AIMS Biophysics, 6(1): 1–22.
DOI: 10.3934/biophy.2019.1.1
Received: 05 November 2018
Accepted: 07 January 2019
Published: 10 January 2019

http://www.aimspress.com/journal/biophysics

Review

Challenges in understanding the structure/activity relationship of Aβ oligomers

Albert W. Pilkington IV[1] and Justin Legleiter[1,2,3,*]

[1] The C. Eugene Bennett Department of Chemistry, West Virginia University, 217 Clark Hall, P.O. Box 6045, Morgantown, West Virginia 26505, United States
[2] Blanchette Rockefeller Neurosciences Institutes, West Virginia University, 1 Medical Center Dr., P.O. Box 9303, Morgantown, West Virginia 26505, United States
[3] Department of Neuroscience, West Virginia University, 1 Medical Center Dr., P.O. Box 9303, Morgantown, West Virginia 26505, United States

* **Correspondence:** Email: justin.legleiter@mail.wvu.edu; Tel: +13042930175; Fax: +13042934904.

Abstract: A major hallmark of Alzheimer's disease (AD) is the accumulation and deposition of fibrillar aggregates of the amyloid-β (Aβ) peptide into neuritic plaques. These amyloid deposits were thought to play a central role in AD; however, the correlation between plaque load and disease is weak. Increasing evidence supports the notion that a variety of small, globular aggregates of Aβ, referred to broadly as Aβ oligomers (AβO), may in fact be the primary culprits associated with neurotoxicity. Evaluation of AβO structure and physiological activity is complicated by their metastability, heterogeneity, complex aggregation pathways, and dependence on experimental conditions. Numerous different types of oligomers have been reported, and these have been associated with varying degrees of toxicity and modes of interaction. Here, we briefly review AβOs with a focus on their formation, structure, and biophysical methods applied to their investigation.

Keywords: Alzheimer's disease; β-amyloid; oligomers; neurodegeneration; protein aggregation

1. Introduction

Alzheimer's disease (AD) is a fatal neurodegenerative disorder that is the most prevalent form of dementia. The neuropathological and neurochemical hallmarks of AD include: Synaptic loss and selective neuronal cell death; decreases in markers for certain neurotransmitters; and abnormalities in

neurons and their processes as well as in the extracellular space. Two of the main features associated with AD are neurofibrillary tangles comprised of the protein tau and cerebrovascular, diffuse, and neuritic plaques composed predominantly of the amyloidogenic peptide amyloid-β (Aβ). These proteinaceous deposits of tau and Aβ consist of stable amyloid fibrils, which are β-sheet rich fibrous protein aggregates. Similar deposition of amyloid is associated with numerous other diseases [1].

In the early 90's, the amyloid cascade hypothesis was introduced, which postulated that Aβ aggregation and deposition directly lead to neuronal death, resulting in AD [2]. Yet, the correlation between plaques and cognitive dysfunction in AD has been questionable for years [3–7], and with Aβ-directed therapeutic strategies failing in numerous clinical trials, the role of Aβ in AD progression is being re-evaluated [8–11]. This has led to an enhanced research focus on diffuse, soluble aggregates of Aβ. Various small, globular aggregates of Aβ, referred to broadly as Aβ oligomers (AβO), were detected in AD patients a few decades ago [12–14], and these AβO were originally classified as being intermediates toward the formation of amyloid fibrils. Over the years, increasing evidence points toward AβOs playing a central role in AD, as AβOs correlate more strongly with AD progression in patients and animal models [15–19]. For example, AβO formation and buildup occurs early compared with plaque buildup (much earlier than plaque deposition) in the AD brain [20–22]and CSF [23], which has led to extensive efforts to develop assays to detect AβOs for potential application as biomarkers [24–31].

An extensive body of evidence has linked high levels of AβOs in the brain to a variety of pathogenic consequences associated with AD (summarized in Table 1). As such, there has been significant effort made to characterize AβO formation, structure, and biochemical/biophysical characteristics (such as interactions with other proteins and lipids) in the hope that underlying modes of AβO-related toxicity could be revealed [32–37]. As AβOs are metastable, highly heterogeneous in nature, and can form via a variety of different pathways, this remains a challenging task, yet progress has been made. Here, we review AβOs with a focus on biophysical characterization of their formation and structure.

Table 1. Toxic mechanisms associated with AβOs.

Toxic effects of AβOs	Model system(s)	References
Reduction in neural plasticity	mice, rat	[37–40]
Stimulation of tau phosphorylation	cortical neurons (rat), hippocampal neurons, neuroblastoma, primary neurons, Tg-Mice	[41–45]
Choline acetyltransferase Inhibition	cholinergic cell lines	[46,47]
Oxidative stress	cortical neurons, hippocampal neurons, *in vitro*, neuroblastoma	[48–51]
Endoplasmic reticulum stress	cortical astrocytes, cortical neurons (rat), fibroblasts, Tg-mice	[43,52,53]
Receptor disturbance	cortical neurons, hippocampal neurons	[54–57]
Insulin resistance	cortical neurons, hippocampal neurons, rat	[40,42,57,58]
Synapse deterioration	hippocampal neurons, pyramidal neurons (rat), Tg-mice	[21,35,54,59]
Axonal transport	cortical neurons, hippocampal, in vitro, Tg-mice	[60–62]
Astrocytes/microglia effects	cortical astrocytes, Tg-mice	[44,52,63,64]

Continued on next page

Toxic effects of AβOs	Model system(s)	References
Cell cycle disruption	cortical neurons, Tg-mice	[65,66]
Selective neuron death	Mice	[38,67]
Inhibition of long-term potentiation	Mice	[37,40]
Calcium dysregulation	cortical neurons (rat), hippocampal neurons, primary neurons	[43,45,48,52]
Modulation of metal toxicity	neuroblastoma	[49]
Cytoskeleton disruption	primary neurons	[50]
Modulation of Receptor/Channel Activity	hippocampal neurons	[48]

2. The Aβ Peptide

Aβ is an approximately 4 kDA peptide (typically 40–42 amino acids long) that is derived from the transmembrane portion of the amyloid precursor protein (APP; Figure 1). The production of Aβ is achieved by the sequential cleavage of APP by two membrane-bound endoprotease activities, β- and γ-secretase. The two predominant Aβ peptides produced are 40 and 42 amino acids in length, and these peptides are referred to as $A\beta_{40}$ and $A\beta_{42}$ respectively. Aβ is amphipathic in nature (having a predominately hydrophilic N-terminus and a predominately hydrophobic C-terminus), which is thought to drive its aggregation. As the C-terminal end of Aβ coincides with the transmembrane portion of APP, $A\beta_{42}$ has a larger hydrophobic domain, making it more fibrillogenic compared to $A\beta_{40}$ and deposits to a much greater extent in the brain [68–70]. Only about 10% of APP is processed via this Aβ producing pathway. Most APP is cleaved by the α-secretase, generating a series of much more benign peptide fragments.

The hydrophilic N-terminal region of Aβ can adopt both an α-helical or β-sheet structure dependent on solution conditions, for example pH [71,72]. The hydrophobic C-terminal end of Aβ has a propensity to adopt β-sheet structure upon aggregation independent of solvent conditions [71,72]. Beyond its amphipathic nature, several other domains have been identified in Aβ. The different polyomorphic fibril structures of Aβ are comprised of bundled β-sheets with backbones orthogonal to the fiber axis creating a cross-β structure [73], and two β-strand forming domains (residues 11–21 and 29–39 respectively) that are separated by a turn/bend region (around residues 23–26) identified through various experimental and computational studies [74–77]. The central region of Aβ (residues 16–21), contained within one of the β-strands, has enhanced amyloidogenic properties and represents a hydrophobic core [78].

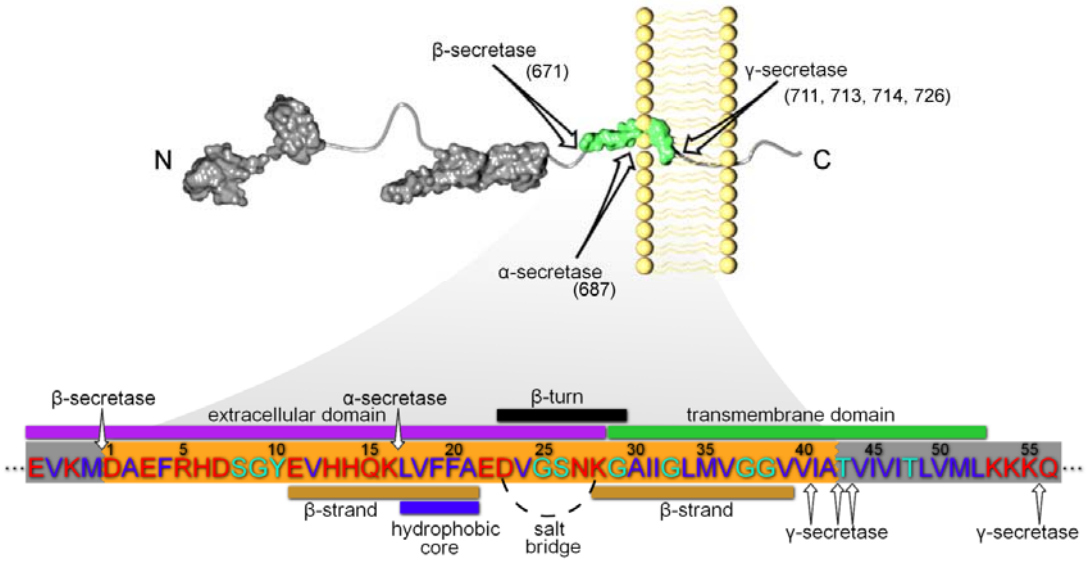

Figure 1. APP processing and subsequent Aβ amino acid sequence with specific domains of interest specified. Proteolytic cleavage of APP is initiated at residue 671 by β-secretase followed by either non-amyloidogenic processing, cleavage at residue 687 by α-secretase, or amyloidogenic processing, cleavage at residue 711, 713, 714, or 726 by γ- secretase. The amino acid sequence below highlights the region of APP from which Aβ is produced with the numbering referring the residues in Aβ. The orange highlighted region represents the intact $A\beta_{42}$ sequence. Hydropathy indexes of individual residues are color coded according to hydrophobic (blue), slightly hydrophobic (light green), and hydrophilic (red). Regions of interest and cites of secretase activity within the APP and Aβ are indicated.

3. Aβ aggregation—a complex mechanism

The aggregation of Aβ (and other amyloid-forming proteins) is typically characterized in terms of fibril formation (Figure 2). Aβ fibril formation occurs via a complex aggregation pathway. Fundamentally, AβOs can be subdivided into species that are intermediates in fibrils formation (referred to as being on pathway) or species that do not directly lead to fibrils (referred to as being off pathway). This is a contributing factor to the immense heterogeneity observed in AβO populations as will be discussed in more detail later. In general, amyloid formation proceeds via a nucleation dependent polymerization mechanism [79–81]. With this mechanism, aggregation initially occurs via a slow nucleation phase (often called the lag phase) that involves the formation of a thermodynamically unfavorable critical nucleus that is associated with a transition from a native to non-native protein conformation. For Aβ, the critical nucleus is likely a multimeric species [82–84]. Once the critical nucleus has formed, an elongation or growth phase (characterized by a relatively rapid extension of fibril aggregates) occurs. While numerical models can extract important parameters (lag phase times, elongation rates, critical nucleus size) from experimental data [85], the actual aggregation pathway toward fibrils can be complicated. For example, other on pathway intermediates, like protofibrils, are also observed in Aβ aggregation. Protofibrils are amyloid-like, elongated aggregates with filament-like morphologies and are late-stage intermediate precursors on

the aggregation pathway to fibrils. A key aspect that facilitates on and off pathway aggregation routes and complicates investigations of AβOs is that they possess structural plasticity and are metastable and transient in nature.

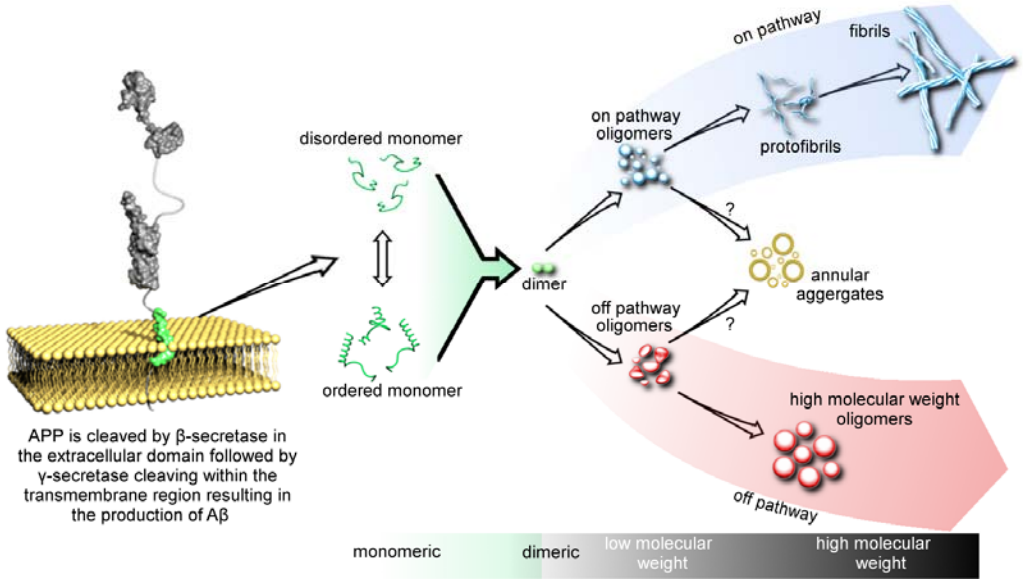

Figure 2. Production and aggregation Aβ. Aβ is a cleavage product of APP, a transmembrane protein. Monomeric Aβ transitions between ordered and disordered states. Once dimerization occurs, subsequent aggregation occurs either on pathway or off pathway with respect to fibril formation. The aggregates associated with these different pathways increase in molecular weight from the left to the right of the schematic. Fibrillization can proceed via several potential pathways that can populate various intermediate aggregate states, including oligomers and protofibrils. Off-pathway oligomers of various size may also form. Annular aggregates of Aβ can also form and are thought to potentially be associated with forming pore-like structures.

Further complicating the issue is the observation that Aβ can aggregate into a variety of morphologically distinct fibril structures, referred to as polymorphs [86–90]. This phenomenon is predicated on subtle changes of environmental conditions associated with aggregation, and as a result, preparatory protocols employed in experiments determines the resulting Aβ fibril morphology [87]. While polymorphic aggregates are readily observed with *in vitro* studies using synthetic Aβ, polymorphic structures have been observed in amyloids derived from tissue, and it is thought that variations in Aβ aggregate morphologies may play an important role in AD [91,92]. For example, polymorphic aggregates and fibrils may result in distinct biological activities and levels of toxicity that could underlie variations in AD [76], and distinct fibril structures can be directly associated with individual AD patients and clinical phenotype [93,94].

A complicating factor in evaluating and comparing studies aimed at elucidating AβO formation, structure, and physiological impact is divergent experimental conditions, such as Aβ preparation protocols, heavily influences experimental outcomes. In terms of oligomers, the emergence of distinct fibril structure strongly suggests that there would also be distinct oligomeric precursors

associated with their formation. Indeed, distinct AβO species can be observed within *in vitro* aggregation assays under conditions that result in fibril polymorphs [95] (Figure 3). For studies conducted with synthetic peptide, there are a variety of protocols used to solubilize Aβ (Table 2). Typically, these protocols consist of a disaggregation step and a reconstitution step. The disaggregation steps usually involves the use of hydrogen bond disrupting solvents, i.e., hexafluoroisopropanol (HFIP) or trifluoroacetic acid (TFA), that break down pre-existing aggregates within lyophilized stocks of Aβ. These solvents are often removed under vacuum, leaving a peptide film. The reconstitution step involves dissolving these peptide films into a solvent that facilitates dilution into an appropriate buffer. Dimethyl sulfoxide (DMSO) is often used, creating a concentrated stock that is diluted into the desired buffer. Disaggregation and reconstitution can also be facilitated by changes in pH. Sometimes reconstitution is performed directly into the final buffer. Importantly, variations in preparatory protocols indeed result in different populations of oligomers (Table 2 and Figure 3), and these variations can complicate direct comparisons between different reports within the literature. Further complicating the issue, there are often distinctions observed between studies performed with synthetic Aβ and naturally derived Aβ [96]. In many studies aimed at elucidating activity of AβOs, specific preparations are used to obtain a particular population of oligomer species. These are then directly applied to different model systems, ranging from cell culture to animal models. However, there is often a lack of effort to verify that once added to the model system (which can often be a pronounced change in chemical environment) that these AβO species do not dissociate or aggregate into a different AβO or Aβ aggregate. To truly relate specific AβOs to a neurotoxic activity, effort should be invoked to attempt additional controls of this type.

Table 2. Representative disaggregation, reconstitution, and miscellaneous protocols for the preparation of Aβ and observed AβOs.

Classification	Disaggregation	Reconstitution	Miscellaneous	Result	References
ADDLs	None	F12 Media 4 °C	Centrifuged 14,000 ×g for 10 mins	5–6 nm by AFM (height)	[38]
ADDLs	HFIP	DMSO at 5 mM Aβ	Sonicate 5 mins, dilute with DMEM/F12 Media	A11+	[97]
Globulomers	HFIP	DMSO at 5 mM Aβ	Sonicate 10 mins, dilute PBS + 0.05% SDS	16–56 kDa by SDS-PAGE	[98]
Globulomers	HFIP	DMSO at 5 mM Aβ	Dilute PBS + 0.05% SDS, Dialyze	38–48 kDa by SDS-PAGE	[99]
Aβ*56	HFIP	DMSO at 5 mM Aβ	Sonicate 20 min, PBS + 0.2% SDS incubate 6 h, dilute and incubated 18 h; centrifuge 3000 ×g, dialysis	56 kDa by Native-PAGE	[100]
AβOs	HFIP	DMSO	F12 Media incubated at 4 °C for 24 h	1–4 nm by AFM	[101,102]
AβOs	HFIP/NH_4OH	10 mM Tris-HCl	Addition of Zn^{2+}	10–12 nm by AFM	[97]
AβOs	TFA/HFIP (2Xs)	2 mM NaOH	PBS Centrifuge 386,000 ×g	2.5 nm by AFM	[87,95]

Continued on next page

Classification	Disaggregation	Reconstitution	Miscellaneous	Result	References
AβOs	TFA salt/2 mM NaOH, pH ~10.5, 1 min sonication	PBS	-	3–12 nm by AFM	[103]
AβOs	10% NH_4OH (w/v) sonicated 5 mins, lyophilized	60 mM NaOH	-	1–10 nm by DLS	[104]
AβOs LMW	-	DMSO	Sonicate 1 min, centrifuge 16,000 ×g, SEC, PBS, PICUP crosslinking	4–26 kDa by SDS-PAGE	[105]

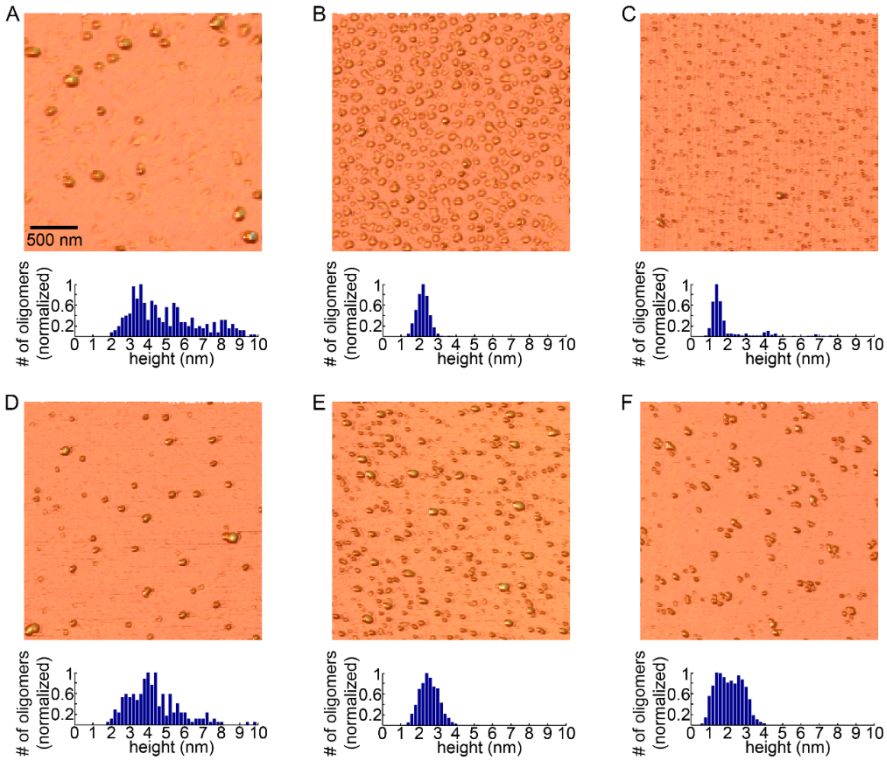

Figure 3. Heterogeneity of AβOs. A series of atomic force microscopy images and size analysis, i.e. height histograms, of AβOs formed from synthetic Aβ that had been prepared by some of the protocols described in Table 2. The AβOs were prepared in the following way: (A) protocol provided in the AggreSure β-amyloid kits available from AnaSpec which consists of a reconstitution step directly into Tris buffer with bath sonication; (B) 10% NH_4OH disaggregation buffer, followed by additional treatment with HFIP, and reconstitution in 2 mM NaOH (pH > 11) with subsequent dilution into Tris buffer; (C) 10% NH_4OH disaggregation buffer, and reconstitution in 60 mM NaOH (pH > 11) with subsequent dilution into HEPES buffer; (D) HFIP disaggregation, reconstitution in DMSO, followed by dilution into PBS; (E) TFA disaggregation with sonication, an additional HFIP disaggregation step, reconstitution with NaOH, and dilution into PBS; (F) No disaggregation step and direct reconstitution in to phosphate buffer.

4. Classifying AβOs

Enormous efforts have been extended in identifying the most toxic/disease-relevant AβO species and the relevant underlying structure [9,106,107]. This is a daunting endeavor due to the transient nature and extensive heterogeneity associated with AβOs [108–110]. It is possible that different AβO species may activate different deleterious changes associated with AD [9,106,107]; however, there may also be relatively benign AβO species or even experimental artifacts [96,108,111]. Unraveling the physiological activity of AβOs may require separate analysis of these different species, which may be altered by preparatory protocols. The ability of smaller AβO (like dimers and trimers) to further aggregate into large, more stable synaptotoxic assemblies [107] must be accounted for in assessing the toxic effects of specific AβO species. That is, upon the addition of preparations of AβO to cell culture or other models, AβOs may further assemble into other higher order species that may influence the associated toxic effects. Despite the inherent complexity of this endeavor, progress has been made. Aβ trimers have been linked to playing a role in inducing pathological conformational changes in tau [112]. However, crosslinked Aβ dimers were shown not to be toxic themselves, but rather contributed to toxicity by further assembling into larger assemblies [113]. A 56 kDa SDS-stable Aβ0 (referred to as Aβ*56) has been identified as a prominent specie in the AD brain [16], CSF [22], and in transgenic mouse models of AD [32,114]. In terms of an actual biological activity, Aβ*56 interacts with N-methyl-D-aspartate receptors (NMDARs), increasing NMDAR-dependent Ca^{2+} influx and activation of Ca^{2+}/calmodulin-dependent kinase IIα (CAMKIIα) [115]. Activation of CAMKIIα correlates with enhanced site-specific phosphorylation and mis-sorting of tau [115]. Smaller AβOs, namely dimers and trimers, do not appear to elicit these specific effects.

There appears to be some common themes emerging with respect to toxicity. Toxic AβOs appear to react with oligomer specific antibodies like A11 (generic for amyloid oligomers in general, [116]) and NU4 (specific for AβOs) [117]; whereas, nontoxic AβOs demonstrate reactivity with anti-fibril antibodies like OC [116]. Importantly, toxic AβOs appear to be unrelated to plaques [116,118]. AβOs related to amyloid plaques temporally, spatially, and structurally are nontoxic [118]. A number of toxic AβO species are larger than 50 kDa [21,54,118], like the previously mentioned Aβ*56 [32]. Smaller AβOs appear to be less toxic or even benign [21,54,118,119], except for their ability to further aggregated into larger assemblies. This has led to distinguishing between high molecular weight (HMW) and low molecular weight (LMW) oligomers [120]. Aggregation mechanisms differentiating between the eventual formation of HMW and LMW AβOs already appear to deviate at the dimer stage [121]. Furthermore, LMW and HMW Aβ oligomers differentially impact synapses and memory [122,123]; although, LMW AβOs are not always associated with memory dysfunction [116,117]. HMW AβOs are the predominant Aβ species in the native soluble protein fraction of AD brains [124]. These HMW species in the AD brain sometimes appear to be constructed from smaller ~7 kDa Aβ species [125]. Neurohistopathological and biochemical analyses of AβOs in the temporal cortex of AD brains implicated an Aβ dodecamer (~55 kDa) [126]. HMW AβOs bind cultured synapses [21,54,118], induce reactive oxygen species (ROS) production [123], and disrupt memory function [116,117]. With respect to the previously defined aggregation pathways, LMW AβOs are typically on pathway to fibril formation; HMW AβOs are off-pathway [127,128]. This is consistent with HMW AβOs being potent, as off pathway AβOs appear more toxic [129]. Collectively, these observations point to the complex interplay between different AβO species and their specific activity with respect to neurotoxicity.

Table 3. Methods used for analyzing AβOs.

Technique	Features of AβOs Obtained
Atomic Force Microscopy (AFM)	Morphology, population distributions
Electron Microscopy (EM)	Morphology, population distributions
Ion-Mobility Mass Spectroscopy (IM-MS)	Secondary structure, multimeric configurations
Nuclear Magnetic Resonance 2D (NMR)	Secondary structure, multimeric configurations, atomic resolution structure
Electron Paramagnetic Resonance (EPR)	Secondary structure, multimeric configurations, atomic resolution structure
Powder X-ray Diffraction (PXRD)	Atomic resolution structure
Small Angle X-ray Scattering (SAXS)	Atomic resolution structure
Single Crystal X-ray Diffraction (SCXD)	Atomic resolution structure
SDS-Page	Size distribution of multimers

5. Characterizing AβO structure and activity

To facilitate structure/activity analysis, significant efforts have been made to obtain structural details of AβOs using a variety of methods (Table 3). This is required to fully elucidate the modes of interaction of AβOs with other biomolecules and related toxic mechanisms [32,33,35–37,130]. Structural characterization of specific AβOs is challenging due to their transient nature and heterogeneity. AβOs can exhibit conformational plasticity that can be heavily influenced by environmental factors, further complicating such analysis. Despite these inherent challenges, efforts have been made to separate, isolate, and characterize distinct AβO species obtained from synthetic Aβ or from AD brain tissue and cell cultures [18,32,35–37,114,129,131–136]. With regard to HMW and LMW AβOs, they are separable in vitro by size exclusion chromatography [137] or ultrafiltration with a 50 kDa molecular weight cutoff [21,54,118]. Quick characterization of AβOs can be accomplished by chromatographic techniques, SDS PAGE, and by the use of a variety of oligomer specific antibodies (Table 4).

Table 4. Anti-AβO specific antibodies.

Antibody	Epitope	References
A11	Soluble amyloid oligomers	[17,138]
NAB61	Dimeric, oligomeric, higher order aggregates	[139]
NU-1	ADDLs specific	[140]
NU-2	ADDLs weak binding; no Aβ monomer staining	[140]
NU-4	ADDL trimer, tetramer, and 12–24 mer specific	[140]

One strategy that has been used to overcome obstacles associated with characterizing AβOs has been to stabilize oligomers via cross-linking. Such methods can lead to not only structural but also functional characterization of AβOs. Photo-induced crosslinking (PICUP) was initially used to stabilize and characterize LMW AβOs of $A\beta_{40}$ and $A\beta_{42}$ [33,141–143].While $A\beta_{40}$ primarily formed an equilibrium of dimers, trimers, and tetramers, $A\beta_{42}$ aggregated into pentamers/hexamers that further assembled into protofibrils [143]. Using mutated $A\beta_{42}$ (F10, Y42), AβOs ranging from dimer to dodecamers were stabilized using PICUP [141], opening up the ability to perform structure/activity analysis on AβOs up to 50 kDa in size. AβOs can also be stabilized using dityrosine crosslinking,

which occurs under elevated copper concentrations and oxidative stress [144]. Both of these conditions have been linked to AD, suggesting that the crosslinking associated with this method may be more physiologically relevant [145,146]. In fact, dityrosine crosslinked proteins are observed in amyloid plaques and CSF from AD patients [145]. Dityrosine linkages are associated with copper-mediated stabilization of AβOs [147]. Copper stabilization is effective enough to allow for 3D structural characterization of AβOs by small-angle x-ray scattering [148], and the copper to Aβ ratio could push aggregation toward ellipsoidal oligomers of 38 peptides (excess copper) or fibrils (excess Aβ) [119].

Another method to overcome the metastability of Aβ species and control the aggregation process is to complex/fuse Aβ sequences within other protein. Aβ sequences are often based on previously identified regions of the peptide that have been identified as playing a role in Aβ aggregation (Figure 1). Such a strategy has been successful in studying monomeric structure of Aβ sequences [149]. With careful design, this method has been applied to stabilizing oligomers derived from Aβ fragments for structural characterization. Fusion of $A\beta_{18–41}$ with the CDR3 loop region of a shark Ig new antigen receptor single variable domain antibody resulted in the formation and stabilization of tightly associated Aβ dimers, which could pair to form tetramers [150]. This dimer had a compact structure rather than a β-turn/β-sheet structure. Several engineered peptide macrocycles that incorporate Aβ sequences ($A\beta_{15–23}$ and $A\beta_{17–36}$) have been designed to contain aggregation to the oligomeric state, and these systems have been structurally characterized by X-ray crystallography and NMR spectroscopy [151–153]. Collectively, this macrocycle strategy has revealed numerous dimers, trimers, tetramers, and higher order oligomer species that display a variety of β-sheet based structural heterogeneity.

Due to the complex nature of Aβ aggregation, techniques that allow for distinguishing and characterizing distinct morphological features of AβOs within heterogeneous aggregation reactions are of enormous benefit. Both atomic force microscopy (AFM) and electron microscopy (EM) provide this capability. In particular, AFM has emerged as a particularly useful technique in studying AβO formation and morphology [95,101,134,135,154–160]. As AFM can be operated in solution, it has the ability to observe and track the behavior of individual AβOs on surfaces under physiological buffer conditions [158,160]. The surfaces used in AFM experiments have become progressively more biologically relevant and include lipid membranes [135,161–164]. With regard to Aβ aggregation on lipid membranes, in solution AFM studies have demonstrated the formation of distinct oligomeric aggregates associated with point mutation in Aβ [165], the formation of pore-like AβO morphologies [154–156], that preparation history influences AβO formation on bilayers [95], and that mechanical changes occur in bilayers associated with the presence of AβOs [166]. AFM based force spectroscopy has even been used to understand the energetics of AβO formation and stability [167,168].

With the recent development of high-speed AFM in solution, insights into the dynamics and fate of individual AβOs has been achieved [128,169]. Using high-speed AFM to track the dynamics of PICUP-stabilized LMW AβOs demonstrated that these AβOs were highly dynamic in structure, fluctuating between single and multi-globular assemblies [169]. Direct visualization of Aβ aggregation with high-speed AFM imaging demonstrates that LMW AβOs much more quickly transition to form fibrils with distinct morphologies compared with HMW AβOs [128]. Despite being classified as being off-pathway, HMW AβOs can still contribute to fibril formation by serving as a reservoir of Aβ. That is, HMW AβOs may dissociate into smaller LMW AβO that seed

fibrillization [128]. This again points to the necessity to track the fate of AβO species when evaluating their physiological activities. For example, the LMW AβOs that are dissociation products of HMW AβOs may actually be more toxic [170].

Another technique that is capable of characterizing heterogeneous populations of AβOs is ion mobility mass spectrometry (IM-MS). These IM-MS studies ascertained qualitative differences in AβO structure associated with $A\beta_{40}$ and $A\beta_{42}$ [131,132]. $A\beta_{40}$ tetramers displayed an enclosed ring-shaped configuration that would inhibit additional contacts required to assemble into larger AβO species [131,132]. $A\beta_{42}$ tetramers preferentially had a bent structure that would provide oligomer ends capable of additional contacts and enabling further aggregation. Indeed, $A\beta_{42}$ was capable of forming larger donut-shaped dodecamers.

Another strategy to perform structural analysis of AβOs is utilizing specific conditions to stabilize them via a kinetic trap. Such an approach has been successfully used to enable NMR spectroscopy of AβOs [171,172]. By incubation of Aβ at 4 ℃ and freeze-trapping with liquid nitrogen, heterogeneous, spherical AβOs were analyzed with 2D NMR and shown to possess in-register parallel β-strand structure similar to fibrils [172]. Based on NMR analysis, $A\beta_{42}$ pentamers stabilized at a low 4 ℃ and 10 mM salt concentration were disordered [171]. These $A\beta_{42}$ pentamers displayed enhanced toxicity compared with protofibrils or fibrils [171]. An atomic model of $A\beta_{42}$ oligomers consisting of approximately 15–24 peptides has been proposed from a combination of biophysical techniques [173]. These oligomers were prepared by disaggregation in HFIP followed by resuspension in dilute ammonium hydroxide, preventing fibril formation. These were not end-stage AβOs, as subsequent dilution in PBS resulted in fibril formation. Powder X-ray diffraction patterns of the AβOs were consistent with helical β-sheet pairs wrapped together into a super-helix. This wrapping results in a hole along the super-helix axis, which is consistent with proposed toxic mechanisms in which Aβ forms pathogenic pores. Another proposed structure, based on site-directed spin labeling and electron paramagnetic resonance, shares similarities with this structure [174]. This study used $A\beta_{42}$ fused to GroES-ubiquitin that formed stable oligomers that were A11 positive. Based on the EPR data, an AβO model was proposed that consisted of a β-sheet with three antiparallel strands with these strands being arranged head to tail. These sheets are further packed face to back as a group of four.

Even just tracking AβO formation has been challenging. A number of straight forward spectroscopic assays are well-established to track formation and kinetic parameters of fibrils (e.g., ThT), but methods to easily track AβO formation have been lacking. Recently, the use of 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) dyes have been utilized to fluorescently detect the AβOs *in vitro* [175,176]. These BODIPY fluorescent probes have high quantum yields with the capability of selectively binding to AβOs [177]. Importantly, increases and decreases in the "BD-Oligo" dye fluorescence also correlated with increasing and decreasing intensities of A11 staining, directly relating this signal to an established methods to detect AβOs [175]. In addition, the BD-Oligo dye can be used in parallel with ThT assays that detect fibril formation, allowing for the direct investigation of the correlation between oligomer and fibril formation [175]. A BODIPY-based probe (BAP-1) has also facilitated the direct visualization of Aβ plaques in transgenic mice [177], and rational modifications of BAP-1 also allowed for near-infrared selective detection of tau neurofibrillary tangles [178]. The ability to rationally modify BODIPY dyes for fluorescent detection of specific aggregate species, the tunability of their spectroscopic properties, and their insensitivity to solvent and pH changes have made BODIPY dyes a recently promising avenue for high throughput studies of AβO formation and stability.

6. Conclusion

While the aggregation of Aβ has been extensively studied, there is still much to understand at the molecular level about AβO formation, structure, and activity. Due to the transient nature of AβOs, their morphological heterogeneity, and the continuing debate concerning specific toxic aggregate species associated with AD, structural details and physiological activities of the variety of AβOs still need to be fully elucidated. The exact mechanisms associated of how AβOs lead to cellular dysfunction and death have not fully been explained. Understanding these phenomenon may prove crucial in the effectiveness of therapeutic strategies based on manipulating Aβ production, clearance, and aggregation. Here, we highlighted some specific features of AβOs and techniques that have provided insight into their structure and formation. While this review is far from exhaustive, we hope that collectively they provide a compelling argument toward the importance of understanding the nature of AβOs, highlight some of the intrinsic obstacles associated with studying AβOs, and provide some insight into methods that will play a role in pushing our knowledge of AβOs further.

Acknowledgments

Previous support from the National Science Foundation (NSF#1054211), and the Alzheimer's Association (NIRG-11-203834) is gratefully appreciated.

Conflict of interest

The authors declare no conflicts of interest.

References

1. Knowles TP, Vendruscolo M, Dobson CM (2014) The amyloid state and its association with protein misfolding diseases. *Nat Rev Mol Cell Biol* 15: 384–396.
2. Hardy JA, Higgins GA (1992) Alzheimers disease—the amyloid cascade hyopothesis. *Science* 256: 184–185.
3. Arriagada PV, Growdon JH, Hedleywhyte ET, et al. (1992) Neurofibrillary tangles but not senile plaques parellel duration and severity of Alzheimers disease. *Neurology* 42: 631–639.
4. Terry RD, Masliah E, Salmon DP, et al. (1991) Physical basis of cognitve alterations in Alzheimers disease—synapse loss is the major correlate of cognitive impairment. *Ann Neurol* 30: 572–580.
5. Viola KL, Sbarboro J, Sureka R, et al. (2015) Towards non-invasive diagnostic imaging of early-stage Alzheimer's disease. *Nat Nanotechnol* 10: 91–98.
6. Hyman B, Tanzi R (1992) Amyloid, dementia and Alzheimer's disease. *Curr Opin Neurol Neurosur* 5: 88–93.
7. Cummings BJ, Pike CJ, Shankle R, et al. (1996) b-Amyloid deposition and other measures of neuropathology predict cognitive status in Alzheimer's disease. *Neurobiol Aging* 17: 921–933.
8. Cummings JL, Morstorf T, Zhong K (2014) Alzheimer's disease drug-development pipeline: Few candidates, frequent failures. *Alzheimers Res Ther* 6: 37.

9. Goure WF, Krafft GA, Jerecic J, et al. (2014) Targeting the proper amyloid-β neuronal toxins: A path forward for Alzheimer's disease immunotherapeutics. *Alzheimers Res Ther* 6: 42.
10. Karran E, Hardy J (2014) A critique of the drug discovery and phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer disease. *Ann Neurol* 76: 185–205.
11. Karran E, Hardy J (2014) Antiamyloid therapy for Alzheimer's disease—are we on the right road? *New Engl J Med* 370: 377–378.
12. Frackowiak J, Zoltowska A, Wisniewski HM (1994) Nonfibrillar β-amyloid protein is associated with smooth-muscle cells of vessel walls in Alzheimer disease. *J Neuropath Exp Neur* 53: 637–645.
13. Oda T, Pasinetti GM, Osterburg HH, et al. (1994) Purification and characterization of brain clusterin. *Biochem Bioph Res Co* 204: 1131–1136.
14. Oda T, Wals P, Osterburg HH, et al. (1995) Clusterin (apoJ) alters the aggregation of amyloid β peptide (Aβ(1-42)) and forms slowly sedimenting Aβ complexes that cuase oxidative stress. *Exp Neurol* 136: 22–31.
15. Esparza TJ, Zhao H, Cirrito JR, et al. (2013) Amyloid-β oligomerization in Alzheimer dementia versus high-pathology controls. *Ann Neurol* 73: 104–119.
16. Gong YS, Chang L, Viola KL, et al. (2003) Alzheimer's disease-affected brain: Presence of oligomeric A β ligands (ADDLs) suggests a molecular basis for reversible memory loss. *P Natl Acad Sci USA* 100: 10417–10422.
17. Kayed R, Head E, Thompson JL, et al. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 300: 486–489.
18. Noguchi A, Matsumura S, Dezawa M, et al. (2009) Isolation and characterization of patient-derived, toxic, high mass amyloid β-protein (Aβ) assembly from Alzheimer disease brains. *J Biol Chem* 284: 32895–32905.
19. Pham E, Crews L, Ubhi K, et al. (2010) Progressive accumulation of amyloid-β oligomers in Alzheimer's disease and in amyloid precursor protein transgenic mice is accompanied by selective alterations in synaptic scaffold proteins. *FEBS J* 277: 3051–3067.
20. Gyure KA, Durham R, Stewart WF, et al. (2001) Intraneuronal Aβ-amyloid precedes development of amyloid plaques in Down syndrome. *Arch Pathol Lab Med* 125: 489–492.
21. Lacor PN, Buniel MC, Chang L, et al. (2004) Synaptic targeting by Alzheimer's-related amyloid β oligomers. *J Neurosci* 24: 10191–10200.
22. Lesne SE, Sherman MA, Grant M, et al. (2013) Brain amyloid-β oligomers in ageing and Alzheimer's disease. *Brain* 136: 1383–1398.
23. Georganopoulou DG, Chang L, Nam JM, et al. (2005) Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. *P Natl Acad Sci USA* 102: 2273–2276.
24. Bruggink KA, Jongbloed W, Biemans EALM, et al. (2013) Amyloid-β oligomer detection by ELISA in cerebrospinal fluid and brain tissue. *Anal Biochem* 433: 112–120.
25. Englund H, Gunnarsson MD, Brundin RM, et al. (2009) Oligomerization partially explains the lowering of Aβ42 in Alzheimer's disease cerebrospinal fluid. *Neurodegener Dis* 6: 139–147.
26. Fukumoto H, Tokuda T, Kasai T, et al. (2010) High-molecular-weight β-amyloid oligomers are elevated in cerebrospinal fluid of Alzheimer patients. *FASEB J* 24: 2716–2726.
27. Gao CM, Yam AY, Wang X, et al. (2010) Aβ40 Oligomers identified as a potential biomarker for the diagnosis of Alzheimer's disease. *PLoS One* 5: e15725.

28. Herskovits AZ, Locascio JJ, Peskind ER, et al. (2013) A luminex assay detects amyloid-β oligomers in Alzheimer's disease cerebrospinal fluid. *PLoS One* 8: e67898.
29. Holtta M, Hansson O, Andreasson U, et al. (2013) Evaluating amyloid-β oligomers in cerebrospinal fluid as a biomarker for Alzheimer's disease. *PLoS One* 8: e66381.
30. Jongbloed W, Bruggink KA, Kester MI, et al. (2015) Amyloid-β oligomers relate to cognitive decline in Alzheimer's disease. *J Alzheimers Dis* 45: 35–43.
31. Santos AN, Ewers M, Minthon L, et al. (2012) Amyloid-β oligomers in cerebrospinal fluid are associated with cognitive decline in patients with Alzheimer's disease. *J Alzheimers Dis* 29: 171–176.
32. Lesne S, Koh MT, Kotilinek L, et al. (2006) A specific amyloid-β protein assembly in the brain impairs memory. *Nature* 440: 352–357.
33. Ono K, Condron MM, Teplow DB (2009) Structure-neurotoxicity relationships of amyloid β-protein oligomers. *P Natl Acad Sci USA* 106: 14745–14750.
34. Quist A, Doudevski L, Lin H, et al. (2005) Amyloid ion channels: A common structural link for protein-misfolding disease. *P Natl Acad Sci USA* 102: 10427–10432.
35. Shankar GM, Bloodgood BL, Townsend M, et al. (2007) Natural oligomers of the Alzheimer amyloid-β protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway. *J Neurosci* 27: 2866–2875.
36. Shankar GM, Li S, Mehta TH, et al. (2008) Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* 14: 837–842.
37. Townsend M, Shankar GM, Mehta T, et al. (2006) Effects of secreted oligomers of amyloid β-protein on hippocampal synaptic plasticity: A potent role for trimers. *J Physiol* 572: 477–492.
38. Lambert MP, Barlow AK, Chromy BA, et al. (1998) Diffusible, nonfibrillar ligands derived from Aβ1-42 are potent central nervous system neurotoxins. *P Natl Acad Sci USA* 95: 6448–6453.
39. Walsh DM, Klyubin I, Fadeeva JV, et al. (2002) Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* 416: 535–539.
40. Zhao J, Li A, Rajsombath M, et al. (2018) Soluble Aβ Oligomers Impair Dipolar Heterodendritic Plasticity by Activation of mGluR in the Hippocampal CA1 Region. *iScience* 6: 138–150.
41. De Felice FG, Wu D, Lambert MP, et al. (2008) Alzheimer's disease-type neuronal tau hyperphosphorylation induced by Aβ oligomers. *Neurobiol Aging* 29: 1334–1347.
42. Ma QL, Yang F, Rosario ER, et al. (2009) β-amyloid oligomers induce phosphorylation of tau and inactivation of insulin receptor substrate via c-Jun N-terminal kinase signaling: Suppression by omega-3 fatty acids and curcumin. *J Neurosci* 29: 9078–9089.
43. Resende R, Ferreiro E, Pereira C, et al. (2008) ER stress is involved in Aβ-induced GSK-3β activation and tau phosphorylation. *J Neurosci Res* 86: 2091–2099.
44. Tomiyama T, Matsuyama S, Iso H, et al. (2010) A mouse model of amyloid-β oligomers: their contribution to synaptic alteration, abnormal tau phosphorylation, glial activation, and neuronal loss *in vivo*. *J Neurosci* 30: 4845–4856.
45. Zempel H, Thies E, Mandelkow E, et al. (2010) A Oligomers Cause Localized Ca^{2+} Elevation, Missorting of Endogenous Tau into Dendrites, Tau Phosphorylation, and Destruction of Microtubules and Spines. *J Neurosci* 30: 11938–11950.

46. Heinitz K, Beck M, Schliebs R, et al. (2006) Toxicity mediated by soluble oligomers of β-amyloid(1-42) on cholinergic SN56.B5.G4 cells. *J Neurochem* 98: 1930–1945.
47. Nunes-Tavares N, Santos LE, Stutz B, et al. (2012) Inhibition of choline acetyltransferase as a mechanism for cholinergic dysfunction induced by amyloid-β peptide oligomers. *J Biol Chem* 287: 19377–19385.
48. De Felice FG, Velasco PT, Lambert MP, et al. (2007) Aβ oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. *J Biol Chem* 282: 11590–11601.
49. Longo VD, Viola KL, Klein WL, et al. (2000) Reversible inactivation of superoxide-sensitive aconitase in Aβ1-42-treated neuronal cell lines. *J Neurochem* 75: 1977–1985.
50. Sponne I, Fifre A, Drouet B, et al. (2003) Apoptotic neuronal cell death induced by the non-fibrillar amyloid-β peptide proceeds through an early reactive oxygen species-dependent cytoskeleton perturbation. *J Biol Chem* 278: 3437–3445.
51. Tabner BJ, El-Agnaf OMA, Turnbull S, et al. (2005) Hydrogen peroxide is generated during the very early stages of aggregation of the amyloid peptides implicated in Alzheimer disease and familial British dementia. *J Biol Chem* 280: 35789–35792.
52. Alberdi E, Wyssenbach A, Alberdi M, et al. (2013) Ca^{2+}-dependent endoplasmic reticulum stress correlates with astrogliosis in oligomeric amyloid β-treated astrocytes and in a model of Alzheimer's disease. *Aging Cell* 12: 292–302.
53. Nishitsuji K, Tomiyama T, Ishibashi K, et al. (2009) The E693Δ mutation in amyloid precursor protein increases intracellular accumulation of amyloid β oligomers and causes endoplasmic reticulum stress-induced apoptosis in cultured cells. *Am J Pathol* 174: 957–969.
54. Lacor PN, Buniel MC, Furlow PW, et al. (2007) Aβ oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease. *J Neurosci* 27: 796–807.
55. Roselli F (2005) Soluble β-Amyloid$_{1-40}$ induces NMDA-dependent degradation of postsynaptic density-95 at glutamatergic synapses. *J Neurosci* 25: 11061–11070.
56. Snyder EM, Nong Y, Almeida CG, et al. (2005) Regulation of NMDA receptor trafficking by amyloid-β. *Nat Neurosci* 8: 1051–1058.
57. Zhao WQ, De Felice FG, Fernandez S, et al. (2007) Amyloid β oligomers induce impairment of neuronal insulin receptors. *FASEB J* 22: 246–260.
58. De Felice FG, Vieira MN, Bomfim TR, et al. (2009) Protection of synapses against Alzheimer's-linked toxins: insulin signaling prevents the pathogenic binding of Aβ oligomers. *Proc Natl Acad Sci USA* 106: 1971–1976.
59. Koffie RM, Meyer-Luehmann M, Hashimoto T, et al. (2009) Oligomeric amyloid-β associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. *P Natl Acad Sci USA* 106: 4012–4017.
60. Decker H, Lo KY, Unger SM, et al. (2010) Amyloid-Peptide oligomers disrupt axonal transport through an NMDA receptor-dependent mechanism that is mediated by glycogen synthase kinase 3 in primary cultured hippocampal neurons. *J Neurosci* 30: 9166–9171.
61. Pigino G, Morfini G, Atagi Y, et al. (2009) Disruption of fast axonal transport is a pathogenic mechanism for intraneuronal amyloid β. *P Natl Acad Sci USA* 106: 5907–5912.
62. Poon WW, Blurton-Jones M, Tu CH, et al. (2011) β-Amyloid impairs axonal BDNF retrograde trafficking. *Neurobiol Aging* 32: 821–833.

63. Hu J, Akama KT, Krafft GA, et al. (1998) Amyloid-β peptide activates cultured astrocytes: Morphological alterations, cytokine induction and nitric oxide release. *Brain Res* 785: 195–206.
64. Jimenez S, Baglietto-Vargas D, Caballero C, et al. (2008) Inflammatory response in the hippocampus of PS1M146L/APP751SL mouse model of Alzheimer's disease: Age-dependent switch in the microglial phenotype from alternative to classic. *J Neurosci* 28: 11650–11661.
65. Bhaskar K, Miller M, Chludzinski A, et al. (2009) The PI3K-Akt-mTOR pathway regulates a oligomer induced neuronal cell cycle events. *Mol Neurodegener* 4: 1–18.
66. Varvel NH, Bhaskar K, Patil AR, et al. (2008) Aβ oligomers induce neuronal cell cycle events in Alzheimer's disease. *J Neurosci* 28: 10786–10793.
67. Kim HJ, Chae SC, Lee DK, et al. (2003) Selective neuronal degeneration induced by soluble oligomeric amyloid β protein. *FASEB J* 17: 118–120.
68. Roher AE, Lowenson JD, Clarke S, et al. (1993) β-Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. *P Natl Acad Sci USA* 90: 10836.
69. Näslund J, Schierhorn A, Hellman U, et al. (1994) Relative abundance of Alzheimer Aβ amyloid peptide variants in Alzheimer disease and normal aging. *P Natl Acad Sci USA* 91: 8378.
70. Vandersteen A, Hubin E, Sarroukh R, et al. (2012) A comparative analysis of the aggregation behavior of amyloid-β peptide variants. *FEBS Lett* 586: 4088–4093.
71. Barrow CJ, Yasuda A, Kenny PTM, et al. (1992) Solution conformations and aggregational properties of synthetic amyloid β-peptides of Alzheimer's disease: Analysis of circular dichroism spectra. *J Mol Biol* 225: 1075–1093.
72. Barrow CJ, Zagorski MG (1991) Solution structures of β peptide and its constituent fragments: Relation to amyloid deposition. *Science* 253: 179–182.
73. Inouye H, Fraser PE, Kirschner DA (1993) Structure of β-crystallite assemblies formed by Alzheimer β-amyloid protein analogs—analysis by x-ray diffraction. *Biophys J* 64: 502–519.
74. Torok M, Milton S, Kayed R, et al. (2002) Structural and dynamic features of Alzheimer's Aβ peptide in amyloid fibrils studied by site-directed spin labeling. *J Biol Chem* 277: 40810–40815.
75. Antzutkin ON, Balbach JJ, Leapman RD, et al. (2000) Multiple quantum solid-state NMR indicates a parallel, not antiparallel, organization of β-sheets in Alzheimer's β-amyloid fibrils. *P Natl Acad Sci USA* 97: 13045–13050.
76. Petkova AT, Leapman RD, Guo ZH, et al. (2005) Self-propagating, molecular-level polymorphism in Alzheimer's β-amyloid fibrils. *Science* 307: 262–265.
77. Tycko R (2006) Solid-state NMR as a probe of amyloid structure. *Protein Peptide Lett* 13: 229–234.
78. Pastor MT, Kuemmerer N, Schubert V, et al. (2008) Amyloid toxicity is independent of polypeptide sequence, length and chirality. *J Mol Biol* 375: 695–707.
79. Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem* 75: 333–366.
80. Xue WF, Homans SW, Radford SE (2008) Systematic analysis of nucleation-dependent polymerization reveals new insights into the mechanism of amyloid self-assembly. *Proc Natl Acad Sci USA* 105: 8926–8931.
81. Murphy R (2007) Kinetics of amyloid formation and membrane interaction with amyloidogenic proteins. *BBA-Biomembranes* 1768: 1923–1934.

82. Ghosh P, Vaidya A, Kumar A, et al. (2016) Determination of critical nucleation number for a single nucleation amyloid-β aggregation model. *Math Biosci* 273: 70–79.
83. Garai K, Sahoo B, Sengupta P, et al. (2008) Quasihomogeneous nucleation of amyloid β yields numerical bounds for the critical radius, the surface tension, and the free energy barrier for nucleus formation. *J Chem Phys* 128: 045102.
84. Novo M, Freire S, Al-Soufi W (2018) Critical aggregation concentration for the formation of early Amyloid-β(1-42) oligomers. *Sci Rep* 8: 1783.
85. Xue C, Lin TY, Chang D, et al. (2017) Thioflavin T as an amyloid dye: Fibril quantification, optimal concentration and effect on aggregation. *Roy Soc Open Sci* 4: 160696.
86. Kodali R, Wetzel R (2007) Polymorphism in the intermediates and products of amyloid assembly. *Curr Opin Struc Biol* 17: 48–57.
87. Kodali R, Williams AD, Chemuru S, et al. (2010) Aβ(1-40) forms five distinct amyloid structures whose β-sheet contents and fibril stabilities are correlated. *J Mol Biol* 401: 503–517.
88. Meinhardt J, Sachse C, Hortschansky P, et al. (2009) Aβ(1-40) fibril polymorphism implies diverse interaction patterns in amyloid fibrils. *J Mol Biol* 386: 869–877.
89. Tycko R (2015) Amyloid polymorphism: Structural basis and neurobiological relevance. *Neuron* 86: 632–645.
90. Colletier JP, Laganowsky A, Landau M, et al. (2011) Molecular basis for amyloid-β polymorphism. *P Natl Acad Sci USA* 108: 16938–16943.
91. Crowther RA, Goedert M (2000) Abnormal Tau-containing filaments in Neurodegenerative Disease. *J Struct Biol* 130: 271–279.
92. Lu JX, Qiang W, Yau WM, et al. (2013) Molecular structure of β-amyloid fibrils in Alzheimer's disease brain tissue. *Cell* 154: 1257–1268.
93. Qiang W, Yau WM, Lu JX, et al. (2017) Structural variation in amyloid-β fibrils from Alzheimer's disease clinical subtypes. *Nature* 541: 217–221.
94. Paravastu AK, Qahwash I, Leapman RD, et al. (2009) Seeded growth of β-amyloid fibrils from Alzheimer's brain-derived fibrils produces a distinct fibril structure. *P Natl Acad Sci USA* 106: 7443–7448.
95. Yates EA, Legleiter J (2014) Preparation protocols of Aβ(1-40) promote the formation of polymorphic aggregates and altered interactions with lipid bilayers. *Biochemistry* 53: 7038–7050.
96. Teplow DB (2013) On the subject of rigor in the study of amyloid β-protein assembly. *Alzheimers Res Ther* 5: 39.
97. Lee MC, Yu WC, Shih YH, et al. (2018) Zinc ion rapidly induces toxic, off-pathway amyloid-β oligomers distinct from amyloid-β derived diffusible ligands in Alzheimer's disease. *Sci Rep* 8: 1–16.
98. Ryan DA, Narrow WC, Federoff HJ, et al. (2010) An improved method for generating consistent soluble amyloid-β oligomer preparations for in vitro neurotoxicity studies. *J Neurosci Meth* 190: 171–179.
99. Barghorn S, Nimmrich V, Striebinger A, et al. (2005) Globular amyloid β-peptide1-42 oligomer—A homogenous and stable neuropathological protein in Alzheimer's disease. *J Neurochem* 95: 834–847.
100. Thibaudeau TA, Anderson RT, Smith DM (2018) A common mechanism of proteasome impairment by neurodegenerative disease-associated oligomers. *Nat Commun* 9: 1097.

101. Stine WB, Dahlgren KN, Krafft GA, et al. (2003) In vitro characterization of conditions for amyloid-b peptide oligomerization and fibrillogenesis. *J Biol Chem* 278: 11612–11622.
102. Stine WB, Jungbauer L, Yu C, et al. (2011) Preparing synthetic Aβ in different aggregation states, In: Roberson ED (editor.), *Alzheimer's Disease and Frontotemporal Dementia. Methods in Molecular Biology (Methods and Protocols)*, Totowa, NJ: Humana Press, 13–32.
103. Benninger RJ, David T (1983) An improved method of preparing the amyloid β-protein for fibrillogenesis and neurotoxicity experiments. *Brain Res Rev* 287: 173–196.
104. Ryan TM, Caine J, Mertens HDT, et al. (2013) Ammonium hydroxide treatment of Aβ produces an aggregate free solution suitable for biophysical and cell culture characterization. *PeerJ* 1: e73.
105. Bitan G, Lomakin A, Teplow DB (2001) Amyloid β-protein oligomerization: Prenucleation interactions revealed by photo-induced cross-linking of unmodified proteins. *J Biol Chem* 276: 35176–35184.
106. Lesne SE (2013) Breaking the code of amyloid-β oligomers. *Int J Cell Biol* 2013: 950783.
107. Sengupta U, Nilson AN, Kayed R (2016) The role of amyloid-β oligomers in toxicity, propagation, and immunotherapy. *EBioMedicine* 6: 42–49.
108. Benilova I, Karran E, De Strooper B (2012) The toxic Aβ oligomer and Alzheimer's disease: An emperor in need of clothes. *Nat Neurosci* 15: 349–357.
109. Ferreira ST, Lourenco MV, Oliveira MM, et al. (2015) Soluble amyloid-β oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. *Front Cell Neurosci* 9: 191.
110. Cline EN, Bicca MA, Viola KL, et al. (2018) The amyloid-β oligomer hypothesis: Beginning of the third decade. *J Alzheimers Dis* 64: S567–S610.
111. Brody DL, Jiang H, Wildburger N, et al. (2017) Non-canonical soluble amyloid-β aggregates and plaque buffering: Controversies and future directions for target discovery in Alzheimer's disease. *Alzheimers Res Ther* 9: 62.
112. Sherman MA, LaCroix M, Amar F, et al. (2016) Soluble conformers of Aβ and Tau alter selective proteins governing axonal transport. *J Neurosci* 36: 9647–9658.
113. O'Malley TT, Oktaviani NA, Zhang D, et al. (2014) Aβ dimers differ from monomers in structural propensity, aggregation paths and population of synaptotoxic assemblies. *Biochem J* 461: 413–426.
114. Cheng IH, Scearce-Levie K, Legleiter J, et al. (2007) Accelerating amyloid-β fibrillization reduces oligomer levels and functional deficits in Alzheimer disease mouse models. *J Biol Chem* 282: 23818–23828.
115. Amar F, Sherman MA, Rush T, et al. (2017) The amyloid-β oligomer Aβ*56 induces specific alterations in neuronal signaling that lead to tau phosphorylation and aggregation. *Sci Signal* 10: eaal2021.
116. Liu P, Reed MN, Kotilinek LA, et al. (2015) Quaternary structure defines a large class of amyloid-β oligomers neutralized by sequestration. *Cell Rep* 11: 1760–1771.
117. Knight EM, Kim SH, Kottwitz JC, et al. (2016) Effective anti-Alzheimer Aβ therapy involves depletion of specific Aβ oligomer subtypes. *Neurol Neuroimmunol Neuroinflamm* 3: e237.
118. Velasco PT, Heffern MC, Sebollela A, et al. (2012) Synapse-binding subpopulations of Aβ oligomers sensitive to peptide assembly blockers and scFv antibodies. *ACS Chem Neurosci* 3: 972–981.

119. Ryan TM, Roberts BR, McColl G, et al. (2015) Stabilization of nontoxic Aβ-oligomers: Insights into the mechanism of action of hydroxyquinolines in Alzheimer's disease. *J Neurosci* 35: 2871–2884.
120. Ferreira IL, Ferreiro E, Schmidt J, et al. (2015) Aβ and NMDAR activation cause mitochondrial dysfunction involving ER calcium release. *Neurobiol Aging* 36: 680–692.
121. Barz B, Liao Q, Strodel B (2018) Pathways of amyloid-β aggregation depend on oligomer shape. *J Am Chem Soc* 140: 319–327.
122. Brito-Moreira J, Lourenco MV, Oliveira MM, et al. (2017) Interaction of amyloid-β (Aβ) oligomers with neurexin 2 and neuroligin 1 mediates synapse damage and memory loss in mice. *J Biol Chem* 292: 7327–7337.
123. Figueiredo CP, Clarke JR, Ledo JH, et al. (2013) Memantine rescues transient cognitive impairment caused by high-molecular-weight Aβ oligomers but not the persistent impairment induced by low-molecular-weight oligomers. *J Neurosci* 33: 9626–9317.
124. Upadhaya AR, Lungrin I, Yamaguchi H, et al. (2012) High-molecular weight Aβ oligomers and protofibrils are the predominant Aβ species in the native soluble protein fraction of the AD brain. *J Cell Mol Med* 16: 287–295.
125. Mc Donald JM, O'Malley TT, Liu W, et al. (2015) The aqueous phase of Alzheimer's disease brain contains assemblies built from similar to 4 and similar to 7 kDa Aβ species. *Alzheimers Dement* 11: 1286–1305.
126. Savioz A, Giannakopoulos P, Herrmann FR, et al. (2016) A study of Aβ oligomers in the temporal cortex and cerebellum of patients with neuropathologically confirmed Alzheimer's disease compared to aged controls. *Neurodegener Dis* 16: 398–406.
127. Breydo L, Kurouski D, Rasool S, et al. (2016) Structural differences between amyloid β oligomers. *Biochem Bioph Res Co* 477: 700–705.
128. Watanabe-Nakayama T, Ono K, Itami M, et al. (2016) High-speed atomic force microscopy reveals structural dynamics of amyloid β(1-42) aggregates. *P Natl Acad Sci USA* 113: 5835–5840.
129. Matsumura S, Shinoda K, Yamada M, et al. (2011) Two distinct amyloid β-protein (Aβ) assembly pathways leading to oligomers and fibrils identified by combined fluorescence correlation spectroscopy, morphology, and toxicity analyses. *J Biol Chem* 286: 11555–11562.
130. Miller Y, Ma B, Nussinov R (2010) Polymorphism in Alzheimer Aβ amyloid organization reflects conformational selection in a rugged energy landscape. *Chem Rev* 110: 4820–4838.
131. Bernstein SL, Dupuis NF, Lazo ND, et al. (2009) Amyloid-β protein oligomerization and the importance of tetramers and dodecamers in the aetiology of Alzheimer's disease. *Nat Chem* 1: 326–331.
132. Economou NJ, Giammona MJ, Do TD, et al. (2016) Amyloid β-protein assembly and Alzheimer's disease: Dodecamers of Aβ42, but not of Aβ40, seed fibril formation. *J Am Chem Soc* 138: 1772–1775.
133. Shamitko-Klingensmith N, Boyd JW, Legleiter J (2016) Microtubule modification influences cellular response to amyloid-β exposure. *AIMS Biophysics* 3: 261–285.
134. Yates EA, Cucco EM, Legleiter J (2011) Point mutations in Aβ induce polymorphic aggregates at liquid/solid interfaces. *ACS Chem Neurosci* 2: 294–307.
135. Yates EA, Owens SL, Lynch MF, et al. (2013) Specific domains of Aβ facilitate aggregation on and association with lipid bilayers. *J Mol Biol* 425: 1915–1933.

136. Glabe CG (2008) Structural classification of toxic amyloid oligomers. *J Biol Chem* 283: 29639–29643.
137. Chromy BA, Nowak RJ, Lambert MP, et al. (2003) Self-assembly of Aβ(1-42) into globular neurotoxins. *Biochemistry* 42: 12749–12760.
138. Glabe CG (2006) Common mechanisms of amyloid oligomer pathogenesis in degenerative disease. *Neurobiol Aging* 27: 570–575.
139. Lee EB, Leng LZ, Zhang B, et al. (2006) Targeting amyloid-β peptide (Aβ) oligomers by passive immunization with a conformation-selective monoclonal antibody improves learning and memory in Aβ precursor protein (APP) transgenic mice. *J Biol Chem* 281: 4292–4299.
140. Lambert MP, Velasco PT, Chang L, et al. (2007) Monoclonal antibodies that target pathological assemblies of Aβ. *J Neurochem* 100: 23–35.
141. Hayden EY, Conovaloff JL, Mason A, et al. (2017) Preparation of pure populations of covalently stabilized amyloid β-protein oligomers of specific sizes. *Anal Biochem* 518: 78–85.
142. Ono K, Li L, Takamura Y, et al. (2012) Phenolic compounds prevent amyloid β-protein oligomerization and synaptic dysfunction by site-specific binding. *J Biol Chem* 287: 14631–14643.
143. Bitan G, Kirkitadze MD, Lomakin A, et al. (2003) Amyloid β-protein (Aβ) assembly: Aβ40 and Aβ42 oligomerize through distinct pathways. *P Natl Acad Sci USA* 100: 330–335.
144. Al-Hilaly YK, Williams TL, Stewart-Parker M, et al. (2013) A central role for dityrosine crosslinking of Amyloid-β in Alzheimer's disease. *Acta Neuropathol Commun* 1: 83.
145. Bush AI (2013) The metal theory of Alzheimer's disease. *J Alzheimers Dis* 33: S277–S281.
146. Butterfield DA, Boyd-Kimball D (2018) Oxidative stress, amyloid-β peptide, and altered key molecular pathways in the pathogenesis and progression of Alzheimer's disease. *J Alzheimers Dis* 62: 1345–1367.
147. Smith DP, Ciccotosto GD, Tew DJ, et al. (2007) Concentration dependent Cu^{2+} induced aggregation and dityrosine formation of the Alzheimer's disease amyloid-β peptide. *Biochemistry* 46: 2881–2891.
148. Ryan TM, Kirby N, Mertens HDT, et al. (2015) Small angle X-ray scattering analysis of Cu^{2+}-induced oligomers of the Alzheimer's amyloid β peptide. *Metallomics* 7: 536–543.
149. Takano K, Endo S, Mukaiyama A, et al. (2006) Structure of amyloid β fragments in aqueous environments. *FEBS J* 273: 150–158.
150. Streltsov VA, Varghese JN, Masters CL, et al. (2011) Crystal structure of the amyloid-β p3 fragment provides a model for oligomer formation in Alzheimer's disease. *J Neurosci* 31: 1419–1426.
151. Liu C, Sawaya MR, Cheng PN, et al. (2011) Characteristics of amyloid-related oligomers revealed by crystal structures of macrocyclic β-sheet mimics. *J Am Chem Soc* 133: 6736–6744.
152. Pham JD, Chim N, Goulding CW, et al. (2013) Structures of oligomers of a peptide from β-amyloid. *J Am Chem Soc* 135: 12460–12467.
153. Spencer RK, Li H, Nowick JS (2014) X-ray crystallographic structures of trimers and higher-order oligomeric sssemblies of a peptide derived from $A\beta_{17–36}$. *J Am Chem Soc* 136: 5595–5598.
154. Bhatia R, Lin H, Lal R (2000) Fresh and nonfibrillar amyloid b protein(1-42) induces rapid cellular degeneration in aged human fibroblasts: Evidence for AbP-channel-mediated cellular toxicity. *FASEB* 14: 1233–1243.

155. Lin H, Bhatia R, Lal R (2001) Amyloid b protein forms ion channels: Implications for Alzheimer's disease pathophysiology. *FASEB* 15: 2433–2444.
156. Lin H, Zhu YJ, Lal R (1999) Amyloid-b protein (1–40) forms calcium-permeable, Zn^{2+}-sensitive channel in reconstituted lipid vesicles. *Biochemistry* 38: 11189–11196.
157. Rhee SK, Quist AP, Lal R (1998) Amyloid b protein-(1-42) forms calcium-permeable, Zn^{2+}-sensitive channel. *J Biol Chem* 273: 13379–13382.
158. Parbhu A, Lin H, Thimm J, et al. (2002) Imaging real-time aggregation of amyloid β protein (1-42) by atomic force microscopy. *Peptides* 23: 1265–1270.
159. Legleiter J, (2011) Assessing Aβ aggregation state by atomic force microscopy, In: Roberson ED (editor.), *Alzheimer's Disease and Frontotemporal Dementia: Methods and Protocols*, 57–70.
160. Kowalewski T, Holtzman DM (1999) In situ atomic force microscopy study of Alzheimer's β-amyloid peptide on different substrates: New insights into mechanism of β-sheet formation. *Proc Natl Acad Sci USA* 96: 3688–3693.
161. Hane F, Drolle E, Gaikwad R, et al. (2011) Amyloid-β aggregation on model lipid membranes: An atomic force microscopy study. *J Alzheimers Dis* 26: 485–494.
162. Legleiter J, Fryer JD, Holtzman DM, et al. (2011) The modulating effect of mechanical changes in lipid bilayers caused by apoE-containing lipoproteins on Aβ induced membrane disruption. *ACS Chem Neurosci* 2: 588–599.
163. Yip CM, Elton EA, Darabie AA, et al. (2001) Cholesterol, a modulator of membrane-associated Ab-fibrillogenesis and neurotoxicity. *J Mol Biol* 311: 723–734.
164. Yip CM, McLaurin J (2001) Amyloid-b assembly: A critical step in fibrillogensis and membrane disruption. *Biophys J* 80: 1359–1371.
165. Pifer PM, Yates EA, Legleiter J (2011) Point mutations in Aβ result in the formation of distinct polymorphic aggregates in the presence of lipid bilayers. *PLoS One* 6: e16248.
166. Burke KA, Yates EA, Legleiter J (2013) Amyloid-forming proteins alter the local mechanical properties of lipid membranes. *Biochemistry* 52: 808–817.
167. Hane F, Tran G, Attwood S, et al. (2013) Cu^{2+} affects amyloid-β (1-42) aggregation by increasing peptide-peptide binding forces. *PLoS One* 8: e59005.
168. Kim BH, Palermo NY, Lovas S, et al. (2011) Single-molecule atomic force microscopy force spectroscopy study of Aβ-40 interactions. *Biochemistry* 50: 5154–5162.
169. Banerjee S, Sun Z, Hayden EY, et al. (2017) Nanoscale dynamics of amyloid-β42 oligomers as revealed by high-speed atomic force microscopy. *ACS Nano* 11: 12202–12209.
170. Yang T, Li S, Xu H, et al. (2017) Large soluble oligomers of amyloid β-protein from Alzheimer brain are far less neuroactive than the smaller oligomers to which they dissociate. *J Neurosci* 37: 152–163.
171. Ahmed M, Davis J, Aucoin D, et al. (2010) Structural conversion of neurotoxic amyloid-β(1-42) oligomers to fibrils. *Nat Struct Mol Biol* 17: 561–556.
172. Chimon S, Shaibat MA, Jones CR, et al. (2007) Evidence of fibril-like β-sheet structures in a neurotoxic amyloid intermediate of Alzheimer's β-amyloid. *Nat Struct Mol Biol* 14: 1157–1164.
173. Stroud JC, Liu C, Teng PK, et al. (2012) Toxic fibrillar oligomers of amyloid-β have cross-β structure. *P Natl Acad Sci USA* 109: 7717–7722.
174. Gu L, Liu C, Stroud JC, et al. (2014) Antiparallel triple-strand architecture for prefibrillar Aβ42 oligomers. *J Biol Chem* 289: 27300–27313.

175. Teoh CL, Su D, Sahu S, et al. (2015) Chemical fluorescent probe for detection of Aβ oligomers. *J Am Chem Soc* 137: 13503–13509.
176. Jameson LP, Dzyuba SV (2013) Aza-BODIPY: Improved synthesis and interaction with soluble Aβ1-42 oligomers. *Bioorg Med Chem Lett* 23: 1732–1735.
177. Ono M, Watanabe H, Kimura H, et al. (2012) BODIPY-based molecular probe for imaging of cerebral β-amyloid plaques. *ACS Chem Neurosci* 3: 319–324.
178. Verwilst P, Kim HR, Seo J, et al. (2017) Rational design of *in vivo* Tau tangle-selective near-infrared fluorophores: expanding the BODIPY universe. *J Am Chem Soc* 139: 13393–13403.

AIMS Press