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Journal

Organic Letters, 19(13)

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Publication Date

2017-07-07

DOI

10.1021/acs.orglett.7b01445

Peer reviewed

HHS Public Access

Author manuscript

Org Lett. Author manuscript; available in PMC 2018 July 07.

Published in final edited form as:

Org Lett. 2017 July 07; 19(13): 3462–3465. doi:10.1021/acs.orglett.7b01445.

X-ray Crystallographic Structure of a Compact Dodecamer from a Peptide Derived from $A\beta_{16–36}$

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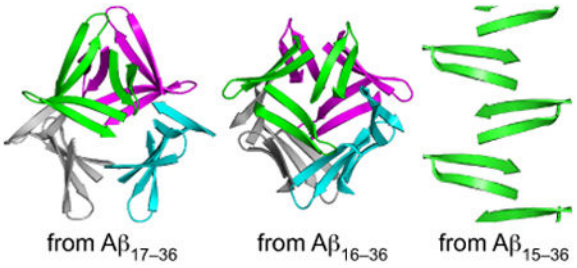
Abstract

The assembly of the β-amyloid peptide, Aβ, into soluble oligomers is associated with neurodegeneration in Alzheimer's disease. The Aβ oligomers are thought to be composed of β-hairpins. Here, the effect of shifting the residue pairing of the β-hairpins on the structures of the oligomers that form is explored through X-ray crystallography. Three residue pairings were investigated using constrained macrocyclic β-hairpins in which $A\beta_{30–36}$ is juxtaposed with $A\beta_{17–23}$, $A\beta_{16–22}$, and $A\beta_{15–21}$. The $A\beta_{16–22}$–$A\beta_{30–36}$ pairing forms a compact ball-shaped dodecamer composed of fused triangular trimers. This dodecamer may help explain the structures of the trimers and dodecamers formed by full-length Aβ.

Graphical abstract



β-Hairpins are emerging as key building blocks of amyloid oligomers.[1] The twisted shape, exposed hydrogen-bonding edges, and hydrophobic surfaces of β-hairpins impart a unique propensity to self-assemble. Characterization of the assemblies that form at high-resolution has been challenging, because the resulting amyloid oligomers are heterogeneous and polymorphic. The potential of β-hairpins to fold in a variety of ways may further contribute to heterogeneity and polymorphism. There is a desperate need for high-resolution structural models of the oligomers formed by amyloidogenic peptides and proteins. Here we report the

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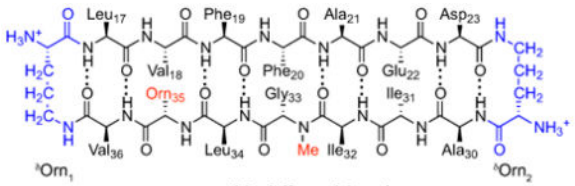
Supporting Information: The Supporting Information is available free of charge on the ACS Publications website.
Details of the synthesis and characterization of peptides **2**, **3**, and **4**.
Crystallographic data in CIF and PDB format.[13]

Notes: The authors declare no competing financial interest.

X-ray crystallographic structure of a compact ball-shaped dodecamer derived from the β-amyloid peptide, Aβ, that is composed of fused trimers.

Several research groups have generated models of Aβ oligomers composed of either β-hairpins or β-sheets.[2] The Härd group proposed an assembly of six β-hairpins, arranged in a barrel-like structure.[2b] Our own laboratory described the X-ray crystallographic structures of trimers, hexamers, and dodecamers formed by macrocyclic β-hairpin peptide **1** derived from $A\beta_{17–36}$.[2c] Peptide **1** contains the heptapeptide β-strands Gly_{33} further blocks uncontrolled assembly and reduces the propensity of the peptide to aggregate. Peptide **1** assembles into trimers that further assemble into spherical dodecamers (PDB 4NTR). The interface between the dodecamers constitutes a hexamer in which two trimers pack together. We subsequently reported that a homologue of peptide **1** containing the loop of the β-hairpin assembles in a similar fashion.[2e]



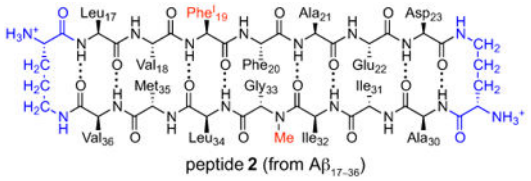
peptide **1** (from $A\beta_{17–36}$)

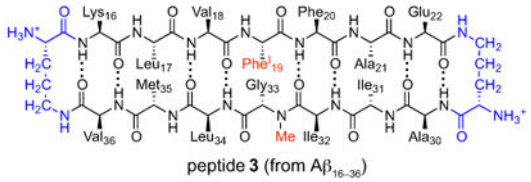
In the current study, we set out to explore how altering the residue pairing of the β-hairpin associated with peptide **1** alters the resulting supramolecular assembly. We envisioned a scenario in which $A\beta_{15–23}$ is free to adopt three pairings with $A\beta_{30–36}$: one in which $A\beta_{17–23}$ pairs with $A\beta_{30–36}$, one in which $A\beta_{16–22}$ pairs with $A\beta_{30–36}$, and one in which $A\beta_{15–21}$ pairs with $A\beta_{30–36}$. Figure 1 illustrates this concept. These shifts in pairing sequentially pull Lys_{16} and Gln_{15} into the upper β-strand while pushing Asp_{23} and Glu_{22} out of the β-strand and into the loop.

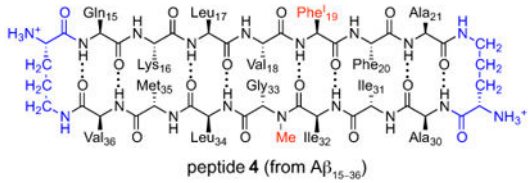
$A\beta_{17–23}$ and $A\beta_{30–36}$ connected by two 5-linked ornithine β-turn mimics ($^{\delta}$Orn) to form a macrocycle.[3] In designing peptide **1**, we replaced the native hydrophobic Met_{35} with the polar isostere ornithine (α-linked) to enhance solubility and reduce the propensity of the peptide to aggregate. An *N*-methyl on We designed macrocyclic β-hairpin peptides **2**, **3**, and **4** to explore the concept embodied in Figure 1 and characterized the resulting assemblies by X-ray crystallography.[4] We incorporated the native Met_{35} into each of these peptides, rather than the α-linked ornithine isostere, to better mimic the native β-hairpins. We replaced Phe_{19} with *para*-iodophenylalanine (Phe^{I}) to facilitate crystallographic phase determination by single wavelength anomalous diffraction phasing.[5] We synthesized, crystalized, and determined the crystallographic structures of peptides **2**–**4** using procedures that we have previously reported.[6]

Peptide **2** assembles in an identical fashion to peptide **1**, forming triangular trimers that further assemble into spherecal dodecamers (Figure 2). In each trimer, three monomers occupy the edges of an equilateral triangle (Figure 2A). The $A\beta_{17–23}$ β-strands of the monomers come together, hydrogen bonding to each other and to three water molecules that sit in the center of each trimer. The $A\beta_{30–36}$ β-strands of the monomers form the outer edges

of the trimer. The side chains of Leu_{17}, Phe^{I}_{19}, and Val_{36} of one monomer pack against the side chains of Ala_{21}, Asp_{23}, Ile_{32}, and Leu_{34} from an adjacent monomer at the three vertices of the trimer. Four trimers further assemble in a tetrahedral arrangement into a loosely packed hollow dodecamer (Figure 2B).[7] The diameter of the dodecamer spans 4–6 nm, depending on the points of measure, while its central cavity spans *ca.* 1.4 nm. The side chains of Phe^{I}_{19}, Leu_{34}, and Val_{36} line the cavity. The dodecamers further pack to form the lattice, with each interface between two dodecamers constituting a sandwich-like hexamer (Figure 2C). The side chains of Phe_{20}, Glu_{22}, and Ile_{31} pack against one another in the interior of the hexamer.







Peptide **3** assembles into compact ball-shaped dodecamers that differ from those formed by peptide **2** (Figure 2D). The dodecamer formed by peptide **2** comprises discrete triangular trimers, while the dodecamer formed by peptide **3** comprises fused triangular trimers. Each trimer in the dodecamer formed by peptide **3** shares three edges with the three adjacent trimers. As a result, the trimers are not discrete entities within the ball-shaped dodecamer, but instead are fused like the benzene rings of naphthalene or graphite (Figure S1).[8]

Two types of trimers make up the ball-shaped dodecamer formed by peptide **3** (Figures 2E and F). The two types of trimers differ in the placement of the $A\beta_{16–22}$ and $A\beta_{30–36}$ β-strands. The $A\beta_{16–22}$ β-strands of the monomers hydrogen bond to each other within the trimer depicted in Figure 2E, while the $A\beta_{30–36}$ β-strands hydrogen bond to each other within the trimer depicted in Figure 2F. The outer edges of the trimer depicted in Figure 2E lie within three different trimers, like the one depicted in Figure 2F. Conversely, the outer edges of the trimer depicted in Figure 2F lie within three different trimers, like the one depicted in Figure 2E. Three water molecules occupy the center of the trimer depicted in Figure 2E. The three *N*-methyl groups occupy the center of the trimer depicted in Figure 2F, in lieu of three water molecules.

The ball-shaped dodecamer formed by peptide **3** is hollow, like the dodecamer formed by peptide **2**. The diameter of the ball-shaped dodecamer spans 3–4 nm depending on the points of measure, while its central cavity spans *ca.* 1.0 nm. The side chains of Phe^{I}_{19} line the cavity, while the exterior surface of the dodecamer displays the side chains of Lys_{16}, Val_{18}, Phe_{20}, Glu_{22}, Ala_{30}, Ile_{32}, Leu_{34}, and Val_{36}. Unlike the dodecamers formed by peptide **2**, the dodecamers formed by peptide **3** do not form sandwich-like hexamers. Instead these dodecamers pack hexagonally and stack like cannonballs.

The dodecamers formed by peptides **2** and **3** share similar themes in self-assembly, as both are composed of triangular trimer subunits. Mapping the triangular subunits of each dodecamer onto an octahedron highlights these similarities, as well as key differences (Figure 3). In the dodecamer formed by peptide **2**, the four trimers occupy four of the bonding network in the dodecamer formed by peptide **2**. The dodecamer formed by peptide **3** is more densely packed than the dodecamer formed by peptide **2**. Assembly of peptide **3** into a dodecamer buries *ca.* 10,800 $Å^2$ of surface area, whereas assembly of peptide **2** into a dodecamer buries only *ca.* 8,100 $Å^2$. It is not obvious from their sequences or structures why peptide **3** forms a more compact dodecamer that differs from that of peptide **2**.

In contrast to the discrete oligomers formed by peptides **2** and **3**, peptide **4** forms a fibril-like assembly (Figure 4). Each monomer of peptide **4** hydrogen bonds with the two neighboring monomers along the fibril axis. The interface between monomers constitutes a parallel β-sheet with three intermolecular hydrogen bonds in which Leu_{17}, Val_{18}, and Phe^{I}_{19} pair with $^{\delta}Orn_2$, Ala_{30}, and Ile_{31}. The *N*-methyl group on Gly_{33} eight triangular faces of the octahedron. The interfaces between the trimers define the remaining four triangular faces. In the dodecamer formed by peptide **3**, each monomer occupies one edge of the octahedron, and each trimer defines one of the eight triangular faces of the octahedron. At each of the six vertices of the octahedron, four monomers of peptide **3** form an eight-stranded β-barrel-like opening. An analogous opening is absent in the dodecamer formed by peptide **2**. The hydrogen-bonding network that helps stabilize both dodecamers is more extensive in the dodecamer formed by peptide **3**, which contains 36 additional intermolecular hydrogen bonds beyond those that compose the hydrogen- blocks formation of a fully hydrogen-bonded interface, prying apart the β-sheets and requiring a water molecule to bridge a hydrogen bond between the NH group of Phe_{20} and the carbonyl group of Ile_{31}. Each monomer is flipped upside down with respect to the neighboring monomers in the fibril such that the surfaces of the monomers are displayed in an alternating pattern along the surface of the fibril: one monomer displays its top surface, the next monomer displays its bottom surface, and so on down the fibril (Figure S2).

The fibril-like assemblies formed by peptide **4** are not flat; instead they zig-zag in the *x*-*z* plane as depicted in Figure 4B. The fibrils stack along the *x*-axis, creating densely packed layers in the *x*-*z* plane. The layers run in opposite directions to one another. Within each layer, all of the *N*-methyl groups point in the same direction. In the green layers in Figures 4A and B, the *N*-methyl groups point in the negative *z* direction, while in the cyan layers, the *N*-methyl groups point in the positive *z* direction (Figure S3). The layers pack tightly through hydrophobic interactions, with the top surface of a monomer in one layer packing against the bottom surface of its neighbor in the adjacent layer. This heterofacial packing of

residues contrasts the exclusively homofacial packing of residues in the dodecamers formed by peptides **2** and **3**. It is not obvious why peptide **4** forms fibril-like assemblies in the crystal lattice, instead of the trimers and dodecamers formed by peptides **2** and **3**.

The different assemblies of peptides **2**–**4** reflect the rich and diverse modes of β-hairpin self-assembly and illustrate their propensity to form both fibril-like and oligomeric assemblies. The assembly of β-hairpins into dodecamers comprising triangular trimer subunits offers an alluring high-resolution model for the enigmatic oligomers reported for full-length Aβ. Selkoe *et al.* reported that Aβ trimers inhibit long-term potentiation.[9] Ashe *et al.* reported that putative dodecamers of Aβ, termed Aβ*56, cause memory deficits in a mouse model of Alzheimer's disease.[10] The putative Aβ*56 dodecamers appear to be composed of trimer subunits. The trimers and dodecamers formed by peptides **2** and **3** provide two models of how Aβ may oligomerize in Alzheimer's disease. The formation of trimeric oligomers is also a common theme of full-length peptides and proteins associated with other amyloid diseases.[11] We have also reported trimeric assemblies and related higher-order oligomers formed by peptides derived from α-synuclein and β_2-microglobulin.[12]

Shifting the residue pairings of the two β-strands within a β-hairpin may dramatically alter the self-assembly. The approach of systematically varying the residue pairings in constrained macrocyclic β-hairpins has revealed a compact ball-shaped dodecamer containing fused trimers and stabilized by an extensive network of hydrogen bonds. The importance of residues 15–36 in the aggregation of full-length Aβ makes peptides **2**–**4** relevant models for the assembly of full-length Aβ. We envision that full-length Aβ may be able to fold and assemble in a similar fashion. We do not yet understand the relationship between the sequence of a β-hairpin and its mode of assembly. This gap in understanding represents an exciting frontier in supramolecular assembly.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institutes of Health (Grant GM097562). P.J.S. thanks the UCI Training Program in Chemical and Structural Biology for training grant support (T32 GM108561) as well as ARCS Foundation Orange County for additional support.

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4. Peptides **2**–**4** mimic only three of the six potential arrangements of β-strands associated with the $A\beta_{17–36}$, $A\beta_{16–36}$, and $A\beta_{15–36}$ β-hairpins. The other three arrangements of β-strands cannot be achieved with these macrocyclic β-sheets.
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7. The asymmetric unit of the crystal lattice formed by peptide **2**contains 16 peptide molecules, which assemble into four crystallographically unique dodecamers, each containing four unique peptidemolecules. The four dodecamers are very similar in structure.
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13. Crystallographic coordinates of peptides **2**, **3**, and **4** were deposited into the Protein Data Bank (5V65, 5V63, and 5V64).

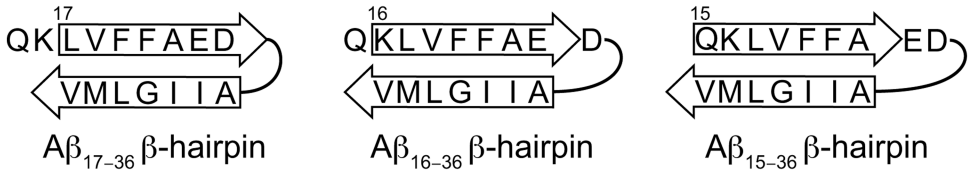

Figure 1.
Cartoons of three different β-hairpins formed by $A\beta_{15–36}$, with different residue pairings.

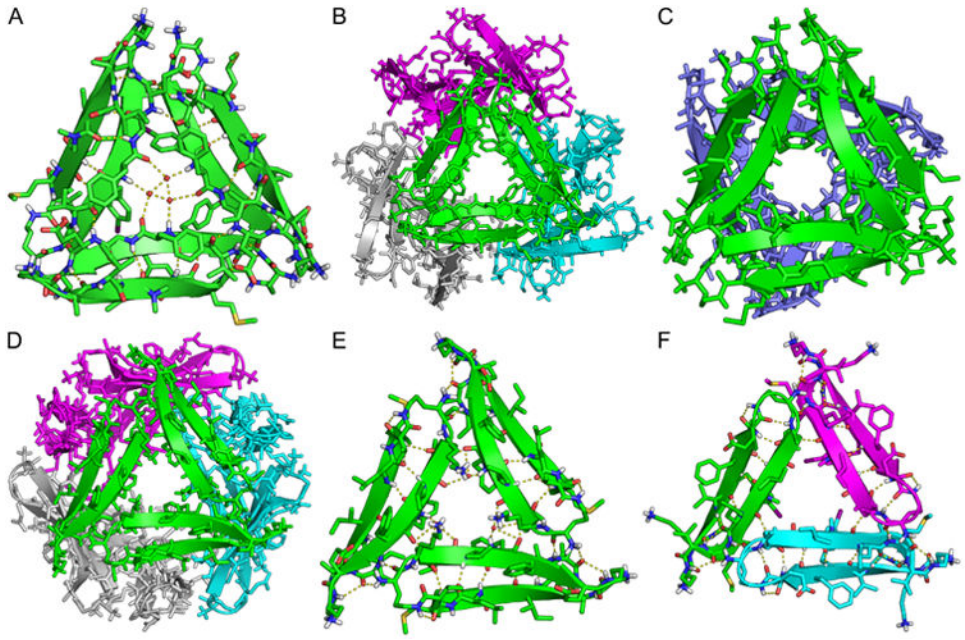

Figure 2.

Supramolecular assembly of peptide **2** (A-C, PDB 5V65) and peptide **3** (D-F, PDB 5V63). (A) Triangular trimer formed by peptide **2**. (B) Dodecamer comprising four triangular trimers formed by peptide **2**. (C) Sandwich-like hexamer comprising two triangular trimers that constitutes the interface between two dodecamers formed by peptide **2**. (D) Ball-shaped dodecamer formed by peptide **3**. (E and F) The two types of triangular trimers within the ball-shaped dodecamer formed by peptide **3**.

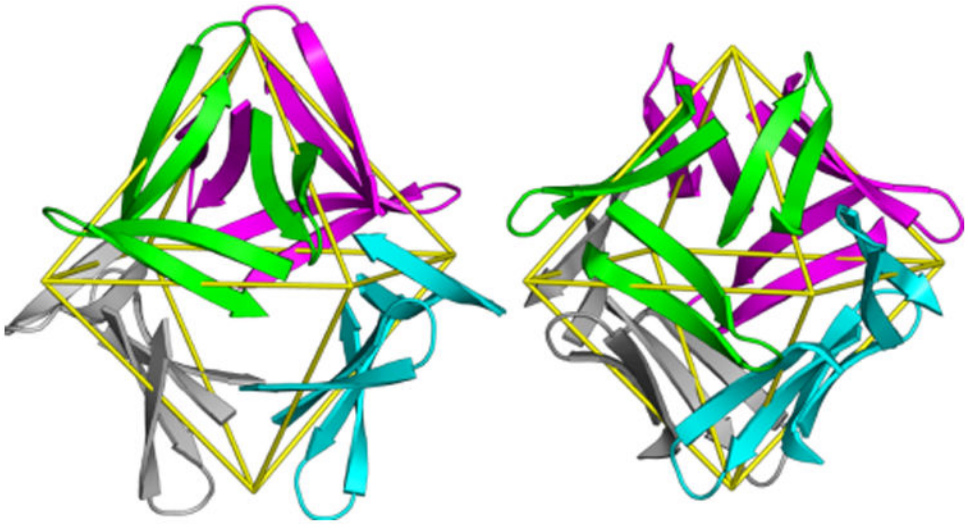

Figure 3.

Dodecamers formed by peptides **2** (left) and **3** (right) superimposed on octahedra (yellow). Both dodecamers are depicted on the same scale.

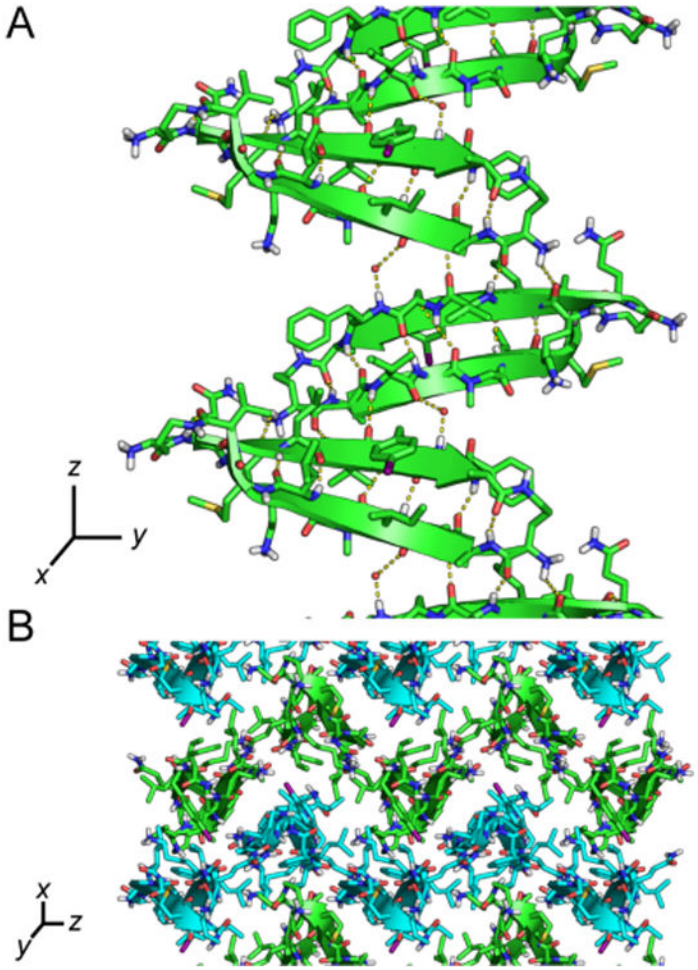

Figure 4.

Fibril-like assembly formed by peptide **4** (PDB 5V64). (A) Top view of the fibril formed by peptide **4**. (B) Side view of four layers of fibrils colored to highlight the zig-zag.