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X-Ray Crystallography Reveals Parallel and Antiparallel β-Sheet Dimers of a β-Hairpin Derived from Aβ16–36 that Assemble to Form Different Tetramers

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Abstract

High-resolution structures of oligomers formed by the β-amyloid peptide, Aβ, are important for understanding the molecular basis of Alzheimer's disease. Dimers of Aβ are linked to the pathogenesis and progression of Alzheimer's disease, and tetramers of Aβ are neurotoxic. This paper reports the X-ray crystallographic structures of dimers and tetramers, as well as an octamer, formed by a peptide derived from the central and *C*-terminal regions of Aβ. In the crystal lattice, the peptide assembles to form two different dimers—an antiparallel β-sheet dimer and a parallel β-sheet dimer—that each further self-assemble to form two different tetramers—a sandwich-like tetramer and a twisted β-sheet tetramer. The structures of these dimers and tetramers derived from Aβ serve as potential models for dimers and tetramers of full-length Aβ that form *in vitro* and in Alzheimer's disease brains.

Graphical Abstract

sandwich-like tetramer   twisted β-sheet tetramer

Keywords

Amyloid; Aβ; Oligomer; Dimer; Tetramer; Crystal Structure; Alzheimer's Disease

INTRODUCTION

Interactions among β-sheets are ubiquitous in protein folding and protein-protein interactions. The self-assembly of β-sheets is particularly important in the aggregation of amyloidogenic peptides and proteins to form oligomers and fibrils. Understanding how β-sheets fold and assemble to form amyloid oligomers and fibrils is fundamental to peptide and protein science and is also important for understanding devastating diseases such as Alzheimer's disease, Parkinson's disease, and type II diabetes. Recent cryo-EM structural studies of amyloid fibrils have revealed a rich tapestry of β-sheet assemblies composed of continuous extended networks of parallel β-sheets that fold and intricately pack together. [1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16] The structures of amyloid oligomers remain more elusive. X-ray crystallographic studies of fragments of amyloidogenic peptides and proteins, as well as NMR studies of full-length proteins, have provided clues about amyloid oligomer structures indicating that many amyloid oligomers are composed of antiparallel β-sheets and packed hydrophobic cores. [17,18,19,20,21,22,23,24,25,26]

The β-amyloid peptide, Aβ, assembles to form oligomers that vary in size and shape and are thought to be important in the pathogenesis and progression of Alzheimer's disease. [27,28] Elucidating the structures of these oligomers is central to understanding the molecular basis of Alzheimer's disease. Recently, our laboratory has elucidated a wealth of X-ray crystallographic structures of oligomers formed by peptides designed to mimic Aβ β-hairpins. In studying these peptides, we have discovered new structural motifs for Aβ oligomers including triangular trimers, barrel-like and sandwich-like hexamers, ball-shaped dodecamers, and large annular pore-like structures. [29,30,31,32,33,34] These structures have revealed the novel and unpredictable ways that β-hairpin peptides containing Aβ sequences can fit together to form oligomers, and may also help shed light on the structures of oligomers that full-length Aβ forms in Alzheimer's disease.

In the current paper, we report the X-ray crystallographic structures of two different β-sheet dimers—one antiparallel β-sheet dimer and one parallel β-sheet dimer—formed by peptide **1**, a β-hairpin derived from $A\beta_{16-36}$. In the crystal lattice, these two dimers self-assemble to form different tetramers. The antiparallel dimer self-assembles with another antiparallel dimer to form a sandwich-like tetramer stabilized by a tightly packed hydrophobic core. In contrast, the parallel dimer self-assembles with another parallel dimer to form a tetramer composed of a highly twisted eight-stranded β-sheet. The parallel tetramer is stabilized by hydrophobic packing and edge-to-edge hydrogen bonding between the two parallel dimers. These oligomer structures further illustrate the remarkable ways in which β-hairpins derived from Aβ can interact to form oligomers, and add to the diversity of oligomers that β-hairpins can form.

RESULTS AND DISCUSSION

Design of peptide 1.

Peptide **1** is designed to mimic a β-hairpin formed by $A\beta_{16-36}$ (Figures 1A and B). Peptide **1** contains Aβ residues 16–36 linked together at the *N*- and *C*-termini with a δ-linked ornithine turn unit and also contains a cross-strand disulfide bond in place of Ala_{21} and Ile_{31} to stabilize the β-hairpin structure.[35] The disulfide bond at this position maintains the hydrophobic character of this region of the peptide. Peptide **1** also contains an *N*-methyl group on Gly_{33} to block uncontrolled aggregation of the peptide. These design features facilitate crystallization of the peptide. Variants of peptide **1** without the cross-strand disulfide bond or without the *N*-methyl group did not crystallize in any of the 864 different crystallization conditions tested. To facilitate determination of the X-ray crystallographic phases, peptide **1** also contains a *para*-iodo group on Phe_{19}.[36]

X-ray crystallographic structure of peptide 1.

The X-ray crystallographic structure of peptide **1** reveals that the peptide folds to form a twisted β-hairpin and that the β-hairpins assemble to form oligomers. The asymmetric unit of the X-ray crystallographic structure contains 11 copies of peptide **1**. Each copy folds to form a β-hairpin that is composed of an $A\beta_{16-23}$ β-strand and an $A\beta_{27-36}$ β-strand connected by an $A\beta_{24-28}$ loop (Figure 1C). The peptide backbones of the β-strands share comparable geometries for all 11 copies of peptide **1** in the asymmetric unit, while the loop regions vary (Figure 1D).

In the crystal lattice, peptide **1** forms assemblies that may be thought of as oligomers. Peptide **1** forms two different β-sheet dimers—an antiparallel β-sheet dimer and a parallel β-sheet dimer. Both dimers comprise four-stranded β-sheets. The antiparallel dimer further assembles with another antiparallel dimer to form a sandwich-like tetramer. The parallel dimer further assembles with another parallel dimer to form a twisted β-sheet tetramer. Two of these twisted β-sheet tetramers further assemble to form an octamer. An additional antiparallel dimer, that is not part of a tetramer, rests upon the octamer in the crystal lattice. The following describes these oligomers in detail.

Antiparallel dimer.

The antiparallel dimer formed by peptide **1** consists of two peptide monomers arranged in an antiparallel orientation in which the $A\beta_{16-23}$ β-strand of one monomer is across from the $A\beta_{16-23}$ β-strand of the other monomer (Figure 2A). Five hydrogen-bonding interactions between the amide backbones of the two monomers stabilize the antiparallel dimer: Phe_{19}^{I} and Cys_{21} on one monomer pair with Cys_{21} and Phe_{19}^{I} on the other monomer; a water molecule bridges Asp_{23} on one monomer and Leu_{17} on the other monomer.

Parallel dimer.

The parallel dimer formed by peptide **1** consists of two peptide monomers arranged in a parallel orientation in which the $A\beta_{16-23}$ β-strand of one monomer is across from the $A\beta_{16-23}$ β-strand of the other monomer (Figure 2B). Four hydrogen bonds between the amide backbones of the two monomers stabilize the parallel dimer: Phe_{20} and Cys_{21} on one monomer pair with Phe_{19}^{I} and Phe_{20} on the other monomer; a water molecule bridges Phe_{19}^{I} on one monomer and Leu_{17} on the other monomer.

The antiparallel dimer and the parallel dimer each self-assemble to form different tetramers. The antiparallel dimer assembles with another antiparallel dimer to form a sandwich-like tetramer, whereas the parallel dimer assembles with another parallel dimer to form a highly twisted β-sheet tetramer. The twisted β-sheet tetramer further assembles with another twisted β-sheet tetramer to form an octamer.

Sandwich-like tetramer formed by the antiparallel dimer.

Two antiparallel dimers further assemble to form a sandwich-like tetramer (Figure 3A). The sandwich-like tetramer is stabilized by packing between the two antiparallel dimers to create a hydrophobic core (Figure 3B). In the hydrophobic core, the side chains of Val_{18}, Phe_{20}, Ile_{32}, Leu_{34}, and Val_{36} from four copies of peptide **1** pack together, creating a dense core containing 20 hydrophobic amino acid side chains. The hydrophilic $A\beta_{25-27}$ loops extend off of the sandwich-like tetramer and do not make any significant contacts.

Twisted β-sheet tetramer formed by the parallel dimer.

Two parallel dimers further assemble in a parallel orientation to form a highly twisted β-sheet tetramer comprising an eight-stranded β-sheet (Figures 4A and B). In the twisted β-sheet tetramer, the $A\beta_{27-36}$ β-strand of one dimer is across from the $A\beta_{27-36}$ β-strand of the other dimer. Four hydrogen bonds between the amide backbones of the two parallel dimers stabilize the twisted β-sheet tetramer: Gly_{29} and Cys_{31} on one parallel dimer pair with Lys_{28} and Ala_{30} on the other parallel dimer. The twisted β-sheet tetramer is further stabilized by hydrophobic packing between the two parallel dimers to create a hydrophobic core (Figure 4C). In the hydrophobic core, the side chains of Phe_{19}^{I}, Phe_{20}, Cys_{21}, Val_{24}, Ala_{30}, Cys_{31}, Ile_{32}, Leu_{34}, and Val_{36} of each parallel dimer pack together, creating a dense core containing 18 hydrophobic amino acid side chains.

Octamer.

Two twisted β-sheet tetramers further assemble to form an octamer. The inner four β-hairpin monomers of the octamer create a continuous hydrogen-bonding network containing 12 intermolecular hydrogen bonds between the peptide backbones (Figure 5A). Packing between hydrophobic residues on the inner four β-hairpin monomers further stabilizes the octamer. The side chains of Leu_{17}, Phe_{19}^{I}, Phe_{20}, Ile_{32}, Leu_{34}, Met_{35}, and Val_{36} pack together, creating a core containing 22 hydrophobic amino acid side chains.

The dimers, tetramers, and octamer are all part of the same crystal lattice.

The assembly of peptide **1** into a crystal lattice reveals the ways in which the peptide can self-assemble with other copies of the peptide to form oligomers. The oligomers described above are all part of the same crystal lattice. The asymmetric unit of the X-ray crystallographic structure of peptide **1** contains 11 copies of the peptide (Figure 6A). The asymmetric unit contains two crystallographically unique twisted β-sheet tetramers that comprise the octamer (Figure 6B, cyan strands). The octamer is flanked by three additional copies of peptide **1**: Two copies — by a symmetry operation — comprise the sandwich-like tetramer (Figure 6B, magenta strands). One copy — by a symmetry operation — forms an antiparallel dimer that rests upon the octamer in the crystal lattice (Figure 6B, yellow strands).

Oligomers of $A\beta_{40}$ and $A\beta_{42}$ in SDS-PAGE.

Dimers and tetramers of synthetic or expressed Aβ have been observed to form *in vitro*. In SDS-PAGE, $A\beta_{40}$ predominantly forms two oligomers that migrate at molecular weights consistent with a dimer and tetramer, in addition to the monomer (Figure 7). $A\beta_{42}$ predominantly forms two oligomers that migrate at molecular weights consistent with a trimer and tetramer, in addition to the monomer (Figure 7). The structures of these oligomers are unknown. The parallel and antiparallel dimers formed by peptide **1** provide two potential structural models for the dimer formed by $A\beta_{40}$ in SDS-PAGE. Furthermore, the sandwich-like tetramer and twisted β-sheet tetramer formed by peptide **1** provide potential structural models for the tetramers formed by $A\beta_{40}$ and $A\beta_{42}$ in SDS-PAGE.

Crystallographically based models of two $A\beta_{12-40}$ tetramers.

We envision that the full-length Aβ peptide can assemble in the same fashion as peptide **1** to form sandwich-like tetramers and twisted β-sheet tetramers. To better understand what a sandwich-like tetramer (dimer of antiparallel dimers) and a twisted β-sheet tetramer (dimer of parallel dimers) containing full-length Aβ might look like, we modeled $A\beta_{12-40}$ into the crystallographic coordinates of each tetramer.[37] We first appended the *N*- and *C*-terminal regions 12–15 (VHHQ) and 37–40 (GGVV) onto the crystallographic coordinates of the four peptide **1** monomers that comprise each tetramer, and mutated all modified residues back to the native residues. We then performed replica-exchange molecular dynamics (REMD) to generate realistic conformations the *N*- and *C*-terminal regions of the β-hairpins (Figures 8A and B).[38,39] In these models, the β-hairpins constitute the cores of the tetramers, and the *N*- and *C*-termini surround the cores. The REMD simulations shows that both tetramers can accommodate the *N*- and *C*-terminal residues without steric clashes and

suggests that full-length Aβ could form a sandwich-like tetramer or a twisted β-sheet tetramer.

The structures of the dimers and tetramers formed by peptide **1** provide new models for Aβ oligomers. Aβ dimers are thought to have special significance in the pathogenesis and progression of Alzheimer's disease. Aβ plaques from Alzheimer's disease patients contain cross-linked Aβ dimers that are composed of different Aβ alloforms.[40] Aβ dimers appear to be the building blocks of large, mildly cytotoxic oligomers with molecular weights ranging from 150–650 kDa.[41] Aβ dimers also promote phosphorylation and aggregation of the microtubule-associated protein tau, which is also involved in Alzheimer's disease progression.[42] The structures of these dimers are unknown. The parallel and antiparallel β-sheet dimers formed by peptide **1** provide two potential structural models for the dimers observed in Alzheimer's disease brains.

Aβ tetramers have been observed in protein extracts from Alzheimer's disease brains, but their exact roles in the pathogenesis and progression of the disease is less clear than that of dimers.[43,44] Aβ tetramers prepared *in vitro* are toxic toward both neuroblastoma cells and cultured hippocampal neurons.[45,46] Furthermore, covalently stabilized Aβ tetramers prepared using photo-induced cross-linking of unmodified proteins (PICUP) interact with the cell membranes of hippocampal neurons.[47] The structures of the Aβ tetramers in these studies are unknown. The sandwich-like tetramer and twisted β-sheet tetramer formed by peptide **1** provide two potential structural models for the tetramers observed in Alzheimer's disease brains, as well as the tetramers prepared *in vitro*.

CONCLUSIONS

The structures of the dimers, tetramers, and octamer formed by peptide **1** contribute to the rich structural landscape of amyloidogenic peptides and proteins. CryoEM structures of fibrils formed by amyloidogenic peptides and proteins such as Aβ, islet amyloid polypeptide, tau, α-synuclein, and human prion protein have revealed that the peptides and proteins adopt highly convoluted shapes and pack together to form twisted filaments.[8,10,12,13,14,15,16] The twisted shapes of the oligomers formed by peptide **1** are distinct from the twists of fibrils and filaments. The structure of the sandwich-like tetramer formed by peptide **1** (PDB 6WXM) is reminiscent of the Aβ tetramer reported by Streltsov *et al.* (PDB 3MOQ), as well as the tetramer formed by transthyretin (*e.g.*, PDB 1TTC).[21,48] It is also evocative of the Aβ tetramer and octamer structural models from Ciudad *et al.* (PDB 6RHY).[26] Prominent features of these structures include antiparallel β-hairpins that pack together in a sandwich-like fashion to form a hydrophobic core, much like the sandwich-like tetramer formed by peptide **1**.

The parallel and antiparallel β-sheet dimers, the sandwich-like and twisted β-sheet tetramers, and the octamer formed by peptide **1** add to the diversity of Aβ-derived oligomers observed by our laboratory. In studying, β-hairpin peptides that contain the central and *C*-terminal regions of Aβ, we have discovered a variety of different structures (Figure 9). These structures reveal the intricate ways that Aβ β-hairpins can fit together to form compact oligomers that are stabilized by edge-to-edge hydrogen bonding interactions and

hydrophobic cores. We believe the variety of structures we have observed exemplifies the heterogeneity of oligomers formed by Aβ *in vitro* and in the brain. Investigating the exact relationship between the oligomer structures we have observed crystallographically and the structures of Aβ oligomers in Alzheimer's disease is an active area of research in our laboratory, and we will report our findings from these studies in due course.

METHODS

Synthesis of peptide **1**, X-ray crystallographic procedures, Aβ oligomer preparation, SDS-PAGE and silver staining, and replica-exchange molecular dynamics were performed as described previously.[29,30,31,32,33] These procedures are restated in detail in the Materials and Methods section in the Supporting Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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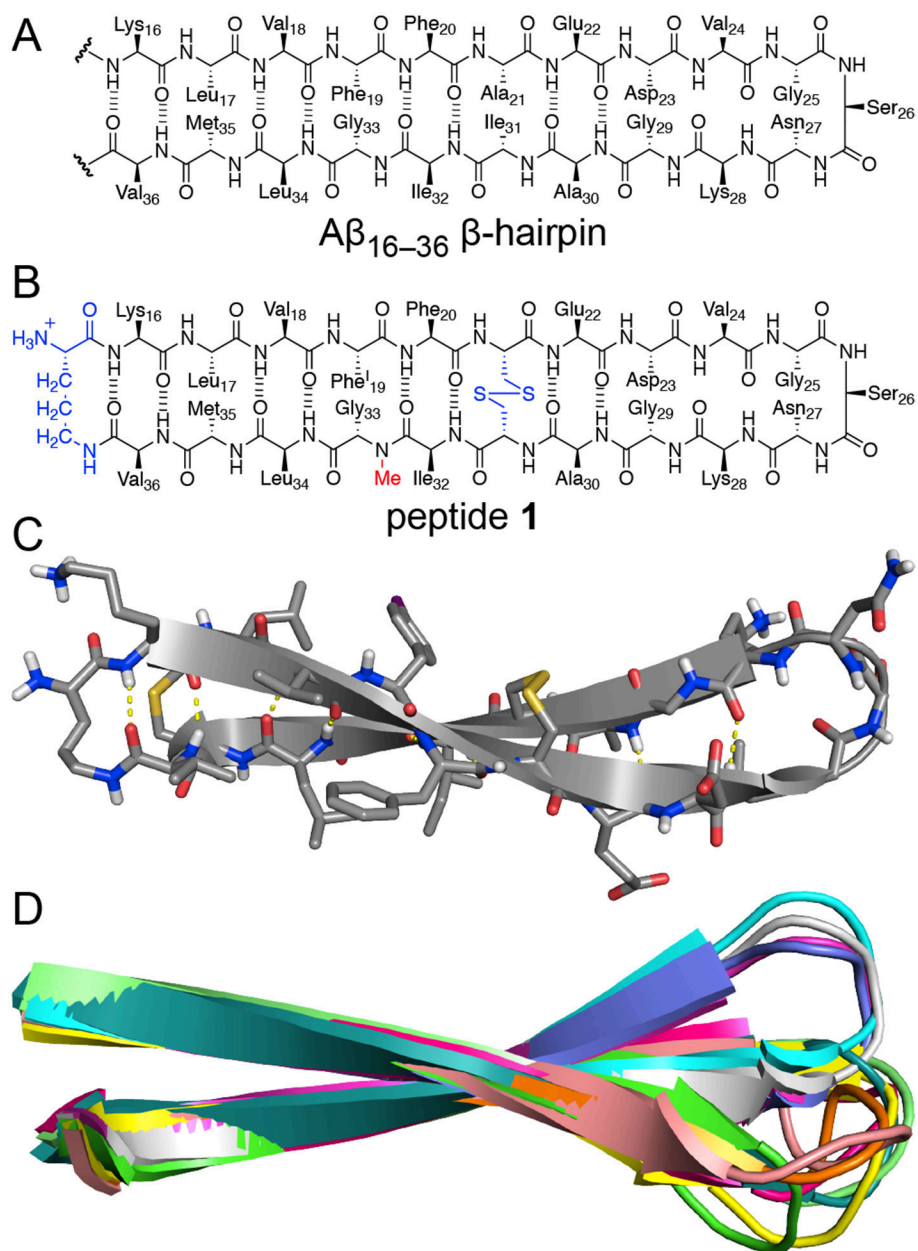
Figure 1.
Peptide **1**. (A) Chemical structure of an Aβ$_{16-36}$ β-hairpin. (B) Chemical structure of peptide **1**. (C) X-ray crystallographic structure of a representative β-hairpin monomer formed by peptide **1** (PDB 6WXM). (D) Overlay of the 11 peptide **1** β-hairpins in the asymmetric unit.
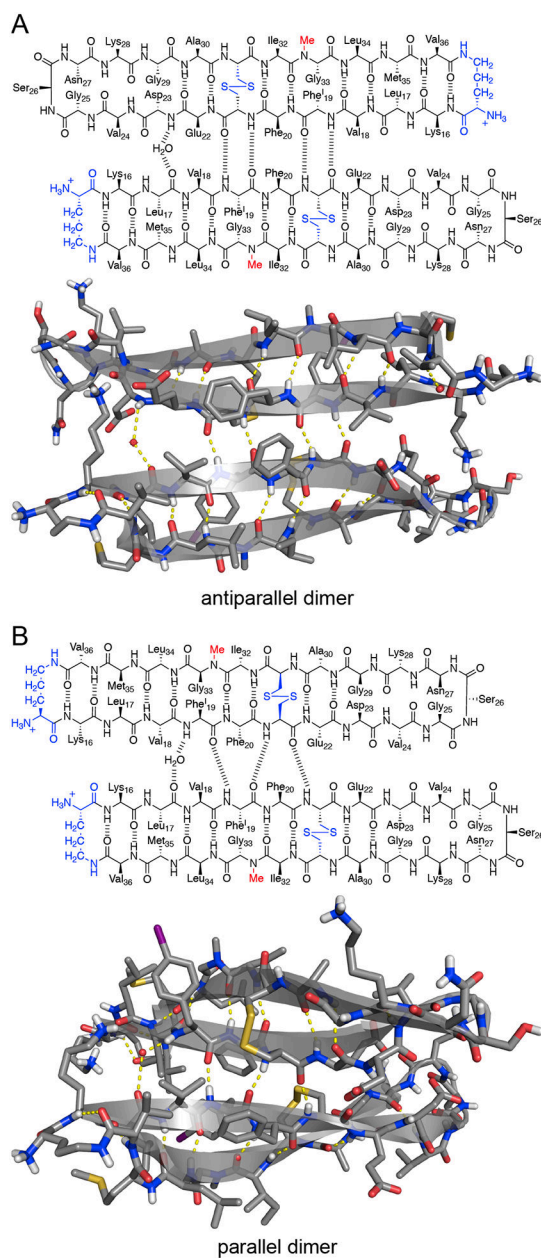
Figure 2.
Dimers formed by peptide **1**. (A) Chemical structure (top) and X-ray crystallographic structure (bottom) of the antiparallel dimer formed by peptide **1**. (B) Chemical structure (top) and X-ray crystallographic structure (bottom) of the parallel dimer formed by peptide **1**.
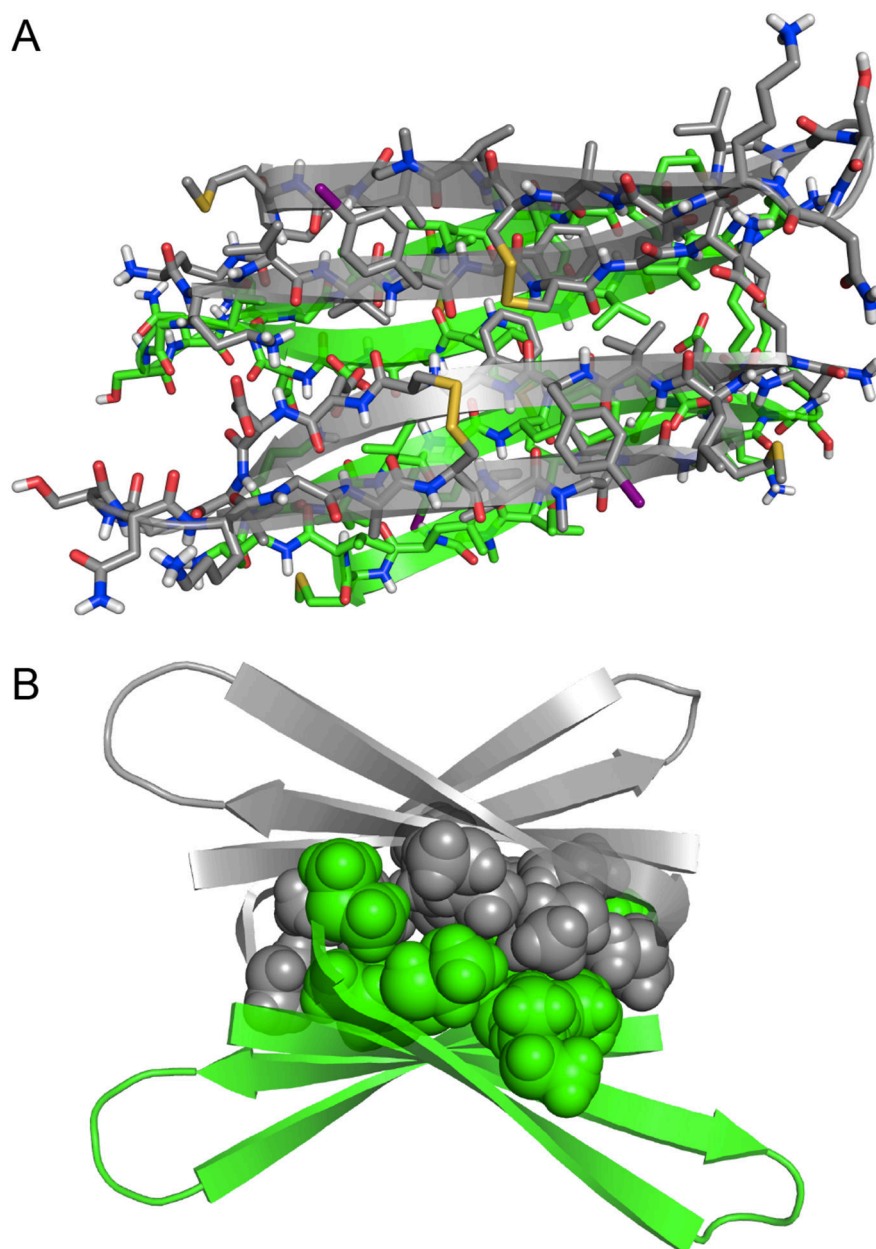
Figure 3.
X-ray crystallographic structure of the sandwich-like tetramer (dimer of antiparallel dimers) formed by peptide **1**. (A) Cartoon and stick model of the sandwich-like tetramer. (B) Cartoon and sphere model of the sandwich-like tetramer. The residues that comprise the hydrophobic core are shown as spheres.
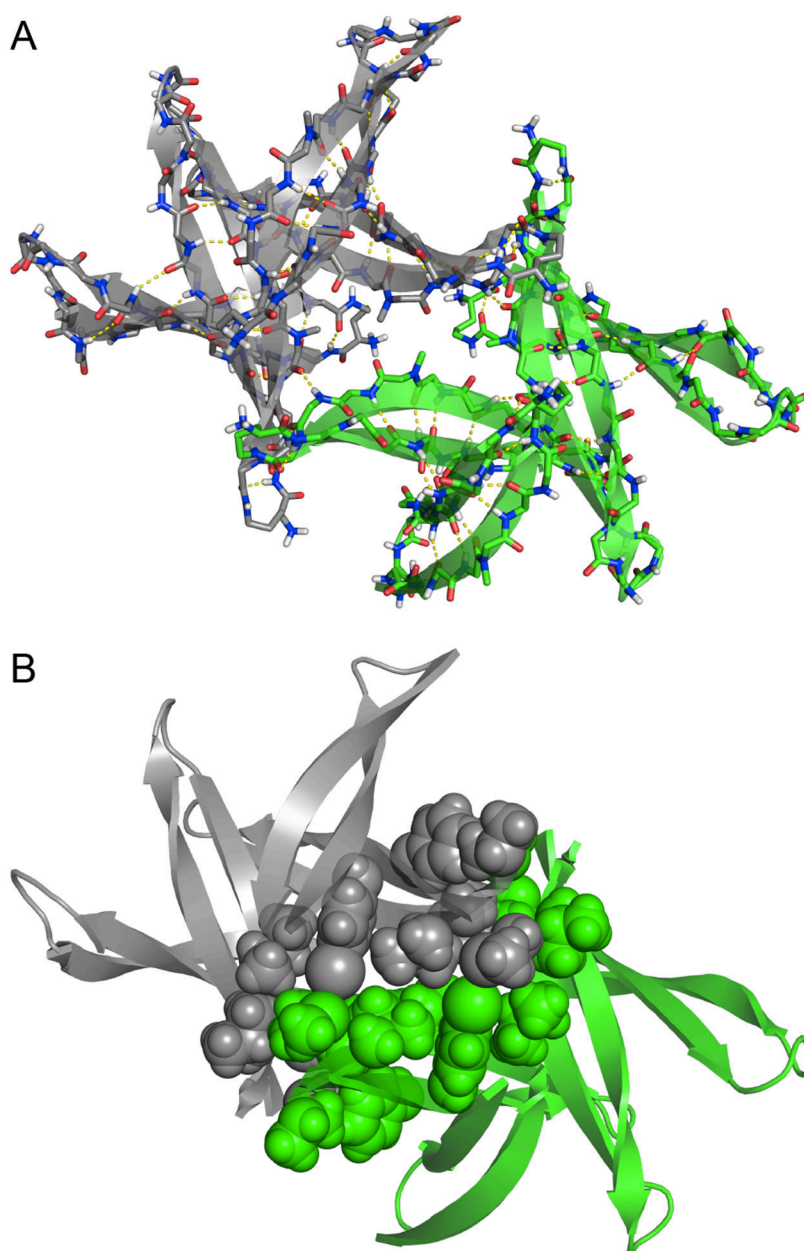
Figure 4.
The twisted β-sheet tetramer formed by the parallel dimer. (A) Chemical structure. (B) X-ray crystallographic structure of the twisted β-sheet tetramer (cartoon and stick model; the side chains are omitted for clarity). (C) X-ray crystallographic structure of the twisted β-sheet tetramer (cartoon and sphere model; the residues that comprise the hydrophobic core are shown as spheres).

Figure 5.
X-ray crystallographic structure of the octamer (dimer of twisted β-sheet tetramers) formed by peptide **1**. (A) Cartoon and stick model of the octamer (side chains are omitted for clarity). (B) Cartoon and sphere model of the octamer illustrating the hydrophobic packing between the two twisted β-sheet tetramers. The side chains of the hydrophobic core are shown as spheres.
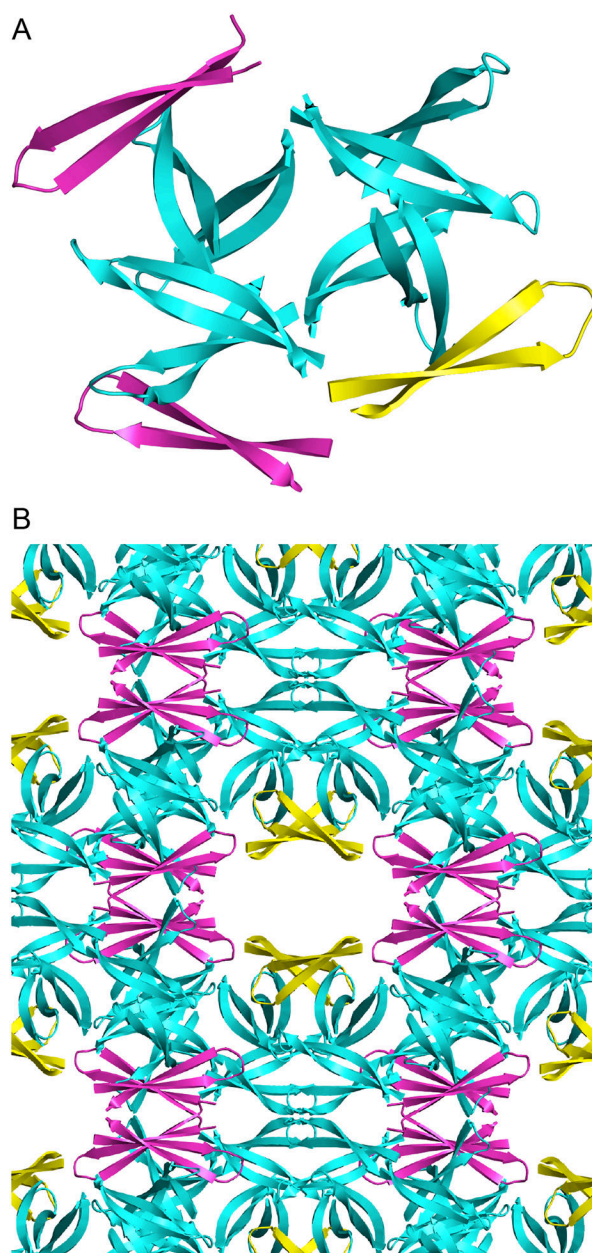
Figure 6.
(A) The asymmetric unit of the X-ray crystallographic structure of peptide **1**. (B) The crystal lattice of peptide **1**, illustrating the relationship between the sandwich-like tetramer (magenta) and the twisted β-sheet tetramers that comprise the octamer (cyan). An antiparallel dimer that rests on the octamer is shown in yellow.
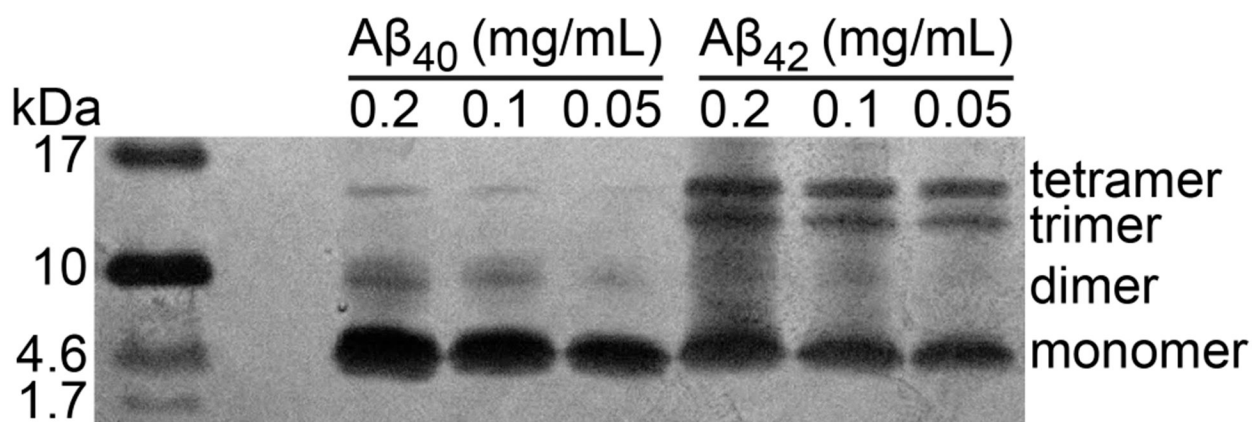
Figure 7.
Silver-stained SDS-PAGE of recombinantly expressed $A\beta_{40}$ and $A\beta_{42}$ illustrating the oligomers that the peptides form *in vitro*. A 5-μL aliquot of each peptide concentration in a serial dilution was run on the gel.
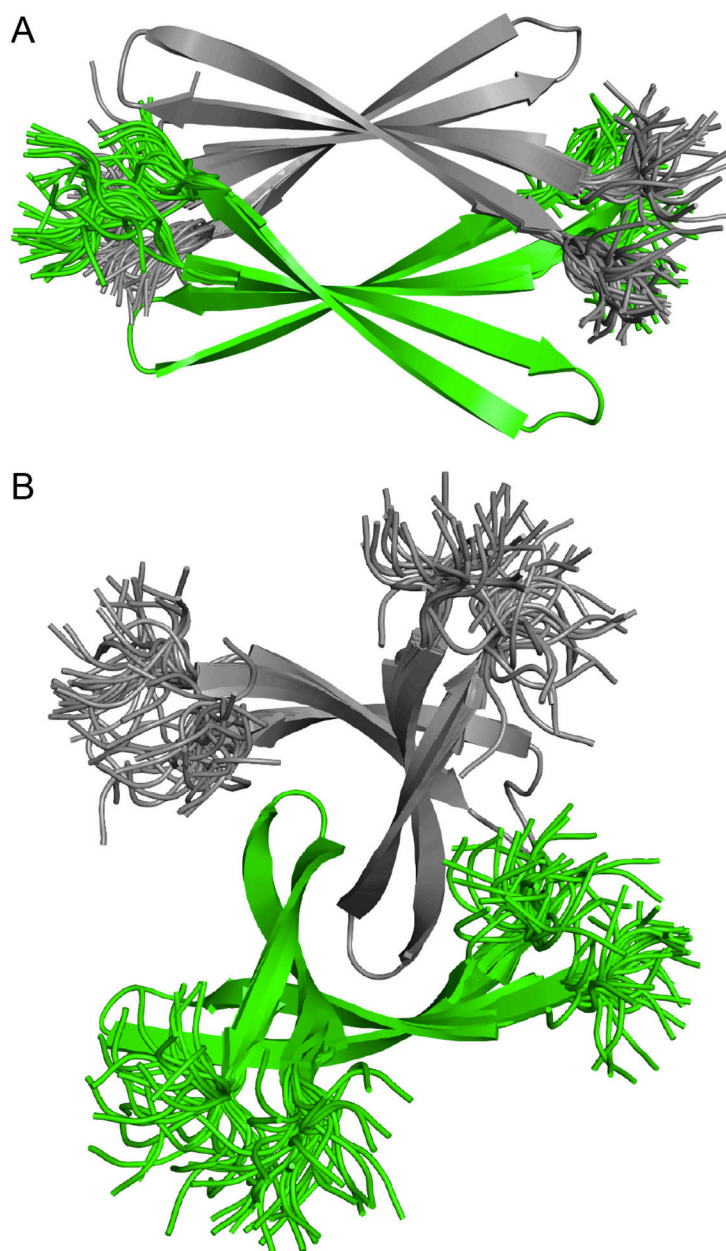
Figure 8.
Crystallographically based models of an $A\beta_{12-40}$ sandwich-like tetramer (A) and an $A\beta_{12-40}$ twisted β-sheet tetramer (B). Superpositions of 32 structures generated by replica-exchange molecular dynamics.
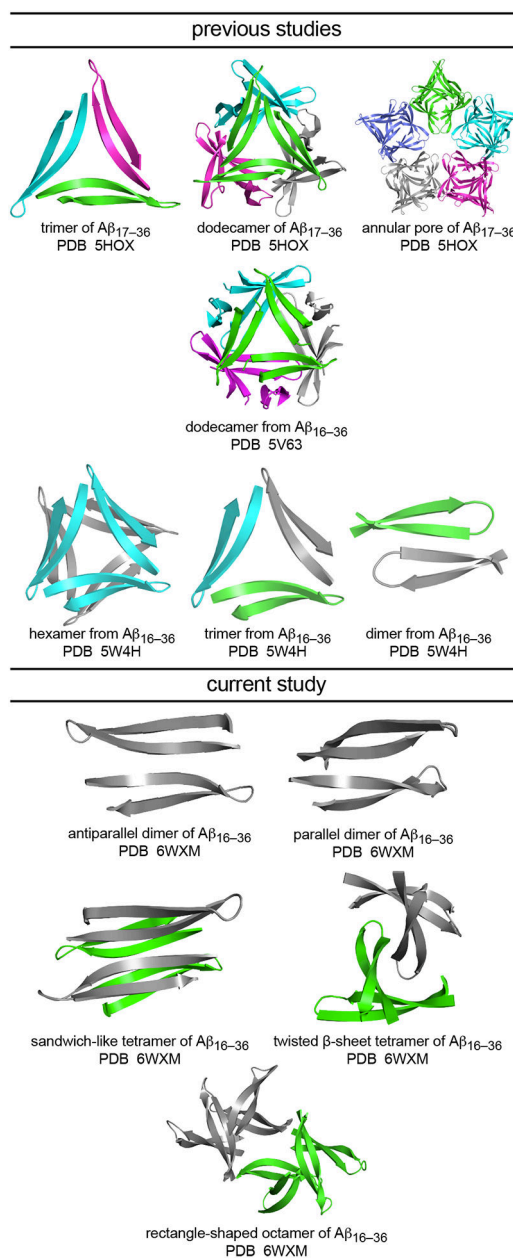
Figure 9.
Representative oligomers of Aβ-derived peptides observed in our laboratory by X-ray crystallography.