# Polymorphism in Alzheimer A$\beta$ Amyloid Organization Reflects Conformational Selection in a Rugged Energy Landscape

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## 1. Introduction

Amyloids can have normal biological functions.[1−3] However, amyloidogenic proteins can also form unwanted oligomeric or polymeric aggregates when disturbed from their native functional states. Such aggregates are associated with numerous neurodegenerative diseases, such as diabetes type II,[4] Alzheimer (AD), Parkinson, Huntington, and prion ("mad cow") diseases, amyotrophic lateral sclerosis, and Down's syndrome (DS).[5−9] Increasing evidence from studies in human, transgenic mice, cultured cells, wild type rodent, and in vitro systems indicates that soluble oligomers of amyloidogenic proteins are both responsible for amyloidosis[10,11] and are the toxic agent.[12−14] Some data suggest that their final large aggregates can also lead to cytotoxicity.[15,16]

Yifat Miller received her Ph.D. degree in Computational Physical Chemistry from the Hebrew University of Jerusalem in 2007 under the supervision of Professor R. Benny Gerber, using molecular dynamics simulations to investigate the chemistry of atmospheric molecules. She is currently a NIH postdoctoral fellow in the laboratory of Professor Ruth Nussinov at the CCR Nanobiology Program, National Cancer Institute. Her current research interest focuses on the structural variability of potential conformations of Alzheimer's A$\beta$ under various conditions and the self-assembly mechanism leading to ordered fibril formation.

Ordered aggregates can extend into $\beta$-strand enriched fibrils, regardless of their initial native conformational states. Experimental and computational approaches revealed structural details of these organizations. Amyloid structural models were obtained by different methods: spectroscopy,[17−23] solid-state NMR (ssNMR),[24−29] EPR,[30,31] hydrogen/deuterium exchange,[15,27,32] cryo-electron microscopy (cryo-EM) and electron microscopy (EM),[33−36] X-ray fiber diffraction and X-ray diffraction,[37−41] and peptide design.[42] Apart from a handful of peptides that were successfully crystallized providing insight into the ordered amyloid arrangement,[43] amyloid structural elucidation has been fraught with difficulties. The insolubility of amyloid fibrils has made crystallization and solution NMR virtually impossible. Under these circumstances, ssNMR has been the method of choice for structural determination, providing insight into the nature of $\beta$-sheet organization.[44] ssNMR data of A$\beta$ segments coupled with atomistic molecular dynamic simulations have further been useful in addressing the driving forces for targeted associations.[45,46]

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Ruth Nussinov received her Ph.D. in 1977, from the Biochemistry Department at Rutgers University, and did postdoctoral work in the Structural Chemistry Department of the Weizmann Institute. Subsequently she was at the Chemistry Department at Berkeley, the Biochemistry Department at Harvard, and the NIH. In 1984, she joined Tel Aviv University. In 1990, she became a Professor in the Department of Human Genetics, at the Medical School. In 1985, she accepted a concurrent position at the National Cancer Institute of the NIH, where she is a Senior Principal Investigator heading the Computational Structural Biology Group. She has authored over 350 scientific papers. Her interests largely focus on protein folding, protein−protein interactions, amyloid conformations, and large multimolecular associations with the goal of understanding the protein structure−function relationship.

To date, the key question of the mechanism through which amyloids lead to cytotoxity is still a major challenge.[47−51] As in folding, it is well established that in amyloid fibrils, nucleation and kinetics depend not only on inherent features such as the amino acid composition, sequence, and length, but also on environmental conditions such as temperature,[52] concentration, pH,[53] metal ions, agitation, and enhanced aggregation on the lipid bilayer surface. At the same time, the self-assembly mechanism that leads to ordered fibril formation is not fully understood. Open questions relate to (1) how the monomeric peptides assemble into oligomers; (2) which segments of a long peptide constitute the recognition motifs and as such play key roles in amyloid fibril formation;[54] (3) how the β-strands arrange relative to one another; (4) are there favored organizations between the β-sheets, and if so (5) what are these and what are the intermolecular interactions between the layers that stabilize these; and, finally, (6) what are the pathways and the intermediate states that are involved in seed and fibril formation. These combine to contribute to one of the most difficult issues that are related to protein aggregation, that is, aggregate polymorphism; the aggregates can have different preferred fibril architectures depending on (even slight) changes in any of these sequence or environmental factors.

Amyloid β peptide (Aβ peptide) has served as a paradigm for studies of amyloid formation and conformations. The full-length Aβ peptide has 40−42 residues when cut from its precursor protein. Aβ aggregates are observed in brain tissues of Alzheimer's patients,[55] and it is generally believed that Aβ peptide oligomerization is a major mechanism leading to the neuron-cell death.[15] This Review focuses on polymorphic Aβ and other amyloid conformations. It compiles the conformations of the full-length $A\beta_{1-42}$/$A\beta_{1-40}$ and its fragments, and presents amyloid assemblies in terms of the energy landscape. In addition, it describes how, via conformational selection and population shift as the primary molecular recognition mechanism,[56] monomeric Aβ states whose shapes are compatible can assemble into organized fibril geometries.[56−58] Together, this leads to an overview of the structural variability and the underlying mechanisms of fibril polymorphism. Understanding the mechanisms and the range of structural features of the aggregates are of crucial importance for effective drug design to reduce aggregate formation.

## 2. Full-Length β-Amyloid and Its Fragments: A Warehouse of Sequences Prone to Amyloid Formation and Polymorphism

Most of the information on oligomeric assemblies is obtained from the full-length β-amyloid peptide $A\beta_{1-42}$/$A\beta_{1-40}$.[15,25,59−69] The sequence of the human $A\beta_{1-42}$ peptide is 1-DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA-42. Both the $A\beta_{1-42}$ and the shorter $A\beta_{1-40}$ peptides derive from cleavage of the transmembrane amyloid precursor protein (APP) by β- and γ- secretases.[70] There are variations in proteolytic pathways, and thus different fragments can exist in vivo. The cleavage by α- and γ- secretases leads to $A\beta_{17-42}$ in the so-called "non-amyloidogenic cleavage".[9,71]

Experimentally,[53] different full-length $A\beta_{1-40}$ conformations are preferred in the fibril at different pH values. Petkova et al.[72] observed that the distinct fibril morphologies reflect the variation in the molecular structure of $A\beta_{1-40}$ at the protofilament level. As shown in Figure 1A, subtle variations in fibril growth conditions such as an agitated or quiescent environment translate into morphological changes of the dominant amyloid structures; these are captured in the amyloid seeds and self-propagate.[72] In a recent comprehensive study of $A\beta_{1-40}$ fibril polymorphism, using cryo-EM and three-dimensional recognition techniques, Meinhardt et al.[73] have shown that structural persistence and morphological

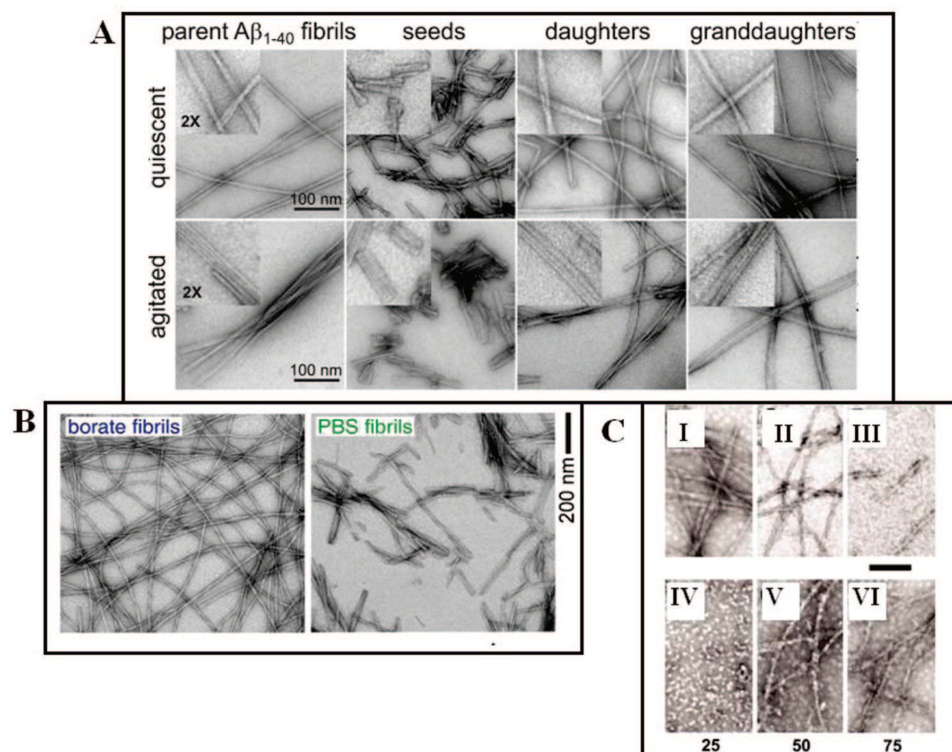


**Figure 1.** Polymorphism of full-length A$\beta$ peptides observed in experiments. (A) Transmission electron microscopy (TEM) images of amyloid fibrils formed by the A$\beta_{1-40}$ peptide, prepared by incubation of A$\beta_{1-40}$ solutions under quiescent dialysis conditions. Reprinted with permission from ref 72. Copyright 2005 Science/AAAS. (B) Cryo electron microscopy (cryo-EM) of the structural persistence and morphological diversity of A$\beta_{1-40}$ fibrils grown either in sodium borate (pH 7.8) at 22 °C or in phosphate-buffered saline (PBS) (pH 7.4) at 37 °C. Both samples encompass evidently more than one fibril morphology; Reprinted with permission from ref 73. Copyright 2009 Elsevier. (C) Electron microscopy (EM) images of morphologies of A$\beta_{1-42}$ fibrils. Reprinted with permission from ref 15. Copyright 2005 National Academic of Sciences, USA.

diversity can be observed in A$\beta_{1-40}$ fibrils grown under the same buffer solutions (Figure 1B). However, with two more residues, the longer A$\beta_{1-42}$ peptide displays even larger variability than A$\beta_{1-40}$ in the monomeric conformation and in fibril morphology. The fibril organization (and neurotoxicity) of A$\beta_{1-42}$ peptides also change when mixing WT M35L and F19G mutants (Figure 1C).[15] Zhang et al.[36] suggested an A$\beta_{1-42}$ conformation with the C-terminus forming the inside wall of a hollow core and the N-terminus playing a role in the fibrilization.

In addition to full-length A$\beta$ peptides, a number of in vitro-generated $\beta$-amyloid fragments were also observed to form amyloid fibrils. The sequences cover all A$\beta$ peptide regions: the N-terminus, middle, and the C-terminus regions. An early study indicated that A$\beta_{18-28}$, A$\beta_{16-28}$, A$\beta_{14-28}$, A$\beta_{12-28}$, and A$\beta_{1-28}$ fragments were sufficient to form amyloids; however, A$\beta_{20-28}$ did not yield a clear amyloid structure.[74,75] Other studies extended the fragment list to include A$\beta_{11-25}$,[76,77] A$\beta_{16-22}$,[78] A$\beta_{10-23}$,[79] and A$\beta_{14-23}$.[80,81] Figure 2A and B provides EM images of the A$\beta_{1-28}$ and A$\beta_{10-23}$ amyloids, respectively. For A$\beta_{1-28}$, an assortment can be seen, including linear, curvilinear, and rosette. The amyloid fibril of A$\beta_{10-23}$ has a diameter around 3 nm and a length of 500 nm, and for the amyloid fibril of A$\beta_{1-28}$ the diameter is in the range of 6−8 nm and the length in the range of 120−160 nm. Among these, the A$\beta_{16-22}$ is one of the most studied fragments by both experimental[78,82] and computational approaches.[83−90] The sequence of A$\beta_{16-22}$ contains the KLVFF core. Starting from the KLVFF, Tjernberg et al.[91] systematically synthesized 10 peptides with one more residue on each side (A$\beta_{6+x,30-x}$, where $0 \leq x \leq 9$). Under their experimental conditions, apart from three peptides (KLVFF, QKLVFFA, and HQKLVFFE), all peptides formed amyloid fibrils with different morphologies.[91]

Extension from 1−28 to 1−30, 1−33, 1−36, and 1−39 presented fibril morphologies similar to the full-length A$\beta$.[92] These A$\beta$ sequences are often used to represent full-length A$\beta$ in studies of amyloid formation and toxicity, particularly those including A$\beta_{25-35}$ and A$\beta_{10-35}$, which are believed to be the major responsible species.[67,93−99] It appears that A$\beta_{25-35}$ could be more toxic than A$\beta_{1-42}$, leading to a rapid lysis of red blood cells[97] and causing more extensive oxidative damage.[100] The short version of the A$\beta_{25-35}$, A$\beta_{26-33}$, also forms thin long amyloids with a uniform diameter of 5 nm[101] (Figure 2C). A$\beta_{10-35}$ has a supramolecular structure similar to the full-length.[67] Using computational approaches, Ma and Nussinov studied the A$\beta_{10-35}$ fragment as a model to study the full-length A$\beta$, and were the first to discover the U-turn conformation in A$\beta$.[46,83]

The C-terminal region (residues 17−42) is mostly hydrophobic. One study observed that the transmembrane part of A$\beta_{29-42}$ forms amyloids faster than the full-length one, with the order of A$\beta_{29-42}$ > A$\beta_{1-42}$ > A$\beta_{1-32}$ > A$\beta_{1-28}$;[102] on the other hand, another study found that this peptide scarcely formed filaments. The few fibril structures that could be visualized had a diameter 2−3 nm and an average length of 50 nm.[79] Hilbich et al.[79] explored X-43 peptides (i.e., peptides with an additional residue at the C-terminus). They found that A$\beta_{4-43}$, A$\beta_{8-43}$, A$\beta_{10-43}$, and A$\beta_{12-43}$ form more stable, tightly packed structures. They further observed that if the

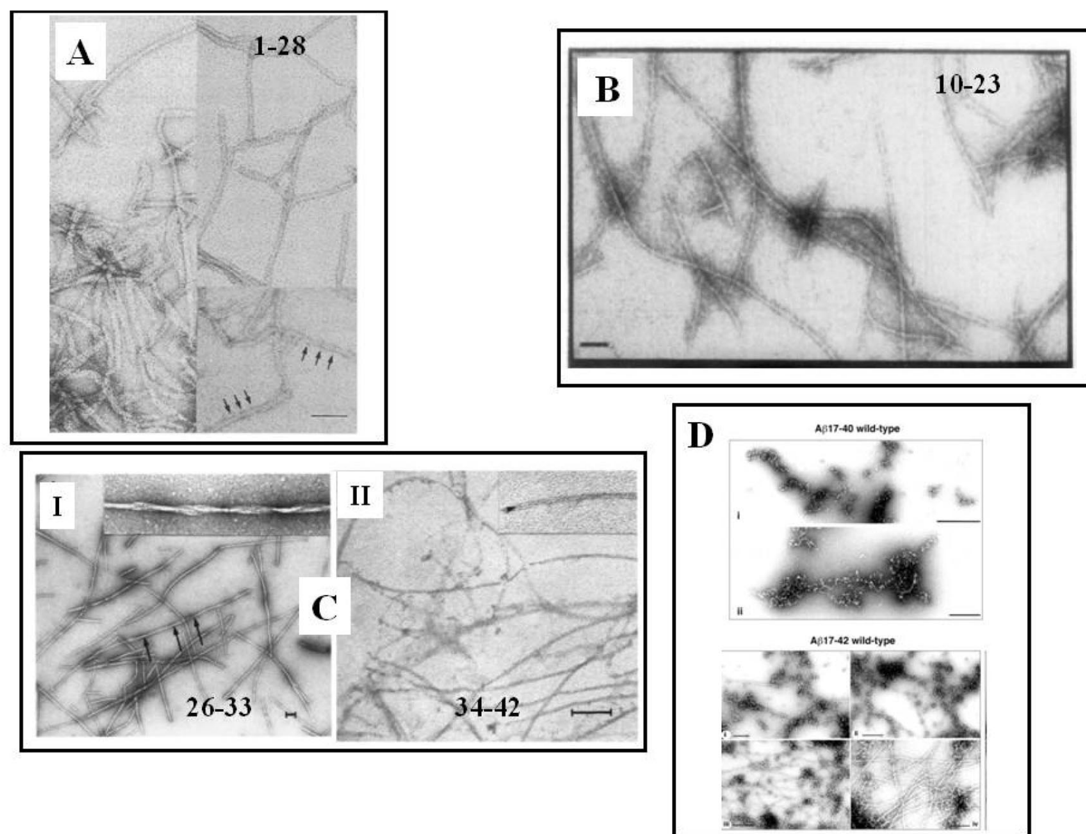


**Figure 2.** Polymorphism of Aβ peptide fragments as observed in EM images. (A) $A\beta_{1-28}$ fibrils, negatively stained, are periodically stained along some of their edges (indicated by arrows). Fibril ends appear to splay or unfurl. Reprinted with permission from ref 75. Copyright 1987, directly permission from Dennis J. Selkoe. (B) $A\beta_{10-23}$ filaments prepared in vitro. The filaments are irregularly twisted around each other. Reprinted with permission from ref 79. Copyright 1991 Elsevier. (C) $A\beta_{26-33}$ and $A\beta_{34-42}$ fibrils. $A\beta_{26-33}$ fibrils are thin and uniform as compared to those of $A\beta_{34-42}$ fibrils and appears to be comprised of fibril pairs. At low magnification, $A\beta_{34-42}$ shows two types of assemblies: twisted fibrils and untwisted narrow fibrils. At higher magnification, twisted fibrils and periodic deposition of enhanced staining (indicated by arrows) are observed. Reprinted with permission from ref 101. Copyright 1990 American Chemical Society. (D) $A\beta_{17-40}$ form protofibrils (top) and a mature fibril of $A\beta_{17-42}$ (bottom). Reprinted with permission from ref 103. Copyright 2003 American Society for Biochemistry and Molecular Biology.

peptides are suspended in aqueous solvents, the N-terminal can also promote amyloid formation: the fibril formed by the $A\beta_{12-43}$ peptide is less stable than the one formed by the $A\beta_{1-43}$.[79] $A\beta_{34-42}$ formed rigid fibrils of varying lengths ranging from 10 to more than 300 nm; the single fibrils were slab-like with no apparent twist and had a width of 9 nm. The assembly of several of these smaller fibrils likely constitutes twisted fibers (Figure 2C).[44,101] Generally, it seems that amyloid fibrils formed from C-terminal regions are less smooth than those from the N-terminal region. Apart from the $A\beta_{34-42}$ fragment, the nonsmooth features of the p3 peptide fibrils ($A\beta_{17-40}$/$A\beta_{17-42}$)[103] (Figure 2D) further support the potential structural variability of the C-terminal, which could relate to its hydrophobic nature promoting polymorphism (see section 3.3).

Overall, in addition to fiber diffraction, which over the years has provided a wealth of useful structural information,[104] computational studies of monomeric and oligomeric structures of various fragments have clearly shown structural flexibility and oligomerization complexity, for example, $A\beta_{10-35}$,[83,105] $A\beta_{16-35}$,[83,106] $A\beta_{17-42}$,[107,108] $A\beta_{10-35}$ monomers,[109−114] $A\beta_{21-30}$,[115−118] $A\beta_{13-23}$,[91] $A\beta_{1-28}$,[119,120] and $A\beta_{14-23}$.[91]

## 3. Molecular Structures Underlying Amyloid Polymorphism

The structural regularity of amyloids is not as good as that of the highly ordered crystals; nonetheless, X-ray fibril diffraction has provided important structural features. Examples of fibril structure variability of $A\beta_{11-25}$ and the full-length $A\beta_{1-40}$ obtained by X-ray diffraction are shown in Figure 3A. Early diffractions revealed that all amyloids, regardless of their sequences and secondary structures in the native folded states, convert to β-sheets. The β-strand backbone orientation is perpendicular to the fibril axis, which is termed cross-β structure (Figure 3C). The distance between the β-strands is ∼0.5 nm, permitting favorable backbone hydrogen bonds, and the layers are usually separated by ∼1 nm allowing closely packed β-sheet association (Figure 3B).[77] With these basic features unchanged, the amyloid polymorphism derives from the way the β-strands associate into fibrils. There are three major structural features that may decide the overall amyloid fibril morphologies: (1) differences in backbone orientation; (2) differences in backbone conformation; and (3) differences in the way in which the oligomers, with almost identical structure, associate. The combination of these three factors can give rise to an enormous variation in conformational

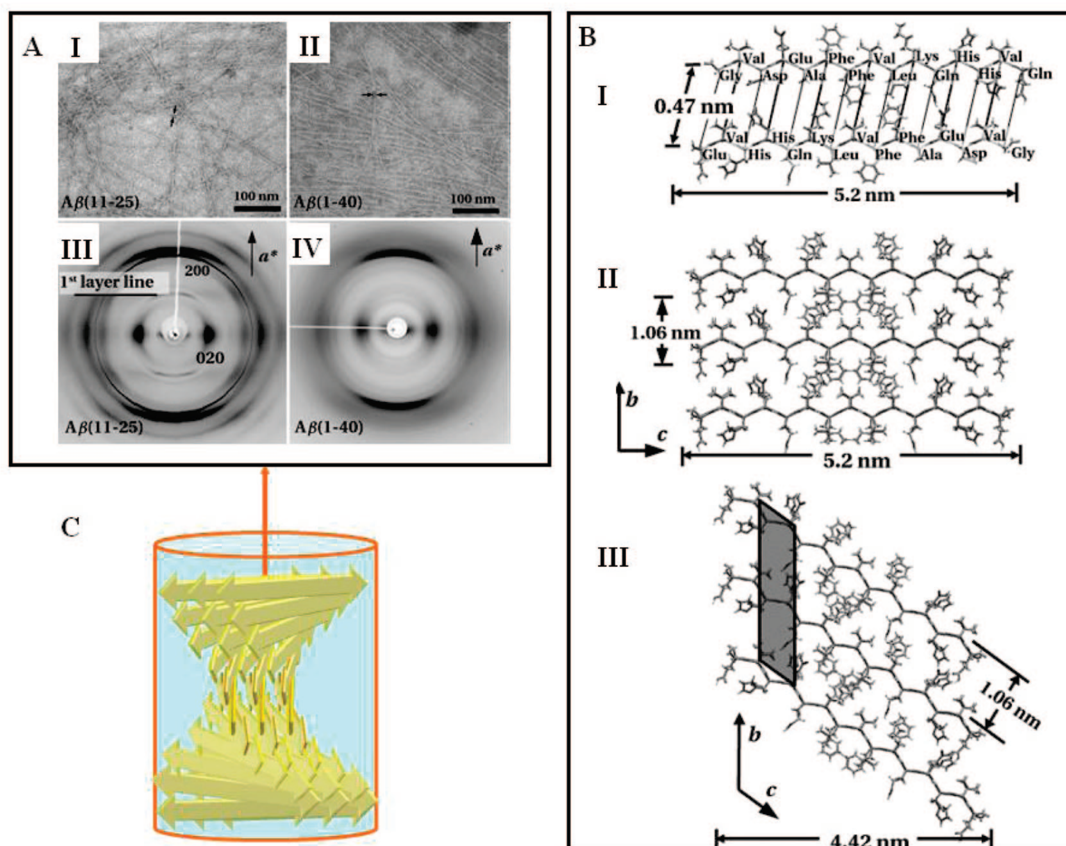


**Figure 3.** (A) EM images and X-ray diffraction patterns of cross-$\beta$ ribbons for A$\beta_{11-25}$ (I) and (III) and A$\beta_{1-40}$ (II) and (IV). The measured widths of the fibrils are ~5 nm for A$\beta_{11-25}$ and ~7 nm for A$\beta_{1-40}$ (see arrows in I and II). These are wide-angle X-ray patterns; $a^*$ is the vertical fibril axis;. Reprinted with permission from ref 77. Copyright 2003 Elsevier. (B) Model of A$\beta_{11-25}$: (I) Antiparallel organization. The 0.47 nm spacing ($a$-direction) is controlled by hydrogen bonding, and the molecules are ~5 nm in length. (II) Direct stacking that would generate an orthorhombic unit cell. (III) Stacking with a slip of 0.697 nm (one $\beta$-sheet crimp; one structural repeat in the $c$-direction) parallel to the $c$ axis. This stacking arrangement gives rise to monoclinic unit cell. Reprinted with permission from ref 77. Copyright 2003 Elsevier. (C) Illustration of cross-$\beta$ arrangement in amyloids; the peptide backbone $\beta$-strands are perpendicular to the fibril axis (arrow).

detail and, consequently, in seed oligomer and fibril morphology. This section demonstrates and discusses these structural features in detail.

## 3.1. Polymorphic Backbone Organizations within and between Sheets in Amyloid Fibrils: Potential Combinations

The detailed structural variations of tightly packed ("steric-zipper") segments of amyloid-forming peptides[43] and the amyloid backbone associations can be applied to other short peptides like A$\beta$ fragments. In principle, there are thousands of possible patterns of intra- and inter-residue amyloid fibril backbone strand organizations for in-register and out-of-register interactions. Figure 4 highlights several representative in-register alignments with double-layered $\beta$-strand segment arrangements within and between sheets. Figure 4A−D presents organizations where each layer has parallel strands; within these, parallel orientations between the two layers are illustrated in Figure 4A and B and antiparallel orientations between the two layers of the segment in Figure 4C and D. Figure 4E−H presents antiparallel arrangements in each layer, with Figure 4E−G illustrating parallel orientation between the two layers and Figure 5H an antiparallel orientation. Other possible models of backbone organization can consist of parallel dimers (or trimers, or variable oligomer sizes, etc.) associated in an antiparallel orientation.

Short segments are more convenient systems, because their smaller sizes make them easier to study experimentally and computationally; at the same time, the basic principles are likely to be unchanged. Most studied A$\beta$ segments[25,44,64−68,74−76,78−80,91−96,101] are organized in an in-register, parallel arrangement; however, some present a preferred antiparallel organization (enumerated in Table 1). While only some of the potential organizations have been observed, others may also exist, except that their populations may be too low to be observed by experiment.

Short fragments (less than 15 residues length) tend to have antiparallel $\beta$-strands. On the basis of X-ray diffraction and Ala[16]A$\beta_{1-28}$ mutant substitution comparison, the $\beta$-strands' orientation of A$\beta_{1-28}$ fibrils was proposed to be all-antiparallel (as in Figure 4H).[75] As shown in Figure 3B, the A$\beta_{11-25}$ has antiparallel $\beta$-strands within the sheet; however, the sheet−sheet orientation is parallel (as shown in Figure 4G).[77] For the A$\beta_{14-23}$, the antiparallel conformation has also been proposed from NMR information.[80] For A$\beta_{16-22}$, which is one of the most studied fragments, both experimental and computational studies suggest antiparallel organization.[83−90] In the C-terminal region, A$\beta_{34-42}$ was also shown by ssNMR to be antiparallel.[44] IR spectroscopy indicates that amyloid

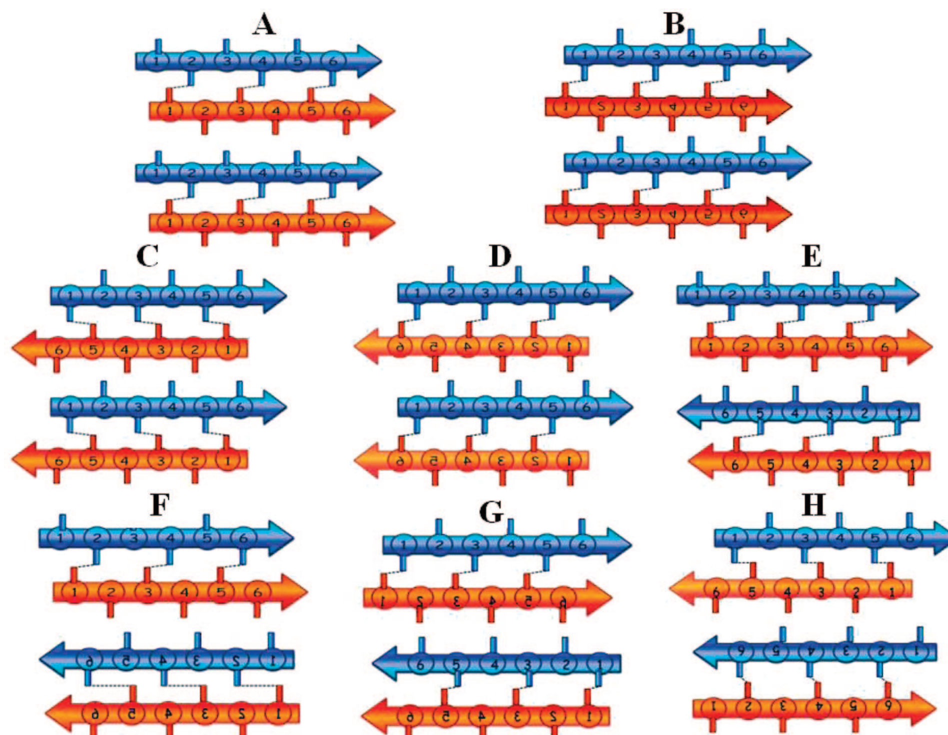


**Figure 4.** Potential architectures of strand organizations in amyloid fibrils. Interactions (in dotted lines) are shown between the first layer (blue) and the second layer (red) for (A) parallel to parallel, face-to-face orientation; (B) parallel to parallel, face-to-back orientation; (C) parallel to antiparallel, face-to-face orientation; (D) parallel to antiparallel, face-to-back orientation; (E) antiparallel to antiparallel, face-to-face orientation, parallel to parallel between layers; (F) antiparallel to antiparallel, face-to-back orientation, parallel to parallel between layers; (G) antiparallel to antiparallel, face-to-back orientation, parallel to parallel between layers; and (H) antiparallel to antiparallel, face-to-back orientation, antiparallel to antiparallel between layers.

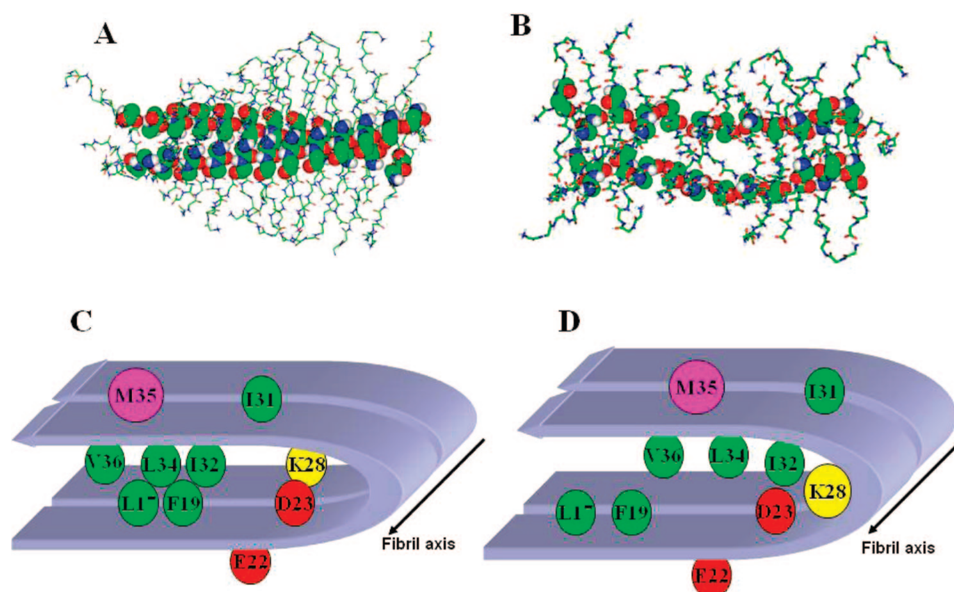


**Figure 5.** Illustration of $A\beta_{25-35}$ in parallel (A) and antiparallel (B) arrangements. The sticks are peptide backbone, and the balls are Ile residues. Parts (C) and (D) illustrate the U-turn structures of Ma−Nussinov−Tycko (ref 46) and Lührs et al. (ref 15), respectively.

fibrils of $A\beta_{29-42}$ and $A\beta_{10-23}$ have antiparallel $\beta$-sheet and less ordered structure (loops or $\beta$-turns).[79]

Polymorphism can thus arise from different $\beta$-strand orientation and side-chain registration. For example, $A\beta_{16-22}$ can have two stable antiparallel orientations (Figure 4G and H)[83] as both have in register side-chain interactions. Recent experiments also revealed an out-of-register, one residue shift in the antiparallel orientation.[82] $A\beta_{25-35}$, a central Aβ region fragment, may have both parallel (Figure 5A) and antiparallel (Figure 5B) orientations; both arrangements are probably stabilized by hydrophobic interactions.[121] However, the parallel orientation could be more ordered due to a strong steric zipper match of central Ile residues (Figure 5A and B); Ile was proposed to promote amyloid formation in general.[122] As indicated in Figure 5, $A\beta_{25-35}$ gives rise to different morphologies depending on whether it is oriented in parallel or antiparallel. A similar scenario arises from parallel or antiparallel arrangements of the peptide hI-$APP_{20-29}$, corresponding to the amyloid of the SNNFGAILSS region of the human islet amylin polypeptide.[123] There,

**Table 1. Features of the Full-Length Aβ Amyloid, Aβ Segments, and $\beta_2$M (K3)**

| segment | length of segment (number residues) | percentage of hydrophobic residues | percentage of hydrophilic residues | percentage of charged residues | net charge of monomer's segment | configuration of amyloid fibril | ref |
|---|---|---|---|---|---|---|---|
| $A\beta_{1-42}/A\beta_{1-40}$ | 40/42 | 48 | 40 | 21 | −3 | parallel | 15, 25, 68 |
| $A\beta_{10-35}$ | 26 | 46 | 42 | 19 | −1 | parallel | 24, 67, 93−96 |
| $A\beta_{17-42}$ | 26 | 62 | 19 | 12 | −1 | parallel | 107, 108 |
| $A\beta_{14-23}$ | 10 | 50 | 50 | 30 | −1 | antiparallel | 80 |
| $A\beta_{34-42}$ | 9 | 78 | 0 | 0 | 0 | antiparallel | 101 |
| $A\beta_{1-28}$ | 28 | 32 | 61 | 32 | −3 | antiparallel | 75 |
| $A\beta_{16-22}$ | 7 | 71 | 29 | 29 | 0 | antiparallel | 78 |
| $A\beta_{11-25}$ | 15 | 47 | 47 | 27 | −2 | antiparallel | 76 |
| $A\beta_{1-9}$ | 9 | 22 | 67 | 44 | −2 | disorder (random coil) | 25 |
| $A\beta_{24-30}$ | 7 | 29 | 43 | 14 | +1 | loop | 25, 64, 65 |
| K3 | 22 | 41 | 32 | 18 | −2 | parallel | 150 |

ssNMR measurements presented two different structural forms of amylin fibrils, responsible for type II diabetes.[123]

## 3.2. Polymorphism in the U-Turns in Amyloid Fibrils

Full-length and other long Aβ fragments, especially those containing central and C-terminal regions (for example, $A\beta_{10-35}$), mostly have parallel β-strands orientation. However, the tendencies for a U-turn backbone conformation in the central region provide yet another variable feature for Aβ peptides. The flexibility of the turn region allows the Aβ peptide to adopt slightly different turn types leading to different amyloid morphologies.

Two models of the three-dimensional structures of $A\beta_{1-42}$/$A\beta_{1-40}$ fibrils were proposed and verified experimentally.[46] The first, Ma−Nussinov−Tycko model,[46,68] presents a double layer structure with residues 10−22 and 30−40 forming β-strands and residues 23−29 a bend or loop. The two β-strands form two separate, in-register, parallel β-sheets, which can interact with one another due to the intervening bend segment (Figure 5C). The second model by Lührs et al.[15] presents a parallel single layer structure of $A\beta_{1-42}$ fibrils based on hydrogen/deuterium exchange NMR data, combined with side-chain packing constraints from pairwise mutagenesis, ssNMR, and high-resolution cryo-EM (Protein Data Bank code, 2BEG). Observations based on these experiments indicated that residues 1−16 are structurally disordered and residues 17−26 and 31−42 form two β-strands, $\beta_1$ and $\beta_2$, respectively, connected by a U-turn spanning four residues, 27−30 (Figure 5D). This model also suggested that parallel oligomers associate in parallel. The difference between the two U-turns is that in the Ma−Nussinov−Tycko model it is tighter, thus allowing the C-terminal residues to interact with up to N-terminal residue Tyr10;[46,68] on the other hand, the Lührs' turn is looser, with Lys28 shifted, and consequently the C-terminal region can only reach residue Leu17.[15]

Conformational differences in the U-turn can be expected to lead to polymorphic sheet registration and β-strand organization at the termini. For the Aβ case, although both models suggest parallel sheets, the two models differ in the U-turn conformation, leading to different extents of disorder in the N-terminal. In Tycko's model, the U-turn segment extends over 7 residues and in Lührs' only over 4. As to the N-terminal, the disordered segment in Tycko's model includes 8 residues, whereas in Lührs' it is 16, because the N-terminal projects into the solvent much beyond the C-terminal, thus without tight vdW interactions. This also affects the sheet−sheet registration. Thus, polymorphism in parallel $A\beta_{1-42}/A\beta_{1-40}$ (or fragments that include the U-turn segment and the two β-strands, such as $A\beta_{10-35}$) appears to be largely governed by the U-turn conformational variability, and an interplay between various factors can also expected to play a role in polymorphism. Consistently, a combined experimental and simulations study revealed varied U-turn shapes forming by different segment sizes: $A\beta_{x-42}$ ($x = 29-31$).[124] Recent simulations presented variations for $A\beta_{10-40/10-42}$[125] and $A\beta_{1-42}$[126−128] due to the U-turn conformation.

It is frequently noted in the literature that the amyloid fibril is a supramolecular structure composed of several protofibrils wound around each other mostly in a left-handed twist along their main axis.[35,129−137] Different U-turn conformations can affect the twist angles leading to different oligomer and fibril morphologies. Presumably, a difference in the U-turn conformation exhibits a difference in the interstrand angle; a change of even a fraction of a degree can significantly change the twist. The change will not present a right-handed twist, but will present different EM morphologies.

## 3.3. Polymorphism in Association of β-Sheet Oligomers

Apart from the large structural variations described above, small changes in side-chain orientation or in the environment (e.g., pH, temperature, concentration, salt, agitation, synthetic versus brain-seeded) may lead β-sheet oligomers to associate in different ways, leading to amyloid polymorphism with similar molecular organizations. This has been illustrated in $A\beta_{1-40}$ fibril morphologies. Recently, polymorphism of $A\beta_{1-40}$ at the protofibril level had been obtained by Meinhardt et al.[73] They classified 12 single $A\beta_{1-40}$ amyloid fibrils differing in the fibril width and twist angles, all forming under the same solution conditions. As shown in Figure 6A, the differences derive from the protofibril associations with altered contact areas. One of the distinct features of the polymorphism observed by Meinhardt et al.[73] is that 2-fold symmetry is mostly conserved for all 11 amyloid fibril models (except fibril model 12) constructed from cryo-EM (not shown here). Fibril model 12 has a different symmetry and organization: even though it still consists of two protofibrils, they are offset with respect to the central fibril axis, alternating from the left-handed to the right-handed and with different mechanical properties.[73] A larger deviation from the 2-fold symmetry of $A\beta_{1-40}$ fibril morphologies has been observed by Paravastu et al.[138] They found two $A\beta_{1-40}$ fibril morphologies differing in several features: the overall symmetry (2-fold versus 3-fold); the conformation of the

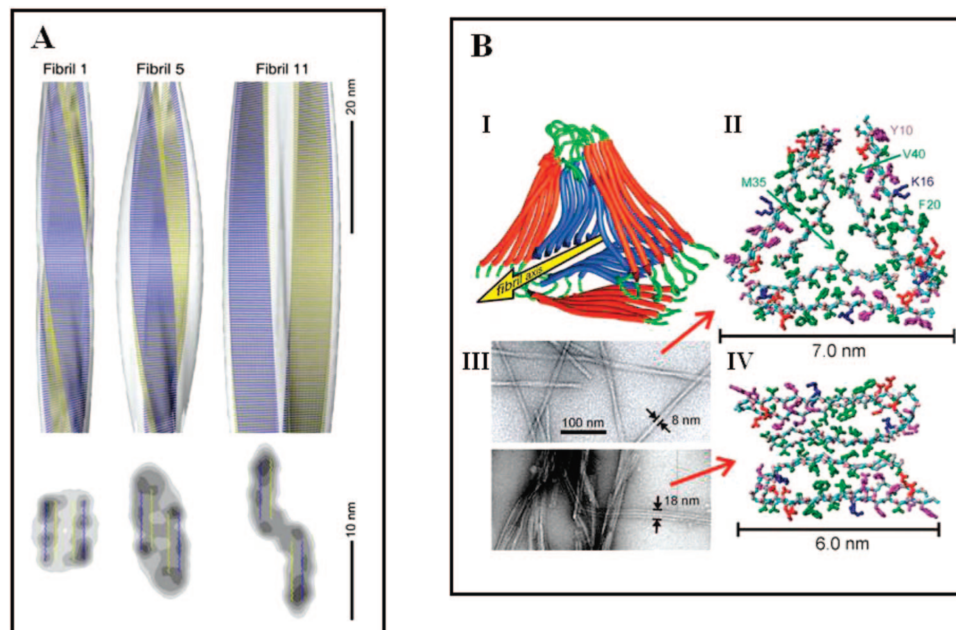


**Figure 6.** Polymorphism of the A$\beta_{1-40}$ peptide based on different associations of the protofibrils. (A) A structural model of protofilament core topology of fibrils 1, 5, and 11, observed from Meinhardt et al.[73] Side view of the fibrils with two protofilament cores modeled into the density (top) and contoured density cross sections of the fibrils superimposed with two protofilament cores (bottom). Each protofilament core comprises a pair of two $\beta$-sheet regions: interface (yellow) and outside (blue). Reprinted with permission from ref 73. Copyright 2009 Elsevier. (B) Experiment-based structural models of A$\beta_{9-40}$. (I) A ribbon presentation of the lowest-energy model for fibrils with twisted morphology. (II) Atomic representation, viewed down the fibril axis. Hydrophobic, polar, negatively charged, and positively charged amino acid side-chains are green, magenta, red, and blue, respectively. Backbone nitrogen and carbonyl oxygen atoms are cyan and pink. (III) Comparison of twisted (upper) and striated ribbon (lower) fibril morphologies in negatively TEM images. (IV) Atomic representation of a model for striated ribbon fibrils developed previously by Petkova et al.[68,72] Reprinted with permission from ref 138. Copyright 2008 National Academic of Sciences, USA.

non-$\beta$-strand (U-turn/loop) segment; and the quaternary contacts (Figure 6B).[138] Recently, Paravastu et al.[139] have shown that the predominant molecular structure in brain-seeded with A$\beta_{1-40}$ fibrils differs from the structure of purely synthetic fibrils that were previously characterized.[68,138] Fandrich et al.[140] defined polymorphism based on variations in (1) the protofilament number, (2) the protofilament arrangement, and (3) protofilament substructures.

Nonetheless, overall, A$\beta_{1-40}$ fibrils present regular smooth amyloid morphologies. Many smaller fragments, particularly those containing C-terminal residues (like the A$\beta_{17-42}$ and A$\beta_{34-42}$ fibrils, Figure 2), are much more irregular as compared to the full-length A$\beta_{1-40}$/A$\beta_{1-42}$. The potential variability of the fibril morphologies for these amyloids is expected to be much more diverse.

## 4. Other Protein/Peptide Sequences Also Have Polymorphic Fibrils Structures

Polymorphism in amyloid fibrils has also been observed for other proteins, for both designed model systems and disease-related proteins. For example, Verel et al.[141] demonstrated polymorphism in a de novo designed peptide model system (Ac-SIRELEARIRELELRIG-NH2), which is expressed by different arrangements of the $\beta$-sheets in the oligomer. Depending on the pH, the model peptide can shift the registration of residues and still keep an antiparallel $\beta$-sheet organization. Disease-related amyloid polymorphism can be illustrated by the cross-seeding fibrilization of the Q/N-rich protein, which leads to pathological diversity in Huntington disease with the morphologies differing with the segment size.[142]

Among the other species that form amyloid fibrils is $\beta_2$-microglobulin ($\beta_2$M), which is a component of type I major histocompatibility antigen. In patients on long-term hemodialysis, $\beta_2$M can aggregate into amyloid fibers and cause dialysis-related amyloidosis.[143-149] The ssNMR structure[150] of a 22 amino acid (Ser20-Lys41) region (the so-called K3) that may have a role in amyloid formation of $\beta_2$M was investigated using a series of peptides obtained by cleaving with Achromobacter protease I.[151] Interestingly, K3 also shares similar U-turn shape in the $\beta$-sheet organization with a parallel orientation.[150] K3 was also shown to form ion-conducting channels in membranes.[51] Other key regions in $\beta_2$M shown to be important in fibril formation have also been investigated by Radford,[152,153] Eisenberg,[154-156] and Miranker[157] and their co-workers. $\beta_2$M segments also exhibit polymorphism. The K3 peptide may form two types (f218 and f210, which has more $\beta$-sheet and is thinner and longer) of amyloid fibrils differing in their amino acid contacts between the $\beta$-sheets, and both fibrils were reproduced by seeding, showing the template-dependent propagation of a fibril's conformation.[158] Upon repeated self-seeding, f218 fibrils were gradually transformed into f210 fibrils, revealing the conformational maturation to higher $\beta$-sheet content. Full-length $\beta_2$M has been shown to form at least three different subunit interfaces in the fibril structures assembled from globular tetrameric units.[159] The elegant cryo-EM maps (Figure 7) illustrate three-dimensional reconstructions of type A (Figure 7A and C) and B (Figure 7B and D) forms of the homopolymeric assembly of $\beta_2$M. The two fibril types have the same underlying organization that differs only in the orientation of the two stacks, either parallel or antiparallel.[159]

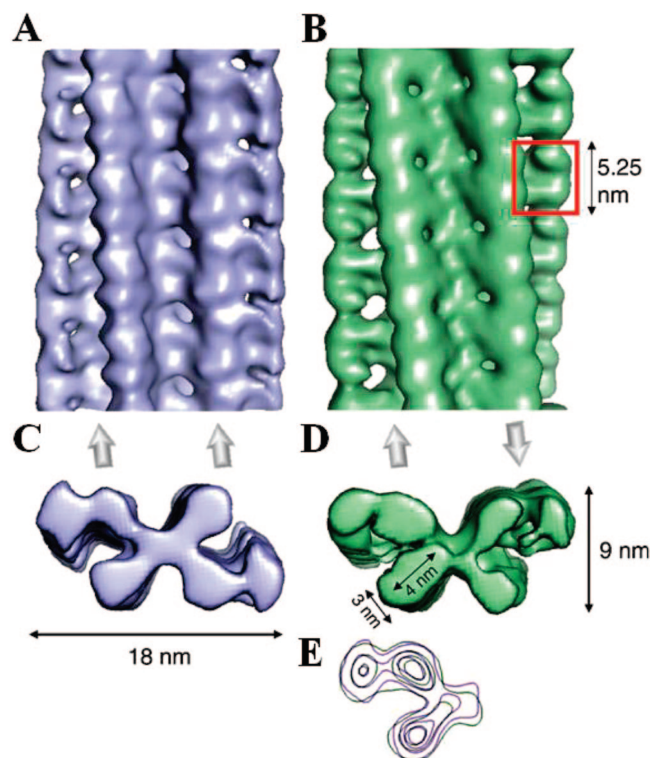


**Figure 7.** Variability in $\beta_2$-microglobulin fibril structure. Reprinted with permission from ref 159. Copyright 2009 Elsevier. Three-dimensional reconstructions of the type A and type B forms of $\beta_2$-microglobulin fibrils. Side views of an A-type fibril (A) and a B-type fibril (B). One dimeric density unit is indicated by a red box in (B). The directions of the half-fibrils are indicated by arrows below the maps. Cross sections of the A type (C) and B-type fibrils (D) show that the structures are formed of crescent-shaped units stacked back-to-back. (E) Superposed contour plots of the A (lilac) and B (green) repeat units, showing that the two fibril types have the same underlying organization that differs only in the orientation of the two stacks, either parallel or antiparallel.

Amyloid polymorphism has also been observed for the prion. Yeast prion variants have been identified on the basis of stabilities and phenotypic intensity[160−163] relating to prion strains.[164−166] Fibril structural polymorphism has been proposed as the culprit in the species barriers[167,168] with the amyloid-forming domain in prion filaments exhibiting polymorphic variation.[169] Prion amyloid structural variations have been observed with differences in long-range axial repeats, diameter, and/or the number of protofilaments.[169] Polymorphic prion structures may form due to conformational self-seeding characteristics, kinetics, and stability.[170] Fibrils that are conformationally less stable produce shorter pieces upon fragmentation.[171] The more stable fibril core regions were also identified.[172]

Amyloid polymorphism has also been observed for the islet amyloid polypeptide (IAPP) amylin, by experiment[123,173,174] and simulations.[175] Amyloid formation by amylin leads to cell death and is related to type II diabetes. It was demonstrated that conformational polymorphism of IAPP peptides in different microenvironments correlates with cellular toxicity and proteasomal inhibitory activity.[123,173,174] The peptide hIAPP$_{20-29}$, $^{20}$NNFGAILSS$^{29}$, may have two amyloid-like forms, which have distinct structures at the molecular level. One form has parallel $\beta$-strands and the other antiparallel, with both stabilized by hydrophobic contacts with similar energy.[123,173,174] Hydrophobic interactions can weaken under high hydrostatic pressure. However, fragments 1−19 and 1−29 of IAPP are resistant to pressure, suggesting more densely packed aggregate structures.[123,173,174]

## 5. Sequences and Physicochemical Properties That Can Affect Amyloid Fibril Architectures

Amyloid fiber growth involves folding and association via conformational selection of peptide monomers.[57,70,176,177] Prediction of the aggregation rate of different A$\beta$ segments may be performed by a model based on the physicochemical properties of the segment.[178] The $\beta$-sheet in A$\beta$ segments, while defined by interstrand hydrogen bonding, owes its stability mainly to intra- and intersheet side-chain packing.[179] The presence of intermolecular hydrogen-bonding interactions along the entire length of the peptides further stabilizes the oligomers and is critical in determining the fibril structure.[180] As can be seen from Table 1, some of the A$\beta$ peptide segments adopting $\beta$-strand conformations form amyloid fibrils in a parallel orientation and others in an antiparallel orientation. This section examines the properties of these fragments that can affect their preferred architecture such as segment length, hydrophobicity, hydrophilicity, and charge. Table 1 also summarizes the characteristics of full-length A$\beta$ and its segments and the K3 segment of $\beta_2$M for which there are ssNMR data.

Different lengths of A$\beta$ segments have different morphologies. As can be seen from Table 1, there is a general trend: longer segments prefer a parallel orientation. Segments with more than 15 residues are organized in parallel; under 15 residues they prefer an antiparallel arrangement probably due to repulsion by the charged termini in the parallel organization, which would need to be overcome. For short peptides, the antiparallel arrangements can be favored by better backbone hydrogen bonding, backbone dipole−dipole interaction, and charge−charge interaction among the terminus residues. However, longer fragment such as A$\beta_{1-28}$ favors antiparallel arrangement, while the parallel arrangement is favored by the short non-A$\beta$ GNNQQNY sequence.[170,181−184] Obviously, not only the length determines the stability of the fibril, but also the nature of the residues along the segment that interacts. For example, the available 15 microcrystal structures of cross-$\beta$ spines exhibit parallel arrangements for peptides free of any charged residues.[43]

### 5.1. Hydrophobicity Can Increase Amyloid Polymorphism

As can be seen from Table 1, the percentage of the hydrophobic residues is above 40% for all $\beta$-strand segments presented here, and some of these segments are also enriched in hydrophilic residues. There are two hydrophobic rich regions in A$\beta_{1-40}$/A$\beta_{1-42}$ peptides, segments 17−22 (KLFFA) and 30−42 (AIIGLMVGGVVIA). ssNMR indicates that A$\beta_{10-35}$, A$\beta_{1-40}$, and A$\beta_{1-42}$ fibrils are organized in parallel, whereas A$\beta_{16-22}$, A$\beta_{34-42}$, and A$\beta_{14-23}$ are antiparallel. Most of these consist of hydrophobic segments believed to be crucial for aggregation.[70,185]

Hydrophobic interactions are the driving force for protein−protein association, including protein aggregation;[122] they largely control the stability and therefore determine the organization. In $\beta$-sheets, stabilization by hydrophobic interactions could maximize when the interactions are between stacked identical residues, which is the case in a parallel organization.[186] For example, 15 interactions between in-register hydrophobic residues of two $\beta$-strands stabilize

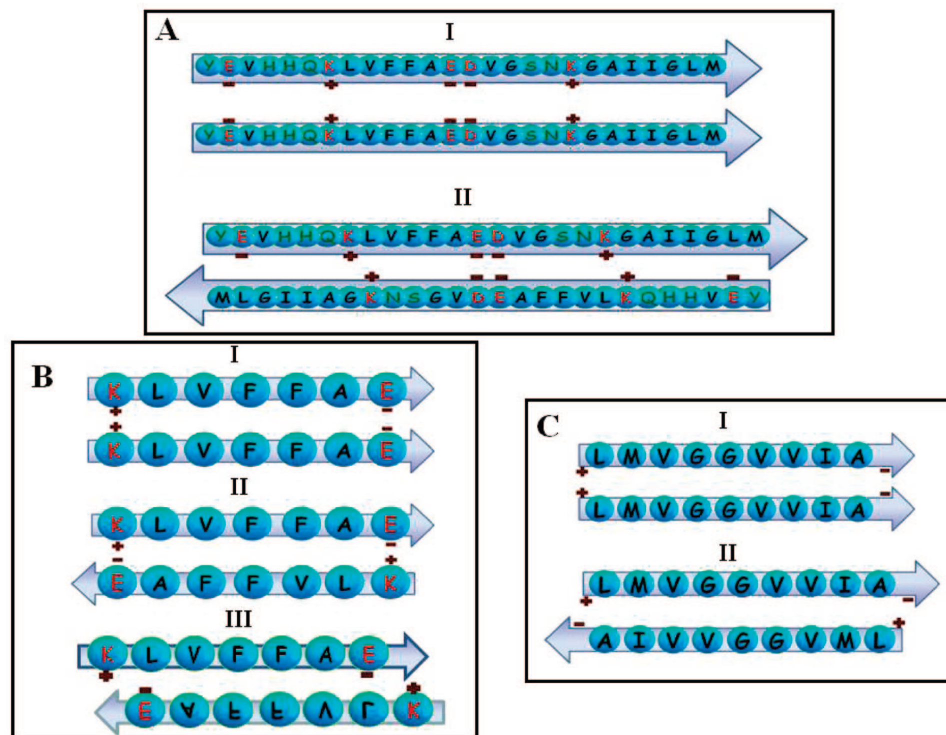


**Figure 8.** Illustration of the organization of the hydrophobic residues (black) and the charged residues (red), and their charge interactions (brown) in parallel and antiparallel alignment for (A) A$\beta_{10-35}$, (B) A$\beta_{16-22}$, and (C) A$\beta_{34-42}$ fragments.

the parallel organization of A$\beta_{10-35}$, whereas there are only six interactions between hydrophobic residues in the antiparallel organization (Figure 8A). Parallel $\beta$-sheet organization juxtaposes the hydrophobic segments of neighboring peptide chains, while an antiparallel organization does not. Therefore, the preferred orientation of the $\beta$-strands for A$\beta_{10-35}$ is parallel.

A$\beta_{16-22}$ is stabilized by hydrophobic interactions between consecutive $\beta$-strands along the fibril axis. It seems that both parallel and antiparallel orientations are favored for A$\beta_{16-22}$, with both arrangements presenting central hydrophobic sequence, including the strongest Phe–Phe hydrophobic interaction. However, A$\beta_{16-22}$ has positively charged Lys at the N-terminus and negatively charged Glu at the C-terminus. Parallel $\beta$-sheet organization will create electrostatic repulsion destabilizing the fibril, whereas an antiparallel $\beta$-sheet organization simultaneously juxtaposes the central hydrophobic segment and minimizes the electrostatic energy (Figure 8B). Ma and Nussinov[83] studied the stability of A$\beta_{16-22}$ and concluded that for the antiparallel model the hydrophobic interactions appear to dominate in this short segment. Nonetheless, as discussed in the next section, because hydrophobic interactions in the parallel A$\beta_{16-22}$ organization are strong, they can overcome the electrostatic destabilization induced by flexible side-chains at the termini, leading to a parallel A$\beta_{16-22}$ arrangement, as seen from Figure 8B. Thus, while both organizations are likely to exist, the antiparallel one presents higher population. A similar picture is presented by A$\beta_{34-42}$. Because it consists of a single hydrophobic segment with a positively charged N-terminus and negatively charged C-terminus, hydrophobic contacts maximize in either parallel or antiparallel structures, while electrostatic interactions would be expected to favor the antiparallel structure (Figure 8C).

Hydrophobic interactions are nonspecific, which could underlie the diversity of possible interaction patterns. Therefore, amyloids dominated by hydrophobic interactions could easily present polymorphic behavior. In the case of A$\beta_{16-22}$, an in-register antiparallel organization (Figure 8BII) has been shown to be preferred. However, a new arrangement with one residue shifted and the backbone flipped (Figure 8BIII) is possible under acidic conditions.[82] It can be seen that both arrangements in Figure 8BII and BIII present favorable hydrophobic interactions; however, the antiparallel one residue-shifted arrangement (Figure 8BIII) may have better aromatic interactions derived from two nearby Phe residues. The importance of aromatic interactions between two $\beta$-sheets in protein aggregation has been emphasized by Gazit and co-workers[187] who suggested that aromatic interactions play a key role in molecular recognition, self-assembly, and amyloid formation.[187] Makin and co-workers[188] further confirmed that aromatic residues that are capable of $\pi-\pi$ stacking are important for amyloid fibril formation.

Apart from the role of the hydrophobic effect in the polymorphic character of the $\beta$-strands arrangement within $\beta$-sheets, the diversity of hydrophobic interactions between protofibrils can also contribute to amyloid polymorphism. In the case of A$\beta_{1-40}$, the highly hydrophobic C-terminal region provides many possible interaction interfaces for 2-fold or 3-fold arrangement patterns (Figure 6).[73,138]

## 5.2. Charged Residues Require a Specific Match, thus Decreasing Amyloid Diversity

Electrostatics can play a crucial role in A$\beta$ fibrils.[75,76,189,190] At first glance, the charged nature of many amyloid-forming peptides can increase the solubility and thus decrease their intrinsic ability to form $\beta$-sheet based aggregates. However, in certain cases, charged residues may help the peptides to form amyloids, as indicated by the full-length A$\beta$ peptide, which forms ordered fibrils much more easily than the shorter and more hydrophobic aggregative A$\beta_{17-42}$.

Table 1 provides the net charge of the segments. Some A$\beta$ segments have $\beta$-strands that are negatively charged, some are positively charged, and others have a net charge of zero. It appears that $\beta$-strand organization in parallel or antiparallel does not depend on the segment net charge. The percentage of the charged residues in the segment does not determine the arrangement of the $\beta$-strands either. However, charge−charge interactions between sheets are important for fibril formation and residues with complementary charges promote fibril formation. Therefore, the positions of the charged residues along the sequence of the segment are important for the stabilization of the amyloid fibril and affect the arrangement of the $\beta$-strands.

A parallel organization of A$\beta$ forms homostacking (identical amino acids stacked on top of each other). Stacking of chemically similar charged residues in a parallel orientation creates electrostatic repulsion. In contrast, the antiparallel orientation is favored by electrostatic interactions between the C- and N- termini of neighboring molecules in a $\beta$-sheet (e.g., the positive amino and negative carboxylate groups at the ends of uncapped A$\beta_{34-42}$ as illustrated in Figure 8) or by oppositely charge residues (e.g., positively charged Lys and negatively charged Glu side chains in capped A$\beta_{16-22}$ as described in Figure 8). Thus, preferred fibril architecture maximizes the number of hydrophobic and attractive electrostatic interactions.

Because hydrophobic interactions are nonspecific, the attractive electrostatic interactions limit the variation of the amyloid arrangement. The A$\beta_{11-25}$ fragment is a good example in this case. As compared to A$\beta_{1-40}$, the A$\beta_{11-25}$ fragment can form a fibril, which yields a better quality and higher resolution X-ray diffraction data (Figure 3A),[77] indicating lower structural fibril diversity. Expanded from A$\beta_{16-22}$, the charged pattern in A$\beta_{11-25}$ increases the weight of electrostatic interactions as compared to the (unchanged) hydrophobic interactions (Figure 3B). In the N-terminus, two His residues (His13, His14) increase the positive charge in the Lys16 region, while Asp23 added to Glu22 leads to a more negative C-terminus. As a result, the strong electrostatic interactions make the in-register antiparallel alignment of A$\beta_{11-25}$ highly favorable, with other alternatives hard to compete. Clearly, the strong electrostatic interaction contributed to the structural uniformity of the A$\beta_{11-25}$ amyloid.

## 5.3. Steric Zipper Could Increase or Decrease Amyloid Diversity

Preferred fibril architecture is also stabilized by tight van der Waals (vdW) packing between $\beta$-sheets. Steric zippers (that is, tight packing) appear a generic structural motif of the amyloid protofilament,[170,191−193] because good geometrical fit provides favorable vdW interactions and constrains side-chain movement. For example, in the amyloid-like GNNQQNY fibril, the interactions are maximized by very tight side-chain packing.[170,192] In the case of this all-polar sequence, the strong steric zipper match provides a dominant amyloid-like crystal, making an atomic resolution structure possible.

Studies of the stability of the islet amyloid polypeptide segments NFGAIL and NFGAILSS[194] have shown that interactions between hydrophobic residues and between aromatic residues dominate. However, recently other arrangements with good geometric fit between $\beta$-sheet layers have also been shown to be possible,[123] suggesting that for the islet amyloid polypeptide segment NFGAILSS, a tight steric zipper packing can occur in more than one way, increasing polymorphism. Recently, Park et al.[195] studied the polymorphism for the steric zipper patterns of various short peptides while considering the kinetics and the thermodynamics as driving forces for the structural selection.

For the A$\beta_{11-25}$, a steric zipper can contribute to decreased polymorphism. The strong electrostatic interactions prefer the in-register antiparallel $\beta$-sheet. Considering only electrostatic interactions, the amyloid structure of A$\beta_{11-25}$ can have an arrangement as in Figure 4H, where electrostatic interactions are also maximized between sheets. Early work indeed suggested this architecture for the 11−24 region in A$\beta_{1-28}$.[75] However, due to a better steric match between parallel $\beta$-sheets, the A$\beta_{11-25}$ adopts the Figure 4G arrangement (also see Figure 3B) to allow optimized hydrophobic, electrostatic, and steric zipper (vdW) fit.

# 6. Polymorphism in Metal-Binding Sites in Alzheimer A$\beta$ Amyloid: $Zn^{2+}$, $Cu^{2+}$, $Fe^{3+}$, and $Al^{3+}$

The most prevalent metal ions in biological systems, such as $Zn^{2+}$, $Cu^{2+}$, and $Fe^{3+}$, are known to be essential for normal brain function and development.[196,197] Disrupted cellular homeostasis of these ions is thought to play a central role in the aggregation and neurotoxicity of A$\beta$.[198,199] These metal ions are markedly enriched in A$\beta$ plaques[200−210] and may act as seeding factors. In contrast, while a higher concentration of $Al^{3+}$ has been established in AD patients' brain,[211−214] the mechanism of its pathogenesis in AD is still debated.[215−218] Nonetheless, experiments clearly demonstrated that $Al^{3+}$ ions do play a role in A$\beta$ aggregation and in neurotoxicity.[219−224] Interactions with these metal ions may explain the increased involvement of soluble toxic oligomeric species.[225] To date, the conformation of A$\beta$ complexes with metal ions is still a major challenge. Open questions include (1) which residues in the A$\beta$ peptide interact with the metal ion; (2) are there different conformations with different residues coordinating the metal ions; and (3) among these, which are preferred. Characterization of the intrinsic conformational properties of A$\beta_{1-40}$/A$\beta_{1-42}$ oligomers complexed with metal ions is important to understand the effect of metal ions on A$\beta$ aggregation. So far, A$\beta$ binding sites for $Zn^{2+}$ and $Cu^{2+}$ were studied extensively, but there is lack of data for $Fe^{3+}$.

Polymorphism can be observed in $Cu^{2+}$ complexes with A$\beta$ amyloid. Possible $Cu^{2+}$ coordinating residues include histidines (His6, His13, His14), tyrosine (Try10), aspartate or glutamate (Asp1, Glu3, Asp7, Glu11, Glu22, Asp23), methionine (Met35), deprotonated amides of peptide backbone, or carbonyl groups.[206,226−231] The most common residues are located at the N-terminus of A$\beta$: the binding site for $Cu^{2+}$ was proposed to consist of the three histidines (His6, His13, and His14) and Tyr10.[205,226,232−238] Further, studies demonstrated that the $Cu^{2+}$ binding site can involve Asp1[239,240] or Glu11.[240] A recent study of the $Cu^{2+}$ binding site in the rat A$\beta_{1-28}$ fragment revealed that $Cu^{2+}$ binds to Asp1, His6, and His14.[241] His13 is absent in rat A$\beta_{1-28}$. Recent simulations suggested that $Cu^{2+}$ coordinates with His13 and His14 of each two A$\beta$ peptides.[242] These studies suggest various possible conformers. We therefore conclude that A$\beta$ amyloid with different experimentally detected $Cu^{2+}$ binding sites depends on the populations of the various morphologies under different conditions.

The zinc binding site in A$\beta$ is also located at the disordered N-terminal (Asp1−Lys16). Seven potential N-terminal residues can bind $Zn^{2+}$: Asp1, Glu3, His6, Asp7, Glu11, His13, and His14. As for the $Cu^{2+}$ binding site, in the case of $Zn^{2+}$,

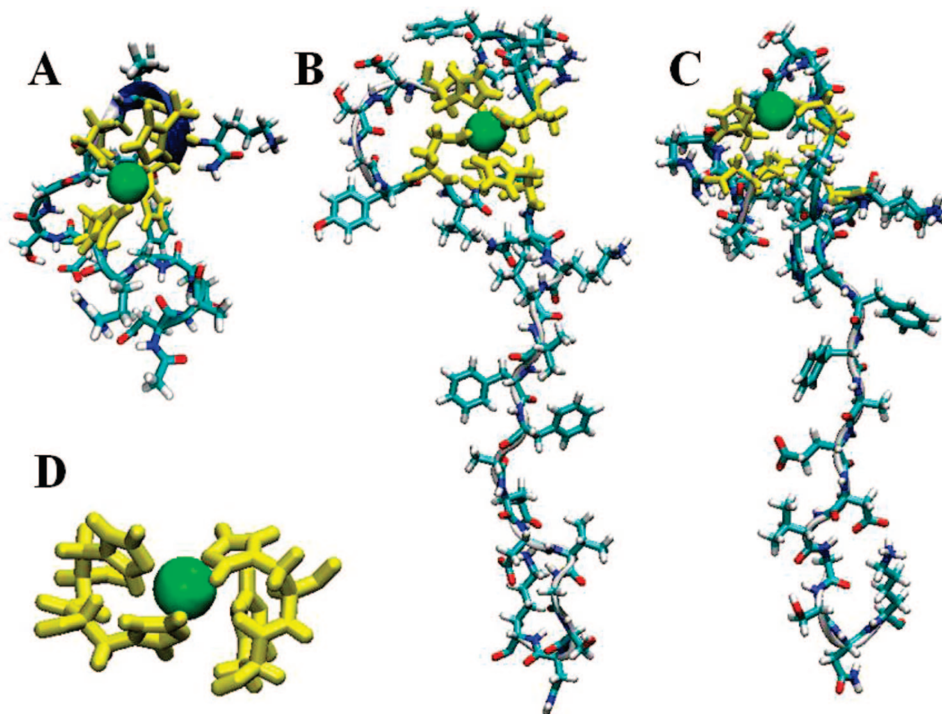


**Figure 9.** Various conformations of $Zn^{2+}-A\beta$ monomer complexes: (A) NMR structural model for $Zn^{2+}-A\beta_{1-16}$ (ref 252), (B) NMR structural model for human $Zn^{2+}-A\beta_{1-28}$ (ref 257), (C) NMR structural model for rat $Zn^{2+}-A\beta_{1-28}$ (ref 257), and (D) X-ray spectroscopy model of $Zn^{2+}$ binding to His13 and His14 of 2 adjacent Aβ peptides (ref 258).

the three histidines (His6, His13, and His14) at the N-terminal segment coordinate with $Zn^{2+}$.[226,227,237,243−260] Recently, three different models of $Zn^{2+}$ binding to Aβ were observed by experiments: First, the NMR structures of $Zn^{2+}-A\beta_{1-16}$ in aqueous solution at pH 6.5 and 7.4 showed that $Zn^{2+}$ is bound to these three histidines and to the two carboxylic groups of Glu11.[252] Second, an NMR study on $Zn^{2+}-A\beta_{1-28}$ in sodium dodecyl sulfate (SDS) micelles at pH 7.5 and a temperature of 298 K proposed a tetra-coordination of $Zn^{2+}$ by His6, Glu11, His14, and Asp1 at the N-terminus for rat $A\beta_{1-28}$, and penta-coordination of $Zn^{2+}$ by His6, Glu11, His13, His14, and Asp1 at the N-terminus for human $A\beta_{1-28}$.[257] The third model is based on X-ray absorption spectroscopy (XAS) in which $Zn^{2+}-A\beta$ complexes are coordinated with four histidines (His13 and His14 of two monomers), indicating that each metal ion is shared by two Aβ peptides.[258] Aβ oligomers coordinated with $Zn^{2+}$ can be modeled using experimental data as shown in Figure 9. See an additional recent reference in the note added in proof. We therefore propose that $Zn^{2+}$ can coordinate the N-terminal domain in different $A\beta_{1-42}$ oligomer morphologies. The prevailing organization and the probability to detect certain morphologies depend on experimental conditions such as temperature, $Zn^{2+}$ concentration, and pH. Oligomer stability depends also on its size, in particular when dealing with $Zn^{2+}$ in the N-terminal.

Much less is known about the preferential regions of $Al^{3+}$ binding to Aβ. Fasman[261] proposed that $Al^{3+}$ coordinates with Asp, Ser, Tyr, and Glu in Aβ. Recently, Ricchelli et al.[262] suggested that $Al^{3+}$ may coordinate with residues in both the hydrophilic segment (1−16) and the hydrophobic sequence 20−35 in the Aβ peptide, and thus $Al^{3+}$ binding site is less restricted than that of $Zn^{2+}$ or $Cu^{2+}$, which are confined to the N-terminal. Thus, polymorphic $Al^{3+}$ Aβ complexes are also expected.

## 7. Polymorphism in Soluble Aβ Oligomers Leads to Different Mechanisms of Toxicity

Amyloid toxicity is the outcome of $Ca^{2+}$ conductance through the membrane, and, as described below, polymorphic Aβ oligomers can differ in their mechanisms of toxicity. Aβ toxicity was first identified by formation of $A\beta_{1-40}$ cation selective channels in planar lipid bilayers fused with liposomes and in acidic bilayers.[263] The $A\beta_{1-40}$ channel in the solvent-free membrane patch presented multiple cation selectivity and sensitivity to tromethamine, which suggested that in cells the ion fluxes would disrupt cellular homeostasis.[263] Toxic $Ca^{2+}$ conductance through unregulated pores or ion channels formed by Aβ or its fragments was also supported by other studies[264−267] and appears universal for amyloidogenic peptides. The channels are polymorphic[48,50] and differ from conventional ion channels.[268] Even though it is currently believed that small oligomers play critical roles in ion-channel formation, the relationship between amyloid deposition and cellular toxicity is still controversial, and the similarity to bacterial pore-forming toxins is challenging to study.[269]

Other soluble Aβ forms, which are broadly named as amyloid β-derived diffusible ligand (ADDLs), have different morphologies and were proposed to have different mechanisms. Unlike ion channels observed in bilayers, ADDLs were shown to be linked to neuron toxicity, mostly via binding to protein receptors.[270] ADDLs are highly ordered, binding to a specific subset of postsynaptic proteins. In particular neural signal transduction pathways, ADDLs can impair synaptic plasticity and associated memory dysfunction during the early stage of AD.

In 2005,[271] it was observed that a homogeneous and stable globular amyloid β-peptide oligomer can be easily prepared in vitro. This globular amyloid β-peptide was present in the

brains of patients with AD and in A$\beta_{1-42}$-overproducing transgenic mice. The pure, water-soluble globular 60 kDa oligomer was reported to have 12 A$\beta$ monomers. This "globulomer" is a persistent structural entity apparently formed independently of the fibril aggregation pathway. Unlike ADDLs, the A$\beta_{1-42}$ globulomer binds specifically to dendritic processes of neurons but not glia in hippocampal cell cultures and completely blocks long-term potentiation in rat hippocampal slices. Similar to ADDLs, the globulomer also relates to early AD. In 2006, another species smaller than the globular 60 kDa oligomer, A$\beta$*56, was reported as a soluble oligomer causing memory deficiency in middle-aged mice.[225] However, on the basis of biochemical properties and immunospecificity, it was argued to be unlikely to bind to receptor proteins, and instead to impair memory independently of plaques or neuronal loss.[225] A larger soluble oligomer, "amylospheroids" (ASPDs),[272,273] was also observed, with sizes around 10−15 nm. These spherical A$\beta$ assemblies have high-mass (more than 100 kDa) and can be detected in AD and Lewy body (DLB) brains, using ASPD tertiary structure-dependent antibodies. ASPDs bind presynaptic targets on mature neurons with different toxicity from other ADDLs. Finally, cellular prion proteins (PrPCs) were recently identified as high-affinity cell-surface receptor for soluble A$\beta$ oligomers on neurons,[274] but the interaction does not require infectious PrPSc conformation.

Even though many studies argue that soluble A$\beta$ oligomers such as ADDLs are off fibril formation pathways, they may nonetheless share some structural similarity with protofibrils. An NMR study of the intermediates of globulomer formation, the so-called preglobulomer, suggested parallel in register $\beta$-sheets in the C-terminal region, even though the N-terminal region has different conformation.[275]

Overall, the observations described above lead to the conclusion that the inherent polymorphic nature of A$\beta$ assemblies can be associated with different toxic behavior. Consequently, there is a need to delineate the structural variability underlying the formation and stabilities of soluble A$\beta$ oligomers.

## 8. The Energy Landscape of Amyloidogenic Proteins and Their Assemblies Is Rugged

The native-like states of amyloidogenic proteins are unstable in environments favoring aggregation, leading to a broad conformational heterogeneity. Among the native-like states, some display amyloid-favored populations. While these conformations may have higher-energy and consequently exist at low concentrations, they may be complementary to a fibrilar state surface. These binding-favored conformers would associate via the "conformational selection" mechanism. Binding stabilizes these conformations, and shifts the population of the monomers toward these binding-favored conformers, propagating the binding reaction.[57,58,176,177,276−280] The larger are the aggregates, the more stable are the associations (Figure 10). Amyloid formation follows the conformational selection and population shift mechanism now accepted as a general paradigm in molecular recognition.[56] This view is based on a dynamic energy landscape picture of protein folding and protein binding. The kinetics of the amyloid formation process depends on the relative stabilities of the states, and the barrier heights that need to be crossed in the conformational transitions, which are the outcome of environmental conditions.

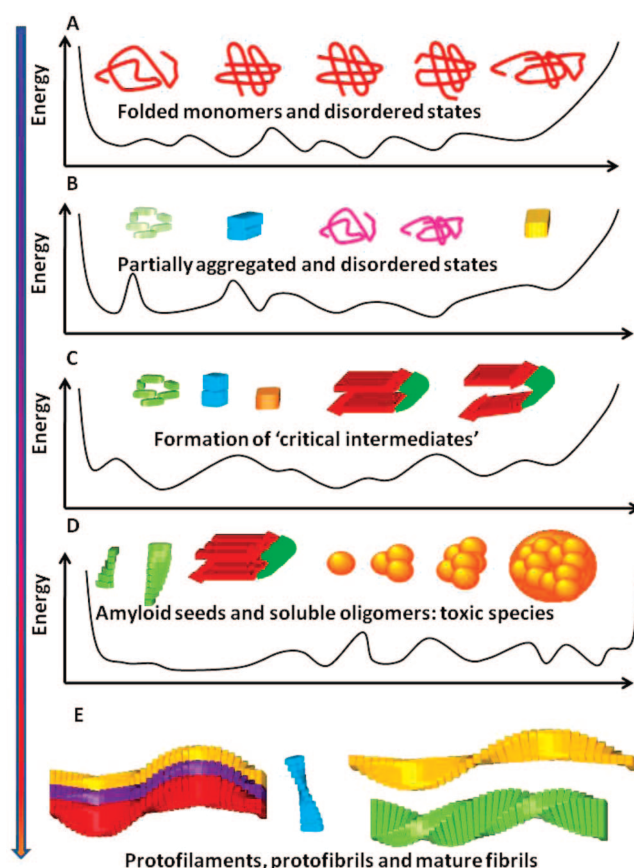


**Figure 10.** Polymerization of A$\beta$ peptides involves continuous hierarchical redistributions of the polymorphic ensembles on a rugged energy landscape. For illustration, we present five aggregation phases starting from the native states and ending in the fibril forms. The arrow on the left indicates the evolution of the aggregated states with time: (A) The conformational ensemble of the "normal" folded monomers (three schematic figures in the middle) and partially disordered or unfolded states (the right and the left schematic figures). (B) Under certain conditions, folded monomers assemble toward polymorphic seeds or partially aggregated states (green, blue, and yellow boxes), redistributing the conformational ensemble. The native disordered or unfolded states (red schematic figures) may still have a large population. (C) "Critical intermediates" may be different partially aggregate states (green, blue, and yellow boxes) and very early stage of small oligomers (two right schematic figures). (D) Certain "critical intermediates" can lead to both different amyloid seeds (three left schematic figures) and different soluble ADDL-like oligomers (orange balls). The soluble ADDL-like oligomers are prior to amyloidogenic protofibril formation. Polymorphic seeds and polymorphic soluble oligomers present different toxic mechanisms (discussed in section 7). (E) The different fibril forms are the most stable states of the A$\beta$ peptide amyloid: protofilament fragments (blue), protofibril forms (yellow and green), and mature fibril (left schematic figure).

Protein conformations and their associations have been optimized by evolution toward specific biological functions. Thus, it is expected that certain states are favored over others due to (some) energy gaps between the favored and the less-favored states; this, however, is not the case in amyloid formation, which is a misfolded, diseased state presenting a rugged energy landscape. Because the toxic non-native amyloidogenic conformations are not under selection pressure to preferentially assemble into a specific oligomer, the outcome is an extensive polymorphic range. As the heterogeneous seeds and fibrils grow, the ordered polymorphic aggregates will increasingly stabilize. Metal ions, such as

$Zn^{2+}$, $Cu^{2+}$, and $Fe^{3+}$, known to promote amyloid formation, further complicate these scenarios. Each A$\beta$ conformer can coordinate metal ions via different residues; because the differences in the stabilities may be not large, the complexity of the landscape, which reflects the polymorphic range, vastly increases.

The nature of the rugged energy landscape of amyloidogenic peptides provides a conceptual framework for polymorphic stages in protein aggregation. As shown in Figure 10, we may describe five protein aggregation phases, with hierarchical redistribution of the ensembles. The "normal" folded stage (Figure 10A) and partially unfolded or aggregated state (Figure 10B) have been universally regarded as necessary steps. However, where to position the "soluble oligomers" with respect to the main fibril forming pathway is still unclear. Even though small amyloidogenic oligomers are on the pathway to seed and larger protofibril formation, ADDL-like species were suggested to be off pathway (for example, as depicted by Roychaudhuri et al.[281]). Such view overlooks common features of ADDLs and other $\beta$-sheet-rich polymer forms.[275] Here, we call attention to the possible existence of certain "critical intermediates" (Figure 10C), which can lead to both seeds and other soluble ADDL-like oligomers (Figure 10D). From this point of view, all soluble oligomers are polymorphic forms distributed on the energy landscape prior to (proto-)fibril formation (Figure 10E). Thus, in principle, a drug can inhibit such critical intermediates, preventing seed and other ADDL-like oligomer formation.

Conformational selection and population shift is the key mechanism in biomolecular recognition,[56] and adding monomers and small oligomers to amyloid seeds in fibril growth[177] is a molecular recognition event.[54] Within such general framework, the "dock and lock" mechanism for the deposition of soluble A$\beta$ onto amyloid was proposed to be mediated by two distinct kinetic processes. In the first "dock" phase, the addition of A$\beta$ to the amyloid seeds can be reversible, while in the second "lock" phase, the deposited peptide becomes irreversibly associated with the template in a time-dependent manner.[282] In particular, in 2001 Massi and Straub combined the "dock−lock" mechanism with the energy landscape to explain the A$\beta$ amyloid growth,[283] thus unifying a number of schemes proposed to describe fibril elongation, is in good agreement with experimental observations for A$\beta$ amyloid[283] and prion growth.[284]

Extensive molecular dynamics simulations of A$\beta_{16-22}$ in water[88] and urea[285] indicated that both hydrophobic interactions and backbone hydrogen bonds are important for A$\beta_{16-22}$ oligomer stability. The kinetics of dissociation of the A$\beta_{16-22}$ oligomer[286] and oligomer growth[287] both involve dock and lock stages. Importantly, it was found that the mobile structured oligomers undergo large conformational changes to accommodate the added monomer, a clear indication of mutual conformation selection process.[287]

Simulation of fibril growth of other fragments, A$\beta_{35-40}$ (MVGGVV),[288] A$\beta_{10-40}$,[289] and A$\beta_{10-40}$ with an Asp23Tyr mutation,[290] pointed to a two-stage mechanism.[282,283] The simulations of A$\beta_{10-40}$ emphasize that the docking transition appears to be continuous and it occurs without free energy barriers or intermediates. Still, it should be distinguished here between short peptides and proteins, short peptides being random coil in solution. During the docking transition, incoming A$\beta$ monomers are disordered on the fibril edge. The locking stage is characterized by the rugged free energy landscape. The locking step takes place when an incoming A$\beta$ peptide forms a parallel $\beta$-sheet structure on the fibril edge. Because the $\beta$-sheets formed by locked A$\beta$ peptides are typically off-register, the structure of the locked phase differs from the structure of the fibril interior.[289] Polymorphic forms can also derive from a variation of pre-existing seeds. Finally, docking[291−293] and the role of conformational ensembles in docking associations[56] are general paradigm in protein and biomolecular recognition.

## 9. Conclusions

Experimental and computational studies of A$\beta$ amyloids suggest that for any given segment there are one or more preferred parallel and (or) antiparallel structural states. The preferred organizations of the A$\beta$ fragments do not appear to present straightforward "rules" with respect to length, hydrophobicity, charge, etc. Because polymorphism is presented by different A$\beta$ segments, clearly a combination of these segments would lead to polymorphic full-length A$\beta$, although the relative populations in the full sequence are likely to be different. This leads us to argue that the observed polymorphism for the disease-related species derives from combinations of the preferred fragment architectures. Because these are strongly affected by the environment, the trade-off among these fragment-interactions and the key nucleation sites may determine the polymorphic oligomer (and fibril) outcome, particularly with respect to parallel/antiparallel backbone alignments.

How the A$\beta$ peptides assemble and form toxic entities and what is the mechanism of toxicity are major questions that persist in Alzheimer research. To address such questions, it is essential to have working structural models of small A$\beta$ oligomers. Two types of models of the three-dimensional structures of A$\beta$ oligomers have been reported from computational and experimental studies. The first is the Ma−Nussinov−Tycko model,[46,68] and the second is by Lührs et al.,[15] indicating polymorphism derived from different turn structures. Additional amyloid organizational motifs containing a U-turn have been reported.[150,191,192] Architectures for the same sequence, which include such a U-turn in their structure, and have similar parallel organization, may be different, due to different turn conformations. The resulting side-chain packing can affect the left-handed twist along the main axis of the amyloid fibril, leading to different fibril morphologies. Nonetheless, it is important to bear in mind that these may only present the prevailing organizations under the experimental conditions; subpopulations with different morphologies may not be captured. The further association of the oligomeric forms leads to combinatorial increase of polymorphic populations at the supramolecular and matured fibril levels. Polymorphism can also be demonstrated by metal ion binding to A$\beta$ oligomers. Because metal ions can coordinate with different residues in each structural model, the variety of the morphologies can increase dramatically.

It behooves us to emphasize, however, that here we review polymorphism focusing on larger scale fibril organization. We do not discuss polymorphism as relating to small side-chain conformational variability and its effects on the aggregated states. This is because current experimental data, based on ssNMR, cryo-EM, fiber diffraction, and mutational studies, are unable to provide such high resolution detail. We further only touched on the effects of pathways and intermediate states because experimental data are scant.

Different pathways can lead to different preferred conformational states; however, pathways are strongly dependent on conditions, for example, concentration, sequence effects, pH, and temperature.[121,294−298] Our focus on Aβ-derived peptides may miss structural contributions like hydrogen-bond ladders, as, for example, for in-parallel stacked Asn or Gln, which also greatly enhances the stability of a parallel orientation.[186]

Nevertheless, the Aβ-derived peptides provide an excellent example toward the understanding of amyloid polymorphism. While here we highlighted hydrophobic interactions as contributing to an increase in polymorphic amyloid behavior, electrostatic interactions could decrease the variability, and steric zipper (vdW) effects may increase or decrease polymorphism depending on the geometric match. Eventually, for amyloid polymorphism, both dominant and minor forms should all be treated in addressing the mechanism of aggregation and toxicity, because it is always possible that minor species are the toxic agents. Therefore, comprehension of polymorphism should assist in therapeutic agents design.

Currently, it is increasingly recognized that amyloids present a vast range of conformational states. Their energy landscape is rugged. Complementary protein conformations are selected from equilibrium of low-energy and higher-energy conformations. This "selected-fit",[280] or conformational selection mechanism,[57,58,176,177,276−279] is based on a dynamic energy-landscape picture of protein folding. The polymorphic differences may or may not be large; polymorphism can be expressed in altered β-strands arrangements, altered turn conformations, altered sheet−sheet registrations, differences in the locations and fragment sizes of regions that are disordered, different sheet organizations, and different supramolecular packing. Because these have not been optimized by evolution for a particular function, the differences in stabilities between the states are likely to be small, with the distribution of the populations easily shifting under even slight changes in cellular conditions. Because amyloids are involved in human disease, comprehension of the range of (preferred) states on their own and when interacting with metal ions is vital. Here, we addressed this challenging problem, reviewing available data within this framework, focusing on the Aβ.

Polymorphism raises a key question: how to prevent AD with drug design or other therapeutic approaches. Targeting toxicity in late stage AD may miss the reservoir of polymorphic forms; preventing Aβ production may encounter side effects derived from normal Aβ function loss. If common links among polymorphic forms can be established, then targeting the bottleneck would be more effective. The "critical intermediates" (for example, as in Figure 10C) could be the main AD targets.

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## 11. Note Added in Proof

Since the completion of this review, the polymorphic states of Aβ oligomers coordinated with $Zn^{2+}$, using the experimental data cited in this review in Section 6 and in Figure 9, were described in Miller et al. See ref. 299.

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