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Spotlight

# Atomic Structures of Amyloid-β Oligomers Illuminate a Neurotoxic Mechanism

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**Amyloid-β (Aβ) accumulation in the brain is a cardinal event in Alzheimer's disease (AD), but the structural basis of Aβ-elicited neurotoxicity is unknown. In a recent paper, Ciudad *et al.* elucidate the first atomic structures of Aβ oligomers, which reveal how they form lipid-stabilized pores that might disrupt neuronal membranes and ion homeostasis.**

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of amyloid-β (Aβ) in the brain [1]. However, whether Aβ accumulation is a key toxic event in AD and the precise mechanism of Aβ-elicited neurotoxicity are vigorously debated [1,2]. Structural information about the toxic oligomeric Aβ species that might underlie AD has been difficult to obtain at an atomic level. This information would enhance our understanding of the potential toxic mechanisms and might also enable the design of effective therapeutics that target the pathogenic Aβ species. In a recent groundbreaking study by Natàlia Carulla and coworkers, the first atomic structures of Aβ(1–42) oligomers have been resolved [3]. These elegant findings indicate that membrane-embedded Aβ(1–42) oligomers form edge-conductivity pores that may drive neurotoxicity in AD [3].

Aβ peptides are cleavage products of the amyloid precursor protein which resides predominantly in the plasma membrane of neurons (Figure 1A) [1]. Some Aβ peptides become trapped in the lipid bilayer where they can form porous channels upon oligomerization (Figure 1A) [4]. Others are initially soluble, but can oligomerize and form fibrils over time [1]. However, the concentration of Aβ fibrils in the brain does not correlate tightly with disease severity [1]. Consequently, growing attention has been directed to understanding membrane-associated and soluble Aβ oligomers and their possible neurotoxicity. The amyloid pore hypothesis was first proposed in 1994 based on biophysical studies [5]. In this framework, Aβ is proposed to form pores in neuronal membranes with channel-like activity that enables permeation of cations such as $Ca^{2+}$, $Cs^{+}$, and $Na^{+}$, which promotes neurotoxicity (Figure 1A) [5].

To test the amyloid pore hypothesis, it is important to ascertain the structure of membrane-associated Aβ oligomers at an atomic level. However, Aβ oligomers can be transient and heterogeneous, which presents difficulties for structural studies [6]. They can also adopt distinct toxic or benign structures, and this further complicates their characterization [7]. In a 2016 paper, Carulla and coworkers established an innovative method to prepare stable homogenous Aβ oligomers in membrane-mimicking conditions to examine possible AD-related properties, which they termed β-sheet, pore-forming oligomers ($\beta PFO_{A\beta(1-42)}$) [8]. In the recent follow-up paper [3], the authors present atomic structures of tetrameric and octameric $\beta PFO_{A\beta(1-42)}$ based on information obtained using NMR spectroscopy and mass spectrometry. Moreover, a mechanism of toxicity is proposed based on this structural information, which is supported by electrophysiology and molecular dynamics studies [3].

$\beta PFO_{A\beta(1-42)}$ in dodecylphosphocholine micelles were first characterized via transverse relaxation optimized spectroscopy (TROSY)-type NMR studies. Peak assignments established that $\beta PFO_{A\beta(1-42)}$ contains two distinct types of Aβ(1–42) subunit [3]. Cα and Cβ chemical shifts were then used to derive three-residue average secondary chemical shifts to discern the presence of secondary structure in each distinct $\beta PFO_{A\beta(1-42)}$ subunit. They found that the first type of subunit contained two β-strands, whereas the second type of subunit contained an α-helical structure followed by a β-strand (Figure 1A,B) [3]. Nuclear Overhauser effect spectroscopy (NOESY)-type NMR studies then enabled the resolution of long-range structure and revealed the topology of an Aβ(1–42) tetramer comprising a six-stranded β-sheet core (Figure 1A,B) [3]. The two faces of the β-sheet core are hydrophobic, whereas the edges are hydrophilic [3]. Thus, Ciudad *et al.* actualize the first atomic view of Aβ oligomers (Figure 1A,B) [3].

After resolving the structure of tetrameric $\beta PFO_{A\beta(1-42)}$, it remained uncertain how pore-like behavior might emerge [3,8]. The authors wondered whether $\beta PFO_{A\beta(1-42)}$ might also contain higher-order oligomeric stoichiometries that could drive pore formation. To assess this possibility, they used size-exclusion chromatography coupled to native ion mobility mass spectrometry [3]. The analysis revealed the presence of Aβ(1–42) tetramers and octamers. The tetramers were more abundant and, because no other charge states were detected for other oligomeric species, it was concluded that the octamers were formed by the combination of two tetramers [3].

Next, the authors prepared $\beta PFO_{A\beta(1-42)}$ enriched in octamers to determine their structure. To do so, they worked at high concentrations of $\beta PFO_{A\beta(1-42)}$ (450 μM) and analyzed their structure via native ion mobility mass spectrometry to derive their collision cross-states [3]. This information was then used to inform simulation studies with the assumption that two tetramers join to form an octamer [3]. Based

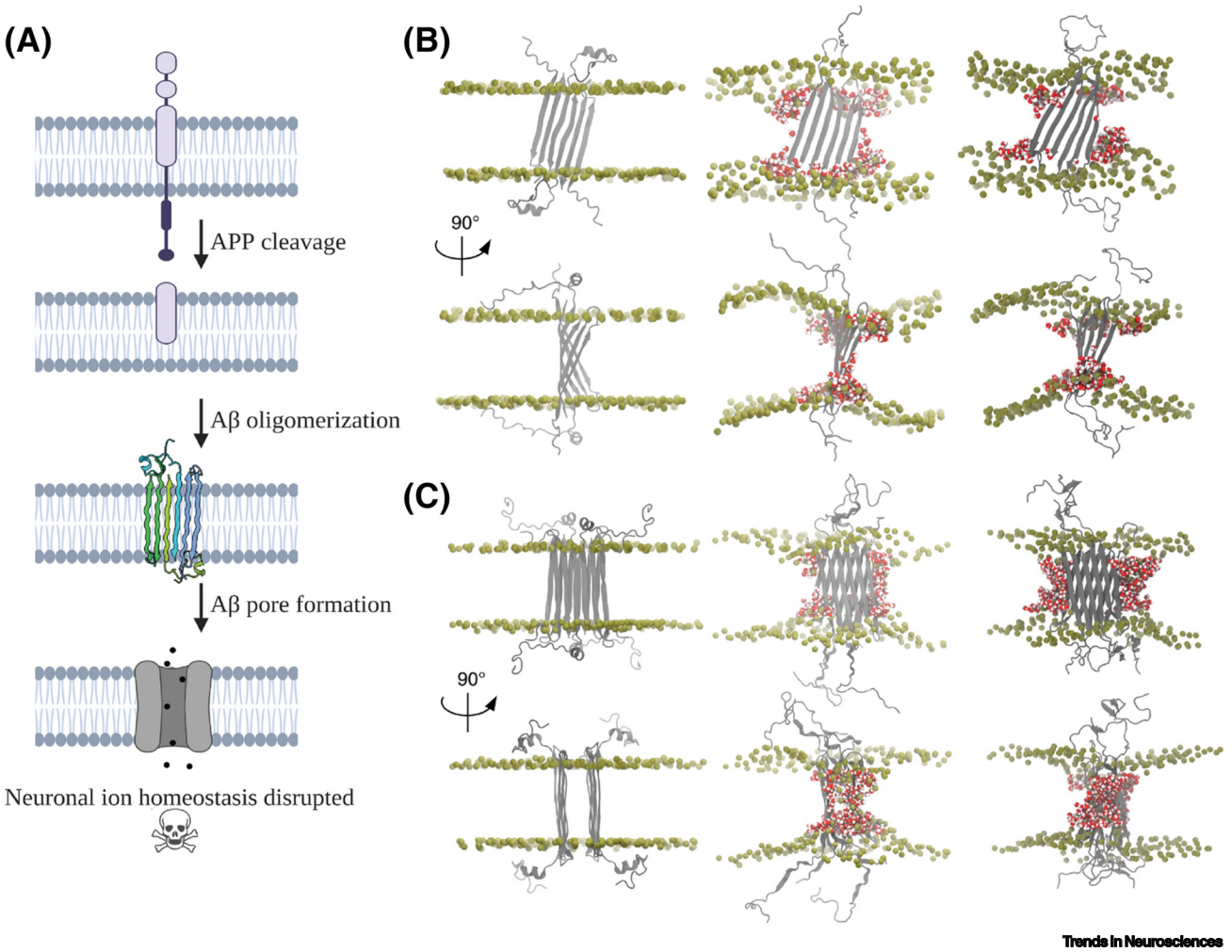


Figure 1. Snapshots of Aβ(1–42) Oligomers. (A) Schematic of a potential toxic mechanism of membrane-bound Aβ oligomers. Once amyloid precursor protein (APP) is cleaved, Aβ(1–42) remains in the membrane and self-associates to form tetramers and octamers that are rich in β-structure. An Aβ(1–42) tetramer is shown; each subunit is depicted in a different color. The hydrophilic edges of the oligomers interact unfavorably with exposed lipid tails, and this elicits lipid headgroup reorientation and the formation of lipid-stabilized pores that alter cellular ion homeostasis. Figure created with BioRender.com. (B) Aβ(1–42) tetramers and (C) Aβ(1–42) octamers in dodecylphosphocholine micelles. Aβ(1–42) is depicted in grey, the dodecylphosphocholine headgroup phosphorous atoms are in tan, and water molecules are in red and white. Images adapted from Figure 5a,b in [3]. Note that the lipid-stabilized, edge-conductivity Aβ(1–42) pores enable water permeation driven by hydrophilic residues residing at the core β-sheet edges of the oligomers.

on the $\beta PFO_{A\beta(1-42)}$ tetramer structure, the authors proposed that the two tetrameric subunits associate to form a β-barrel or β-sandwich structure [3]. Further molecular simulations revealed that the relevant topology for $\beta PFO_{A\beta(1-42)}$ octamers is a β-sandwich (Figure 1C) [3].

The pore-forming behavior of $\beta PFO_{A\beta(1-42)}$ tetramers and octamers was then assessed in planar lipid bilayers via electrical recordings [3]. Remarkably, both $\beta PFO_{A\beta(1-42)}$ tetramers and octamers exhibited pore-like behavior [3]. Moreover, molecular dynamic simulations revealed that $\beta PFO_{A\beta(1-42)}$ tetramers and octamers remained stable in a lipid bilayer with an applied electric field (Figure 1B,C) [3]. Close examination of the $\beta PFO_{A\beta(1-42)}$ structure revealed that hydrophilic residues on the edges on the oligomers are unfavorably exposed to lipid tails of the membrane which, because of their hydrophobicity, leads to lipid reorientation such that the lipid head groups face the hydrophilic edges [3]. The reorientation of the lipid head groups was most apparent near the first β-strand on the first subunit of the $\beta PFO_{A\beta(1-42)}$ oligomer, and this enabled the formation of lipid-stabilized pores (Figure 1B,C) [3]. Notably, the lipid-stabilized protein complex containing the Aβ octamer possessed a markedly higher degree of solvent-accessible

surface and water permeability than did complexes containing the tetramer (Figure 1B,C) [3]. The formation of lipid-stabilized Aβ pores leads to water and ion permeation, which would disrupt neuronal ion homeostasis and might represent a mechanism of Aβ-elicited neurotoxicity in AD (Figure 1A–C) [3].

These painstaking studies provide the first atomic view of Aβ(1–42) oligomers [3]. This valuable information can now be leveraged to further decipher the mechanisms of pathogenesis, design therapeutics, and devise tools to detect biomarkers. One important step to corroborate the results of this study will be to raise antibodies that specifically recognize $\beta PFO_{A\beta(1-42)}$ oligomers to determine whether these structures are found in AD patient tissue. Indeed, it is not yet clear whether this exact form of Aβ oligomer is found in AD patients. Synthetic amyloid fibrils often do not resemble amyloid structures purified from patient brains [9], but whether this mismatch will also be found with oligomers remains to be seen. Furthermore, adding $\beta PFO_{A\beta(1-42)}$ oligomers to neurons and animal models will provide insight into their toxicity *in vivo* and allow further delineation of the toxic mechanism and how it can be mitigated. This approach may empower the design of urgently needed therapeutic strategies for AD. Finally, it will be of great interest to determine whether these specific toxic Aβ oligomers can be targeted by aducanumab [10], an Aβ oligomer- and fibril-specific monoclonal antibody that is under review by the FDA for approval as an AD therapeutic.

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## References

1. Selkoe, D.J. and Hardy, J. (2016) The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* 8, 595–608
2. Kametani, F. and Hasegawa, M. (2018) Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Front. Neurosci.* 12, 25
3. Ciudad, S. *et al.* (2020) Abeta(1–42) tetramer and octamer structures reveal edge conductivity pores as a mechanism for membrane damage. *Nat. Commun.* 11, 3014
4. Arispe, N. *et al.* (1993) Alzheimer disease amyloid beta protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminum. *Proc. Natl. Acad. Sci. U. S. A.* 90, 567–571
5. Arispe, N. *et al.* (1994) The ability of amyloid beta-protein [AbetaP (1–40)] to form $Ca^{2+}$ channels provides a mechanism for neuronal death in Alzheimer's disease. *Ann. N. Y. Acad. Sci.* 747, 256–266
6. Dear, A.J. *et al.* (2020) Kinetic diversity of amyloid oligomers. *Proc. Natl. Acad. Sci. U. S. A.* 117, 12087–12094
7. Ladiwala, A.R. *et al.* (2012) Conformational differences between two amyloid β oligomers of similar size and dissimilar toxicity. *J. Biol. Chem.* 287, 24765–24773
8. Serra-Batiste, M. *et al.* (2016) Aβ42 assembles into specific β-barrel pore-forming oligomers in membrane-mimicking environments. *Proc. Natl. Acad. Sci. U. S. A.* 113, 10866–10871
9. Scheres, S.H. *et al.* (2020) Cryo-EM structures of tau filaments. *Curr. Opin. Struct. Biol.* 64, 17–25
10. Sevigny, J. *et al.* (2016) The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. *Nature* 537, 50–56