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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 ($A\beta$), 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, $A\beta$, in addition to insoluble fibrils. The formation of these $A\beta$ 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 A β 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 β_{42} has been reported thus far (Figure 1F).[34] Carulla and co-workers reported the NMR-based structure of an A β_{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 β_{42} surrounding two antiparallel β -strands of A β_{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 A β . 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 α B crystallin and superoxide dismutase 1 (SOD1) (Figure 1G, H).[38–40] The α B 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 β 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 A β 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 A β β -hairpin that they previously reported.[45] In this analogue, Ala₂₁ and Ala₃₀ are mutated to cysteines to enable formation of a disulfide bridge (Figure 2C). Oligomers formed by this disulfide-stabilized A β β -hairpin mimicked some of the characteristics of oligomers of unmodified A β — 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 A β 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 A β can assemble to form oligomers that recapitulate the properties of biologically relevant A β oligomers. Solid-state NMR spectroscopy revealed that a disulfide-stabilized β -hairpin comprising A β _{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 A β . Intermolecular antiparallel β -sheets form between A β _{34–36} and A β _{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 A β 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 (δ Orn) turn unit and linked by a loop or a second δ 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 A β , 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 A β oligomers, we prepared and studied macrocyclic β -hairpin peptides derived from A β_{17-36} . In 2014, we reported a macrocyclic β -hairpin peptide containing A β_{17-23} and A β_{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 β_{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 β_{17-23} and A β_{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 A β 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 A β 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 A β oligomer mimics to probe biogenic A β oligomers from brain tissue.

We have also studied macrocyclic β -hairpin peptides derived from A β_{16-36} , in which the β -strands adopt a different alignment than the β -hairpin peptides derived from A β_{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 β_{16-22} and A β_{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 $A\beta$. 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 $A\beta$ 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 $A\beta$ 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 β 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 β _{17–36} (Figure 3A, B, D, G), have been used as targets for docking studies of triphenylmethane dyes, fluorescent probes, and therapeutic ligands for A β oligomers.[71–74] Docking simulations of the triphenylmethane dye, crystal violet, with the structure of our covalently-stabilized trimer derived from A β _{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 A β 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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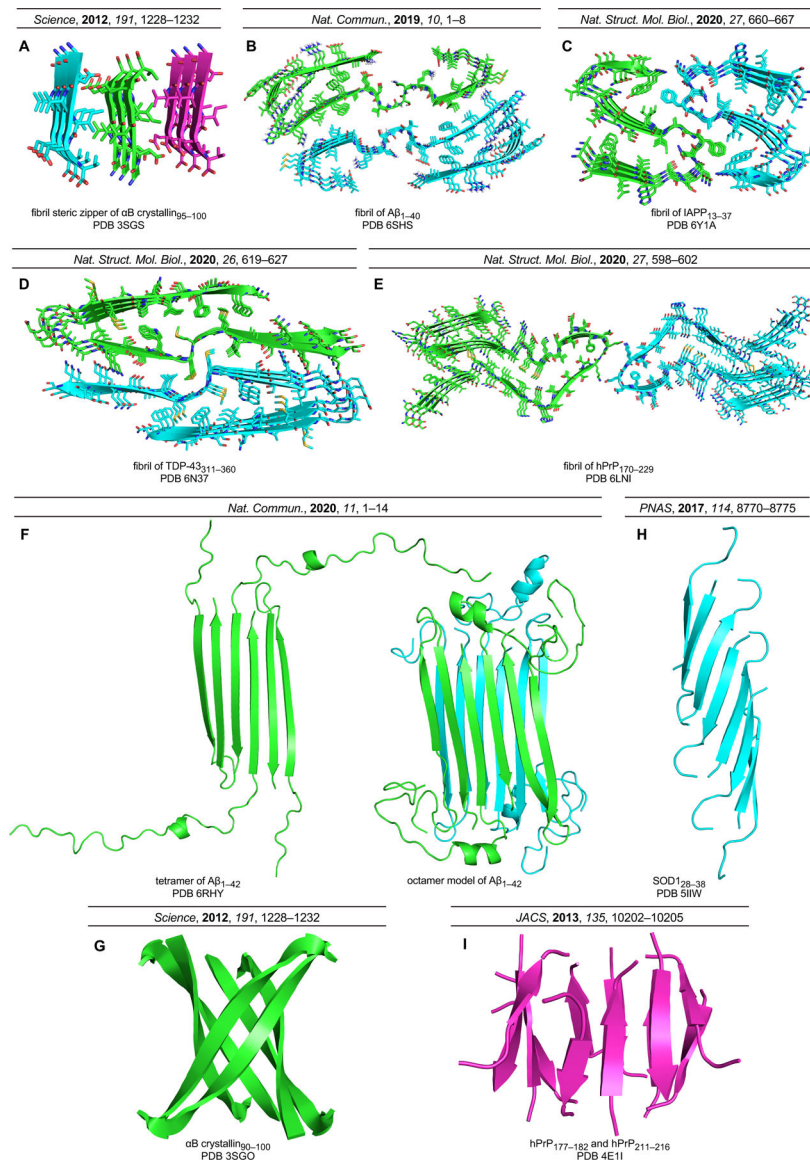
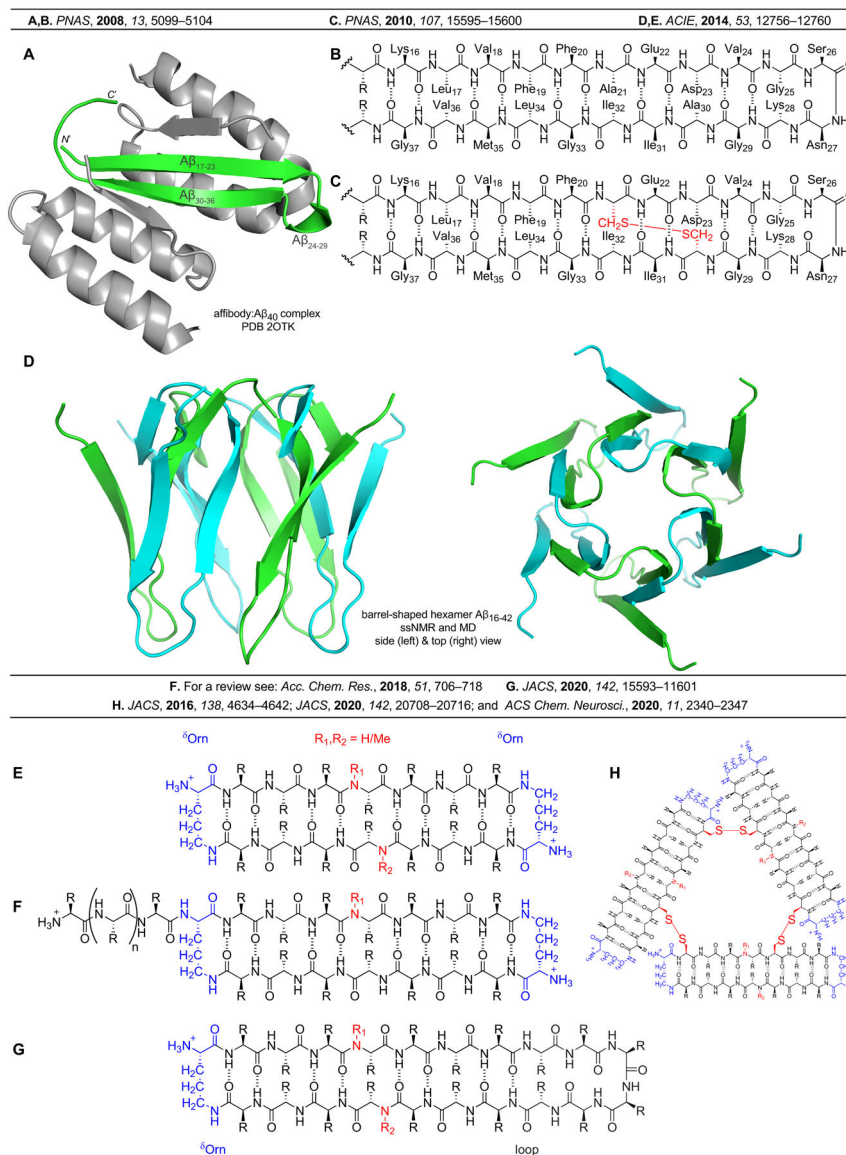
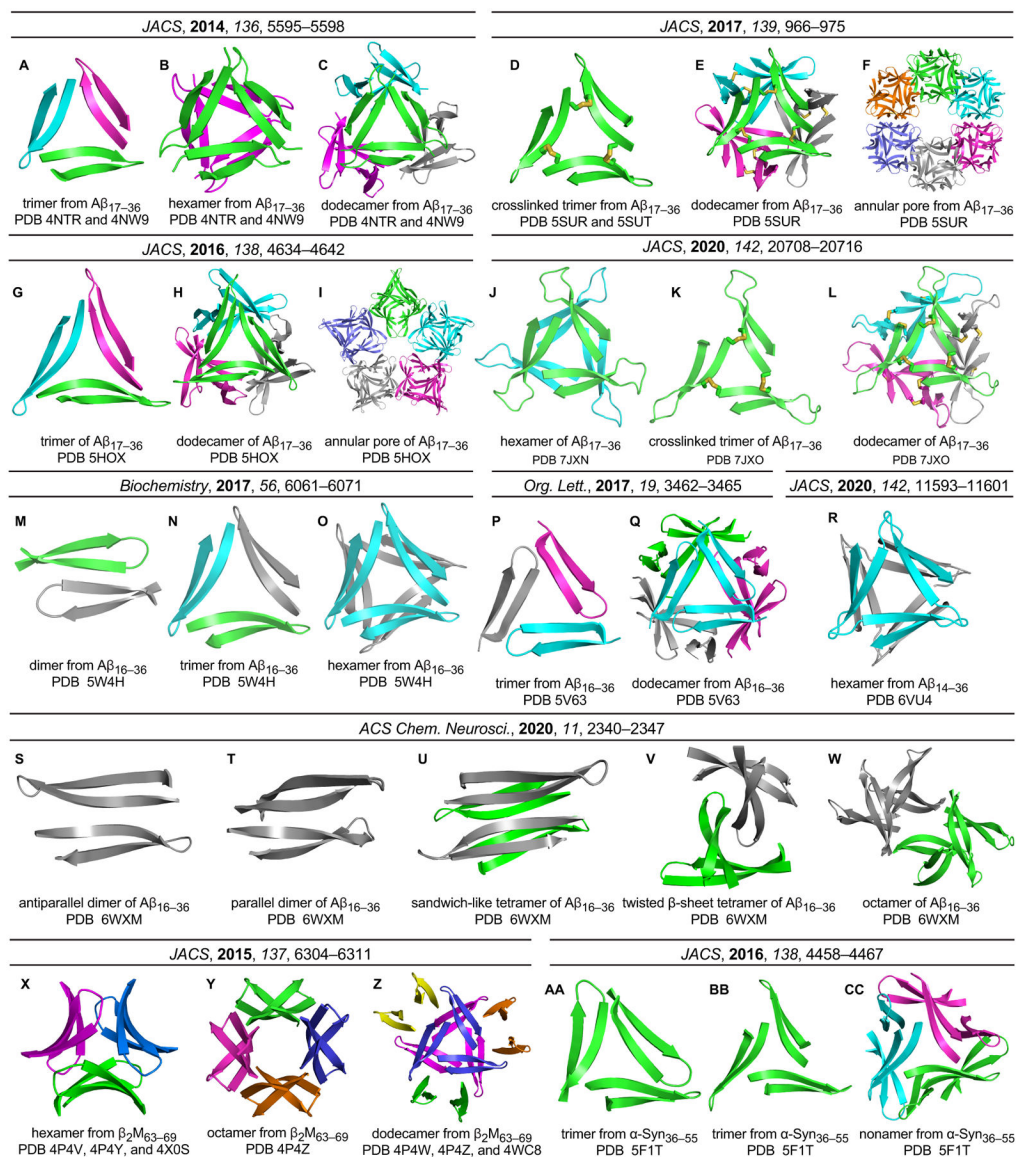


Figure 1. Structures of fibrils and oligomers formed by amyloidogenic peptides. **A.** Fibril-like assembly of α B crystallin₉₅₋₁₀₀; X-ray crystallographic structure. **B-E.** Fibril-like assemblies of $A\beta_{1-40}$, IAPP₁₃₋₃₇, TDP-43₃₁₁₋₃₆₀, and hPRP₁₇₀₋₂₂₉; Cryo-EM structures. **F.** Tetramer and octamer formed by $A\beta_{1-42}$; NMR structure and NMR-based model. **G-I.** Oligomers of α B crystallin₉₀₋₁₀₀, SOD₁₂₈₋₃₈, and hPRP₁₇₇₋₁₈₂ crosslinked with hPRP₂₁₁₋₂₁₆; X-ray crystallographic structures.

**Figure 2.**

A. NMR structure of an Aβ₄₀ β-hairpin stabilized by an affibody. **B.** Alignment of the Aβ₄₀ β-hairpin. **C.** Disulfide stabilization of the Aβ₄₀ β-hairpin. **D.** NMR-based model of a barrel-shaped hexamer formed by a disulfide stabilized Aβ₁₆₋₄₀ 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 A β , β_2 -microglobulin, and α -synuclein. **A-L.** Trimers, hexamers, dodecamers, and annular pores formed by macrocyclic β -hairpin peptides derived from A β_{17-36} . **M-W.** Dimers, trimers, tetramers, hexamers, octamer, and dodecamer derived from A β_{16-36} . **X-Z.** Hexamer, octamer, and dodecamer derived from β_2 -microglobulin. **AA-CC.** Trimers and nonamer derived from α -synuclein.