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Exploring Amyloid Oligomers with Peptide Model Systems

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Abstract

The assembly of amyloidogenic peptides and proteins such as the β-amyloid peptide (Aβ), α-synuclein, huntingtin, tau, and islet amyloid polypeptide (IAPP) into amyloid fibrils and oligomers is directly linked to amyloid diseases, such as Alzheimer's, Parkinson's, and Huntington's diseases, frontotemporal dementias, and type II diabetes. Although amyloid oligomers have emerged as especially important in amyloid diseases, high-resolution structures of the oligomers formed by full-length amyloidogenic peptides and proteins have remained elusive. Investigations of oligomers assembled from fragments or stabilized β-hairpin segments of amyloidogenic peptides and proteins have allowed investigators to illuminate some of the structural, biophysical, and biological properties of amyloid oligomers. Here, we summarize recent advances in the application of these peptide model systems to investigate and understand the structures, biological properties, and biophysical properties of amyloid oligomers.

INTRODUCTION

The assembly and aggregation of peptides and proteins into fibrils and oligomers is a hallmark of amyloid diseases.[1–4] Amyloid diseases are diverse in their prevalence, presentation, and symptoms, encompassing neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and Creutzfeldt-Jakob disease, as well as other diseases, such as type II diabetes and transthyretin amyloidosis.[4–8] Amyloid fibrils are common molecular assemblies associated with amyloid diseases, and are characterized by their insolubility, affinity for Congo red dye and thioflavin T (ThT), cross-β X-ray diffraction pattern, and extended networks of in-register parallel β-sheets.[9–14] The biophysical and structural properties of amyloid fibrils and their roles in disease have been studied extensively (Figure 1A–E).[15–18] Yet, as investigations into amyloid fibrils have proceeded over the last four decades, evidence has increasingly pointed toward amyloid oligomers as the damaging species responsible for disease progression.

Some of the initial evidence for the presence of amyloid oligomers arose from solution-phase biophysical characterization of amyloid plaques isolated from Alzheimer's disease brains.[19,20] These early studies reported the presence of soluble assemblies of the β-amyloid peptide, Aβ, in addition to insoluble fibrils. The formation of these Aβ assemblies and their relevance to disease pathology was supported by subsequent *in vitro* studies, which confirmed their assembly and neurotoxicity, and ultimately led to the formalization of the

hypothesis that amyloid oligomers are causative agents in the neurodegeneration associated with Alzheimer's disease.[21–29]

Oligomers of Aβ are soluble and heterogeneous — varying significantly in their structure, stability, and stoichiometry. Antiparallel β-sheets and β-hairpins are thought to be building blocks of many amyloid oligomers. Amyloid oligomers vary vastly in size, comprising as few as two or three, or as many as dozens or more molecules. Many of these features have been observed for oligomers formed by other amyloidogenic peptides and proteins, such as α-synuclein, polyglutamine, islet amyloid polypeptide (IAPP), and tau.[4,30–33]

Only one atomic-resolution structure of an oligomer formed by the full-length sequence of $A\beta_{42}$ has been reported thus far (Figure 1F).[34] Carulla and co-workers reported the NMR-based structure of an $A\beta_{42}$ tetramer and provided additional evidence for its assembly into an octamer. The tetramer is a six-stranded antiparallel β-sheet comprising two β-hairpins of $A\beta_{42}$ surrounding two antiparallel β-strands of $A\beta_{42}$. Although the disease relevance of this oligomer has not yet been established, the tetramer represents the first high-resolution structure of an oligomer of full-length Aβ. In light of the large number of unique amyloid fibril structures reported and deposited in the Protein Data Bank (PDB), the lack of other high-resolution structures of amyloid oligomers represents an immense gap in our understanding of amyloid diseases.[15–18]

Peptide model systems derived from the sequences of amyloidogenic peptides and proteins have emerged as useful tools to investigate amyloid oligomers and bridge this gap in our understanding. These peptides are designed to mimic the biological and biophysical properties of native amyloid oligomers. Unlike native amyloid oligomers, the oligomers formed by these peptide model systems often have the added benefits of increased homogeneity and stability, facilitating high-resolution characterization of many of the oligomers that form. This review highlights recent investigations of peptide model systems that have helped advance our knowledge of amyloid oligomers.

THE FRAGMENT-BASED APPROACH

X-ray crystallographic investigations of short fragments of amyloidogenic peptides and proteins provide one strategy for studying the molecular interactions governing fibril and oligomer assembly at high resolution. Eisenberg and co-workers reported several high-resolution structures of fibril-forming peptides that are derived from amyloidogenic peptides and proteins.[35–37] Using this fragment-based approach, Eisenberg and co-workers determined the X-ray crystallographic structures of two oligomers composed of eleven-residue peptide fragments derived from αB crystallin and superoxide dismutase 1 (SOD1) (Figure 1G, H).[38–40] The αB crystallin fragment assembles into a cylindrical barrel composed of six antiparallel β-strands, termed a cylindrin by the investigators (Figure 1G). The SOD1 fragment assembles into a corkscrew-like arrangement of antiparallel β-strands (Figure 1H). Surewicz and co-workers determined the structure of a hexamer composed of disulfide-linked antiparallel β-strands comprising two six-residue peptide fragments derived from human prion protein (Figure 1I). [41] Intermolecular hydrogen bonding between

antiparallel β-strands and the close packing of hydrophobic residues are common features that stabilize each of these oligomers.

These fragment-based models are significant, because oligomers of full-length amyloidogenic peptides and proteins are thought to be composed of antiparallel β-sheets and β-hairpins. [42–45] Structures of oligomers assembled from the fragments of amyloidogenic peptides and proteins can serve as models for naturally occurring disease-relevant oligomers formed by full-length amyloidogenic peptides and proteins. Oligomers of full-length amyloidogenic peptides and proteins have not yet yielded to X-ray crystallography or CryoEM. Although CryoEM has emerged as a powerful tool in the structural biology of amyloid fibrils (Figure 1B–E), thus far the oligomers of full-length amyloidogenic peptides and proteins have proven too small or too heterogenous for structural elucidation by CryoEM.[15–18,46,47]

STABILIZED β-HAIRPINS

β-Hairpins are building blocks of some of the oligomers formed by amyloidogenic peptides and proteins.[34,48,49] Model systems consisting of stabilized β-hairpins are valuable tools for studying amyloid oligomers, because they provide control of secondary and tertiary structure while allowing quaternary structure to form through self-assembly. Härd and co-workers demonstrated that three different amyloidogenic peptides and proteins can form β-hairpins and determined the structures of these β-hairpins. In 2008, Härd, Hoyer, and co-workers elucidated the NMR structure of a β-hairpin formed by $A\beta_{40}$ by using an affibody to sequester and stabilize the β-hairpin (Figure 2A).[42] In this β-hairpin, residues 17–23 and 30–36 of Aβ hydrogen bond to form an antiparallel β-sheet, while the intervening residues, 24–29, form a loop (Figure 2B). The remaining *N*- and *C*- terminal residues are unstructured. Härd and co-workers also used affibodies to stabilize and determine the structures of β-hairpins formed by α-synuclein and IAPP.[43,44]

In further studies, Härd and co-workers investigated the biological, biophysical, and structural properties of oligomers formed by a covalently stabilized analogue of the Aβ β-hairpin that they previously reported.[45] In this analogue, Ala_{21} and Ala_{30} are mutated to cysteines to enable formation of a disulfide bridge (Figure 2C). Oligomers formed by this disulfide-stabilized Aβ β-hairpin mimicked some of the characteristics of oligomers of unmodified Aβ — morphology by transmission electron microscopy (TEM), assembly by size-exclusion chromatography (SEC) and SDS-PAGE, and cytotoxicity toward neuronally derived SH-SY5Y cells. These oligomers were also recognized by oligomer-specific antibodies used to recognize native Aβ oligomers isolated from the brains of Alzheimer's patients and transgenic mice. These findings are significant, because they demonstrate that conformationally stabilized β-hairpin monomers of Aβ can assemble to form oligomers that recapitulate the properties of biologically relevant Aβ oligomers. Solid-state NMR spectroscopy revealed that a disulfide-stabilized β-hairpin comprising $A\beta_{16–42}$ forms a barrel-shaped hexamer (Figure 2D). [49] In this oligomer, a hydrophobic core forms at one end of the assembly by the packing of hydrophobic residues from the central and *C*- terminal regions of Aβ. Intermolecular antiparallel β-sheets form between $A\beta_{34–36}$ and $A\beta_{39–42}$ at one end of the barrel; the β-hairpin loops of each monomer comprise the other

end of the barrel. This series of investigations of Aβ β-hairpins illustrates how stabilized β-hairpin peptides can be used to model and study the properties and structures of amyloid oligomers.[40,43,46]

Our laboratory has developed macrocyclic β-hairpin peptides as model systems to learn about the structure, and biological and biophysical properties of the oligomers formed by full-length amyloidogenic peptides and proteins. [50] The macrocyclic β-hairpin peptides consist of two peptide β-strands from the amyloidogenic peptide or protein that are constrained to a macrocycle by a δ-linked ornithine ($^{\delta}$Orn) turn unit and linked by a loop or a second $^{\delta}$Orn turn unit (Figure 2E–G).[51] An *N*-methyl group on one of the β-strands prevents uncontrolled aggregation, and thus facilitates oligomer formation. X-ray crystallographic studies of macrocyclic β-hairpin peptides derived from sequences such as Aβ, β_2-microglobulin, and α-synuclein have revealed the formation of dimers and trimers that further assemble to form tetramers, hexamers, octamers, nonamers, and dodecamers (Figure 3).[51–60] Wetzel and co-workers have developed β-hairpin model systems of polyglutamine derived peptides to better understand the role of polyglutamine folding and aggregation in Huntington's disease using D-Pro-Gly turn units and *N*-methyl amino acids. [61]

In our initial investigations of Aβ oligomers, we prepared and studied macrocyclic β-hairpin peptides derived from $A\beta_{17–36}$. In 2014, we reported a macrocyclic β-hairpin peptide containing $A\beta_{17–23}$ and $A\beta_{30–36}$.[51] X-ray crystallography revealed that this peptide assembles into trimers that further assemble to form a sandwich-like hexamer and a ball-shaped dodecamer (Figure 2E & Figure 3A–C). X-ray crystallographic studies of a homologous macrocyclic β-hairpin, incorporating the $A\beta_{24–29}$ loop, revealed that the peptide assembles to form trimers that further assemble into ball-shaped dodecamers, and five dodecamers further assemble to form an annular pore (Figure 2G & Figure 3G–I).[52] In subsequent studies, we covalently stabilized the trimers formed by the macrocyclic β-hairpin peptide containing $A\beta_{17–23}$ and $A\beta_{30–36}$ with disulfide-bridges (Figure 2H).[53] These covalently stabilized trimers assemble in solution, forming hexamers and dodecamers by SEC and SDS-PAGE. The covalent trimers are toxic to SH-SY5Y cells and are recognized by the amyloid oligomer-specific antibody All, suggesting that they may recapitulate the topology of Aβ oligomers occurring in the Alzheimer's brain.[62] X-ray crystallography revealed that the trimers form a hexamer, a dodecamer, and an annular pore comprising six dodecamers (Figure 3D–F). Recently, we found that incorporation of a cyclohexylalanine residue in place of a phenylalanine residue promotes folding of Aβ derived macrocyclic β-hairpins, further stabilizes trimers formed by the β-hairpins, and promotes formation of hexamers and dodecamers (Figure 3J–L).[56] We are now using antibodies generated against these synthetic Aβ oligomer mimics to probe biogenic Aβ oligomers from brain tissue.

We have also studied macrocyclic β-hairpin peptides derived from $A\beta_{16–36}$, in which the β-strands adopt a different alignment than the β-hairpin peptides derived from $A\beta_{17–36}$. These studies have revealed the assembly of toxic oligomers in both the crystal state and in solution, without the need for covalent stabilization through disulfide bridges.[57] A macrocyclic β-hairpin containing $A\beta_{16–22}$ and $A\beta_{30–36}$ assembles to form dimers and

trimers that further assemble into hexamers that can be observed in SDS-PAGE and by X-ray crystallography (Figure 2E & Figure 3M–O). A related macrocyclic β-hairpin peptide containing $A\beta_{16–22}$ and $A\beta_{30–36}$ assembles in the crystal state to form trimers that further assemble into a dodecamer (Figure 2E & Figure 3P–Q).[58]

Current efforts in our laboratory seek to incorporate more residues from full-length $A\beta_{40}$ or $A\beta_{42}$ into our macrocyclic β-hairpin model systems, to better reflect oligomers formed by full-length Aβ. We recently incorporated $A\beta_{1–14}$ as an *N*-terminally extended "tail" to the hexamer-forming macrocycle comprising $A\beta_{16–22}$ and $A\beta_{30–36}$ (Figure 2F). In studying a series of homologs bearing *N*-terminal tails, we found that residues from the *N*-terminus of Aβ do not disrupt oligomer assembly and likely form an unstructured tail (Figure 3R).[59] X-ray crystallographic studies of a macrocyclic β-hairpin peptide from $A\beta_{16–36}$ that incorporates the $A\beta_{23–29}$ loop revealed the assembly of parallel and antiparallel β-sheet dimers that further assemble to form a sandwich-like tetramer and a twisted β-sheet tetramer, with the latter packing to form an octamer (Figure 2G & Figure 3S–W).[60]

Collectively, our studies of β-hairpin peptides derived from $A\beta_{16–36}$, $A\beta_{17–36}$, and other amyloidogenic peptides and proteins have provided a multitude of distinct oligomer structures and revealed the unique ways in which β-hairpins can assemble to form compact oligomers stabilized by edge-to-edge hydrogen bonding and hydrophobic packing. Other laboratories have also reported various structures of Aβ fibrils, oligomers, and monomer formed by β-hairpins with different β-strand alignments.[32,40,45,46,59] We believe our structures reflect some of the immense variation and heterogeneity in the structures of endogenous amyloid oligomers, because many behave like oligomers of full-length amyloidogenic peptides and proteins in biological and biophysical experiments.

COMPUTATIONAL TOOLS FOR STUDYING AMYLOID OLIGOMERS AND FIBRILS

Molecular modeling can provide valuable insights into amyloid oligomer formation and structure by allowing the visualization, interpretation, and prediction of the conformations, motions, and interactions of the peptides and proteins involved.[4] These simulations allow observation of that which cannot be examined directly through experimentation and can complement experimental studies to provide deeper insights. For example, residues that had to be excluded from the peptide model systems to facilitate characterization by X-ray crystallography can be restored for study in molecular dynamics simulations. Okuno and co-workers thus used dissipative particle dynamics to restore $A\beta_{9–16}$ and $A\beta_{37–42}$ to a dodecamer-forming macrocyclic β-hairpin peptide comprising $A\beta_{17–36}$ (Figure 3H).[52,64] The simulations revealed that residues $A\beta_{37–42}$ can pack to form a stabilizing hydrophobic core in the central cavity of the dodecamer. Our laboratory has similarly made use of replica-exchange molecular dynamics simulations to probe whether residues absent from the design of our macrocyclic β-hairpin peptides can be accommodated by the structures of the oligomers that form.[51,55,57,60]

The protein force fields used in molecular dynamics and other forms of molecular modeling were not developed for amyloid oligomers and have limited ability to accurately model the

conformation, folding, and size of amyloidogenic peptides and proteins.[65–68] Shaw and coworkers used experimental NMR and SAXS data from amyloid oligomers to improve parameters for torsion angles, and protein and water van der Waals interactions, to produce a force field, a99SB-*disp*, that more accurately simulates disordered proteins such as $A\beta_{40}$. [69]

Improved algorithms for simulating the conformations of intrinsically disordered proteins and intrinsically disordered regions also promise to provide enhanced insights into amyloid oligomer formation. Recently, Petersson and co-workers reported the PyRosetta-based algorithms AbinitioVO and FastFloppyTail, which allow for the accurate prediction of protein structure across a wide array of folds and degrees of order.[70] We anticipate that improvements in force fields and algorithms for predicting conformational ensembles will cross-fertilize other studies that use peptide model systems and full-length peptides and proteins and thus contribute to a better understanding of amyloid oligomers.

Molecular docking simulations have guided the development of ligands that bind amyloid oligomers that may ultimately lead to new imaging probes or drugs for Alzheimer's disease or other amyloid diseases. Thus, X-ray crystallographic structures of trimers and hexamers formed by macrocyclic β-hairpin peptides comprising $A\beta_{17–36}$ (Figure 3A, B, D, G), have been used as targets for docking studies of triphenylmethane dyes, fluorescent probes, and therapeutic ligands for Aβ oligomers.[71–74] Docking simulations of the triphenylmethane dye, crystal violet, with the structure of our covalently-stabilized trimer derived from $A\beta_{17–36}$ (Figure 3D) produced a model for molecular recognition that guided structure-activity relationship studies. [71] Our laboratory is currently using the results of these computational and experimental studies to develop novel chemical probes for biogenic Aβ oligomers.

Computational tools are also valuable in identifying amyloidogenic regions of peptides and proteins by identifying features that drive aggregation and assembly, such as hydrophobicity, β-sheet character, a prevalence of aromatic residues, and low-charge content.[75] A number of algorithms, computational tools, and databases have been developed to assess these characteristics for a given peptide or protein sequence.[76] Tools such as TANGO, WALTZ-DB 2.0, and Cordax assess and quantify the aggregation potential of a given sequence.[77–79] Results from this type of primary sequence analysis can supplement and direct structure activity relationship studies of amyloid fibrils and oligomers.[36–38,75] These tools further our understanding of the ever-growing "amyloidome," which extends beyond disease and underlies many normal cellular, bacterial, and fungal processes.[81]

CONCLUSION

The amyloid state of peptides and proteins is an active and fascinating frontier of peptide and protein science for chemical and structural biologists alike. The ever-growing ties between amyloidogenic peptides and proteins and cellular function and disease inspires curiosity, and the resistance of these peptides and proteins to characterization using conventional techniques and tools drives innovation. Until the high-resolution observation of oligomers of full-length amyloidogenic peptides and proteins becomes widely feasible,

peptide model systems that approximate and mimic endogenous oligomers will remain one of the best tools for dissecting their structural, biological, and biophysical properties. The growing understanding of amyloid oligomers provided by these studies will further our knowledge of amyloid diseases and bolster efforts to develop diagnostics and drugs.

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REFRENCES

1. Knowles TPJ, Vendruscolo M, Dobson CM: The amyloid state and its association with protein misfolding diseases. Nat Rev Mol Cell Biol 2014, 15:384–396. [PubMed: 24854788]

2. Chiti F, Dobson CM: Protein misfolding, amyloid formation, and human disease: A summary of progress over the last decade. Annu Rev Biochem 2017, 86:27–68. [PubMed: 28498720]

3**. Ke PC, Zhou R, Serpell LC, Riek R, Knowles TPJ, Lashuel HA, Gazit E, Hamley IW, Davis TP, Fändrich M, et al. : Half a century of amyloids: Past, present and future. Chem Soc Rev 2020, 49:5473–5509. [PubMed: 32632432] This review highlights investigations of pathological, functional, and artificial amyloids in disease, structural biology, microbiology, and engineering. Significant attention is given to structural and biophysical characterization of amyloid fibrils and their assembly.

4**. Nguyen PH, Ramamoorthy A, Sahoo BR, Zheng J, Faller P, Straub JE, Dominguez L, Shea J-E, Dokholyan NV, Simone A De, et al. : Amyloid Oligomers: A Joint Experimental/Computational Perspective on Alzheimer's Disease, Parkinson's Disease, Type II Diabetes and Amyotrophic Lateral Sclerosis. Chem Rev 2021, doi:10.1021/acs.chemrev.0c01122.This review examines *in vitro*, *in vivo*, computational, and pharmacological studies of oligomers formed by Aβ, tau, α-synuclein, IAPP, and superoxide dismutase 1 in Alzheimer's disease, Parkinson's disease, type II diabetes, and amyotrophic lateral sclerosis. The authors highlight how empirical observations of amyloid oligomers have led to and supplemented computational studies of oligomer formation and interactions.

5. Eisenberg D, Jucker M: The amyloid state of proteins in human diseases. Cell 2012, 148:1188–1203. [PubMed: 22424229]

6. Iadanza MG, Jackson MP, Hewitt EW, Ranson NA, Radford SE: A new era for understanding amyloid structures and disease. Nat Rev Mol Cell Biol 2018, 19:755–773. [PubMed: 30237470]

7. Dobson CM, Knowles TPJ, Vendruscolo M: The amyloid phenomenon and its significance in biology and medicine. Cold Spring Harb Perspect Biol 2020, 12:pii: a033878. [PubMed: 30936117]

8. Ruberg FL, Berk JL: Transthyretin (TTR) cardiac amyloidosis. Circulation 2012, 126:1286–1300. [PubMed: 22949539]

9. Li D, Liu C: Structural diversity of amyloid fibrils and advances in their structure determination. Biochemistry 2020, 59:639–646. [PubMed: 31967790]

10. Fitzpatrick AW, Saibil HR: Cryo-EM of amyloid fibrils and cellular aggregates. Curr Opin Struct Biol 2019, 58:34–42. [PubMed: 31200186]

11. Lu JX, Qiang W, Yau WM, Schwieters CD, Meredith SC, Tycko R: Molecular structure of β-amyloid fibrils in Alzheimer's disease brain tissue. Cell 2013, 154:1257–1268. [PubMed: 24034249]

12. Eisenberg DS, Sawaya MR: Structural studies of amyloid proteins at the molecular level. Annu Rev Biochem 2017, 86:69–95. [PubMed: 28125289]

13. Yakupova EI, Bobyleva LG, Vikhlyantsev IM, Bobylev AG: Congo Red and amyloids: History and relationship. Biosci Rep 2019, 39:BSR20181415. [PubMed: 30567726]

14. Xue C, Lin TY, Chang D, Guo Z: Thioflavin T as an amyloid dye: Fibril quantification, optimal concentration and effect on aggregation. R Soc Open Sci 2017, 4:160696. [PubMed: 28280572]

15. Kollmer M, Close W, Funk L, Rasmussen J, Bsoul A, Schierhorn A, Schmidt M, Sigurdson CJ, Jucker M, Fändrich M: Cryo-EM structure and polymorphism of Aβ amyloid fibrils purified from Alzheimer's brain tissue. Nat Commun 2019, 10:1–8. [PubMed: 30602773]

16. Röder C, Kupreichyk T, Gremer L, Schäfer LU, Pothula KR, Ravelli RBG, Willbold D, Hoyer W, Schröder GF: Cryo-EM structure of islet amyloid polypeptide fibrils reveals similarities with amyloid-β fibrils. Nat Struct Mol Biol 2020, 27:660–667. [PubMed: 32541895]

17. Cao Q, Boyer DR, Sawaya MR, Ge P, Eisenberg DS: Cryo-EM structures of four polymorphic TDP-43 amyloid cores. Nat Struct Mol Biol 2019, 26:619–627. [PubMed: 31235914]

18. Wang LQ, Zhao K, Yuan HY, Wang Q, Guan Z, Tao J, Li XN, Sun Y, Yi CW, Chen J, et al. : Cryo-EM structure of an amyloid fibril formed by full-length human prion protein. Nat Struct Mol Biol 2020, 27:598–602. [PubMed: 32514176]

19. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K: Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci USA 1985, 82:4245–4249. [PubMed: 3159021]

20. Selkoe DJ, Abraham CR, Podlisny MB, Duffy LK: Isolation of low-molecular-weight proteins from amyloid plaque fibers in Alzheimer's disease. J Neurochem 1986, 46:1820–1834. [PubMed: 3517233]

21. Burdick D, Soreghan B, Kwon M, Kosmoski J, Knauer M, Henschen A, Yates J, Cotman C, Glabe C: Assembly and aggregation properties of synthetic Alzheimer's A4/β amyloid peptide analogs. J Biol Chem 1992, 267:546–554. [PubMed: 1730616]

22. Podlisny MB, Ostaszewski BL, Squazzo SL, Koo EH, Rydell RE, Teplow DB, Selkoe DJ: Aggregation of secreted amyloid β-protein into sodium dodecyl sulfate-stable oligomers in cell culture. J Biol Chem 1995, 270:9564–9570. [PubMed: 7721886]

23. Podlisny MB, Walsh DM, Amarante P, Ostaszewski BL, Stimson ER, Maggio JE, Teplow DB, Selkoe DJ: Oligomerization of endogenous and synthetic amyloid β-protein at nanomolar levels in cell culture and stabilization of monomer by Congo red. Biochemistry 1998, 37:3602–3611. [PubMed: 9521679]

24. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, et al. : Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. Proc Natl Acad Sci USA 1998, 95:6448–6453. [PubMed: 9600986]

25*. Cline EN, Bicca MA, Viola KL, Klein WL: The amyloid-β oligomer hypothesis: Beginning of the third decade. J Alzheimer's Dis 2018, 64:S567–S610. [PubMed: 29843241] This review summarizes evidence for the roles of Aβ oligomers in neurodegeneration and synaptotoxicity in Alzheimer's disease. The authors highlight *in vitro*, *in vivo*, and clinical evidence that points to Aβ oligomers as the damaging species in Alzheimer's disease.

26. Ferreira ST, Lourenco MV, Oliveira MM, De Felice FG: Soluble amyloid-β oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. Front Cell Neurosci 2015, 9:1–17. [PubMed: 25667569]

27. Benilova I, Karran E, De Strooper B: The toxic Aβ oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci 2012,15:349–357. [PubMed: 22286176]

28. Selkoe DJ, Hardy J: The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med 2016, 8:595–608. [PubMed: 27025652]

29. Kulenkampff K, Perez MW, Sormanni P, Habchi J, Vendruscolo M: Quantifying misfolded protein oligomers as drug targets and biomarkers in Alzheimer and Parkinson diseases. Nat Rev Chem 2021, doi:10.1038/s41570-021-00254-9.

30. Bengoa-Vergniory N, Roberts RF, Wade-Martins R, Alegre-Abarrategui J: Alpha-synuclein oligomers: A new hope. Acta Neuropathol 2017, 134:819–838. [PubMed: 28803412]

31. Hoffner G, Djian P: Monomeric, oligomeric and polymeric proteins in huntington disease and other diseases of polyglutamine expansion. Brain Sci 2014, 4:91–122. [PubMed: 24961702]

32. Jeong HR, An SSA: Causative factors for formation of toxic islet amyloid polypeptide oligomer in type 2 diabetes mellitus. Clin Interv Aging 2015, 10:1873–1879. [PubMed: 26604727]

33. Shafiei SS, Guerrero-Muñoz MJ, Castillo-Carranza DL: Tau oligomers: Cytotoxicity, propagation, and mitochondrial damage. Front Aging Neurosci 2017, 9:1–9. [PubMed: 28174533]

34**. Ciudad S, Puig E, Botzanowski T, Meigooni M, Arango AS, Do J, Mayzel M, Bayoumi M, Chaignepain S, Maglia G, et al. : Aβ(l-42) tetramer and octamer structures reveal edge conductivity pores as a mechanism for membrane damage. Nat Commun 2020, 11:1–14. [PubMed: 31911652] This paper reports the first atomic-resolution structure of a tetramer formed by full-length Aβ42 and provides additional evidence for the formation of an octamer. The structure of the tetramer is the only structure of an oligomer of full-length Aβ that has been deposited in the Protein Data Bank (PDB).

35. Nelson R, Sawaya MR, Balbirnie M, Madsen AØ, Riekel C, Grothe R, Eisenberg D: Structure of the cross-β spine of amyloid-like fibrils. Nature 2005, 435:773–778. [PubMed: 15944695]

36. Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbimie M, Wiltzius JJW, Mcfarlane HT, et al. : Atomic structures of amyloid cross-β-spines reveal varied steric zippers. Nature 2007, 447:453–457. [PubMed: 17468747]

37. Hughes MP, Sawaya MR, Boyer DR, Goldschmidt L, Rodriguez JA, Cascio D, Chong L, Gonen T, Eisenberg DS: Atomic structures of low-complexity protein segments reveal kinked β sheets that assemble networks. Science 2018, 359:698–701. [PubMed: 29439243]

38. Oligomer S, Laganowsky A, Liu C, Sawaya MR, Whitelegge JP, Park J, Zhao M, Pensalfini A, Soriaga AB, Landau M, et al. : Atomic view of a toxic amyloid. Science 2012, 191:1228–1232.

39*. Sangwan S, Zhao A, Adams KL, Jayson CK, Sawaya MR, Guenther EL, Eisenberg DS: Atomic structure of a toxic, oligomeric segment of SOD1 linked to amyotrophic lateral sclerosis (ALS). Proc Natl Acad Sci USA 2017,114:8770–8775. [PubMed: 28760994] This paper reports the structure, biophysical, and biological properties of a corkscrew-like oligomer formed by an eleven-residue peptide fragment derived from SOD1.

40. Sangwan S, Sawaya MR, Murray KA, Hughes MP, Eisenberg DS: Atomic structures of corkscrew-forming segments of SOD1 reveal varied oligomer conformations. Protein Sci 2018, 27:1231–1242. [PubMed: 29453800]

41. Apostol MI, Perry K, Surewicz WK: Crystal structure of a human prion protein fragment reveals a motif for oligomer formation. J Am Chem Soc 2013, 135:10202–10205. [PubMed: 23808589]

42. Hoyer W, Grönwall C, Jonsson A, Ståhl S, Härd T: Stabilization of a β-hairpin in monomeric Alzheimer's amyloid-β peptide inhibits amyloid formation. Proc Natl Acad Sci USA 2008, 105:5099–5104. [PubMed: 18375754]

43. Mirecka EA, Shaykhalishahi H, Gauhar A, Akgül Ş, Lecher J, Willbold D, Stoldt M, Hoyer W: Sequestration of a β-hairpin for control of α-synuclein aggregation. Angew Chemie-IntEd 2014, 53:4227–4230.

44. Mirecka EA, Feuerstein S, Gremer L, Schröder GF, Stoldt M, Willbold D, Hoyer W: β-Hairpin of islet amyloid polypeptide bound to an aggregation inhibitor. Sci Rep 2016, 6:33474. [PubMed: 27641459]

45. Sandberg A, Luheshi LM, Söllvander S, Pereira de Barros T, Macao B, Knowles TPJ, Biverstål H, Lendel C, Ekholm-Petterson F, Dubnovitsky A, et al. : Stabilization of neurotoxic Alzheimer amyloid-β oligomers by protein engineering. Proc Natl Acad Sci USA 2010, 107:15595–15600. [PubMed: 20713699]

46. Renaud JP, Chari A, Ciferri C, Liu WT, Rémigy HW, Stark H, Wiesmann C: Cryo-EM in drug discovery: Achievements, limitations and prospects. Nat Rev Drug Discov 2018, 17:471–492. [PubMed: 29880918]

47. Ragonis-Bachar P, Landau M: Functional and pathological amyloid structures in the eyes of 2020 cryo-EM. Curr Opin Struct Biol 2021, 68:184–193. [PubMed: 33631463]

48. Yu L, Edalji R, Harlan JE, Holzman TF, Lopez AP, Labkovsky B, Hillen H, Barghorn S, Ebert U, Richardson PL, et al. : Structural characterization of a soluble amyloid β-peptide oligomer. Biochemistry 2009, 48:1870–1877. [PubMed: 19216516]

49. Lendel C, Bjerring M, Dubnovitsky A, Kelly RT, Filippov A, Antzutkin ON, Nielsen NC, Härd T: A hexameric peptide barrel as building block of amyloid-β protofibrils. Angew Chemie - Int Ed 2014, 53:12756–12760.

50**. Kreutzer AG, Nowick JS: Elucidating the structures of amyloid oligomers with macrocyclic β-hairpin peptides: Insights into Alzheimer's disease and other amyloid diseases. Acc Chem Res 2018, 51:706–718. [PubMed: 29508987] This review highlights the use of macrocyclic β-hairpin peptides by the Nowick laboratory to mimic and study the structures, and biophysical and biological properties of oligomers formed by amyloidogenic peptides and proteins. X-ray crystallographic studies of these macrocyclic β-hairpin peptides have revealed the formation of dimers and trimers that further assemble to form tetramers, hexamers, octamers, nonamers, and dodecamers.

51. Spencer RK, Li H, Nowick JS: X-ray crystallographic structures of trimers and higher-order oligomeric assemblies of a peptide derived from $A\beta_{17–36}$. J Am Chem Soc 2014, 136:5595–5598. [PubMed: 24669800]

52. Kreutzer AG, Hamza IL, Spencer RK, Nowick JS: X-ray crystallographic structures of a trimer, dodecamer, and annular pore formed by an $A\beta_{17–36}$ β-hairpin. J Am Chem Soc 2016, 138:4634–4642. [PubMed: 26967810]

53. Kreutzer AG, Yoo S, Spencer RK, Nowick JS: Stabilization, assembly, and toxicity of trimers derived from Aβ. J Am Chem Soc 2017, 139:966–975. [PubMed: 28001392]

54. Spencer RK, Kreutzer AG, Salveson PJ, Li H, Nowick JS: X-ray crystallographic structures of oliogmers of peptides derived from β_2-microglobulin. J Am Chem Soc 2015, 137:6304–6311. [PubMed: 25915729]

55. Salveson PJ, Spencer RK, Nowick JS: X-ray crystallographic structure of oligomers formed by a toxic β-hairpin derived from α-synuclein: trimers and higher-order oligomers. J Am Chem Soc 2016,138:4458–4467. [PubMed: 26926877]

56*. Haerianardakani S, Kreutzer AG, Salveson PJ, Samdin TD, Guaglianone GE, Nowick JS: Phenylalanine mutation to cyclohexylalanine facilitates triangular trimer formation by β-hairpins derived from Aβ. J Am Chem Soc 2020, 142:20708–20716. [PubMed: 33237748] This paper reports the X-ray crystallographic structures and solution phase behavior of trimers, hexamers, and dodecamers formed by macrocyclic β-hairpin peptides derived from $A\beta_{17–36}$. Substitution of cyclohexylalanine for phenylalanine facilitates the formation of trimers and their covalent stabilization through disulfide crosslinks.

57. Kreutzer AG, Spencer RK, McKnelly KJ, Yoo S, Hamza IL, Salveson PJ, Nowick JS: A hexamer of a peptide derived from $A\beta_{16–36}$. Biochemistry 2017, 56:6061–6071. [PubMed: 29028351]

58. Salveson PJ, Spencer RK, Kreutzer AG, Nowick JS: X-ray crystallographic structure of a compact dodecamer from a peptide derived from $A\beta_{16–36}$. Org Lett 2017, 19:3462–3465. [PubMed: 28683555]

59*. Samdin TD, Wierzbicki M, Kreutzer AG, Howitz WJ, Valenzuela M, Smith A, Sahrai V, Truex NL, Klun M, Nowick JS: Effects of N-terminal residues on the assembly of constrained β-hairpin peptides derived from Aβ. J Am Chem Soc 2020, 142:11593–11601. [PubMed: 32501687] This paper reports the incorporation of Aβ residues 1–14 as an *N*-terminal "tail" appended to a macrocyclic β-hairpin peptide derived from $A\beta_{16–36}$ that forms a hexamer. The tailed-macrocyclic β-hairpin peptides are synthesized using an orthogonal protecting group strategy that allows incorporation of the *N*-terminal residues, and are characterized by SDS-PAGE and X-ray crystallography.

60. Kreutzer AG, Samdin TD, Guaglianone G, Spencer RK, Nowick JS: X-ray crystallography reveals parallel and antiparallel β-sheet dimers of a β-hairpin derived from Aβ16–36 that assemble to form different tetramers. ACS Chem Neurosci 2020, 11:2340–2347. [PubMed: 32584538]

61. Wetzel R: Exploding the repeat length paradigm while exploring amyloid toxicity in Huntington's disease. Acc Chem Res 2020, 53:2347–2357. [PubMed: 32975927]

62. Kayed R, Head E, Thompson JL, Mcintire TM, Milton SC, Cotman CW, Glabe CG: Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 2003, 300:486–490. [PubMed: 12702875]

63. Ghosh U, Thurber KR, Yau W-M, Tycko R: Molecular structure of a prevalent amyloid-β fibril polymorph from Alzheimer's disease brain tissue. Proc Natl Acad Sci USA 2021, 118:e2023089118. [PubMed: 33431654]

64. Kawai R, Chiba S, Okuwaki K, Kanada R, Doi H, Ono M, Mochizuki Y, Okuno Y: Stabilization mechanism for a nonfibrillar amyloid β oligomer based on formation of a hydrophobic core

determined by dissipative particle dynamics. ACS Chem Neurosci 2020, 11:385–394. [PubMed: 31899612]

65. Piana S, Donchev AG, Robustelli P, Shaw DE: Water dispersion interactions strongly influence simulated structural properties of disordered protein states. J Phys Chem B 2015, 119:5113–5123. [PubMed: 25764013]
66. Man VH, Nguyen PH, Derreumaux P: High-resolution structures of the amyloid-β 1–42 dimers from the comparison of four atomistic force fields. J Phys Chem B 2017, 121:5977–5987. [PubMed: 28538095]
67. Mehrazma B, Rauk A: Exploring amyloid-β dimer structure using molecular dynamics simulations. J Phys Chem A 2019, 123:4658–4670. [PubMed: 31082235]
68. Strodel B: Amyloid aggregation simulations: challenges, advances and perspectives. Curr Opin Struct Biol 2021, 67:145–152. [PubMed: 33279865]
69*. Robustelli P, Piana S, Shaw DE: Developing a molecular dynamics force field for both folded and disordered protein states. Proc Natl Acad Sci USA 2018, 115:E4758–E4766. [PubMed: 29735687] This paper describes the development of the a99SB-*disp* force field, which overcomes limitations of current protein force fields in accurately modeling amyloidogenic peptides and proteins in molecular dynamics simulations.
70. Ferrie JJ, Petersson EJ: A unified de novo approach for predicting the structures of ordered and disordered proteins. J Phys Chem B 2020, 124:5538–5548. [PubMed: 32525675]
71*. Salveson PJ, Haerianardakani S, Thuy-boun A, Yoo S, Kreutzer AG, Demeler B, Nowick JS: Repurposing triphenylmethane dyes to bind to trimers derived from Aβ. J Am Chem Soc 2018, 140:11745–11754. [PubMed: 30125493] This paper reports the development of triphenylmethane dyes as ligands for trimers derived from Aβ. Detailed studies of the interactions of crystal violet and other triphenylmethane dyes with *C*3 symmetric trimers derived from $A\beta_{17–36}$ are described.
72. Lv G, Sun A, Wei P, Zhang N, Yi T: A spiropyran-based fluorescent probe for the specific detection of β-amyloid peptide oligomers in Alzheimer's disease. Chem Sci 2016, 52:8865–8868.
73. Teoh CL, Su D, Sahu S, Yun S, Drummond E: Chemical fluorescent probe for detection of Aβ oligomers. J Am Chem Soc 2015,137:13503–13509. [PubMed: 26218347]
74. Liu H, Qian C, Yang T, Wang Y, Luo J, Zhang C, Wang X, Wang X, Guo Z: Small molecule-mediated co-assembly of amyloid-β oligomers reduces neurotoxicity through promoting non-fibrillar aggregation. Chem Sci 2020,11:7158–7169. [PubMed: 34123000]
75. Meric G, Robinson AS, Roberts CJ: Driving forces for nonnative protein aggregation and approaches to predict aggregation-prone regions. Annu Rev Chem Biomol Eng 2017, 8:139–159. [PubMed: 28592179]
76. Ebo JS, Guthertz N, Radford SE, Brockwell DJ: Using protein engineering to understand and modulate aggregation. Curr Opin Struct Biol 2020, 60:157–166. [PubMed: 32087409]
77. Femandez-Escamilla AM, Rousseau F, Schymkowitz J, Serrano L: Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. Nat Biotechnol 2004, 22:1302–1306. [PubMed: 15361882]
78. Louros N, Konstantoulea K, Vleeschouwer M De, Ramakers M, Schymkowitz J, Rousseau F: WALTZ-DB 2.0: an updated database containing structural information of experimentally determined amyloid-forming peptides. Nucleic Acids Res 2020, 48:D389–D393. [PubMed: 31504823]
79. Louros N, Orlando G, De Vleeschouwer M, Rousseau F, Schymkowitz J: Structure-based machine-guided mapping of amyloid sequence space reveals uncharted sequence clusters with higher solubilities. Nat Commun 2020, 11:1–13. [PubMed: 31911652]
80. Howitz WJ, Wierzbicki M, Cabanela RW, Saliba C, Motavalli A, Tran N, Nowick JS: Interpenetrating cubes in the X-ray crystallographic structure of a peptide derived from $medin_{19–36}$. J Am Chem Soc 2020,142:15870–15875. [PubMed: 32816461]
81. Otzen D, Riek R: Functional amyloids. Cold Spring Harb Perspect Biol 2019, 11:pii: a033860. [PubMed: 31088827]

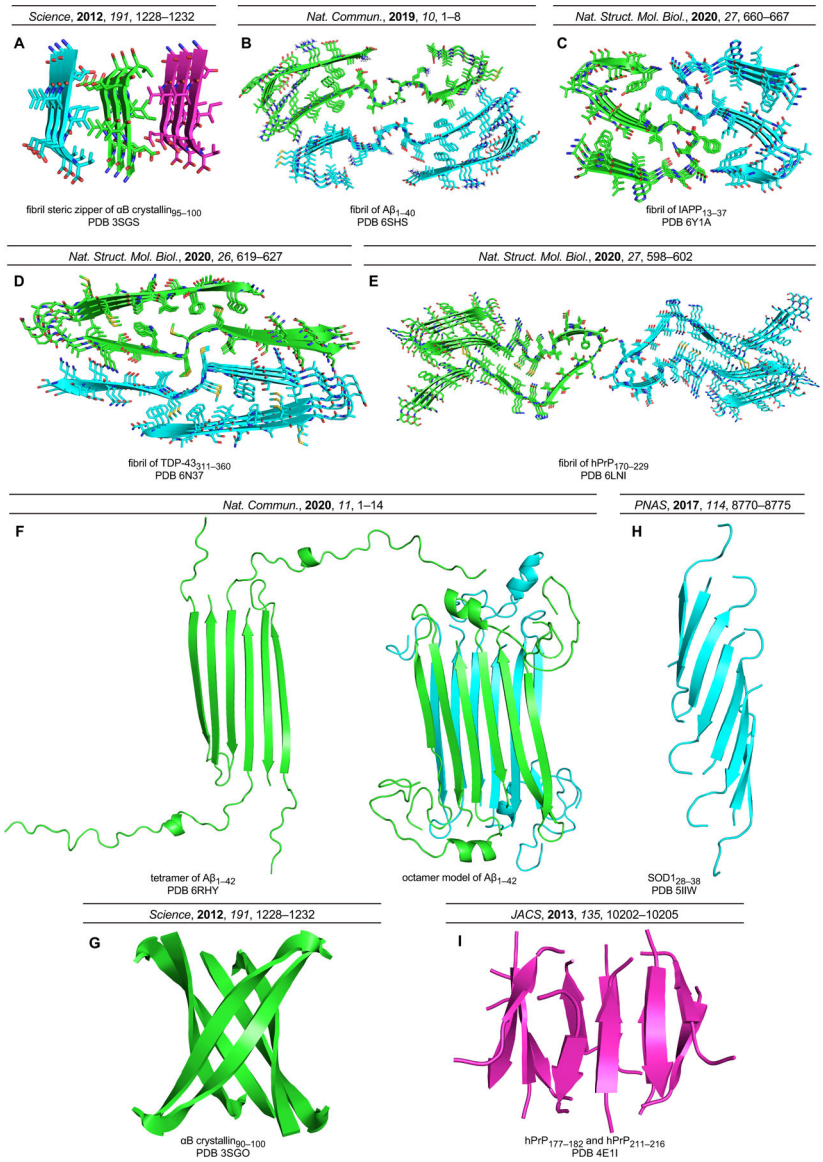

Figure 1.

Structures of fibrils and oligomers formed by amyloidogenic peptides. **A.** Fibril-like assembly of αB crystallin$_{95–100}$; X-ray crystallographic structure. **B-E.** Fibril-like assemblies of Aβ$_{1–40}$, IAPP$_{13–37}$, TDP-43$_{311–360}$, and hPRP$_{170–229}$; Cryo-EM structures. **F.** Tetramer and octamer formed by Aβ$_{1–42}$; NMR structure and NMR-based model. **G-I.** Oligomers of αB crystallin$_{90–100}$, SOD1$_{28–38}$, and hPRP$_{177–182}$ crosslinked with hPRP$_{211–216}$; X-ray crystallographic structures.

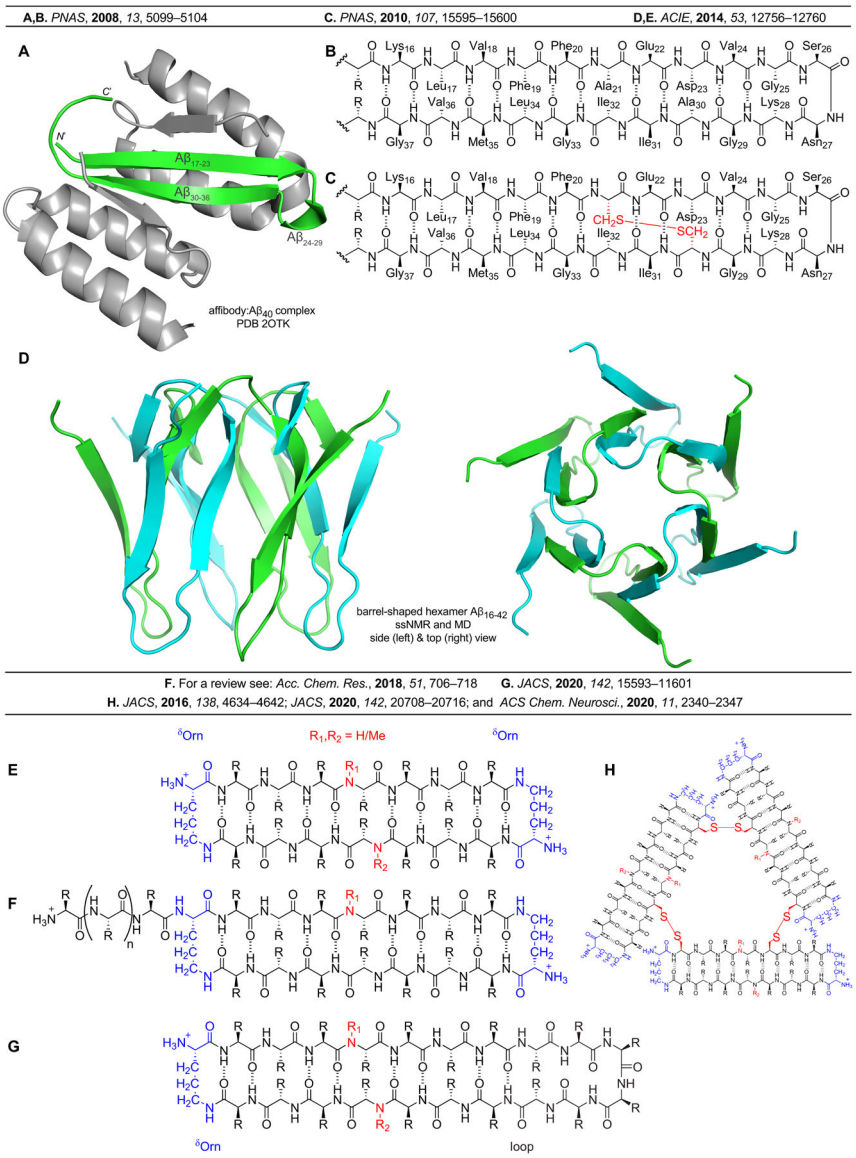

Figure 2.

A. NMR structure of an $A\beta_{40}$ β-hairpin stabilized by an affibody. **B.** Alignment of the $A\beta_{40}$ β-hairpin. **C.** Disulfide stabilization of the $A\beta_{40}$ β-hairpin. **D.** NMR-based model of a barrel-shaped hexamer formed by a disulfide stabilized $A\beta_{16–40}$ β-hairpin. **E-H.** Macrocyclic β-hairpins and disulfide-stabilized β-hairpins derived from amyloidogenic peptides and proteins.

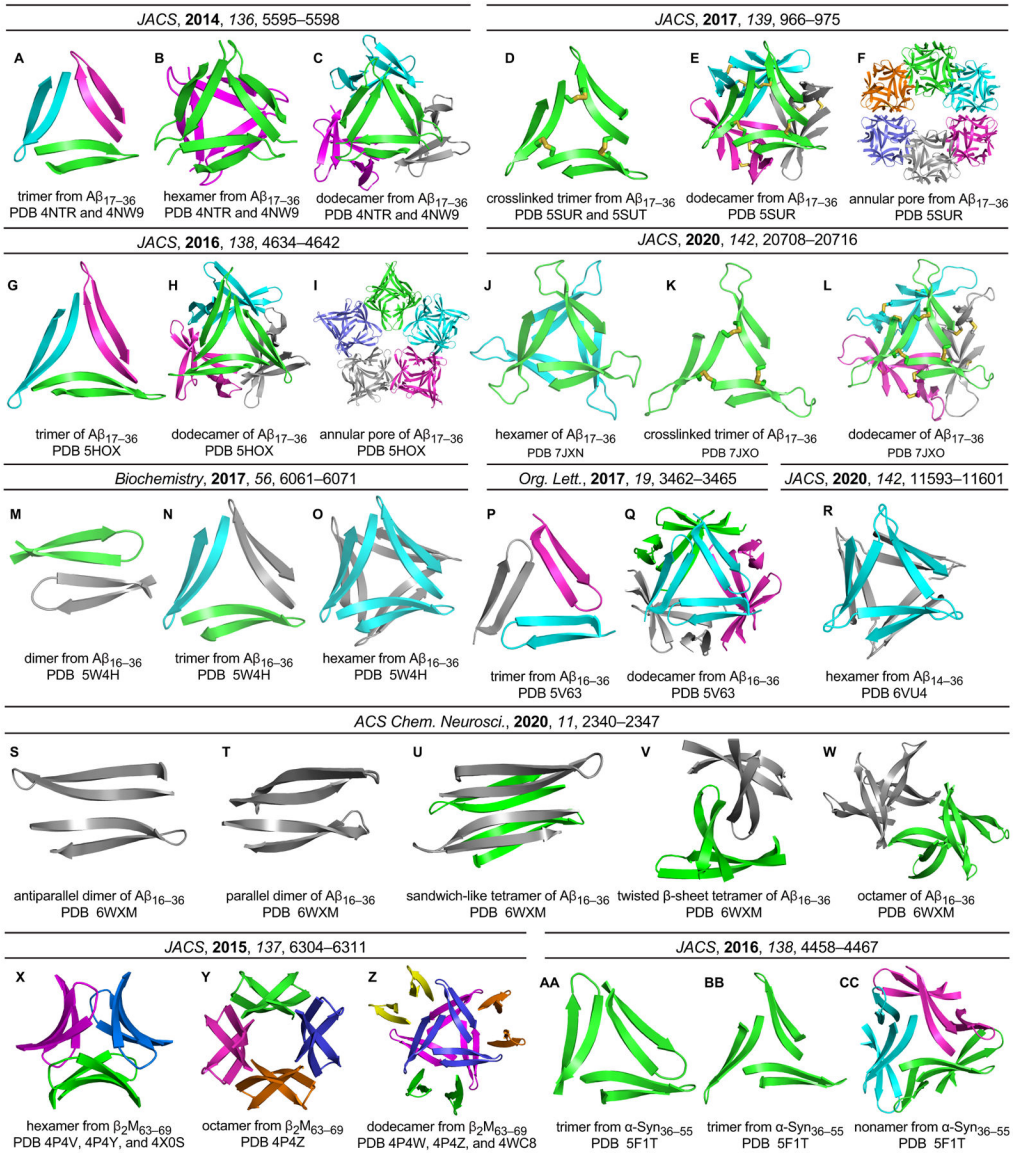

Figure 3.

X-ray crystallographic structures of oligomers formed by macrocyclic β-hairpin peptides derived from Aβ, β_2-microglobulin, and α-synuclein. **A-L.** Trimers, hexamers, dodecamers, and annular pores formed by macrocyclic β-hairpin peptides derived from $A\beta_{17–36}$. **M-W.** Dimers, trimers, tetramers, hexamers, octamer, and dodecamer derived from $A\beta_{16–36}$. **X-Z.** Hexamer, octamer, and dodecamer derived from β_2-microglobulin. **AA-CC.** Trimers and nonamer derived from α-synuclein.