

X-ray Crystallographic Structures of Trimers and Higher-Order Oligomeric Assemblies of a Peptide Derived from $A\beta_{17-36}$

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S Supporting Information

ABSTRACT: A peptide derived from $A\beta_{17-36}$ crystallizes to form trimers that further associate to form higher-order oligomers. The trimers consist of three highly twisted β -hairpins in a triangular arrangement. Two trimers associate face-to-face in the crystal lattice to form a hexamer; four trimers in a tetrahedral arrangement about a central cavity form a dodecamer. These structures provide a working model for the structures of oligomers associated with neurodegeneration in Alzheimer's disease.

Here we report the X-ray crystallographic structures of trimers and higher-order oligomeric assemblies of a peptide derived from the β -amyloid peptide ($A\beta$). Oligomers of $A\beta$ are now thought to play a central role in neurodegeneration in Alzheimer's disease.¹ Selkoe et al. found that small $A\beta$ oligomers disrupt long-term potentiation, with trimers showing the highest disruption.^{1g} Ashe et al. found that a 56 kDa $A\beta$ oligomer, termed $A\beta^*56$, impairs memory.^{1f} The oligomer appears to be a dodecamer composed of four trimers. Understanding the structures of these oligomers is essential to understanding their mechanism of action. Trimers are particularly enigmatic, because their structure cannot be explained by simply pairing monomers.

The hydrophobic central and C-terminal regions of $A\beta$ are known to participate in aggregation to form fibrils and are likely involved in the aggregation of oligomers.² Although many molecular details of the aggregation processes have yet to be elucidated, the formation of β -sheets appears to be involved. While the structures of the fibrils are relatively well understood, the structures of trimers and higher-order oligomers are not known.³

Peptide fragments derived from amyloidogenic peptides and proteins are valuable tools for studying the structures of amyloid fibrils and oligomers.⁴ In the current study, we set out to use a peptide fragment derived from both the central and C-terminal regions of $A\beta$ to elucidate the structures of $A\beta$ oligomers.^{2c,d} We designed peptide **1a** as a mimic of $A\beta_{17-36}$ in which residues 17–23 (LVFFAED) and 30–36 (AIIGLMV) form the β -strands of a β -hairpin (Figure 1). In this structure, the δ -linked ornithine β -turn mimic connecting Asp₂₃ and Ala₃₀ promotes a β -hairpin structure and replaces residues 24–29 (VGSNKG). The δ -linked ornithine connecting Leu₁₇ and Val₃₆ forms a macrocycle to further enforce the β -hairpin structure. We incorporated an *N*-methyl group on Gly₃₃ to help prevent uncontrolled aggregation through edge-to-edge H-bonding between β -sheets.⁵ We replaced Met₃₅ with the

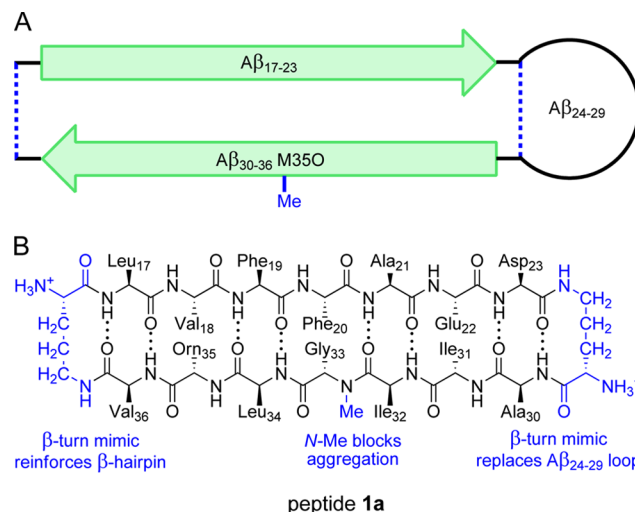


Figure 1. (A) Cartoon illustrating the design of peptide **1a** and the envisioned relationship to $A\beta_{17-23}$, $A\beta_{30-36}$ M35O, the *N*-methyl group, and the δ -linked ornithine β -turn mimic.

hydrophilic isostere ornithine (α -linked) to enhance solubility and further prevent uncontrolled aggregation. We designed an analogue of peptide **1a** containing 4-iodophenylalanine at the Phe₁₉ position (peptide **1b**) for crystallographic phase determination.

The synthesis and crystallization of peptides **1a** and **1b** were straightforward.⁶ The peptides were synthesized using Fmoc-based solid-phase peptide synthesis, solution-phase cyclization, and RP-HPLC purification. Initial crystallization conditions for peptide **1a** were identified using the Hampton Research crystallization kits: Crystal Screen, Index, and PEG/Ion (288 experiments). Conditions with HEPES buffer and Jeffamine M-600 were selected and further optimized (0.1 M HEPES at pH 6.75 with 30% Jeffamine M-600) to give rapid (<24 h) formation of good crystals. Crystal diffraction data were collected at the Advanced Light Source at Lawrence Berkeley National Laboratory with a synchrotron source at 1.0 Å wavelength. Diffraction data were collected to 1.70 Å resolution. Data were scaled and merged using XDS, and phases were determined by isomorphous replacement of the Phe₁₉ 4-iodophenylalanine derivative **1b**.⁷ The structure of peptide **1a** was solved and refined in the R3 space group. The

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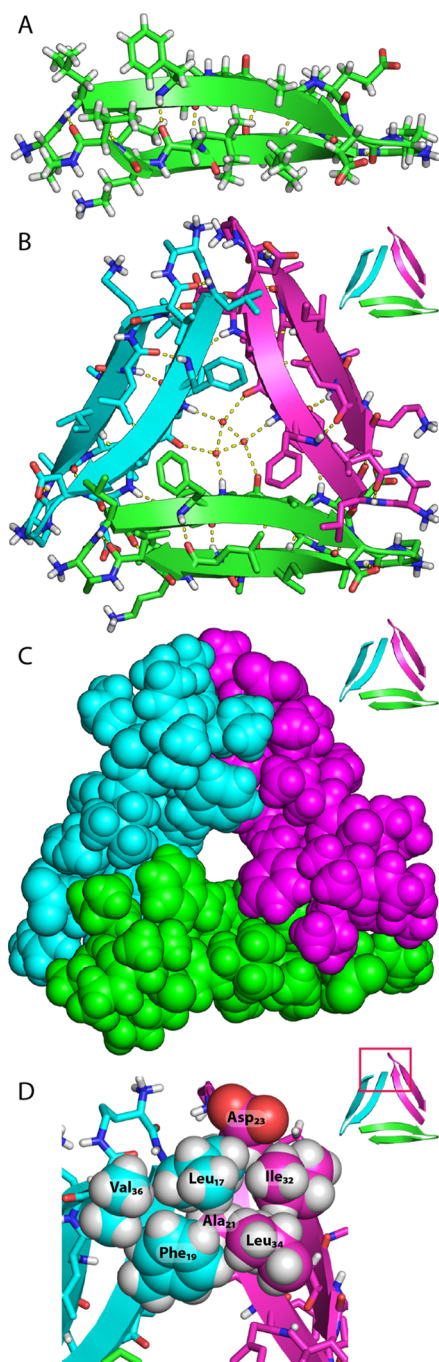


Figure 2. X-ray crystallographic structure of peptide 1a: (A) β -hairpin monomer; (B) trimer, cartoon and stick representation with ordered water; (C) trimer, space-filling representation; (D) detail of trimer interface illustrating the hydrophobic cluster formed by Leu₁₇, Phe₁₉, Ala₂₁, Asp₂₃, Ile₃₂, Leu₃₄, and Val₃₆.

asymmetric unit contains 16 nearly identical monomers (rmsd ≈ 0.2 Å, Figure S3). Coordinates for hydrogens were generated by phenix.refine during refinement.⁸

The X-ray crystallographic structure reveals that peptide 1a folds to form a β -hairpin comprising two heptapeptide β -strands. Eight residues (Leu₁₇, Phe₁₉, Ala₂₁, Asp₂₃, Ala₃₀, Ile₃₂, Leu₃₄, and Val₃₆) make up one surface of the β -hairpin (the LFA face), and six residues (Val₁₈, Phe₂₀, Glu₂₂, Ile₃₁, Gly₃₃, and Orn₃₅) make up the other surface (the VF face). The β -hairpin has a strong twist in which each residue rotates in a right-

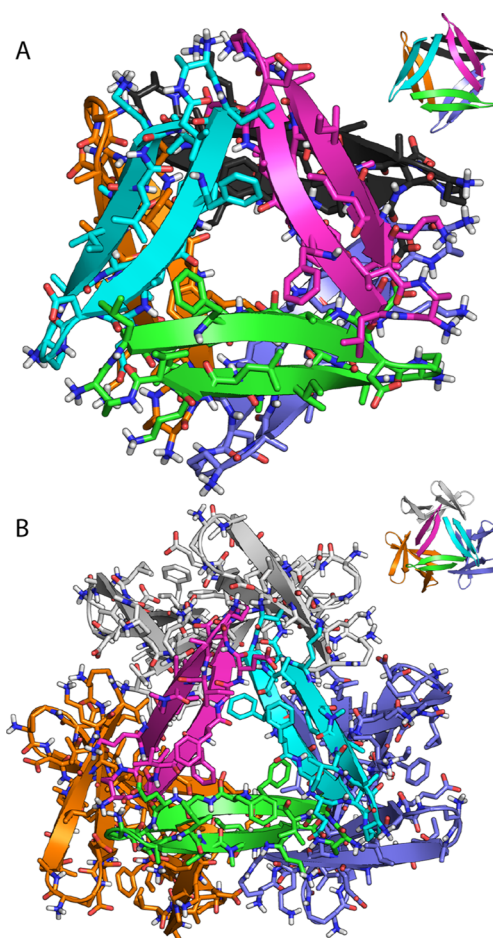


Figure 3. (A) Hexamer and (B) dodecamer observed in the X-ray crystallographic structure of peptide 1a.

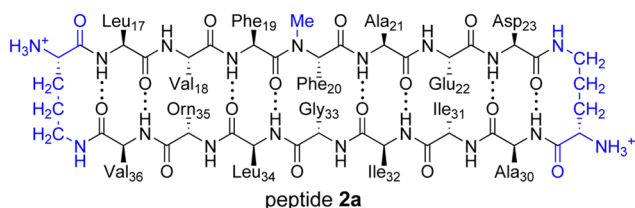
handed fashion $\sim 25^\circ$ along the β -strand axis.⁹ Contacts between the side chains of residues located diagonally across the β -sheet help stabilize the twisted β -hairpin (Phe₁₉-Val₃₆, Ala₂₁-Leu₃₄, and Asp₂₃-Ile₃₂; Figures 2A and S1).

Three β -hairpins assemble in a triangular fashion and interlock to form a trimer, with each β -hairpin making up one edge of the equilateral triangle (Figure 2). The central $A\beta$ strand (17–23) constitutes the inner edges of the equilateral triangle, and the C-terminal strand (30–36) constitutes the outer edges. A hole runs through the center of the triangle, and the NH and C=O groups of Phe₂₀ line the hole. The side chains of Phe₁₉ surround the front of the hole, and the side chains of Phe₂₀ surround the back of the hole. Three ordered water molecules hydrogen bond to the NH and C=O groups of Phe₂₀ and fill the hole in the center of the triangle (Figure 2B).¹⁰ The β -hairpins come together at the corners of the triangle and stabilize the trimer through both polar and nonpolar interactions. The main chains of Val₁₈ and Glu₂₂ H-bond at each corner to create a four-stranded β -sheet (Figure S2). The side chains of Leu₁₇, Phe₁₉, Ala₂₁, Asp₂₃, Ile₃₂, Leu₃₄, and Val₃₆ form an extensive hydrophobic cluster at the corners where the β -hairpins meet. The side chain of Leu₁₇ makes extensive contacts with the side chains of Ala₂₁, Asp₂₃, Ile₃₂, and Leu₃₄ of the adjacent β -hairpin and is buttressed by the side chains of Phe₁₉ and Val₃₆ (Figures 2D and S2).

The trimers assemble loosely in the crystal lattice to form hexamers and dodecamers (Figure 3). The hexamer consists of

two trimers clasped through the VF faces. In the hexamer, the Ile₃₁ residues of the two trimers H-bond through bridging waters. Loose contacts between Phe₂₀ and Ile₃₁ appear to further stabilize the hexamer. The dodecamer consists of four trimers in a tetrahedral arrangement around a central cavity, with the LFA faces lining the cavity. Hydrophobic contacts occur between the Leu₁₇ residues of the trimers. Salt bridges between the Asp₂₃ residues and the δ -linked ornithines preceding Leu₁₇ further stabilize the dodecamer. The contacts stabilizing the hexamers and dodecamers appear to be in opposition, preventing either oligomer from packing tightly.

To test whether trimer formation results from *N*-methylation of Gly₃₃, we prepared and studied peptide **2a**, in which Phe₂₀ is *N*-methylated and Gly₃₃ is not. Peptide **2a** crystallized in conditions similar to those for peptide **1a**. Phases were determined by isomorphous replacement of the Phe₁₉ 4-iodophenylalanine derivative **2b**. The structure of peptide **2a** was solved and refined in the *P*3₂1 space group and contains 12 nearly identical monomers (rmsd \approx 0.3 Å, Figure S4).



The X-ray crystallographic structure of peptide **2a** is nearly identical to that of peptide **1a**. Peptide **2a** crystallizes as a β -hairpin that assembles to form trimers, which further form hexamers and dodecamers. Moving the *N*-methyl group from Gly₃₃ to Phe₂₀ does not significantly alter the structures of the oligomers. The central *A* β strand (17–23) still forms the inner edges of the trimer, and the C-terminal strand (30–36) still forms the outer edges. In the X-ray crystallographic structure of peptide **2a**, the *N*-methyl groups from Phe₂₀ replace the three ordered waters that fill the hole in the center of the triangle (Figure 4). While *N*-methylation of either Phe₂₀ or Gly₃₃ is necessary to prevent aggregation, it does not dictate the formation of the trimer, hexamer, or dodecamer.

The trimer formed by peptides **1a** and **2a** is unlike the structures of fibrils or oligomers previously observed for amyloidogenic peptides or proteins. A similar triangular assembly of three β -hairpins occurs in actinohivin, a 114-amino-acid lectin that binds HIV gp120.¹¹ Actinohivin contains three nearly identical sequences in tandem that form the three β -hairpins and fold into a triangular assembly (Figure 5, PDB 3A07). Each β -hairpin comprises two pentapeptide β -strands connected by a turn of three residues. Although these β -strands are smaller than those of peptide **1a**, they occupy similar positions to residues 17–23 and 30–36 of **1a** in the trimer. Actinohivin binds to mannose-containing glycans on gp120 with high affinity through trivalent interactions of the concave surfaces formed by the three near-repeats. This mode of interaction suggests that trimers of *A* β may bind to molecules on the surface of neurons through trivalent interactions of three β -hairpins formed by *A* β _{17–36}.

We modeled a trimer of Ac-*A* β _{17–36}-NHMe β -hairpins to generate a working model of a trimer of *A* β . We used the crystallographic coordinates of peptide **1a** to generate residues 17–23 (LVFFAED) and 30–36 (AIIGLMV) of the trimer and added loops comprising residues 24–29 (VGSNKG). We

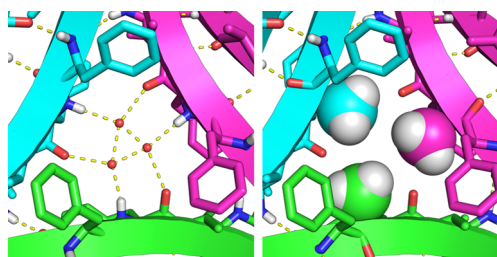


Figure 4. Detail of the X-ray crystallographic structure of the trimers formed by peptides **1a** (left) and **2a** (right). The *N*-methyl groups of **2a** take the place of the ordered water molecules in **1a**.

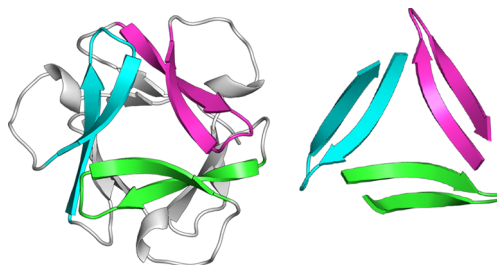


Figure 5. Cartoon representation showing the structural similarities of actinohivin (left) and peptide **1a** trimer (right).

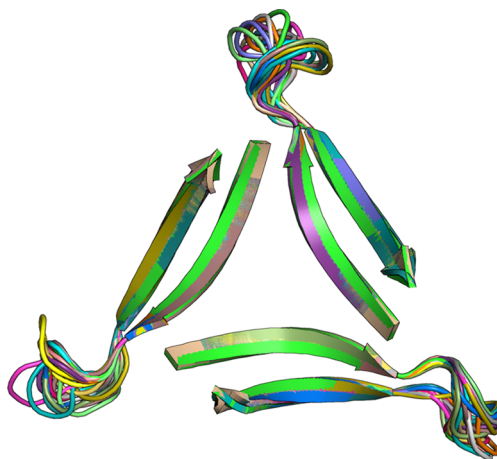


Figure 6. Cartoon representation of 20 low-energy structures for Ac-*A* β _{17–36}-NHMe generated by REMD.

performed replica-exchange molecular dynamics (REMD) in NAMD using the CHARMM22 force field with generalized Born implicit solvent to generate realistic conformations of the loops.¹² Figure 6 illustrates 20 low-energy structures from the simulation. These structures provide a working model in which residues 24–29 act as a loop connecting the 17–23 and 30–36 β -strands. The concave surfaces formed by the loops and the twisted β -strands might serve as binding sites in multivalent biological interactions.

The X-ray crystallographic structures of the trimers and higher-order oligomeric assemblies formed by peptides **1a** and **2a** provide working models for the structures of the trimers and higher-order oligomers of *A* β that are important in neurodegeneration. In this model, *A* β associates to form trimers comprising three β -hairpins in a triangular arrangement. The trimers are stabilized through interactions among the central region of *A* β _{17–23} (LVFFAED). These interactions are buttressed by hydrophobic interactions with the hydrophobic

C-terminal region of $A\beta$ —either $A\beta_{30-36}$ (AIIGLMV) or another segment of the extensive hydrophobic C-terminal sequence. Two trimers can stack to form hexamers. While the hexamers formed by peptides **1a** and **2a** associate along the VF face, there is little distinction between the residues of the VF face and the LFA face, and stacking might occur through interactions among either face. Although the dodecamer in Figure 3b might explain the structure of $A\beta^*56$, an assembly consisting of a stack of four trimers is also possible and may provide greater contact and stabilization. These structures and ideas serve as a starting point for developing and testing hypotheses about the structures and mechanism of action of amyloid oligomers.

■ ASSOCIATED CONTENT

■ Supporting Information

Details of the synthesis and crystallization of peptides **1a**, **1b**, **2a**, and **2b**; X-ray diffraction data collection, processing, and refinement; and modeling of Ac- $A\beta_{17-36}$ -NHMe. Crystallographic data in CIF format. Coordinates for models of Ac- $A\beta_{17-36}$ -NHMe in PDB format. This material is available free of charge via the Internet at <http://pubs.acs.org>. Crystallographic coordinates of **1a**, **1b**, **2a**, and **2b** were deposited into the Protein Data Bank with PDB codes 4NTR, 4NTP, 4NW9, and 4NW8.

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Notes

The authors declare no competing financial interest.

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