

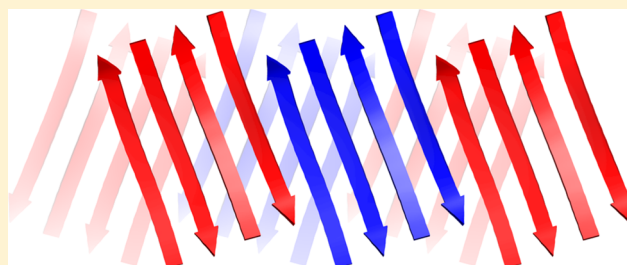
A Fibril-Like Assembly of Oligomers of a Peptide Derived from β -Amyloid

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S Supporting Information

ABSTRACT: A macrocyclic β -sheet peptide containing two nonapeptide segments based on $A\beta_{15-23}$ (QKLVFFAED) forms fibril-like assemblies of oligomers in the solid state. The X-ray crystallographic structure of macrocyclic β -sheet peptide **3** was determined at 1.75 Å resolution. The macrocycle forms hydrogen-bonded dimers, which further assemble along the fibril axis in a fashion resembling a herringbone pattern. The extended β -sheet comprising the dimers is laminated against a second layer of dimers through hydrophobic interactions to form a fibril-like assembly that runs the length of the crystal lattice. The second layer is offset by one monomer subunit, so that the fibril-like assembly is composed of partially overlapping dimers, rather than discrete tetramers. In aqueous solution, macrocyclic β -sheet **3** and homologues **4** and **5** form discrete tetramers, rather than extended fibril-like assemblies. The fibril-like assemblies of oligomers formed in the solid state by macrocyclic β -sheet **3** represent a new mode of supramolecular assembly not previously observed for the amyloidogenic central region of $A\beta$. The structures observed at atomic resolution for this peptide model system may offer insights into the structures of oligomers and oligomer assemblies formed by full-length $A\beta$ and may provide a window into the propagation and replication of amyloid oligomers.



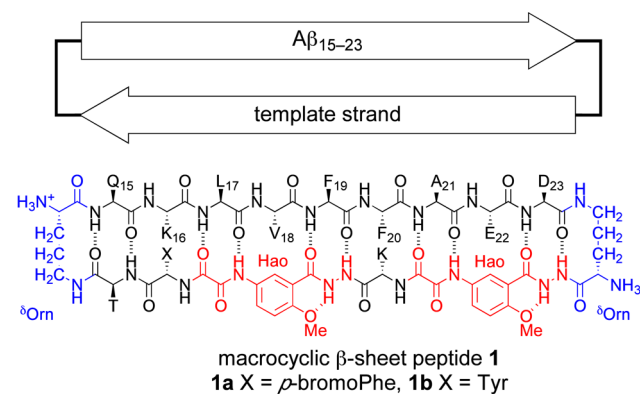
INTRODUCTION

The supramolecular assembly of the β -amyloid peptide $A\beta$ to form fibrils and soluble oligomers has been the subject of intense interest and study over the past two decades. The plaques formed by the 40–42 amino acid $A\beta$ polypeptide in the brain are one of the most distinctive physiological features of Alzheimer's disease, while the more cryptic soluble $A\beta$ oligomers that also form are now thought to be the primary culprits in the devastating neurodegeneration that occurs.¹ In β -amyloid fibrils, the central region of $A\beta$ forms an extended network of β -sheets.² The oligomers also appear to involve β -sheet formation, but their structures are still largely unknown at atomic resolution.^{3,4} Enhanced understanding of the structures and interactions of the oligomers and fibrils offers the promise of preventing and treating Alzheimer's and other amyloid diseases.

The highly amyloidogenic central region of $A\beta$, which includes the hydrophobic pentapeptide sequence LVFFA ($A\beta_{17-21}$) has provided an archetype not only for the assembly of $A\beta$ but also for amyloidogenic peptides and proteins in general.⁵ This region is particularly prone to interaction, and peptides derived from $A\beta_{17-21}$ have been found to inhibit the aggregation of full length $A\beta$.⁶ The hydrophobic residues 17–21 are flanked by cationic and anionic residues K₁₆ and E₂₂, making the heptapeptide sequence KLVFFAE ($A\beta_{16-22}$) especially prone to supramolecular assembly to form fibrils and nanotubes.⁷

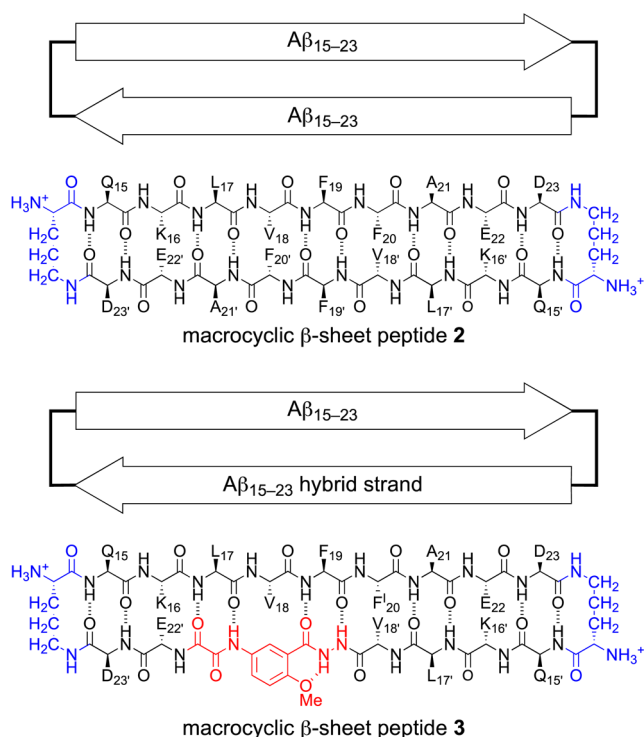
We recently began using macrocyclic β -sheet peptides containing the nonapeptide sequence QKLVFFAED ($A\beta_{15-23}$)

as a model system with which to explore the structures and interactions of amyloid oligomers. We incorporated the $A\beta_{15-23}$ nonapeptide into a 66-membered ring macrocycle containing template and turn units that help enforce a β -sheet structure and block uncontrolled aggregation, and we studied the supramolecular assembly of the resulting macrocyclic β -sheet peptides **1** in the solid state by X-ray crystallography and in aqueous solution by NMR spectroscopy.^{8,9} Macrocyclic β -sheets **1** contain an $A\beta_{15-23}$ peptide strand connected through two δ -linked ornithine turn units (δ Orn) to a template strand that contains two Hao amino acid tripeptide mimics.^{10,11} In the solid



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state, macrocyclic β -sheet peptide 1a forms tetramers, dodecamers, and porelike assemblies of oligomers. In solution, macrocyclic β -sheets 1 form tetramers that differ in structure from those in the solid state. The differences between the solid-state and solution-state tetramers are important, because they reveal polymorphism among amyloid oligomers at atomic resolution and illustrate the importance of environment upon oligomer structure.

In the current study, we envisioned replacing the template strand with $A\beta_{15-23}$ and determining the structures of the oligomers that form. Attempts to synthesize and study macrocyclic β -sheet peptide 2, which embodies this concept, proved fruitless, yielding only an insoluble peptide hydrogel. Incorporating two full strands of $A\beta_{15-23}$ into a macrocycle without a template designed to block aggregation appeared to give a peptide that was highly amyloidogenic. To reduce the amyloidogenicity of the macrocycle, we prepared macrocyclic β -sheet peptide 3, which incorporates a single Hao amino acid in place of the $F_{19}F_{20}A_{21}$ tripeptide segment of macrocyclic β -sheet 2, to give an $A\beta_{15-23}$ hybrid strand. To facilitate phase determination through single anomalous dispersion (SAD) phasing, we incorporated *p*-iodophenylalanine (F^I) in place of F_{20} in the $A\beta_{15-23}$ peptide strand.

Here, we report the X-ray crystallographic structure of macrocyclic β -sheet 3. We compare the solid-state supramolecular assembly to that of the oligomer observed in solution. We describe a new mode of assembly of $A\beta_{15-23}$ in the solid state—a fibril-like assembly of oligomers—that resembles both fibrils and oligomers.

RESULTS

X-ray Crystallographic Structure of Macrocyclic β -Sheet 3. In the solid state, macrocyclic β -sheet 3 forms hydrogen-bonded dimers arranged in a herringbone fashion in offset layers that pack through hydrophobic interactions. The resulting supramolecular assembly differs substantially both from that which we have observed previously for macrocyclic

Table 1. X-ray Crystallographic Data Collection and Refinement Statistics for Macrocyclic β -Sheet Peptide 3

Crystal parameters	
space group	C2
<i>a</i> , <i>b</i> , <i>c</i> (Å)	32.174, 62.852, 20.094
α , β , γ (deg)	90.00, 89.98, 90.00
molecules per asymmetric unit	2
Data collection	
synchrotron beamline	SSRL beamline 7-1
wavelength (Å)	1.00
resolution (Å)	17.56–1.75 (1.81–1.75)
total reflections ^a	14845 (1450)
unique reflections ^a	4060 (398)
completeness (%) ^a	99.2 (97.1)
multiplicity ^a	3.7 (3.6)
R_{merge} (%) ^{a,b}	3.6 (6.3)
$CC_{1/2}$ (%) ^a	99.8 (99.6)
CC^* (%) ^a	100 (99.9)
$I/\sigma(I)$ ^a	25.4 (13.4)
Refinement	
resolution (Å)	1.75
R_{work} (%) ^c	17.9
R_{free} (%) ^d	22.0
RMS bond lengths (Å)	0.010
RMS bond angles (deg)	1.52
ligands	2-methyl-2,4-pentanediol (2)
water	43
Ramachandran favored (%)	100
Ramachandran outliers (%)	0
Wilson <i>B</i> -factor (Å ²)	18.5
average <i>B</i> -factor (Å ²)	22.6
twinning	$-h, -k, l$ ($\alpha = 0.49$)

^aStatistics for the highest resolution shell are shown in parentheses. ^b $R_{\text{merge}} = \sum |I - \langle I \rangle| / \sum I$. ^c $R_{\text{work}} = \sum |F_{\text{obs}} - F_{\text{calc}}| / \sum F_{\text{obs}}$. ^d R_{free} was computed as R_{work} using a cross-validation set of 10% nonredundant data.

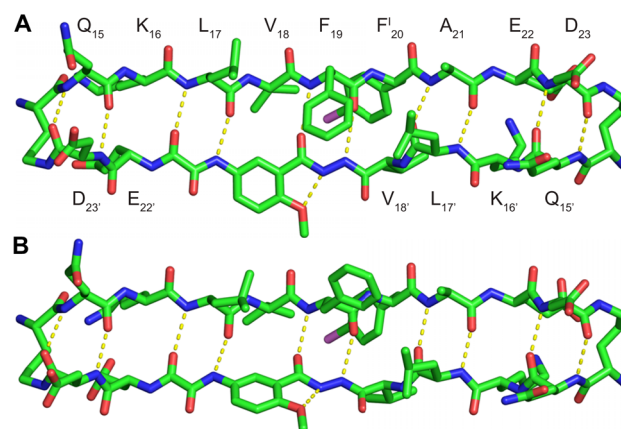


Figure 1. X-ray crystallographic structure of macrocyclic β -sheet peptide 3. Two conformers (A and B) make up the asymmetric unit.

β -sheets 1^{8,9} and that which others have previously observed for $A\beta$.

Macrocyclic β -sheet 3 readily formed crystals under sparse-matrix screening conditions with kits from Hampton Research. Crystals suitable for X-ray crystallography were grown from a 3.5 mg/mL solution with 0.1 M sodium citrate at pH 7.3, 0.1 M ammonium acetate, and 30% 2-methyl-2,4-pentanediol. Crystal diffraction data were collected on beamline 7-1 at the Stanford

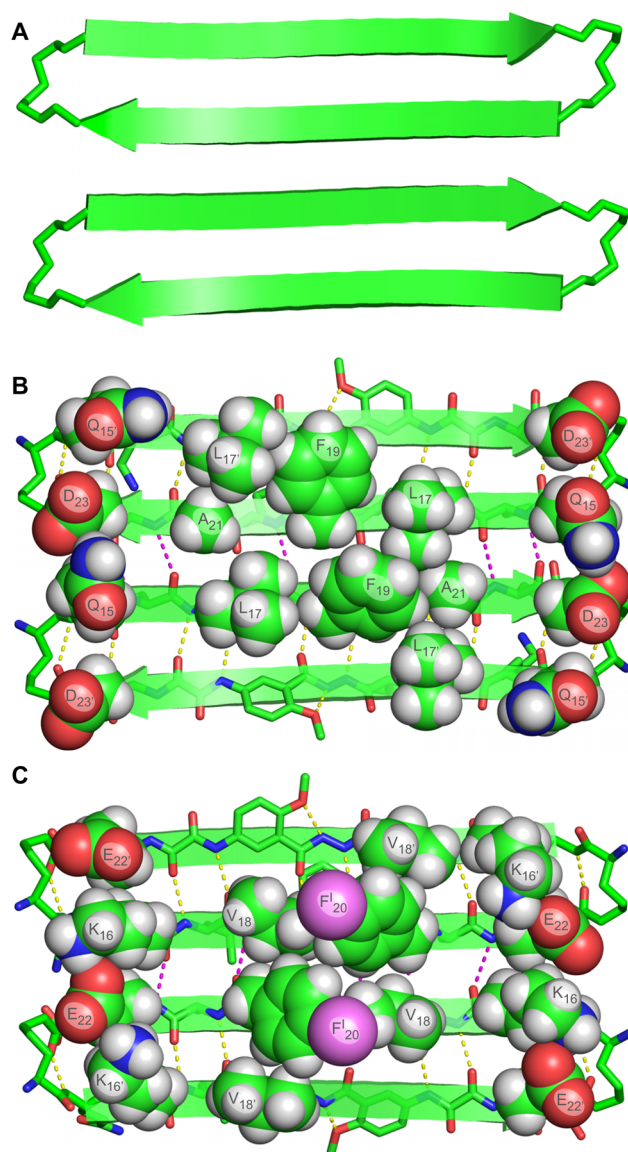


Figure 2. X-ray crystallographic structure of the hydrogen-bonded dimer of macrocyclic β -sheet peptide 3. (A) Cartoon illustration of the hydrogen-bonded dimer. (B) The LFA face of the hydrogen-bonded dimer, bearing the side chains of residues Q_{15} , L_{17} , F_{19} , A_{21} , and D_{23} of the $A\beta_{15-23}$ peptide strands and Q_{15}' , L_{17}' , and D_{23}' of the $A\beta_{15-23}$ hybrid strands. (C) The VF face of the hydrogen-bonded dimer, bearing the side chains of residues K_{16} , V_{18} , F_{20} , and E_{22} of the $A\beta_{15-23}$ peptide strands and K_{16}' , V_{18}' , and E_{22}' of the $A\beta_{15-23}$ hybrid strands.

Synchrotron Radiation Lightsources (SSRL) at 1.00 Å wavelength to 1.75 Å resolution. Data were integrated and scaled with XDS¹² and merged with Aimless.¹³ Iodine locations were determined with HySS in the PHENIX software suite.¹⁴ Initial density maps and phasing were generated with Autosol. Alternating rounds of manual rebuilding with Coot¹⁵ and refinement with phenix.refine were performed. The structure was solved in the C2 space group with 49% pseudomerohedral twinning¹⁶ to give a model with $R_{\text{free}} = 22.0\%$ and $R_{\text{work}} = 17.9\%$ (Table 1). The asymmetric unit contains two molecules of macrocyclic β -sheet 3 and two molecules of 2-methyl-2,4-pentanediol.

Macrocyclic β -sheet 3 crystallizes as a folded monomer in which the $A\beta_{15-23}$ peptide strand and the $A\beta_{15-23}$ hybrid strand form a hydrogen-bonded β -sheet. Two conformers of the macrocycle occur in the asymmetric unit, differing in rotamer

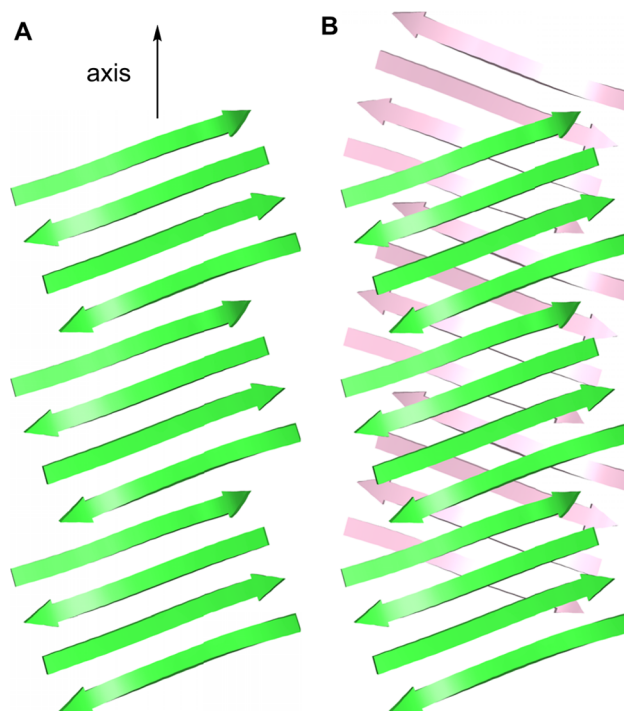


Figure 3. Assembly of hydrogen-bonded dimers in the X-ray crystallographic structure of macrocyclic β -sheet peptide 3. (A) Extended β -sheet that runs the length of the crystal lattice. (B) Packing of the extended β -sheets to form a two-layered structure.

of F_{19} and tilt of the Hao amino acid. The conformers alternate in the crystal lattice, geared together through crystal packing. Figure 1 illustrates the structure of the two conformers of the macrocycle.

Macrocyclic β -sheet 3 forms a hydrogen-bonded dimer, in which the two conformers hydrogen bond to form a four-stranded antiparallel β -sheet. The $A\beta_{15-23}$ peptide strands of the macrocycles make up the dimerization interface and are fully aligned, with residues 15–23 of one of the macrocycles paired with residues 23–15 of the other through eight hydrogen bonds. The side chains of residues Q_{15} , L_{17} , F_{19} , A_{21} , and D_{23} of the $A\beta_{15-23}$ peptide strands and Q_{15}' , L_{17}' , and D_{23}' of the $A\beta_{15-23}$ hybrid strands decorate one of the surfaces of the four-stranded antiparallel β -sheet dimer; the side chains of residues K_{16} , V_{18} , F_{20} , and E_{22} of the $A\beta_{15-23}$ peptide strands and K_{16}' , V_{18}' , and E_{22}' of the $A\beta_{15-23}$ hybrid strands decorate the other surface. Figure 2 illustrates the structure of the hydrogen-bonded dimer and the two surfaces. We term the two surfaces the LFA face and the VF face for the discussion of the higher-order supramolecular assembly of the dimers that follows.

The hydrogen-bonded dimers assemble to form an extended β -sheet that runs the length of the crystal lattice. The $A\beta_{15-23}$ hybrid strands form the interfaces between the dimers. At the interfaces, the $A\beta_{15-23}$ hybrid strands are not fully aligned, but rather are shifted out of alignment by two residues toward the C-termini. As a result of the shift in alignment, the β -strands comprising the β -sheets are not orthogonal to the axis formed by the extended β -sheet, but rather are rotated approximately 20° from orthogonality. The resulting assembly of the dimers resembles a herringbone pattern. Figure 3A illustrates the assembly of the hydrogen-bonded dimers.

The extended β -sheets formed by the hydrogen-bonded dimers pack through the VF faces to form a two-layered structure. The dimers comprising each layer do not overlap

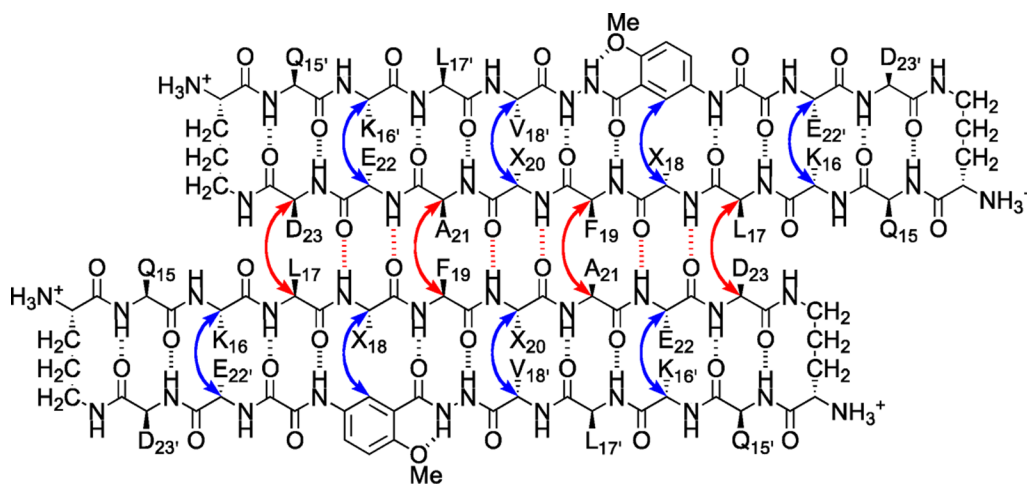


Figure 5. Hydrogen-bonded dimers formed by macrocyclic β -sheet peptides 3–5 in aqueous solution. Key NOEs associated with dimerization and folding are shown with red and blue arrows. (3: $X_{18} = V$, $X_{20} = F^1$; 4: $X_{18} = V$, $X_{20} = F$; 5: $X_{18} = T$, $X_{20} = Y$).

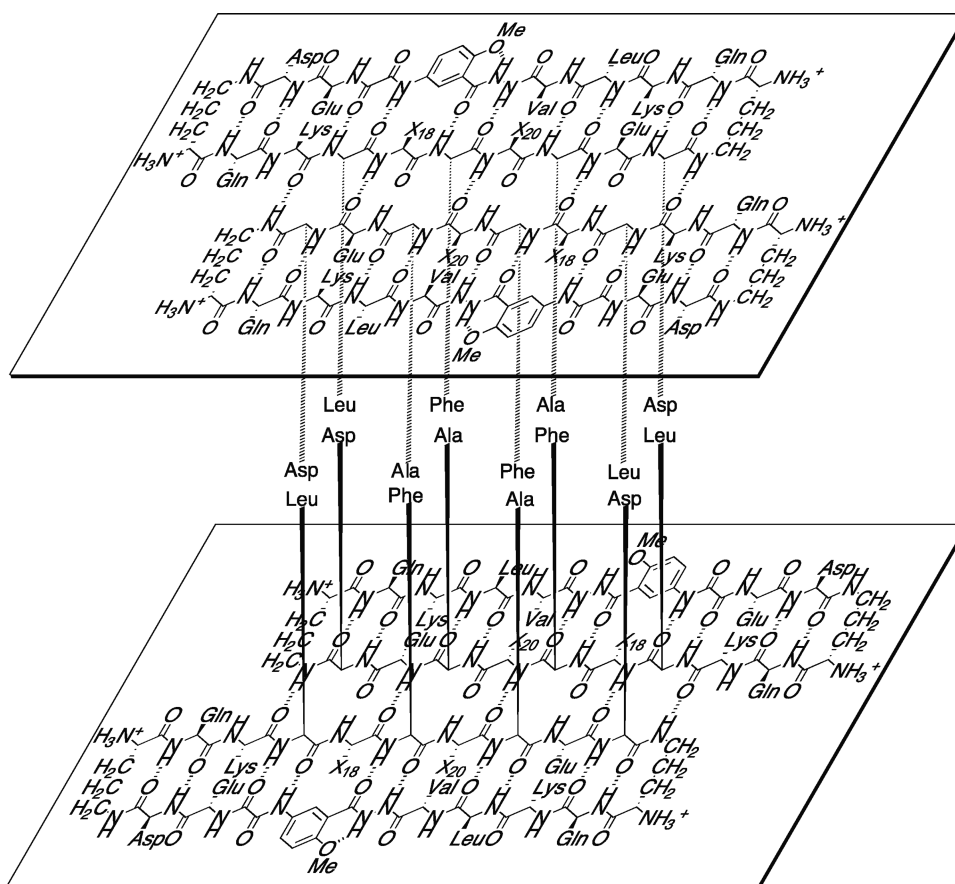


Figure 6. Illustration of the tetramer formed by macrocyclic β -sheet peptides 3–5 in aqueous solution. The tetramer forms as a sandwich-like assembly of two hydrogen-bonded dimers, sandwiched through the LFA faces (3: $X_{18} = V$, $X_{20} = F^1$; 4: $X_{18} = V$, $X_{20} = F$; 5: $X_{18} = T$, $X_{20} = Y$).

4, and 5 exhibit diffusion coefficients of $10.1\text{--}10.7 \times 10^{-7} \text{ cm}^2/\text{s}$ (Table 3). These values are comparable to those that we have observed previously for similar tetramers and are 0.58–0.61 times smaller than that of macrocyclic β -sheet 6, which is monomeric.^{9,23,24}

The tetramer forms as a sandwich of hydrogen-bonded dimers. It is sandwiched through the hydrophobic face that displays L_{17} , F_{19} , and A_{21} , and these residues help create the hydrophobic core of the tetramer (Figure 6). When F_{19} and A_{21} are rendered hydrophilic by hydroxylation in macrocyclic

Table 3. Diffusion Coefficients (D) of Peptides 3–6 at 2.0 mM in D_2O at 298 K

peptide	MW _{monomer} ^a (Da)	MW _{tetramer} ^a (Da)	D ($10^{-7} \text{ cm}^2/\text{s}$)	oligomer state
3	2380	9522	10.1	tetramer
4	2254	9018	10.3	tetramer
5	2272	9090	10.7	tetramer
6	2286	NA	17.4	monomer

^aMolecular weight calculated for the neutral (uncharged) macrocycle.

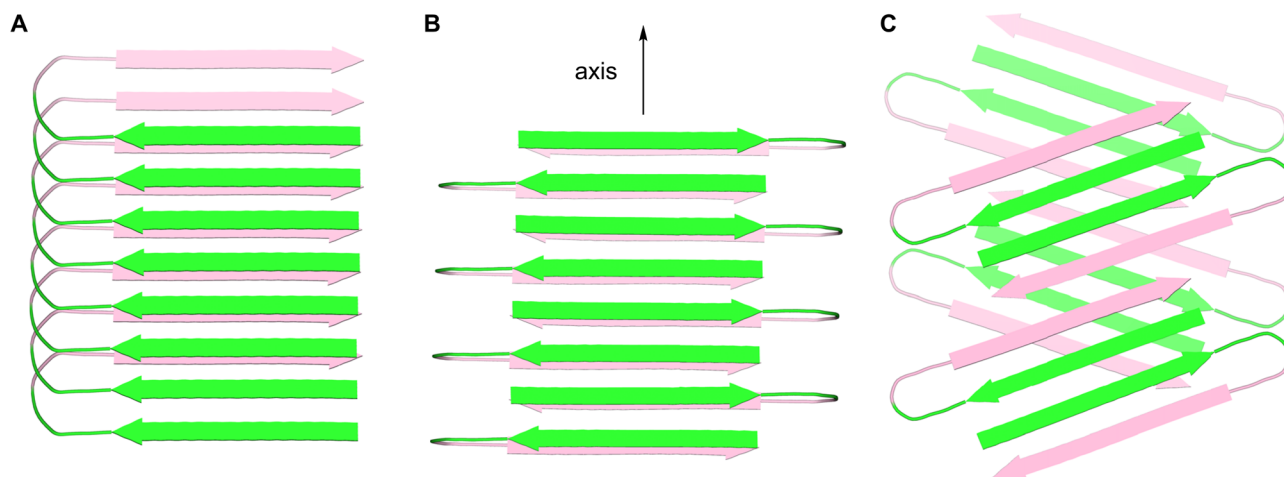


Figure 7. Cartoon representations of fibrils formed by $A\beta$. (A) Parallel β -sheet fibril composed of U-shaped turns in a staggered arrangement, observed for $A\beta_{1-40}$.^{2c} (B) Antiparallel β -sheet fibril composed of U-shaped turns, observed for the Iowa mutant $A\beta_{1-40}$.²⁷ (C) Fibril-like assembly of oligomers composed of β -hairpins, that we propose from the X-ray crystallographic structure of macrocyclic β -sheet peptide 3. The green and pink colors represent the central and C-terminal regions of $A\beta$.

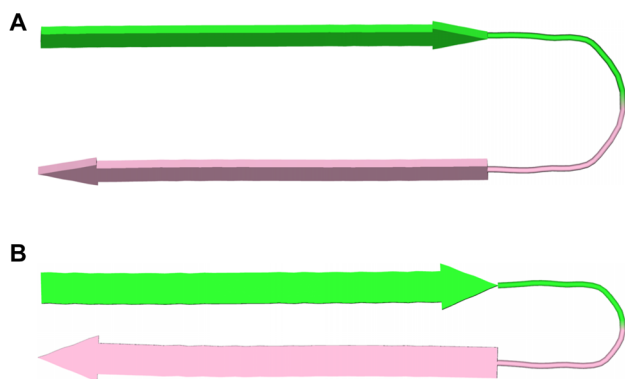


Figure 8. Cartoon representations of U-shaped turns (A) and β -hairpins (B) composed of $A\beta$. In the U-shaped turns, the faces of the β -strands pack together.^{2c,27} In the proposed β -hairpins, the edges of the β -strands hydrogen bond together. The green and pink colors represent the central and C-terminal regions of $A\beta$.

β -sheet 6, the hydrophobic core cannot form and the tetramer is disrupted.⁹ In contrast, when V_{18} and F_{20} are rendered hydrophilic by hydroxylation in macrocyclic β -sheet 5, the hydrophobic core is unaffected and the tetramer is not disrupted.

DISCUSSION

The extended layered β -sheet formed by the hydrogen-bonded dimers of 3 (Figure 3B) resembles the structures of amyloid fibrils.^{25,26} Amyloid fibrils consist of extended β -sheets composed of networks of hydrogen-bonded β -strands running the length of the fibril axis and laminated in pairs through hydrophobic interactions to form two-layered assemblies.² In the fibrils formed by $A\beta_{1-40}$, the hydrophobic central and C-terminal regions of the peptide assemble to form extended β -sheets that run the length of the fibril axis. The β -strands comprising the β -sheets are roughly orthogonal (90°) to the fibril axis. The molecules of $A\beta$ form U-shaped turns, and the hydrophobic central and C-terminal β -strands pack in a face-to-face fashion through hydrophobic interactions. The resulting two-layered β -sheets make up the basic fibril structure, further assembling to form four-layered or triangular fibrils consisting of two or three of these subunits. Although most of the

structures reported for $A\beta_{1-40}$ fibrils involve parallel β -sheets, antiparallel β -sheets have been reported for Iowa mutant β -amyloid fibrils.²⁷ Figure 7A,B illustrates the structures of the parallel and antiparallel two-layered β -sheets reported for $A\beta_{1-40}$. Figure 8A illustrates the structure of the component U-shaped turns that make up these two-layered assemblies.

The assembly formed by the hydrogen-bonded dimers of 3 (Figure 3B) differs notably from amyloid fibrils in that it is composed of discrete oligomeric subunits. While the two-layered β -sheets of the $A\beta_{1-40}$ fibrils contain no subunit larger than the monomer, the fibril-like assemblies formed by macrocyclic β -sheet 3 are composed of oligomers consisting of two β -hairpin-like macrocycles, hydrogen bonded to form a four-stranded antiparallel β -sheet.

The X-ray crystallographic structure of the fibril-like assembly of oligomers formed by macrocyclic β -sheet 3 suggests that alternative fibril assemblies of $A\beta_{1-40}$ or $A\beta_{1-42}$ might also be possible.^{25,26} In a fibril-like assembly of oligomers formed by full-length $A\beta$, the hydrophobic central and C-terminal regions of the peptide could hydrogen bond to form a β -hairpin.²⁸ The β -hairpins could then further assemble to form layered β -sheets through edge-to-edge hydrogen bonding and face-to-face hydrophobic interactions. In this assembly, the β -strands comprising the β -sheets are not orthogonal to the fibril axis, but rather are rotated approximately 20° from orthogonality. To compensate for this rotation, the β -sheets must shift registration by two residues for every two β -hairpins. Figure 7C illustrates a structure of this fibril-like assembly of oligomers; Figure 8B illustrates the structure of the component β -hairpins.

The solid-state and solution-state structures of macrocyclic β -sheets 3–5 show that $A\beta_{15-23}$ can form both aligned and shifted antiparallel β -sheets. In the X-ray crystallographic structure of macrocyclic β -sheet 3, the $A\beta_{15-23}$ peptide strand forms an aligned β -sheet, while the $A\beta_{15-23}$ hybrid strand forms a shifted β -sheet. In the solution-state structure of macrocyclic β -sheets 3–5, the $A\beta_{15-23}$ peptide strand forms a shifted β -sheet. Figure 9 illustrates these modes of supramolecular assembly.

We have previously observed both aligned and shifted antiparallel β -sheets involving $A\beta_{15-23}$ in the solid-state and solution-state structures of macrocyclic β -sheets 1. In the

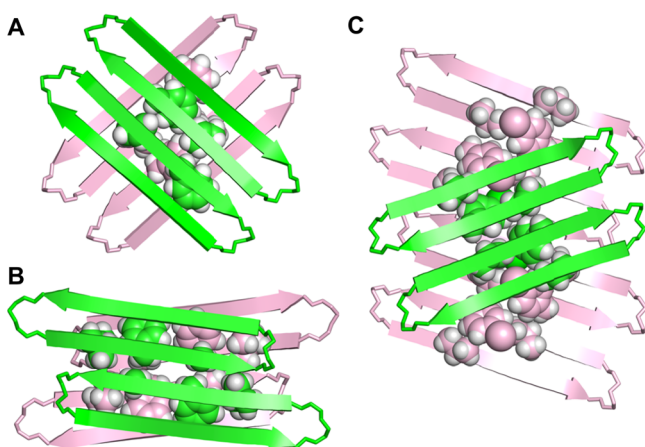


Figure 10. Supramolecular assemblies of macrocyclic β -sheet peptides derived from $A\beta_{15-23}$. (A) Tetramer of 1a observed in the solid state (PDB: 4HVH).⁸ (B) Tetramer of 1b (and 1a) observed in aqueous solution.⁹ (C) Fibril-like assembly of dimers of 3 observed in the solid state.

addition and folding of monomeric $A\beta$. The fibril-like assembly of oligomers can then serve as a reservoir of oligomers, dissociating organized dimers, tetramers, or other small homogeneous assemblies of soluble toxic amyloid oligomers. Such mechanisms for oligomer replication have been seen and discussed previously but have not been observed at atomic resolution.³⁰ The X-ray crystallographic structure of macrocyclic β -sheet 3 may thus provide a window at atomic resolution into a prion-like mechanism of amyloid oligomer propagation.

CONCLUSION

The X-ray crystallographic structure of macrocyclic β -sheet 3 provides new insights into the supramolecular assembly of peptides from β -amyloid, revealing a fibril-like assembly of hydrogen-bonded dimers. The dimers repeat along the fibril axis, to form an extended β -sheet, like in conventional amyloid fibrils. Unlike conventional amyloid fibrils, the β -strands comprising the β -sheets are rotated approximately 20° from orthogonality to the fibril axis, and a two-residue shift in alignment of the β -strands occurs at the juncture between the dimers. The β -sheets are layered and laminated through hydrophobic interactions. The dimers are not layered directly over each other, but rather are offset by two strands. As a result, the fibril-like assembly of dimers is not composed of discrete tetramers.

The fibril-like assembly of oligomers formed by macrocyclic β -sheet 3 offers the intriguing possibility that full-length $A\beta$ may also be able to form similar assemblies, perhaps consisting of β -hairpins formed by the amyloidogenic central and C-terminal regions of $A\beta$. This model further suggests the provocative hypothesis that fibril-like assemblies of $A\beta$ oligomers might catalyze $A\beta$ oligomer formation and replication.

ASSOCIATED CONTENT

Supporting Information

NMR spectra, mass spectra, and HPLC traces for macrocyclic β -sheet peptides 3–6. Crystallographic data in CIF and PDB format for macrocyclic β -sheet 3. The crystal structure of macrocyclic β -sheet 3 was deposited into the Protein Data Bank (PDB) with PDB code 4Q8D. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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(18) Macrocytic β -sheet **5** shows predominantly or wholly the tetramer in the ^1H NMR spectrum at concentrations as low as 0.1 mM in D_2O at 298 K.

(19) The ^1H NMR spectra of macrocytic β -sheets **3** and **4** are broader than that of **5**, suggesting that while the tetramers predominate for all of these peptides, the tetramers formed by the more hydrophobic peptides **3** and **4** may participate in minor equilibria with additional tetramers that differ in structure or oligomers that differ in molecularity.

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