5 (2013).

114) Izuo N, Kume T, Sato M, Murakami K, Irie K, Izumi Y, and Akaike A, *ACS Chem. Neurosci.,* **3**, 674-681 (2012).

115) Murakami K, Irie K, Morimoto A, Ohigashi H, Shindo M, Nagao M, Shimizu T, and Shirasawa T, *J. Biol. Chem.,* **278**, 46179-46187 (2003).

116) LaFerla FM, Green KN, and Oddo S, *Nat. Rev. Neurosci.,* **8**, 499-509 (2007).

117) Umeda T, Tomiyama T, Sakama N, Tanaka S, Lambert MP, Klein WL, and Mori H, *J. Neurosci. Res.,* **89**, 1031-1042 (2011).

118) Gouras GK, Tsai J, Naslund J, Vincent B, Edgar M, Checler F, Greenfield JP, Haroutunian V, Buxbaum JD, Xu H, Greengard P, and Relkin NR, *Am. J. Pathol.,* **156**, 15-20 (2000).

119) Ohyagi Y, *Curr. Alzheimer Res.,* **5**, 555-561 (2008).

120) Soejima N, Ohyagi Y, Nakamura N, Himeno E, Iinuma KM, Sakae N, Yamasaki R, Tabira T, Murakami K, Irie K, Kinoshita N, LaFerla FM, Kiyohara Y, Iwaki T, and Kira J, *Curr. Alzheimer Res.,* **10**, 11-20 (2013).

121) Kulic L, McAfoose J, Welt T, Tackenberg C, Spani C, Wirth F, Finder V, Konietzko U, Giese M, Eckert A, Noriaki K, Shimizu T, Murakami K, Irie K, Rasool S, Glabe C, Hock C, and Nitsch RM, *Transl. Psychiatry,* **2**, e183 (2012).

122) Tomiyama T, Nagata T, Shimada H, Teraoka R, Fukushima A, Kanemitsu H, Takuma H, Kuwano R, Imagawa M, Ataka S, Wada Y, Yoshioka E, Nishizaki T, Watanabe Y,

1 and Mori H, *Ann. Neurol.,* **63**, 377-387 (2008).
2 123) Kondo T, Asai M, Tsukita K, Kutoku Y, Ohsawa Y, Sunada Y, Imamura K, Egawa N,
3 Yahata N, Okita K, Takahashi K, Asaka I, Aoi T, Watanabe A, Watanabe K, Kadoya C,
4 Nakano R, Watanabe D, Maruyama K, Hori O, Hibino S, Choshi T, Nakahata T, Hioki
5 H, Kaneko T, Naitoh M, Yoshikawa K, Yamawaki S, Suzuki S, Hata R, Ueno S-i,
6 Seki T, Kobayashi K, Toda T, Murakami K, Irie K, Klein William L, Mori H, Asada T,
7 Takahashi R, Iwata N, Yamanaka S, and Inoue H, *Cell Stem Cell,* (2013).
8 124) Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, Welch D,
9 Manness L, Lin C, Yu J, Zhu H, Ghiso J, Frangione B, Stern A, Schmidt AM,
10 Armstrong DL, Arnold B, Liliensiek B, Nawroth P, Hofman F, Kindy M, Stern D, and
11 Zlokovic B, *Nat. Med.,* **9**, 907-913 (2003).
12 125) Mori C, Spooner ET, Wisniewsk KE, Wisniewski TM, Yamaguch H, Saido TC, Tolan
13 DR, Selkoe DJ, and Lemere CA, *Amyloid,* **9**, 88-102 (2002).
14 126) Blurton-Jones M, and Laferla FM, *Curr. Alzheimer Res.,* **3**, 437-448 (2006).
15 127) Umeda T, Maekawa S, Kimura T, Takashima A, Tomiyama T, and Mori H, *Acta*
16 *Neuropathol. (Berl).* (2014).
17 128) Williams P, Sorribas A, and Howes MJ, *Nat. Prod. Rep.,* **28**, 48-77 (2011).
18 129) Sinha S, Lopes DH, Du Z, Pang ES, Shanmugam A, Lomakin A, Talbiersky P,
19 Tennstaedt A, McDaniel K, Bakshi R, Kuo PY, Ehrmann M, Benedek GB, Loo JA,
20 Klarner FG, Schrader T, Wang C, and Bitan G, *J. Am. Chem. Soc.,* **133**, 16958-16969
21 (2011).
22 130) Sato M, Murakami K, Uno M, Nakagawa Y, Katayama S, Akagi K, Masuda Y,
23 Takegoshi K, and Irie K, *J. Biol. Chem.,* **288**, 23212-23224 (2013).
24 131) Kenche VB, Hung LW, Perez K, Volitakes I, Ciccotosto G, Kwok J, Critch N, Sherratt
25 N, Cortes M, Lal V, Masters CL, Murakami K, Cappai R, Adlard PA, and Barnham KJ,
26 *Angew. Chem. Int. Ed. Engl.,* **52**, 3374-3378 (2013).
27 132) Xia W, Yang T, Shankar G, Smith IM, Shen Y, Walsh DM, and Selkoe DJ, *Arch.*
28 *Neurol.,* **66**, 190-199 (2009).
29 133) Fukumoto H, Tokuda T, Kasai T, Ishigami N, Hidaka H, Kondo M, Allsop D, and
30 Nakagawa M, *FASEB J.,* **24**, 2716-2726 (2010).
31 134) Esparza TJ, Zhao H, Cirrito JR, Cairns NJ, Bateman RJ, Holtzman DM, and Brody

DL, *Ann. Neurol.,* **73**, 104-119 (2013).

135) Klaver AC, Patrias LM, Finke JM, and Loeffler DA, *J. Neurosci. Methods,* **195**, 249-254 (2011).

136) Savage MJ, Kalinina J, Wolfe A, Tugusheva K, Korn R, Cash-Mason T, Maxwell JW, Hatcher NG, Haugabook SJ, Wu G, Howell BJ, Renger JJ, Shughrue PJ, and McCampbell A, *J. Neurosci.,* **34**, 2884-2897 (2014).

137) Poduslo JF, Curran GL, and Berg CT, *Proc. Natl. Acad. Sci. USA,* **91**, 5705-5709 (1994).

138) Niewoehner J, Bohrmann B, Collin L, Urich E, Sade H, Maier P, Rueger P, Stracke JO, Lau W, Tissot AC, Loetscher H, Ghosh A, and Freskgard PO, *Neuron,* **81**, 49-60 (2014).

Footnotes

§"Conformation" in this context refers to tertiary structure of proteins including oligomers.

§§"Conformer" in this context refers to isomers which are exclusively interconvertible by the single bond rotation without breaking the chemical bonds.

Figure and Table legends

Fig. 1. Aβ-related and -unrelated Biomarkers Associated with Clinical Disease Stages during AD. The Figure 1 in the reference[6)] was modified.

CSF, cerebrospinal fluid; MCI, mild cognitive impairment.

Fig. 2. APP Processing with α-, β-, γ-Secretases to Generate Aβ (Amyloidogenic Pathway) or Truncated Aβ (non-Amyloidogenic Pathway).

sAPPα(β), secreted APPα(β); CTFα(β), C-terminal fragment α(β) of APP; AICD, APP intracellular domain.

Fig. 3. Schematic Aggregation Pathway of Aβ Based on the Dimer and Trimer as a Minimum Unit for Oligomerization.

Aβ forms synaptotoxic oligomers to move into fibrillization (on-pathway), while to remain unchanged (off-pathway).

Fig. 4. Development of Antibody against Toxic Conformer of Aβ42.

(A) A proposed mechanism of the formation of *S*-oxidized radical at Met35 and its stabilization within a C-terminal core to induce long-lasting oxidative stress by a partially cleaved carboxyl radical at Ala42 in Aβ42. (B) Toxic conformation with a "toxic" turn at positions 22 and 23 and non-toxic conformation with a turn at positions 25 and 26 have been identified from solid-state NMR and systematic proline replacement studies. Immunohistochemical studies of anti-toxic turn antibody (11A1) using human AD brain sections. Arrows indicate extracellular Aβ depositions (senile plaques), and arrowheads

1 indicate the accumulation of intracellular Aβ within the cells, respectively. Scale bar
2 represent 100 μm.
3
4 **Table legend**
5 **Table 1.** Synaptotoxic Aβ Oligomers of and Their Biological Activities Together with
6 Antibody Development against These Oligomers.
7 The three formers refer to the intermediates on the on-pathway into fibrillization, while
8 the four latters refer to the assemblies on the off-pathway.
9

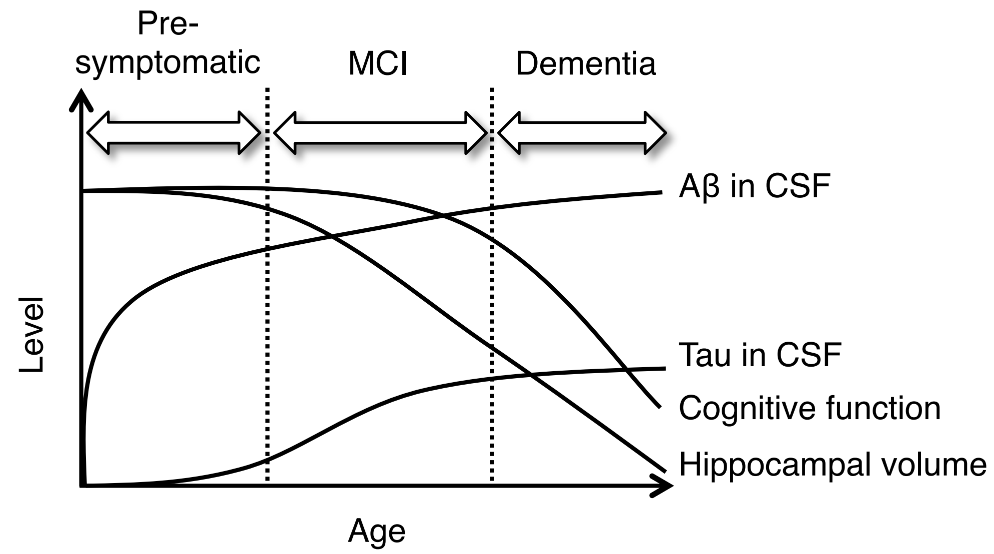


Figure 1 K. Murakami

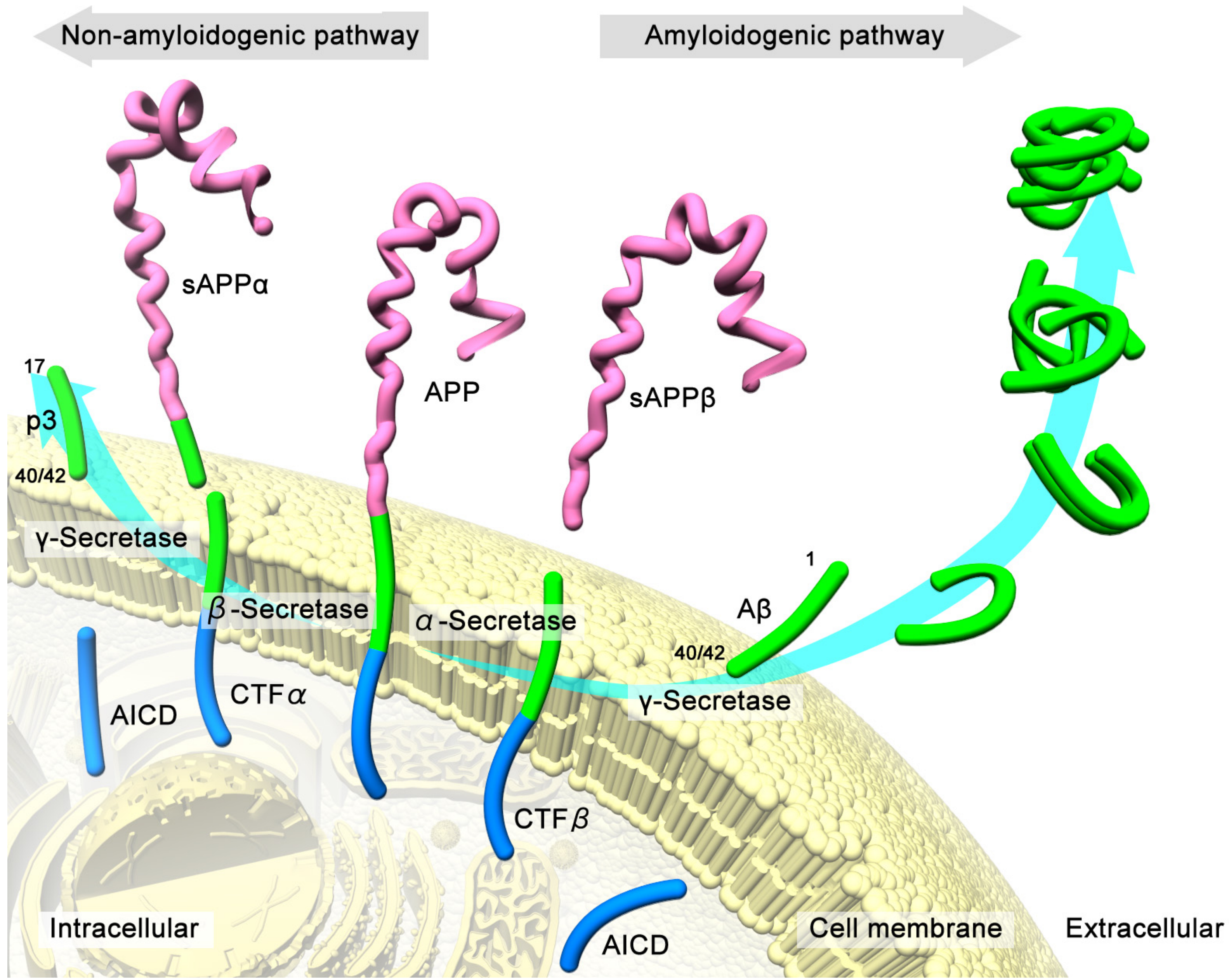


Figure 2 K. Murakami

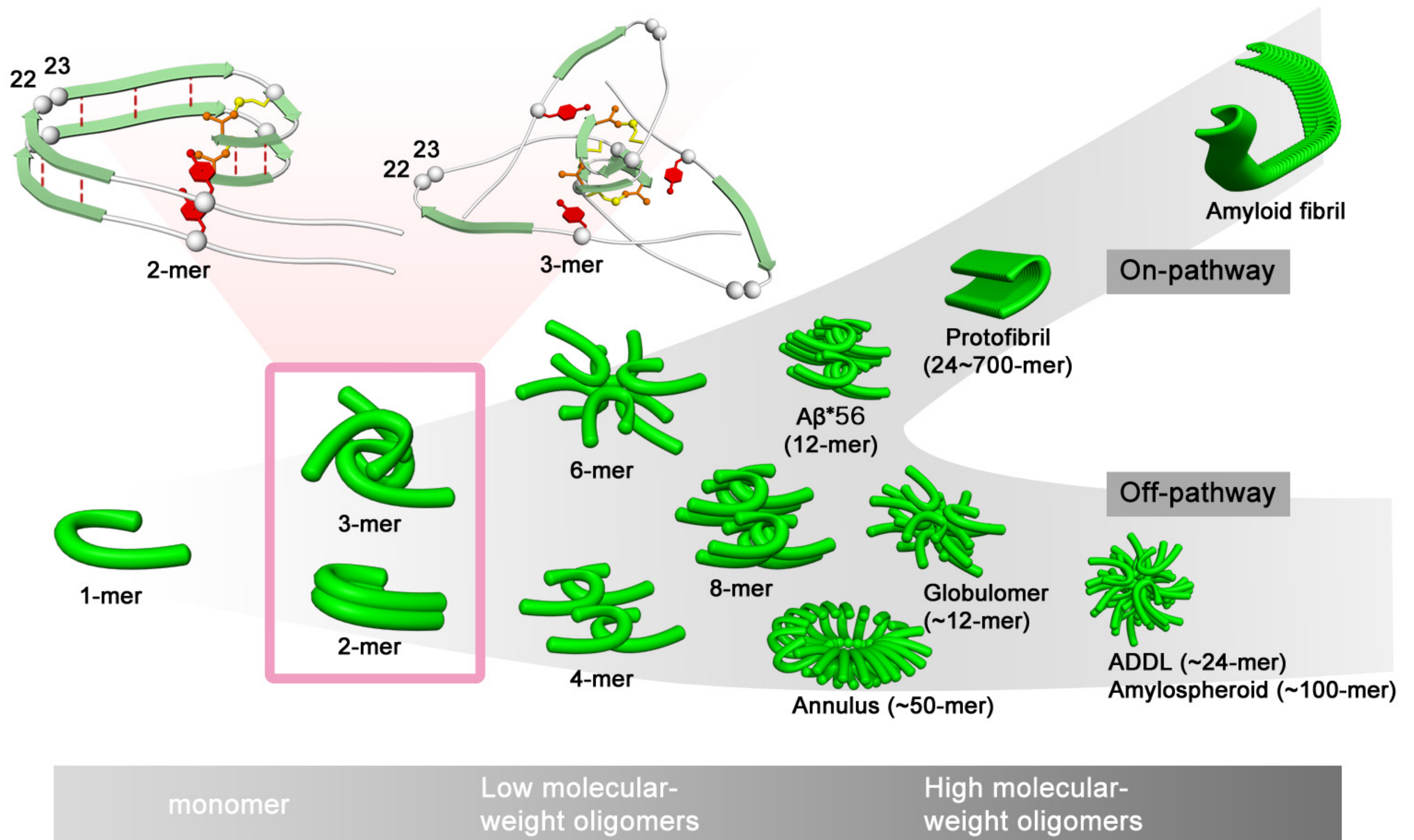
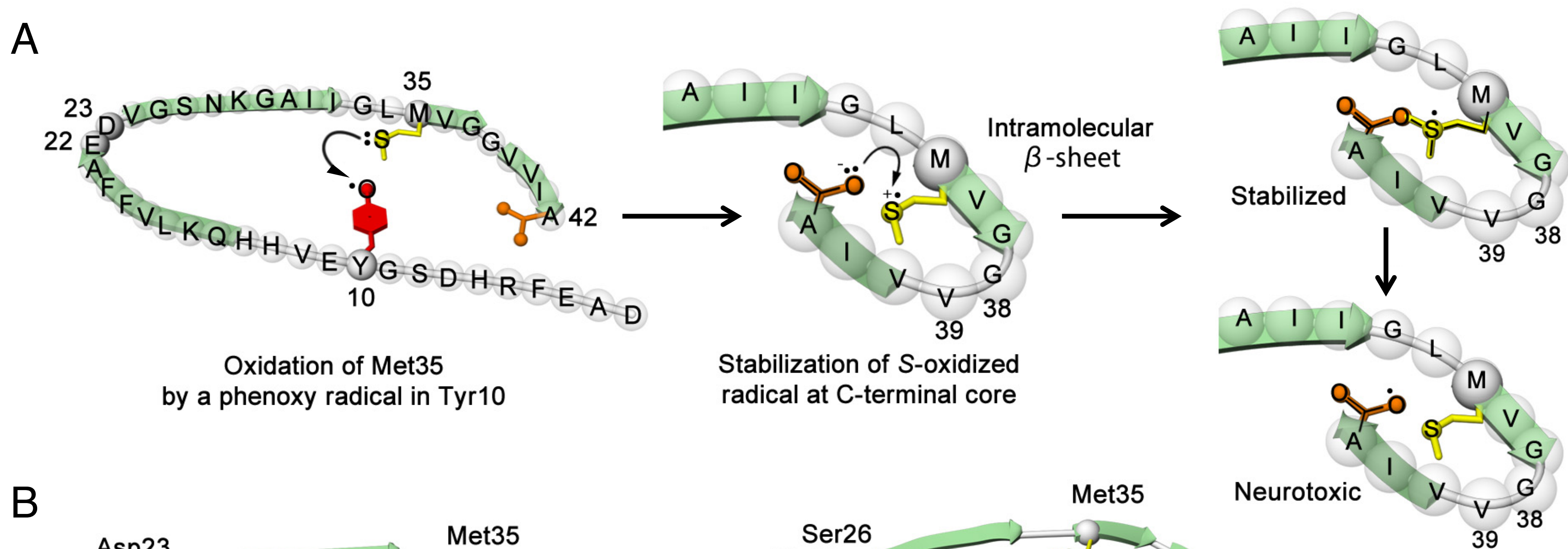
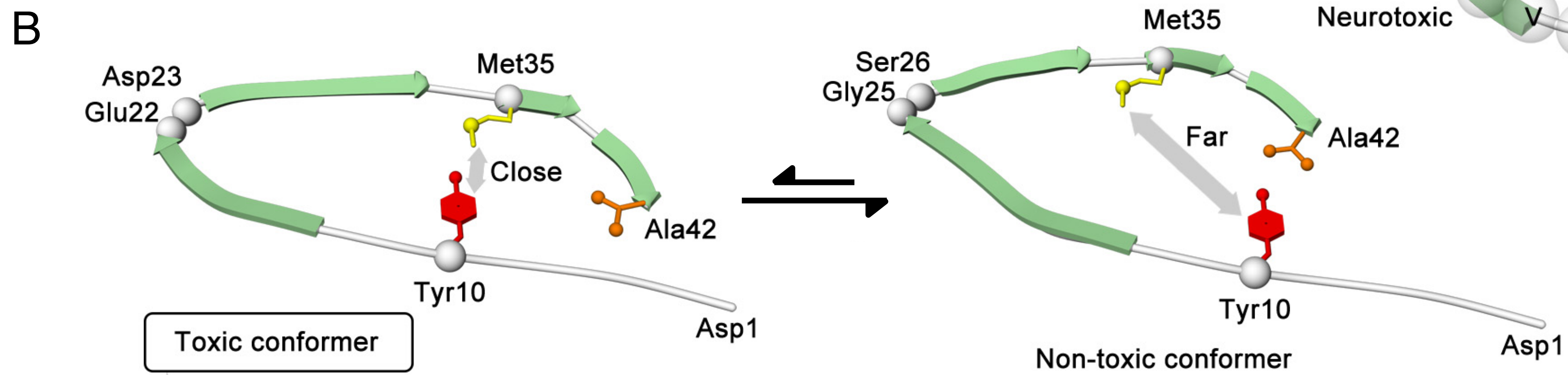
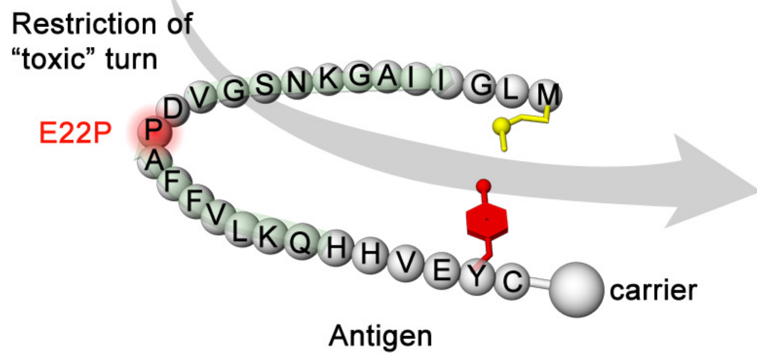


Figure 3 K. Murakami







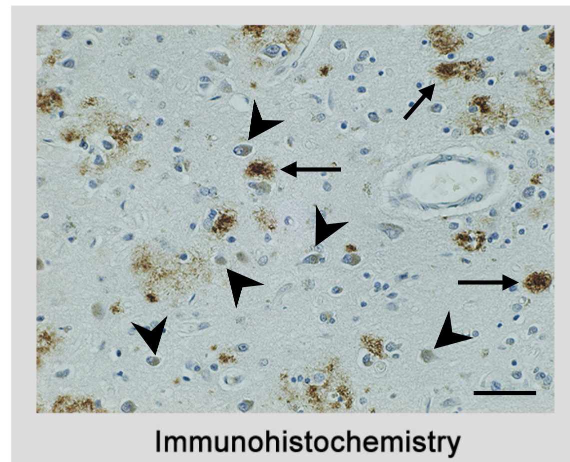


Figure 4 K. Murakami