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Toxic oligomer species of amyloid- β in Alzheimer's disease, a timing issue

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Abstract

A decade following the paradigm-shifting concept that endogenous forms of soluble, non-fibrillar amyloid- β ($A\beta$) might constitute the major bioactive entity causing synaptic loss and cognitive decline in Alzheimer's disease (AD), our understanding of these oligomeric species still remains conspicuously superficial. The current lack of direct evaluation tools for each endogenous $A\beta$ oligomer hampers our ability to readily address crucial question such as: (i) where they form and accumulate, (ii) when they first appear in human brains and body fluids, (iii) what is the longitudinal expression of these putative toxins during the course of the disease, (iv) how do these soluble $A\beta$ assemblies alter synaptic and neuronal function in the brain. Despite these limitations, indirect *ex vivo* measurement and isolation from biological specimens have been possible and have allowed parsing out intrinsic differences between putative endogenous $A\beta$ oligomers. In this review, I integrated recent findings and extrapolated on emerging hypotheses derived from these studies with the hope to provide a clarified view on the putative role of endogenous $A\beta$ oligomers in AD, with a particular emphasis on the timing at which these soluble species might act in the aging and diseased brain.

Keywords

amyloid-beta; oligomer; Alzheimer; brain; cognitive impairment; transgenic

Many neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease or frontotemporal dementias are characterized by the presence of abnormal protein aggregates in the brain. In the case of AD, amyloid plaques (also called senile or neuritic) and neurofibrillary tangles are the two neuropathological lesions that characterize this neurological disorder in postmortem human brain tissue¹. These lesions are respectively formed of insoluble fibrillar species of the amyloid- β peptide ($A\beta$) and the protein tau. While these deposited aggregates have been suspected to be the cause of AD for nearly a

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century, accumulating evidence over the past twelve years seems to suggest that soluble, non-fibrillar multimeric forms of these proteins might initiate the synaptic and neuronal dysfunction associated with the disease^{2–11}. Generally, these molecular species of A β are referred to as A β oligomers (oA β). Species composed of two (dimers), three (trimers) or four (tetramers) A β monomers are often placed under the umbrella term low molecular weight or low-*n* oA β while larger assemblies are termed high molecular weight or high-*n* A β oligomers^{12,13}.

Due to the dynamic nature of these oligomeric forms and the lack of specific molecular tools specific to each assembly, it has proven difficult to study oA β present in human brain tissue, in brains of transgenic mice modeling AD and in cellular systems derived from these animal models. Despite this apparent obstacle and the accompanying ongoing debate surrounding the aforementioned points, several research groups have contributed to the reliable identification of several specific oA β species by combining immunological and biochemical approaches developed for this purpose: dimers^{5,9,10,14}, trimers^{7,9,11,14}, A β *56 a possible dodecamer composed of twelve molecules A β ^{11,14} and annular protofibrils^{15,16}. As a direct consequence of the identification of several oligomeric A β species, the hypothetical pathological cascade of events leading to the development of AD has increased exponentially in its complexity^{12,17}. In addition, multiple lines of evidence recently summarized^{18–20} suggest that tau is mediating the deleterious effects of oA β , and that abnormal changes in tau biology and subcellular localization might be responsible for neuronal dysfunction and cognitive decline^{21–23}.

This paradigm shift also prompted the field to tackle questions that need to be answered in order to further improve our understanding of the series of events that might underlie AD pathogenesis. These questions are: (1) what is(are) the role(s) of A β oligomers in the pathogenesis of the disease, (2) how do these molecular entities disrupt synaptic and neuronal homeostasis, (3) when are A β oligomers active during aging, disease onset and progression. A recently published review from our group tried to summarize the existing knowledge on the production and toxicity of endogenous forms of A β oligomers¹². For those interested in these aspects related to oA β , you are encouraged to read its content as they will not be detailed in this review. Instead, I will try to focus on the third question aforementioned above, by integrating observations from experimental and human studies with a specific emphasis on the timing of the variation in oA β brain levels and how they might provide clues about the biological(s) cascade(s) involved in AD pathophysiology. In doing so, it is important to keep in mind, as a word of caution, that the comparisons made between mouse and human data in this review might be confounded by inherent differences in postmortem interval delays between animal and human studies (5.11 ± 2.26 hours, $N = 88$ in our ROS cohort¹⁴).

What are endogenous oligomeric forms of A β ?

This term is used here to describe sodium dodecyl sulfate (SDS)-resistant multimeric A β assemblies that can be reliably detected in brain or cell protein extracts by combining size-exclusion chromatography coupled with gel electrophoresis and multiple antibodies detecting various domains of A β or various conformations of A β ^{11,16,24,25}. The combined

use of both approaches is thought to minimize the likelihood of mistakenly assign the term oligomer to a molecule that might in fact correspond to a cleavage product of the amyloid protein precursor (APP) or to a heteromeric complex between A β and another protein. Additionally, concerns have emerged regarding the type of solvent used to extract proteins in studies of oA β by independent groups. In this review, I will not concentrate on how oA β were obtained as previous work showed that both water- and detergent-soluble A β oligomers can be detected in mouse and human brain lysates^{11,14,26,27}. It is worth reiterating that the existence of these “endogenous” oA β will only be validated when specific detection tools raised against each form of interest are generated and available to the community. Nonetheless, the generation of conformation-dependent antibodies pioneered by Charles Glabe and his group has led to the proposed classification of these oligomeric forms of A β into two large families²⁸. For the purpose of this review, these families are referred as pre-fibrillar and non-fibrillar assemblies (Figure 1). Pre-fibrillar oA β are multimeric assemblies that lead to the formation of A β fibrils while non-fibrillar oA β are not able to form fibrils. Of note, the exact structural motifs and quaternary structures of these A β oligomers are unknown and will require additional studies.

A β dimers as the molecular building block for pre-fibrillar assemblies?

With these caveats explicitly enunciated, numerous groups have focused their efforts in studying dimeric A β species. There are multiple reasons for this overall particular interest: (1) A β dimers appear to be stable in the presence of detergent such as SDS, (2) the abundance of A β dimers elevates with aging and amyloid burden in brains of transgenic mice used as models of AD and in patients with AD^{5,6,12}, (3) due to this remarkable increase (~20-fold in 26-month-old Tg2576 vs. 10-month-old animals¹²), it is fairly straight forward to detect and measure A β dimers in brain lysates from transgenic mouse and human brains^{5,12,14,29}, (4) A β dimers separated by size exclusion chromatography from AD brain lysates alter the cellular processes of long-term potentiation (LTP) and depression (LTD), and induce cognitive deficits in rodents when infused into the brain^{2,4,5,8}.

In transgenic mouse models of AD such as Tg2576 or J20 in which A β deposition is not very aggressive^{30–32}, A β dimers can be measured in soluble extracts of brain proteins around 10–14 months depending on gender and line. It is puzzling that these ages also correspond to the period of increased amyloid deposition in neocortical areas, perhaps hinting at a link between brain abundance of A β dimers and fibrillar forms of A β ^{12,29,33}. Consistent with this observation, A β dimers can be detected at earlier ages (~7–8 months) in APP transgenic mice displaying more aggressive amyloid deposition such as APP/PS1 or APP23 mice (Lesné *et al.*, unpublished data). In another genetically engineered mouse line, A β dimers appear to be the major species formed in mice overexpressing the E693 mutant of APP³⁴. Identified in 2008, this deletion in the middle region of A β preferentially engages the peptide into forming oligomers instead of fibrils^{34,35}. Given the panel of antibodies used to identify the putative oligomeric A β assemblies, apparent A β dimers were readily detected in TgAPP_{E693} brain tissue. Based on the biochemical segregation of the putative A β trimers detected in 1% Triton X-100 lysates and in 2% SDS lysates, the pair of antibodies used to detect A β peptides in this study (6E10 immunoprecipitation followed by western blotting using the rabbit polyclonal β 001 antibody) and a slight upward shift in the

electrophoretic migration compared to recombinant A β standards, it is very likely that the immunoreactive molecule labeled as A β trimers might in fact correspond to the carboxyl terminal fragment of APP generated by the β -secretase activity, CTF- β . If this assessment were correct, this would indicate that A β trimers are not readily detected in this mouse line. Further, this would also suggest that the E693 mutation is somehow stabilizing low-*n* prefibrillar A β oligomers such as A β dimers thereby preventing their aggregation into fibrils. This hypothesis is consistent with the exclusive segregation of A β _{E693} dimers in the insoluble protein fraction extracted with formic acid³⁴. Interestingly, these oligomeric A β species were immunoreactive to the antibody NU-1 previously shown to detect diffuse and dense-core deposits in human brain tissue³⁶ labeled with the amyloid-binding dye thioflavin S, further suggesting that these oA β are prefibrillar.

In studies using postmortem human brain specimens, soluble dimeric forms of A β seem to be detected in brains of subjects with abundant amyloid loads^{5,14}. This observation is further supported by the examination of the relative oA β levels with respect to neuritic plaque pathology as assessed by the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria. As shown in Figure 2A, CERAD scoring tracked well with amyloid loads detected in the brain region analyzed (inferior temporal gyrus or ITG). Regarding oA β , ITG A β dimers were most abundant in subjects with CERAD scores of 1 (Definite AD) or 2 (Probable AD) compared to those with a CERAD 3 (Possible AD) or 4 (No AD) (Figure 2C). It is worth noting that putative ITG A β trimers and A β *56 levels did not follow this pattern (Figure 2, D–F), suggesting that A β dimers and amyloid plaques could be interconnected in the brain.

Despite this noticeable relationship, the origin of dimeric A β species remains mysterious. Previous studies have revealed that Tg2576 cortical neurons maintained in culture produce mostly trimers and monomers while dimers of A β are produced and secreted in very small amounts (1:20 to 1:15 relative to A β monomers and trimers respectively)^{11,37}. These results contrast sharply with the relative abundance of putative A β dimers detected in the conditioned medium of 7PA2 cells, a stable line of Chinese Hamster Ovary cells overexpressing a mutant form of APP^{9,38}. Perhaps explaining this discrepancy, a recent study from the Blenow group indicated that part of the immunoreactivity of these apparent A β dimers in these cell lines might instead correspond to truncated A β peptides containing part of the APP molecule upstream of the β -secretase site³⁹. This finding was recently confirmed and additionally showed that these N-terminally extended (NTE) A β species can also impair *in vivo* LTP⁴⁰. Although future work will evaluate the physiological relevance of these NTE A β molecules, this report also validated the presence of synaptotoxic A β homodimers in this cell line⁴⁰.

Consistent with recent work using artificially modified synthetic A β dimers^{41,42}, these independent results support the notion that endogenous A β dimers could constitute the molecular building block for fibrillar forms of A β *in vivo*.

A β trimers as the molecular building block for non-fibrillar assemblies?

Although the detection of A β dimers in brain tissue appears to coincide with elevated amyloid loads in transgenic mouse and human brain^{12,14}, this does not seem to be the case for endogenous A β trimers¹⁴. These putative assemblies are defined as such based on: (i) the ability to immunocapture these molecules with 6E10, 4G8, 82E1, 40/42-end specific A β antibodies from conditioned media of APP transgenic cultured neurons or brain lysates enriched in extracellular proteins and (ii) the ability to segregate them from other A β species by SEC^{10,11,14,37}. If this hypothesis were correct, A β trimers could therefore constitute the molecular building block for non-fibrillar A β oligomers. What are then the elements that support this argument?

First, A β trimers appear to be by far the most abundant oligomeric A β species produced and secreted by primary cultured neurons^{11,37}. Interestingly, we did not observe the presence of A β assemblies larger than a putative tetramer (~18kDa) in near-pure or mixed cultures of mouse cortical neurons.

Second, A β trimers are readily detected at low levels as early as 14 days of gestation in brain tissue of Tg2576 mice¹¹ and at 1 year of age in post-mortem human brain tissue¹⁴. Both in mouse and human brain, the abundance of A β trimers increased very slowly but steadily with aging¹¹. Obviously, the presence of these putative A β trimers was detected months or decades prior to A β deposition in APP transgenic mouse and human brain tissue respectively¹². This apparent dissociation in time between A β trimers and amyloid plaque formation proves difficult to reconcile with the hypothesis that A β trimers are pre-fibrillar A β oligomers.

Third, brain levels of A β trimers did not correlate with amyloid burden (Spearman rho = -0.2682, $P > 0.05$, N = 85) in our human studies¹⁴. By contrast, brain levels of A β dimers strongly correlated with amyloid loads (Spearman rho = 0.68, $P < 0.0001$, N = 85).

Fourth, the disassembly of A β oligomers present in 13-month-old Tg2576 mice induced by the solvent hexafluoroisopropanol (HFIP) led to a decrease in 6E10-immunoreactive bands consistent with putative hexamers, nonamers and dodecamers and a parallel elevation of A β trimers by more than 3-fold¹¹. These results suggest that the 28 kDa, 42 kDa and 56 kDa A β species might contain or be composed of A β trimers.

Fifth, the detection of extracellular A β trimers seems to depend on A β production *in vivo*. Although Tg2576 and Tg5469 mice express comparable protein levels of transgene-derived APP, soluble SDS-A β levels are ~10-fold higher in Tg2576 (~20–25 pmol/g) than in Tg5469 (~2 pmol/g) mice^{43,44}. Upon analyzing extracellular-enriched protein fractions from 12-month-old Tg5469 animals, no A β oligomers were detected while A β trimers were readily observed in lysates of age-matched Tg2576 animals⁴³.

Overall, it is clear that additional research is needed to determine whether A β trimers truly exist *in situ*, what governs the formation of A β trimers, where trimers are located and how trimers might alter neuronal function.

Very few studies have been performed to examine the role of A β trimers in AD pathogenesis^{2,7,8,37}. Preparations of conditioned media from 7PA2 cells enriched for putative A β trimers were reported to inhibit LTP in mouse hippocampal slices⁷. Intracerebroventricular injections of either 7PA2- or Tg2576-derived A β trimers in healthy rats were however unable to cause cognitive impairments during the alternating lever cyclic ratio (ALCR) assay despite a noticeable trend². This finding might be inherent to the concentrations used in the study or to the stability of the species tested. In fact, when A β trimers purified from AD brain tissue were applied to cortical primary neurons at low nanomolar concentrations (1–5 nM), these assemblies were bioactive by inducing the phosphorylation of the Src kinase Fyn and its downstream target tau³⁷. Overall, these observations suggest: (1) the formation of A β trimers *in vivo* appears to be dependent on A β production, (2) A β trimers are the first detectable endogenous oligomeric species produced and secreted by neurons.

Importance of trimer-based A β assemblies exemplified by the oligomer

A β *56

While searching to identify the soluble molecules of A β associated with the onset of spatial reference memory deficit observed in young Tg2576 mice prior to the formation of amyloid plaques, the examination of brain lysates from these animals by western blotting revealed apparent trimers (molecular weight of about 13 kDa), hexamers (~27 kDa), nonamers (~40 kDa) and dodecamers (~56 kDa) using antibodies targeting the amino terminus and the central area of the A β peptide such as 6E10 and using the A11 antibody that detects non-fibrillar oligomeric forms of amyloid proteins^{11,25}. Of note, the OC antibody, which recognizes pre-fibrillar oligomers²⁴, does not detect A β *56 (data not shown). This combined epitope specificity therefore categorizes A β *56 as a non-fibrillar A β oligomer. As summarized elsewhere¹², A β *56 was also reported in other APP transgenic lines^{33,45,46}, in APP23 (Lesné & Ashe, unpublished data) and APPPS1 mice (Lesné, unpublished data), and in 7-month-old regulatable tTA-APP mice⁴⁷ confirming that its formation was not restricted to the Tg2576 mouse line. In these animal models, A β *56 was found in two soluble protein fractions, extracellular and membrane extracts^{11,45,47,48}, suggesting that this oligomer was formed extracellularly and that it may interact with a putative surface receptor at the neuronal plasma membrane. A similar profile has been observed in recently published studies with two human cohorts (N = 135), in which A β *56 increased with aging starting in midlife years¹⁴.

Contrasting with these *in vivo* observations, we have not been able to detect A β *56 in cellular protein lysates of APP transgenic primary neurons or in the conditioned media of these cells (Lesné *et al.*, unpublished data). Based on these results, we believe that A β *56 is not produced and secreted by primary neurons^{11,37}. Considering that Tg2576 primary neurons generate and secrete relatively large quantities of apparent A β trimers^{11,37}, one likely explanation as to why A β *56 is not formed *in vitro* might be due to the possibility that this multimeric form A β requires an unidentified cofactor or chaperone “X” in brain tissue in order to promote its formation. The obvious alternative is that the abundance of A β *56 *in vitro* lies below our detection limit (~1–2 pg of recombinant A β). If this cofactor X is indeed

required to generate A β *56, aging should regulate the expression of this cofactor to explain the sudden accumulation of A β *56 at the age of 6 months in Tg2576 brain tissue¹¹ and the rise of A β *56 abundance in brain tissue of subjects in their mid-forties¹⁴. Furthermore, the relative levels of extracellular A β *56 in human brains showed no relation to A β dimers or A β monomers¹⁴. By contrast, the brain abundance of A β *56 was positively correlated with that of trimeric A β . This observation is consistent with the hypothesis that A β trimers are the molecular building block for larger oligomers of A β in the non-fibrillar pathway (Figure 1).

What is the temporal profile of these oligomeric forms of A β in the human brain during aging and AD?

To answer this question, we analyzed the expression of A β oligomers that we can measure reliably and reproducibly (*i.e.* A β dimers, trimers and A β *56) in postmortem brain tissue of 135 subjects aged between 1 and 97 years¹⁴. Of these, 75 individuals had no cognitive impairment (NCI), including young children to the elderly, and 58 subjects were clinically diagnosed with mild memory impairment (MCI) or probable AD. It is important to reiterate here that it is currently believed that AD begins about two to three decades before the onset of symptoms and neuronal loss⁴⁹.

Similarly to APP transgenic mouse models of AD in which A β trimers were present in young mice before any memory decline¹¹, A β trimers were detected in postmortem human brain tissue of children and adolescents¹⁴. In combination with the observation that cultured primary neurons secrete putative A β trimers, the findings in our human studies suggest that A β trimers might be physiologically produced and secreted in the infant and adult brain. If true, trimeric A β could be regarded as the first step in the oligomerization of natively-folded A β monomers since A β trimers are not immunoreactive to either A11 or OC antibodies. With aging, their relative expression gradually increased and became elevated above baseline levels in subjects over 70 years of age¹⁴. In the brain of subjects with mild cognitive impairment (MCI), trimeric A β levels seemed particularly high compared to those measured in age-matched control individuals¹⁴. Unexpectedly, the relative abundance of A β trimers diminished in postmortem AD brain tissue below the levels detected in the NCI group. Although longitudinal studies are needed to confirm these variations, we advance that these changes are suggestive of a transient, perhaps stage-specific, elevation of this A β oligomer¹⁴. We also propose that the apparent decrease in the brain abundance of A β trimers in AD compared to NCI might indicate a switch in the On/Off pathway balance (Figure 1) in favor of prefibrillar A β species, including A β dimers (see below).

Contrasting with A β trimers, brain levels of A β *56 initially appeared below the range of detection in our assay (~1–2 pg of A β) in children and young adults but, unlike A β trimers, abnormally increased in subjects in their forties to culminate in subjects over 80 years of age¹⁴. This elevation coincided with the onset of subtle cognitive decline linked to aging^{50,51}. It is also interesting to note that this age-dependent elevation of A β *56 levels exceeded the respective abundance of A β trimers, suggesting that the formation of A β *56 is favored to the detriment of trimeric A β species (pending that A β *56 is a trimer-based oA β). However, to our initial surprise, soluble or membrane-bound A β *56 levels decreased in what

seemed a step-wise fashion in MCI and AD¹⁴. Since the levels of A β *56 decreased while A β trimers increased in MCI brain tissue, we propose that this change reflects a possible reversal of the trimer-A β *56 equilibrium. With disease progression, the relative brain abundance of both A β *56 and A β trimers returned to baseline or lowered below baseline suggesting a possible collapse of the “Off pathway” or trimer-based oligomerization pathway (Figure 1). If this scenario truly exists, it could allow for a greater pool of soluble A β potentially available for sustaining or exacerbating the “On pathway” or dimer-based oligomerization pathway.

Conversely, A β dimers were not detected in brain tissue of individuals younger than 60 years of age. At older ages, their abundance increased strongly in parallel with the accumulation of amyloid plaques¹⁴. Although the magnitude of the change was lessened compared to the effect observed across aging, we found that A β dimers increased in AD compared to MCI and NCI groups¹⁴ consistent with previous observations⁵.

Toxicity of discrete brain A β oligomers

To my knowledge, very little is known about the toxicity of A β oligomers purified from brain tissue. While numerous reports have observed the presence of putative oA β in brain lysates¹², only two studies have attempted to determine the effects on signaling of discrete oA β species immunopurified from AD brain tissue^{3,37}. In the first study, Jin and colleagues demonstrated that application of apparent A β dimers at ~0.5 nM could induce tau hyperphosphorylation and neurotoxicity in primary hippocampal neurons after 3 days³. Similarly, our own group demonstrated apparent A β dimers selectively induced the hyperphosphorylation of tau on tyrosine 18 following the formation of a signaling complex containing the cellular prion protein PrP^C and the protein kinase Fyn³⁷ previously associated to neuronal death induced by A β ⁵². Neither A β *56 nor protofibrillar A β species of molecular weight approaching 150 kDa were able to activate Fyn in primary neurons³⁷ suggesting that each form of A β oligomers might induce very specific effects in brain cells. Consistent with this hypothesis, we found strong positive correlations between A β *56 and two pathological forms of tau protein (hyperphosphorylated tau at serine 202 and tau conformers immunoreactive with Alz50), and negative correlations between A β *56 and two postsynaptic proteins (drebrin and fyn) in individuals without apparent memory problems for their age category⁵³. By contrast, no association was found between A β dimers or A β trimers and these same tau changes. These observations suggest that A β *56 could induce abnormal changes of tau and neuronal connections early in the pathogenesis of AD. Studies are ongoing to identify the molecular mechanism induced by A β *56 and to confirm whether these effects on tau are also observed in mice and in primary neurons.

This obvious scarcity in available data surrounding this topic is extremely problematic and additional research studies are needed to decipher the role of oA β endogenous to brain tissues.

Hypothetical model of the timing of oligomeric forms of A β

Altogether, these observations posit that the human brain might sustain the attack of (at least) three successive waves of exposure to A β oligomers (*i.e.* A β *56, A β trimers and A β dimers) during aging and AD (Figure 3). In this model, A β *56 would be the main initiator toxin of the first wave during midlife, leading to abnormal changes in tau protein but without causing neuronal death, possibly through compensatory cellular mechanisms. The role of A β *56 could be envisioned as priming the cells for a secondary insult, rendering them more sensitive to the exposure of additional toxins. This model could explain a long silent asymptomatic phase of the disease. The second toxic wave would be mediated by exposure to a rapid accumulation of A β trimers around neurons already stressed by A β *56, triggering additional pathological changes of tau molecules. In this scenario, neurons exposed to the first two waves could no longer avoid cellular death, likely due to the depletion in resources required to maintain compensatory mechanisms. I propose that this phase would coincide with mild cognitive impairment, the preclinical phase of AD, and a nascent neuronal atrophy in sensitive brain regions. The third and final wave of exposure to A β oligomers would be induced by A β dimers, which would act as executioner species in this model. Due to the apparent relationship between A β dimers and dense-core amyloid plaques, these assemblies of A β would induce additional and specific changes of the tau protein and lead to neuronal death in the vicinity of amyloid deposits.

To conclude, if these soluble A β species prove to be the major bioactive toxins initiating the synaptic and neuronal loss associated with Alzheimer's disease, I believe that decoding the precise molecular role of each oA β must constitute a priority. Armed with this new knowledge, we as a field should be in a better position to conceive and develop novel therapeutic strategies aimed at countering cellular processes activated by these A β oligomers. In this context, taking one or multiple drugs at specific ages/clinical phases of the disease might prevent, limit or stop its progression.

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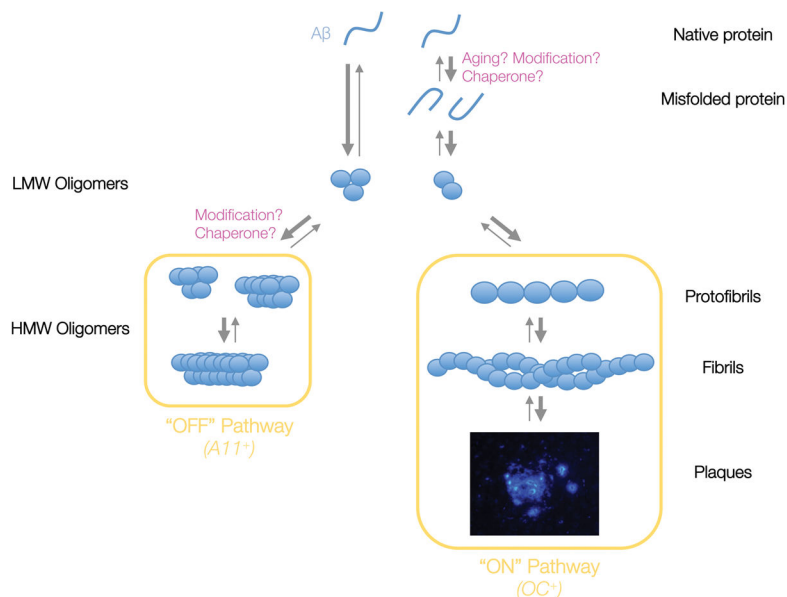


Figure 1. Proposed model of Aβ aggregation

In this model, natively folded Aβ monomers preferentially form Aβ trimers under physiological conditions, whereas dimers are formed following the misfolding of Aβ monomers induced by aging, post-translational modification of the primary sequence of Aβ or a chaperone protein. Both Aβ assemblies constitute the pool of low molecular weight (LMW) or low-n oAβ from which two pathways emerge. The “Off” pathway does not lead to the formation of insoluble fibrillar amyloid and is characterized by its immunoreactivity to the conformation-specific oligomer antibody A11. By contrast, the “On” pathway promotes the formation of fibrillar Aβ and molecules involved in this pathway are immunopositive to the conformation-specific fibril antibody OC. Once a species of Aβ has entered one of these paths, it cannot effectuate a horizontal transfer into the opposite pathway but instead it must be disassembled back into a monomeric state. The thickness of arrows illustrates the favored direction of the putative equilibrium suggested by integrated experimental observations.

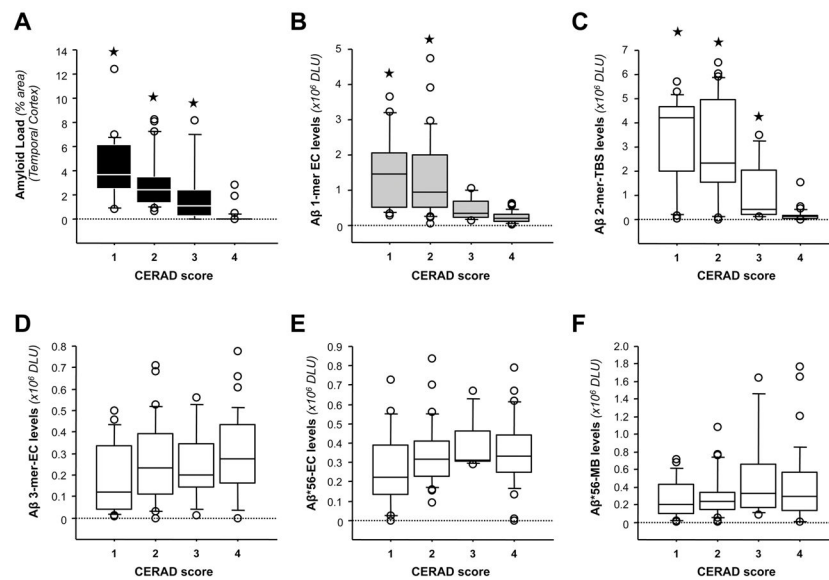


Figure 2. Relationships between specific Aβ oligomers in the brain and CERAD scores (A–B) Relationships between CERAD scores and amyloid load in temporal cortex (A) and soluble Aβ monomers detected in EC fractions (B). CERAD scores: 1, Definite AD; 2, Probable AD; 3, Possible AD and 4, No AD. (C–F) Relationships between CERAD scores and the levels of various oligomeric Aβ species, including Aβ dimers in TBS extracts (C), Aβ trimers in EC fractions (D), and Aβ*56 in EC (E) and MB (F) lysates. (*P < 0.05 vs. the CERAD score 4 group using Kruskal-Wallis test followed by Mann-Whitney U test with Bonferroni corrections). Abbreviations: EC: extracellular-enriched, MB: membrane-enriched; TBS: Tris Buffered Saline; DLU = densitometry light units.)

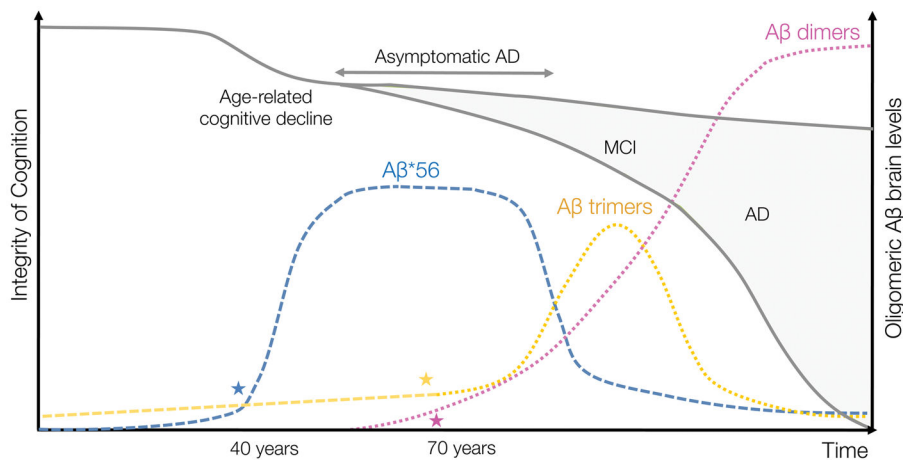


Figure 3. Revised model of endogenous oligomeric A β brain levels in aging and Alzheimer's disease

In this model limited to three readily detectable oA β in human brain tissues (*i.e.* A β dimers, trimers and A β *56), A β *56 is the first assembly to rise abnormally in midlife coinciding with subtle age-related cognitive decline although A β trimers are the first soluble species expressed by neurons. This scenario implies that energetic requirements for forming A β *56 are relatively low. The high expression levels of A β *56 in the brain are sustained over decades possibly contributing to early disruption in neuronal physiology and to the long silent asymptomatic phase of AD. Concomitant to a lowering of brain levels of A β *56, A β trimers accumulate transiently during the initial stage of the symptomatic phase of AD, MCI. This sequence implies that A β trimers are indeed the molecular building block of A β *56 and that the putative cofactor responsible for sustaining A β *56 production declines with disease progression/aging. With the emerging accumulation of prefibrillar and fibrillar A β assemblies, brain levels of “trimer-based” oligomers (*i.e.* A β *56 and A β trimers) decline with disease progression. In this setting, A β dimers contribute to the local toxicity in the vicinity of amyloid plaques, finishing off neurons that might have resisted the toxic waves induced by A β *56 and A β trimers. Stars (★) indicate the ages at which statistically significant changes in the respective levels of oA β were observed.