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Review

Challenges in understanding the structure/activity relationship of Aβ oligomers

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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 AβOs 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]|

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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 Aβ₄₀ and Aβ₄₂ 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, Aβ₄₂ has a larger hydrophobic domain, making it more fibrillogenic compared to Aβ₄₀ 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 polyomorph 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 Aβ42 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 involve 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 ℃  | 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 Sonicate 20 min, PBS + 0.2% SDS incubate 6 h, dilute and incubated 18 h; centrifuge 3000 ×g, dialysis | 38–48 kDa by SDS-PAGE | [99]       |
| Aβ*56         | HFIP           | DMSO at 5 mM Aβ | 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 ℃ for 24 h | 1–4 nm by AFM | [101,102] |
| AβOs          | HFIP/NH₄OH     | 10 mM Tris-HCl | Addition of Zn²⁺ | 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           | (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% NH₄OH disaggregation buffer, followed by additional treatment with HFIP, and reconstitution in 2 mM NaOH (pH > 11) with subsequent dilution into Tris buffer; (C) 10% NH₄OH 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, rather contributed to toxicity by further assembling into larger assemblies [113]. A 56 kDa SDS-stable AβO (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 Ca2+ influx and activation of Ca2+/calmodulin-dependent kinase IIa (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 Aβ40 and Aβ42 [33,141–143]. While Aβ40 primarily formed an equilibrium of dimers, trimers, and tetramers, Aβ42 aggregated into pentamers/hexamers that further assembled into protofibrils [143]. Using mutated Aβ42 (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 Aβ18–41 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 (Aβ15–23 and Aβ17–36) 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
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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 Aβ40 and Aβ42 [131,132]. Aβ40 tetramers displayed an enclosed ring-shaped configuration that would inhibit additional contacts required to assemble into larger AβO species [131,132]. Aβ42 tetramers preferentially had a bent structure that would provide oligomer ends capable of additional contacts and enabling further aggregation. Indeed, Aβ42 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 °C 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, Aβ42 pentamers stabilized at a low 4 °C and 10 mM salt concentration were disordered [171]. These Aβ42 pentamers displayed enhanced toxicity compared with protofibrils or fibrils [171]. An atomic model of Aβ42 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 Aβ42 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.

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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.

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Conflict of interest

The authors declare no conflicts of interest.

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