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Annular Structures as Intermediates in Fibril Formation of Alzheimer A β _{17–42}

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Abstract

We report all-atom molecular dynamics simulations of annular β -amyloid (17–42) structures, single- and double-layered, in solution. We assess the structural stability and association force of A β annular oligomers associated through different interfaces, with a mutated sequence (M35A), and with the oxidation state (M35O). Simulation results show that single layered annular models display inherent structural instability: one is broken down into linear-like oligomers, and the other collapses. On the other hand, a double-layered annular structure where the two layers interact through their C-termini to form an NC-CN interface (where N and C are the N and C termini, respectively) exhibits high structural stability over the simulation time due to strong hydrophobic interactions and geometrical constraints induced by the closed circular shape. The observed dimensions and molecular weight of the oligomers from atomic force microscopy (AFM) experiments are found to correspond well to our stable double-layered model with the NC-CN interface. Comparison with K3 annular structures derived from the β_2 -microglobulin suggests that the driving force for amyloid formation is sequence specific, strongly dependent on side-chain packing arrangements, structural morphologies, sequence composition, and residue positions. Combined with our previous simulations of linear-like A β , K3 peptide, and sup35-derived GNNQQNY peptide, the annular structures provide useful insight into oligomeric structures and driving forces that are critical in amyloid fibril formation.

Keywords

A β annular structure; amyloid conformations; amyloid aggregation; molecular modeling

Introduction

Alzheimer's disease (AD), the most common neurodegenerative disorder, is characterized by extracellular senile plaques and intracellular neurofibrillary tangles in the human brain^{1,2}, leading to progressive memory loss, disability, and eventually death^{3,4}. The major components of these plaques are amyloid- β (A β) peptides consisting of a 40–42-residues cleaved by β - and γ -secretases from the transmembrane amyloid precursor protein (APP). A β fibrils, similar to fibrils associated with other neurodegenerative diseases such as Parkinson and Huntington diseases, have a high content of a cross- β pattern⁵, in which β -strands are arranged perpendicular to the fibrillar axis and form intermolecular parallel β -sheets with typical

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diameters of 10 nm and lengths up to several micrometers⁶. It has been suggested that nearly all native proteins and some synthetic peptides have the ability to form amyloids under appropriate conditions^{7,8}, implying that general principles could govern amyloid fibril formation⁹. Increasing evidence from numerous structural determinations and biophysical experiments suggests that soluble oligomeric intermediates, rather than fully formed fibrils, are likely to be the primary toxic species^{10–12}. When exposed to different environmental conditions (e.g. pH, ionic strength, temperature, solvent), these aggregated intermediates could exhibit multiple distinct morphologies, which are nevertheless still related to each other. Some amyloid fibril morphologies have been shown to be more toxic than others¹³. The different oligomeric morphologies may represent protofibrils at different assembly stages or paths to stable fibrils¹³. Ring-/annular-like aggregates in several amyloidogenic systems such as A β ^{14,15}, the human hormone calcitonin¹⁶, α -synuclein^{17,18}, and the islet amyloid polypeptide have often been observed by electron microscopy (EM) and atomic force microscopy (AFM), as well as computational modeling¹⁹, suggesting that these annular aggregates could serve as another generic form of intermediates in amyloid oligomerization or fibrillization^{14,20}. Many efforts have been exerted to obtain structural information and insight into the mechanism of amyloid formation and its relation to neurotoxicity; but despite considerable advances, much still remains to be understood.

With rapid advances in solid-state NMR spectroscopy and molecular modeling techniques, an extensive set of experimental and computational structures of β -amyloid (A β) has been proposed. Tycko and co-workers reported several quaternary structures of A β fibrils based on various packing symmetries (positions and orientations) of β -sheets relative to one another. The proposed candidate quaternary structures contain two layers of A β ₄₀ molecules stacked in either parallel or antiparallel fashion perpendicular to the fibril axis. In parallel, Ma and Nussinov²¹ independently predicted that the A β peptide amyloid adopts a U-turn bent β -strand-loop- β -strand motif, illustrating the key structural features of the salt bridge between Asp23-Lys28 and the intramolecular hydrophobic cluster between Leu17/Phe19 and Ile32/Leu34, qualitatively agreeing with the Tycko et al. model²². Lührs et al.²³ presented a 3D structure of A β _{17–42} fibrils based on hydrogen/deuterium-exchange NMR data (Fig. 1a). This emerging structure with a U-turn bent β -sheet further validates the computational model of Ma and Nussinov²¹ and is consistent with the experimental model of Petkova et al.²² All those models illustrate the bent β -hairpin structure stabilized by a salt bridge between Asp23 and Lys28, as well as similar sidechain orientation, although each model has a distinct turn structure. Given the fact that all proposed A β fold structures are qualitatively consistent with each other, the next step is to explore how the preformed β -sheets aggregate into relatively large oligomers in solution. Experimental study of A β structural organization and the kinetic pathway in the oligomeric state is a challenging task because of the short-lived and dynamic nature of these intermediates. Computational approaches, complementary to experiments, have provided valuable insight into preformed oligomeric structures at the early stage of amyloid fibril formation at the all-atom level^{24–28}, kinetic proteins/peptides folding and aggregation pathways at the coarse-grained level^{29,30}, as well as better understanding of the correlation between fibril structure and neurotoxicity^{31,32}.

We previously investigated A β linear-like protofibrils in solution using all-atom molecular dynamics (MD) simulations, particularly focusing on the effect of sheet-to-sheet interface on the preformed oligomers³³. Our simulations suggested that double-layered A β oligomers stacked in antiparallel fashion and associated through the C-terminal-C-terminal (CC) interface perpendicular to the fibril axis were energetically more favorable than those with the N-terminal-N-terminal (NN) interface, although both interfaces exhibited structural stability. The CC interface is essentially stabilized by hydrophobic and van der Waals (shape complementarity via M35-M35 contacts) intermolecular interactions, whereas the NN interface is stabilized by hydrophobic and electrostatic interactions. In this work, we extended

previous simulations to A β _{17–42} annular structures in solution since annular structures have often been observed by AFM and EM experiments under certain conditions. These images indicated an outer diameter of 8–12 nm and an inner diameter of the cavity pore of 2.0–2.5 nm^{10,14,34}. Nonetheless, the structural transition/conversion between linear- and annular-like structures is still unclear. Here we investigated different annular A β models with different sizes (single or double layers), interface types (CC or NN association), and sequences (wild-type or mutant), as shown in Table 1 and Fig. 2. The structural and dynamic properties of these annular-like structures were studied in explicit water. Most interestingly, we compared the linear-like and annular-like structures of A β peptides with those of K3 peptides derived from the fragment of Ser20-Lys41 of the β 2-microglobulin³⁵, which has a similar β -strand-turn- β -strand motif in a monomeric structure. Structural comparisons suggest that amyloid structures can be dramatically different in size, structure, and morphology as a result of differences in side-chain packing arrangements, intermolecular driving forces, sequence composition, and residue positions, suggesting that the mechanism leading to distinct morphologies and the aggregation pathways is sequence specific.

Materials and Methods

System setup

Monomeric A β _{17–42} coordinates were generated by averaging 5 NMR-based structures (PDB 2BEG)²³. As shown in Fig. 1, the A β peptide contains two β -strand segments (residues 17–25 and 31–42) that are connected by a “bend” segment with non- β -strand conformation (Ser26-Asn27-Lys28-Gly29-Ala30). The β -strand at the C-terminal consists of a central hydrophobic cluster. To build a single-layered annular structure, we first aligned β -strands of a single peptide to the z axis. Then, the peptide was replicated and translated at a radius distance of R from the center, and every replicated peptide was also rotated so that one of the β -strands was facing the z axis of the annular structure. Because of the circular curvature, the inner and outer distances between two neighboring peptides were about 4.2 and 5.3 Å, respectively. The same procedure is used to build a double-layered annular structure with a separation distance of ~9 Å between two layers. Single-layered annular oligomers comprised 24 monomeric units with initial inner diameters of ~20 Å, while double-layered annular oligomers comprised 36 peptides in the outer layer and 24 peptides in the inner layer with initial inner diameters of ~20 Å. X-ray diffraction data and NMR⁸ have shown that the average distance between amyloid-forming peptides running orthogonal to the fibril axis is ~4.7 Å, with the peptides associated via hydrogen-bonds. Thus, the number of peptides in the annular structure is determined not only by the inner diameter of the annular structure, but also by the distance between two neighboring peptides (i.e. ~4.2–5.5 Å). For example, for a given inner diameter, if the number of peptides is extended to 28, 32, or 36 peptides, the inner distance of two neighboring peptides will be less than 4.2 Å, which leads to a significant steric effect of the packed sidechains. On the other hand, decreasing the number of peptides at a given inner diameter will result in the loss of hydrogen bonding and hydrophobic interactions between sidechains. Thus, the number of peptides is optimized to 24 peptides in single-layered annular models and 36 and 24 peptides in double-layered annular models to maintain appropriate sidechain interactions (hydrogen bonding and hydrophobic interaction) within inner diameters of 20–25 Å. Since the A β _{17–42} peptide has two ordered β -strands located at the N- and C-terminus, respectively, four types of annular structures were obtained: CN and NC corresponding to single-layered structures while NC-CN and CN-NC corresponding to double-layered structures where C and N represent the C-terminal and N-terminal β -strands. For example, the CN annular structure indicates that C-terminal β -strands are facing bulk solvent whereas N-terminal β -strands are facing the solvated pore. Similarly, the double-layered NC-CN annular structure indicates that all N-terminal β -strands are facing either bulk solvent or solvated pore whereas all C-terminal β -strands from inner and outer layers form a dry CC interface associated via intermolecular

contacts. The cross-section of all double-layered models at the interface is well protected from solvent. We present the initial CN and NC-CN annular structures in Fig. 2.

Simulation protocol

The simulation systems (Table 1) differed by layer (single or double), interface (NC-CN or CN-NC), and sequences (wild-type or mutant). Counterions (Cl^- and Na^+) were added to achieve overall neutrality and mimic ion concentration of ~150 mM, close to the physiological value. Model systems were first energy minimized to remove unfavorable contacts and equilibrated for 1 ns. The production runs were carried out in the NPT ensemble (1 atm and 330 K) for 50 ns. Long-range electrostatics interactions were treated by the particle mesh Ewald method, while short-range van der Waals interactions were evaluated by the force-shifted method at 12.0 Å. The integration time step was 2 fs with the SHAKE algorithm applied to constrain bonds involving hydrogen atoms. All simulations were performed with the NAMD program³⁶ using the CHARMM27 force field³⁷. All models were built with the same starting structures but different random seeds for the initial Maxwell-Boltzman distribution of velocities to validate the simulation data. No significant differences in the results were found for the different initial velocities.

Results and Discussion

Single-layered annular structures

Fig. 3 shows snapshots of annular oligomers at different simulation lengths. All annular oligomers were intact closed circles at the beginning of the simulations. The single-layered annular oligomer with the C-terminal facing bulk solvent (the CN model) opened at 5 ns and finally broke down into three subunits consisting of twisted linear-like oligomers. In contrast, the annular oligomer with the N-terminal facing bulk solvent (the NC model) largely collapsed within 20 ns as a result of strong hydrophobic interactions induced by the pore-facing C-terminal β -strands. Although both structures exhibited severe structural deformation, they displayed different oligomerization scenarios: one structure broke down into pieces, and the other collapsed. This could be due to the more hydrophobic residues in the C-terminal segment compared to the N-terminal segment, which experience large deformation when they are exposed to the solvent. We also observed that Na^+ ions were easily attracted to the negatively charged Glu22 side chains in both NC and CN structures, consistent with our recent observations that a single-layered annular A β oligomer with the C-terminal facing the bilayer forms a stable toxic Ca^{2+} -selective conducting channel in membrane³¹.

To quantitatively examine the conformational evolution of the A β oligomers, Fig. 4 shows the root-mean-squared deviation (RMSD) of the backbone atoms with respect to the initial minimized crystal structure between the CN and NC models. The RMSD for the CN model increased sharply during the first 2.5 ns, and then continuously reached 13 Å at the end of 20 ns simulation, consistent with the structure deformation seen in the snapshots of Fig 3b. Although the NC model experienced relatively smaller conformational change than the CN model, it still displayed high instability with a continued increase in RMSD. The overall size of the annular structure is measured by the radius of gyration (R_g). The CN single-layered annular structure displayed a larger R_g value than the NC annular structure because the CN structure was broken down into small pieces while the NC structure collapsed. Interestingly, the secondary structures of β -sheets for both models were well conserved over the course of the simulations (Fig. 7 a–b). Taken together, this structural information indicates that both single-layered annular models are not stable in solution.

Double-layered annular structures

Fig. 5a shows a series of snapshots of the double-layered annular model with an interior NC-CN interface. After initial relaxation, the NC-CN annular structure was able to maintain its circular shape with an inner diameter of ~ 20 Å during 50 ns, despite the disappearance of the perfect annular shape. The calculated RMSD reached an asymptotic value of 10 Å at 12 ns and remained steady after that (Fig. 6a). The size of this channel is comparable to amyloid channels as observed in the AFM images³⁸. In contrast, the CN-NC structure experienced large structural deviation with a continuously increased RMSD of 14 Å at 50 ns. It can be seen from Fig. 5b that the CN-NC structure experienced large twisting motions with many opening/closure events at the outer layer during the simulations. Looking into the cross section of the pore structures, the NC-CN pore was a straight and fat tube (Fig. 8a), which can easily be active in ion conductance³¹. We also observed that the exposed charged E22 at the N-terminus in the NC-CN annular structure was able to selectively attract Na^+ cations. Stable A β annular structures carrying cations might be able to thin host membranes, lower the dielectric barrier, and increase cation conductance³⁹, disrupting the normal ion gradient and causing neuronal dysfunction or cell death⁴⁰. As compared to the NC-CN pore, the CN-NC pore was twisted and contained more disordered A β peptides (Fig. 8b). This could be partly due to unfavorable Glu22-Glu22 repulsive interactions. To explain the influence of the interface on the structural stability of the annular structure, we examined intermolecular interactions at the interface. The NC-CN interface consists of large hydrophobic patches of I31, I41, M35, and V39 in the antiparallel β -strand portions and shape-complementary M35-M35 contacts between adjacent inner and outer layers, while the CN-NC interface consists of the V18-F20 hydrophobic contacts and the K16-E22 salt bridges. At the CN-NC interface the K16-E22 salt bridges are solvent exposed and easily attacked by waters, and the V18-F20 hydrophobic interactions are not sufficient to associate the two layers. This was confirmed by the shape complementarity (S_c)⁴¹, which is used to measure the geometrical match of side-chains that protrude from adjacent inner and outer layers, where $S_c=1.0$ represents a perfect match between the interfaces, while 0.0 represents two unrelated interfaces. As shown in Table 2, the NC-CN interface had the highest S_c value of 0.68, indicating that the side-chains at the interface were more comfortable in interacting with each other, without large unfavorable steric effect. On the other hand, the S_c value was greatly reduced (to 0.58) for the CN-NC interface as a result of weak hydrophobic interactions. However, the secondary structures of β -sheets were well preserved for all annular models within 50 ns simulations (Fig. 7c–f).

We further analyzed the interaction energies between two layers with various interfaces (Fig. 6c). Dimeric structures were extracted from explicit MD trajectories by excluding water molecules at a 10 ps time interval. Each structure was first subjected to energy minimization for 1000 conjugate gradient steps. At the minimized state, the solvation energy was then calculated using the Generalized Born with a simple switching method (GBSW)^{42,43}. Thus, the interaction energy between two dimeric structures was calculated by

$\langle \Delta G_{\text{dimer}} \rangle = E_{\text{dimer}}^{\text{mm}} + G_{\text{dimer}}^{\text{gbsw}} - (E_{\text{monomer1}}^{\text{mm}} + G_{\text{monomer1}}^{\text{gbsw}}) - (E_{\text{monomer2}}^{\text{mm}} + G_{\text{monomer2}}^{\text{gbsw}})$. Energy profiles showed that the NC-CN oligomer (-1715.1 kcal/mol) was much more energetically favorable than the CN-NC (-412.3 kcal/mol) oligomers, suggesting that different surface packings are stabilized by different forces. Lashuel et al¹⁴ reported that A β annular species consisted of 40–60 A β peptides with inner diameters of 15–20 Å, in good agreement with our double-layered NC-CN model consisting of 60 A β peptides with an inner diameter of 22.2 Å.

Effects of sequence variation on the stability of annular structures

To examine the effect of the steric zipper on the structural stability of the NC-CN annular model, we performed two additional MD simulations: one with Ala-mutation (NC-CN-M35A) and the other with the sulfoxidation at M35 positions (NC-CN-M35O). Although backbone

RMSD differences between the wild type and its mutants were small, visual inspection of snapshots showed that both mutants started to bend their initial circular shapes and lose their layer-layer association at 50 ns. It can be seen that as compared to the wild type, when the Met35 was replaced with Ala that has similar hydrophobicity, the interaction energy was only slightly affected by 9% (Fig. 6 and Table 2). Since Ala is smaller and less hydrophobic than the Met residue, Ala substitutions at position 35 in the outer layer lead to either loose packing or lack of contacts with Gly33, Ala35, and Gly37 facing the inner layer. However, when neutral Met35 was oxidized into negatively charged residue, the interaction energy became much less favorable and increased by 27% due to charge-charge repulsion between two β -sheets (Fig. 6). Meanwhile, the favorable side-chain geometry match between two layers is also important for the residues to fit into the interface without disturbing the proper hydrophobic interactions at the CC interface. As compared to the A β wild type, S_c values were reduced to 0.63 and 0.60 for the CC-M35A and CC-M35O mutants, respectively, consistent with snapshots of mutants at 50 ns in Fig. 5. It appears that sulfoxidations at Met35 is more vulnerable to the A β annular structure than M35A mutations because they can not attain sufficient packing interactions, consistent with experimental observation that M35O has a negative effect on the A β structural stability⁴⁴. Overall, this information indicates that the side-chain interactions at the interface are indeed responsible for changes in the structural stability of the A β oligomers. Moreover, as compared to A β linear-like oligomeric double-layered structures³³, the M35A mutations not only disrupted the shape-complementary steric zipper (S_c was reduced to 0.55 as for 0.67 of wild type), but also weakened the original hydrophobic M35-G37 interactions between the inner and outer layers. It thus appears that this steric zipper stabilizes in part the linear-like oligomeric structure, but not the annular structure. This could be due to the geometrical confinement induced by the closed-circular shape, implying that the double-layered A β annular structure has inherently favored structural stability over the linear-like structure at low concentration.

Comparing to A β linear-like structures

Comparison among various A β linear-³³/annular-like models reveals different scenarios in the oligomeric aggregation/disaggregation process, suggesting that any or all of these oligomeric intermediates may contribute to different pathways of protein fibrillation. The NC-CN double-layered annular structure presents the most stable and energetically favorable structure, while other annular oligomers can open and merge within a very short time. We note that the stable double-layered structure with the NC-CN interface does not necessarily imply that this intermediate does not ultimately form fibrils. Rather, it represents an alternative aggregation pathway leading to A β fibril formation. For instance, the CN annular structure breaks into three linear-like subunits at 20 ns. Although these subunits are twisted, they are still able to maintain high content of parallel/double-parallel β -sheet secondary structure calculated by the DSSP method⁴⁵ in the simulaid^{46,47} (Fig. 7b). The backbone H-bonds between peptides in each subunit are straddled, marginally preventing the further dissociation of the subunit. Thus, by increasing the temperature or peptide concentration, over time all annular intermediates will convert to fibrils. Simulation results also show that no matter what topology is applied to the double-layered A β models, the NC-CN interface is always energetically more favorable than the CN-CN interface. In parallel, Olofsson and coworkers⁴⁸ also proposed two structural models for A β_{40} and A β_{42} using HD-exchange NMR data. Both A β models included two β -sheets that stacked perpendicular to the fibril axis with antiparallel arrangements via the CC interface. Tycko and coworkers²² recently presented a minimal quaternary linear-like structure of A β_{1-40} protofibrils using NMR data, in which two intermolecular β -sheets associated through CC sidechain-sidechain hydrophobic interactions in an antiparallel manner. All these models suggest that the CC association is favored over other associations, independent of how the β -sheets are packed in a parallel or antiparallel fashion. Additionally, parallel A β strands within the β -sheet provide extra structural stability through backbone/side-chain hydrogen

bonds, salt bridges, and in-register π - π interactions (i.e. identical positions along the sequence from two neighboring peptides interacting with each other).^{49,50}

Comparing to β_2 -microglobulin peptide

Goto and co-workers³⁵ have recently obtained the 3D structure of a 22-residue peptide (K3) derived from β_2 -m (residues Ser20 to Lys41). Similar to the A β ₁₇₋₄₂ peptide, the K3 peptide adopts the U-shaped β -strand-turn- β -strand motif, where several inward-pointing hydrophobic residues are clustered together to form a hydrophobic core (Phe3, Phe11, Ile16, Val18, Leu20) and three negatively charged residues (Asp15, Glu17, and Asp19) at the same β -strand are pointing outward. We modeled equilibrium structures of K3 oligomers with different organization (single- and double-layers), and morphology (linear-like and annular-like) for the wild type and mutants using all-atom MD simulations⁵¹. For the linear-like morphology, we observe two stable organizations: (i) single-layered parallel-stranded β -sheet and (ii) double-layered parallel-stranded antiparallel β -sheets stacked perpendicular to the fibril axis exclusively through the hydrophobic CN-NC interface (data not shown). As for the A β linear-like structures, although the A β structure with the NC-CN interface is more energetically favorable and more stable than the A β structure with the CN-NC interface, A β structure with the CN-NC interface also displays high structural stability³³. This indicates that A β peptides can be stacked on top of each other with the multiple layers in the lateral direction; that is, A β peptides can form multiple layers through either the NC-CN or the CN-NC interface. In contrast, K3 peptides can only associate two β -sheets through the CN-NC interface. Furthermore, no stable K3 annular structures are observed. Figs. 8c-d showed that both K3 annular structures with the NC-CN and CN-NC interfaces were significantly expanded at 20 ns; especially more disordered K3 peptides were observed at the edge, contacting with the bulk water. The structural instability of the K3 annular morphology is mainly attributed to electrostatic repulsion of three negatively charged residues (Asp15, Glu17, Asp19) projecting from the same β -strand surface (Fig. 1b). However, strong hydrophobic interactions occur through the A β NC-CN interface between two neighboring layers, preventing the dissociation of double-layered A β annular structure. The structural and dynamic comparison of K3 peptides with A β peptides reveals that although different primary amyloidogenic sequences could fold into similar monomeric motifs (the β -strand-turn- β -strand motif in this case), when assembling these motifs into highly ordered aggregates, the final stable amyloid structures can be dramatically different in size, structure, and morphology because of the difference in geometrical side-chain packing arrangements, intermolecular driving forces, and primary sequence compositions and positions, suggesting that the molecular mechanism leading to distinct morphologies and the corresponding aggregation pathways is sequence specific. It should be noted that we only explored in-register A β annular structures, which do not rule out other models with out-of-register chains. Further work of out-of-register packing will be needed to investigate how organization changes alter structure.

Conclusions

We explore the structural stability and dynamics of A β annular structures using all-atom MD simulations in explicit water. Our simulation results reveal that single-layered annular structures are much more vulnerable to structure deformation than double-layered annular structures because single-layered structures can more easily be attacked by the solvent because of the lack of protected interlayered association. A β peptides are found to be able to form stable double-layered annular oligomers through the NC-CN interface for a sufficient time in the MD simulation, as compared to other double-layered annular structures. Consistent with our previous simulations of A β linear-like structures, it appears that the NC-CN interface could be a dominant association pattern when A β oligomers stack in the lateral direction perpendicular to the fibril growth axis. The annular A β NC-CN model is well consistent with (and

complements) the AFM observations with similar inner and outer pore sizes, and molecular weight. This may suggest that annular structures represent alternative amyloid intermediates during the early stages of fibrillar aggregation at low concentrations. Comparisons of the conformational behavior of K3 annular structures derived from the β_2 -microglobulin suggest that the microscopic arrangement and morphology of amyloid fibers are sequence dependent, and govern specific molecular interactions. Because of the limitations of current computer power and simulation methods, further studies of other A β morphologies such as micelles with different peptide-peptide registrations are necessary to provide more complete and detailed information of the aggregation mechanism and pathway.

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(a) A β ₁₇₋₄₂ peptide

(b) K3 peptide

Figure 1. A α and K3 monomers

Single (a) A β (17–42) and (b) K3 peptides labeled with residue names. Color ID: polar residues in green, nonpolar residues in orange, positively charged residues in blue, and negatively charged residues in red.

Figure 2. annular models

Initial single-layered CN annular structure comprising 24 A β peptides: (a) sideview of cross-section and (b) topview of whole structure. Initial double-layered NC-CN annular structures comprising total 60 peptides (24 peptides in the inner layer and 36 peptides in the outer layer): (c) sideview of cross-section of two antiparallel β -sheets and (d) topview of whole structure. The color scale bar indicates the distance of atoms from the center. All images were generated by VMD⁵².

Figure 3. single, snapshots

Snapshots from 20 ns MD simulations for the single-layered $A\beta_{17-42}$ annular structures of the (a) NC and (b) CN. The NC structure collapses while the CN structure is broken down into small pieces. Met35 residues are presented in sphere format in yellow to guide the eye for identifying the N/C terminus.

Figure 4. single, rmsd and rg

Backbone atom root-mean-square deviation (RMSD) and radius of gyration (Rg) for both NC and CN single-layered A β annular structures.

Figure 5. double, snapshots

Snapshots from 50 ns MD simulations for the double-layered annular structures with the interface of (a) NC-CN, (b) CN-NC, (c) NC-CN with M35→Ala mutation, and (d) mutant NC-CN with M35→sulfoxidation. The CN-NC, NC-CN-M35A, and NC-CN-M35O structures are not able to maintain annular shape within 50 ns, as shown by open events occurring at the outer layers of annular structures at 50 ns. Met35 residues are presented in sphere format in yellow to guide the eye for identifying the various interfaces. Color scheme is as follows: polar residues in green, hydrophobic residues in white, negatively charged residues in red, and positively charged residue in blue.

Figure 6. double, rmsd, rg, energy

Structural and energetic analysis of double-layered A β models with different interfaces (i.e. NC-CN and CN-NC) and sequences (i.e. wild type and mutant). The time evolution of (a) backbone RMSDs, (b) radius of gyration, and (c) interaction energies between two facing inner and outer annular layers.

Figure 7. dssp

Secondary structures as a function of time for the (a) NC, (b) CN, (c) NC-CN, (d) CN-NC, (e) NC-CN-M35A, and (f) NC-CN-M35O Aβ₁₇₋₄₂ annular structures, as calculated by the DSSP algorithm⁴⁵ in the Simulaid program⁴⁶.

Figure 8. K3

Annular structures of (a) A β NC-CN at 50 ns, (b) A β CN-NC at 50 ns, (c) K3 NC-CN at 20 ns, and (d) K3 CN-NC at 20 ns. In the lateral view, the front part of the structures has been removed to allow a view of the pore. The pore structures are calculated by the HOLE program⁵³ and displayed by the VMD program⁵². Hydrophobic residues are shown in white, polar residues in green, positively charged residues in blue, and negatively charged residues in red.

Table 1

Structural parameters for all A β models.

Simulations	Initial pore size ($\text{\AA}$)		Number of strands		Number of atoms	Time (ns)
	Inner diameter	Outer diameter	Inner layer	Outer layer		
single-layered						
NC	25.5	62.5	24	—	69,443	20
CN	27.4	65.5	24	—	69,325	20
double-layered						
NC-CN	22.2	90.7	24	36	161,142	50
CN-NC	20.5	89.7	24	36	144,454	50
NC-CN with M35A mutations	22.2	90.7	24	36	128,697	50
NC-CN with M35O sulfoxidations	20.1	90.0	24	36	148,088	50

Table 2

Averaged interaction energies and shape complementary (Sc) between two β -sheets for all double-layered organizations. The mean interaction energy and Sc are averaged from the last 10 ns simulations.

Simulation	Interaction Energy (kcal/mol)	Sc
NC-CN	-1715.1 ± 65.7	0.68 ± 0.02
CN-NC	-412.3 ± 23.4	0.58 ± 0.03
NC-CN with M35A mutations	-1559.7 ± 55.6	0.63 ± 0.01
NC-CN with M35O sulfoxidations	-1245.6 ± 39.1	0.60 ± 0.02