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Out-of-Register A β_{42} Assemblies as Models for Neurotoxic Oligomers and Fibrils

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Abstract

We propose a variant of the recently found S-shaped A β_{1-42} -motif that is characterized by out-of-register C-terminal β -strands. We show that chains with this structure can not only form fibrils that are compatible with the NMR signals, but also barrel-shaped oligomers that resemble the ones formed by the much smaller cylindrin peptides. By running long all-atom molecular dynamics simulations at physiological temperatures with an explicit solvent, we study the stability of these constructs and show that they are plausible models for neurotoxic oligomers. After analyzing the transitions between different assemblies, we suggest a mechanism for amyloid formation in Alzheimer's disease.

Graphical Abstract

Keywords

amyloid formation; structural transitions; molecular dynamics

Introduction

Alzheimer's disease and an increasing number of other illnesses are connected with the presence of amyloid fibrils.^{1–4} The bottleneck in the emergence of mature fibrils is the time needed for self-assembly of monomers into a critical nucleus⁵. In this process, smaller oligomers are formed that, in the case of Alzheimer's disease, appear to be the main

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Supporting Information

The coordinates of the out-of-register fibril model ORF and of the out-of-register barrel trimer tetramer BB4 (made out of either full-sized A β_{1-42} chains or from A β_{11-42}) are available in PDB-format as Supporting Information. We also include as supplemental information in table S1 the Chemical Shift calculated from the first and tenth model of the NMR structure (PDB-ID:2MXU) and their difference to the measured shifts, which are used by us in the article as reference.

neurotoxic agents causing memory loss⁶⁻⁷. It is believed that these oligomers, made of 37–42 residue long A β peptides, damage cells by disrupting cell membranes, and that the cell toxicity and disease stage are related to the different conformation of A β oligomer or fibrils.⁸⁻⁹ However, the exact mechanism of neurotoxicity is not clear.¹⁰⁻¹¹

This is in part because it is a challenge to resolve experimentally the structure of such oligomers, existing in an ensemble of transient and polymorphic forms¹²⁻¹⁵. One of the few experimental models is cylindrin, the eleven-residue segment K11V of α B – crystalline, which in solution can assemble not only into fibrils with the typical in-register β -strands but also into fibril-like structures with out-of-register β -strands,¹⁶ and, most interestingly, into a barrel-shaped hexamer that is characterized by out-of-register antiparallel β -strands arranged as a cylindrical barrel and held together by inter-chain hydrogen bonds. The same barrel motif can also be formed by three tandem-repeats K11V-TR, each built out of two K11V segments linked by two glycine residues, where the tandem repeats fold into a U-shaped conformation.

While the Eisenberg group has proposed that the cylindrin barrel motif is shared by the various toxic oligomers in amyloid diseases,¹⁷ its actual relevance for the pathogenesis of Alzheimer's disease is not clear. Thanh D. et al¹⁸ have proposed cylindrin-like models of tandem repeats of eleven-residue-long A β -fragments (residue 24–34, 25–35, 26–36) held together by two glycine residues, and have studied the stability of these models with a combination of computational modeling and ion-mobility mass spectrometry, electron microscopy and other experimental techniques. However, unlike for such short fragments, it is difficult to form cylindrin-like barrels made of out-of-register antiparallel β -strands from *full-sized* A β -peptides if one assumes for the individual chains the U-shaped structure seen in most of the A β -fibril models in the Protein Data Bank.^{8, 12-15} This is because the phenylalanine rings of residues 19 and 20 do not fit into the core of a β -barrel made of three or four U-shaped chains. The steric constraint disappears for larger oligomers, which, however, will have an unstable core. An exception is the β -barrel hexamer of T. Härd et al¹⁹ for a A β ₁₋₄₂ variant where at positions 21 and 30 alanines are replaced by cysteines leading to additional stabilization of the U-shaped chains and the barrel by disulfide bonds.

It was recently shown that, unlike A β ₁₋₄₀ peptides, the less common but more toxic A β ₁₋₄₂ species can form fibrils made from three-stranded S-shaped chains. Assemblies of A β ₁₋₄₂ chains taking this fold are not restricted by the above described steric constraints.²⁰ As A β ₁₋₄₀ cannot assume the S-shape motif²⁰, one can speculate that the higher toxicity of A β ₁₋₄₂ is related to its ability to assemble into cylindrin-like barrel-shaped oligomers.

As a first step into testing this hypothesis, we will present in this article a variant of the S-shaped A β ₁₋₄₂-motif with out-of-register C-terminal β -strand that, similar to cylindrin, can assemble into both fibrils and barrel-shaped oligomers. The fibril assemblies are compatible with the NMR signals. We show that our constructs are possible models for neurotoxic oligomers, and study their stability at physiological temperatures through long all-atom molecular dynamics simulations with an explicit solvent. Analyzing these trajectories, we propose a mechanism for amyloid formation in Alzheimer's disease.

Methods

Model construction

While the individual chains form two β -strands connected by a loop region in all known wild type $A\beta_{1-40}$ amyloid fibrils,^{12–15} several recent studies have demonstrated the possibility of other chain arrangements for $A\beta_{1-42}$ fibrils.^{20–24} These fibril models are characterized by S-shaped chains, with the N-terminal strand $\beta 1$ made of residues 12–18, the central strand $\beta 2$ of residues 24–33, and the C-terminal strand $\beta 3$ of residues 36–40.²⁰ We have shown in earlier work that this S-shaped conformation is not stable in assemblies of $A\beta_{1-40}$ peptides.²⁵ This is not because the lack of the intra-chain salt bridge between side chain of residue K28 and the main chain of residue A42 that connects the $\beta 2$ and $\beta 3$ strands and cannot be formed in $A\beta_{1-40}$ peptides, but because of the lack of hydrophobic contacts involving the C-terminal residues I41 and A42. As already observed in Ref.²⁵, the $\beta 2$ -turn- $\beta 3$ motif is surprisingly stable. For instance, the hydrogen bonding in this region can change from *one between different chains* to one between the *strands of the same chain*, with only a small decrease in the total number of hydrogen bonds. In a U-shape fibril model, the loss of inter-chain hydrogen bonds would dissolve the fibril; however, with a S-shaped geometry, the fibril will be still stabilized by inter-chain hydrogen bonds connecting the $\beta 1$ -strands. It is this property that allows us to construct our models.

In order to build an out-of-register fibril model that preserves as much of the inter-chain contacts of the in-register fibril as possible, we start with the triple β -stranded $A\beta_{42}$ fibril model (PDB id: 2MXU) resolved by Xiao et al²⁰, which we call in this paper the In-Register-Fibril (IRF) model and show in Figure 1a. The IRF model is built from three-stranded S-shaped chains held together by *inter-chain* hydrogen bonds between neighboring chains. Using AMBERTOOLS²⁶ we alter in each chain the $\beta 2$ -turn- $\beta 3$ segment into a β -turn between residue 27 to 42 while keeping the salt bridge K28-A42, but replacing the inter-chain hydrogen bonds by out-of-register *intra-chain* hydrogen bonds. We tried different possible staggering patterns by minimizing them and testing their stability in molecular dynamics simulations of 20 ns. In this way, we derived an out-of-register structure with the hydrogen bond staggering that is listed in Table 1. As our previous work showed that the critical size for the in-register fibril model IRF is five to six chains,²⁵ we also assembled six copies of this out-of-register structure into a fibril-like hexamer that is kept together by inter-chain hydrogen bonds between $\beta 1$ strands of neighbouring chains. Minimizing and equilibrating by 50 ns molecular dynamics runs led to four models of out-of-register fibrils, out of which we choose the most stable as our candidate structure for an Out-of-Register Fibril (ORF) model shown in Figure 1b. The in-register part, residues 11–26, is colored in blue and the out-of-register sections are shown in yellow. Note that in both fibril models we exclude the first ten N-terminal residues as our previous work had shown that they do not affect the stability of the (in-register) fibril.²⁵

The same out-of-register $A\beta_{1-42}$ chain configuration that is utilized in the out-of-register fibril model is also used to build out-of-register β -barrel trimer (BB3) and tetramer (BB4) models. Different side chain orientations and backbone hydrogen bond patterns allow for a multitude of possible arrangements. We built more than twelve different assemblies and

chose the ones with the smallest fluctuation and lowest Generalized-Born energy. Our most stable tetramer is shown in Figure 1c, with adjacent chains drawn in different color (yellow and purple), and the hydrogen bond patterns again listed in Table 1. While the intra-chain hydrogen bond pattern is the same as in the ORF model, for each chain, either the $\beta 2$ or the $\beta 3$ strand forms in addition *in-register non-staggered inter-chain* hydrogen bonds with a neighbor chain, while the other strand forms *out-of-register inter-chain* hydrogen bonds with its neighbor. We remark that our final structures are for the full-sized chain, and not only residues 11–42 as for the fibril models. We also remark that the binding pattern could in principle also be extended to N-mers with $N > 4$, for instance hexamers that have been shown to be especially stable.^{19, 27–29} However, in the present investigation we focus trimmers and tetramers as the most simple systems.

Coordinates of the out-of-register fibril and barrel structures, generated by the above procedure, are available as supplemental material.

Molecular dynamics set-up

Our models are constructed with the AmberTools tool suite²⁶ and studied by all-atom molecular dynamics using the GROMACS 4.6.7 package³⁰. These simulations rely on the CHARMM36 force field³¹ and TIP3P solvent.³² Restraining the bonds in protein and solvent with the LINCS³³ and SETTLE³⁴ algorithm allows us to set the integration time-step to 2 fs. An NPT ensemble is realized by setting the temperature to 310 K with a v-rescale thermostat³⁵ and the pressure to 1 bar with a Parrinello-Rahman barostat³⁶. The cutoff for electrostatic and van der Waals (vdW) interactions are 1.0 nm and 1.4 nm. Periodic boundary conditions are used and the particle mesh Ewald (PME) method is employed to calculate long-ranged electrostatic interactions.³⁷ For the monomer $A\beta_{1-42}$ peptide, the cubic box edge size is 6.7 nm and there are 9414 water molecules in the box. For the $A\beta_{1-42}$ -tetramer, the box edge size is 13.1 nm and 71832 solvent molecules are included. In all our explicit-solvent molecular dynamics runs, the salt concentration is set to 0.1M. In order to check the reliability of our results, we have studied each model in two independent runs (starting from the same configuration but different initial velocity distributions that correspond to $T=310K$). For the out-of-register fibril model ORF, and the β -barrel models BB3 and BB4, each trajectory lasts for 200 ns. While these trajectories are much shorter than the hours to days on which aggregation phenomena happen *in vitro* and *in vivo*, they allow to compare the relative stability of structures as signals for the decay of pre-formed structures can be often observed within very short times.³⁸

The resulting trajectories are analyzed using the GROMACS tools suite. Binding energies are approximated by g_mmpbsa tools with the non-polar solvent effect estimated in SAV methods.³⁹ NMR signals for our proposed structures are predicted with SHIFTX2 approach⁴⁰ using the web-server <http://www.shiftx2.ca>.

Replica Exchange with Tunneling

Transitions between in-register fibrils and out-of-register fibril involve crossing of high free-energy barriers that require impractically long simulation times when using regular molecular dynamics. In order to alleviate this problem we have recently introduced a new

generalized-ensemble technique named replica-exchange-tunneling (RET) methods.⁴¹ Unlike in temperature-driven replica-exchange sampling, the acceptance probability is not calculated at the time of the exchange move, but only after the exchanged replicas have evolved over a short micro-canonical trajectory. By this procedure, one can “tunnel” through unfavorable “transition” states resulting from the exchange move. For details of the sampling technique, see references^{41–42}.

In the present paper, we use this improved exchange move to enhance conversions between out-of-register and in-register fibrils by building a chain of replicas that differ in how strongly they are biased to either of the two models. For this purpose, we define an energy function $E_{\text{pot}} = E_{\text{phys}} + E_{\text{Go}} + \lambda * E_{\lambda}$, where E_{phy} describes the energy of the “physical” model, E_{Go} the energy of a suitable Go-model biasing toward either the IRG or the ORG model, and E_{λ} describes the similarity of configurations in physical and Go-model. The strength of coupling between the two models is set by the parameter λ and varies between the replicas. Note that the exchange move depends only on the coupling term, i.e., is proportional to

$$\exp(-\beta(\lambda_1 \Delta E_{\lambda}^{(1)} - \lambda_2 \Delta E_{\lambda}^{(2)})),$$

when two configurations are exchanged between replicas with coupling parameters λ_1 and λ_2 . A consequence of this procedure is a walk of replicas through the space of coupling parameters, with at one end maximal bias toward the in-register-fibril structure IRF, and at the opposite end maximal bias toward the out-of-register fibril ORF. In the most straightforward implementation of this approach are data only analyzed for the replica where $\lambda = 0$, i.e., where the physical model is not biased by other terms. However, information from replicas with $\lambda \neq 0$ can be also utilized by using re-weighting techniques⁴². More details on this approach can be found in previous work where we have demonstrated the superior sampling of RET in studies of switching proteins⁴¹ and studies of conformational changes in small oligomers⁴².

Our RET algorithm is implemented in a modified version Gromacs 4.6.5 that is available on request from the authors. In order to speed up our simulations we rely on a combination of the CHARMM36 force field³¹ with a generalized-Born model⁴³ to approximate protein-solvent interaction by an implicit solvent. All peptides form an isolated system within an NPT ensemble, and no periodic boundary conditions are used. For the generalized-Born model, the cut-off in electrostatic interaction is set as 999 nm and the salt concentration to 0.1M. The Go-interaction terms are given by the SMOG energy function as generated by the SOMG-server⁴⁴ at <http://smog-server.org>. We use 32 replicas with $\lambda = (0.015, 0.0135, 0.012, 0.01, 0.008, 0.006, 0.005, 0.004, 0.003, 0.002, 0.001, 0.00075, 0.0005, 0.0003, 0.0001, 0, 0, 0.0001, 0.0003, 0.0005, 0.00075, 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.008, 0.01, 0.012, 0.0135, 0.015)$. Our implementation does not allow one to have all replicas the same temperature, instead the thermostat temperature changes in steps of 0.01K between 330K and 330.31K. Note that the slightly higher temperature reflects our choice of an implicit solvent. In previous work using a similar set-up to study amyloids,⁴² we found

that sampling was optimal 330–340K. All the replicas start from the same initial conformations obtained as the final configurations of a 20 ns long molecular dynamics run at 500K with the β 1-strand restrained, starting from the IRF model (see Figure 1a) and leading to disordered β -2 and β -3 sections. The integration step of RET run is 2 fs and exchange attempts are made every 20ps. Each replica trajectory lasted 100ns but only the last 75 ns are used for analysis. As in previous work⁴² we analyze the energy landscape of the fibril-like hexamer by reweighting data from all replicas to the case of $\lambda=0$, i.e., the case where the “physical” model is not biased by the Go-term.

Results and Discussion

An out-of-register fibril model of A β 42

Changing the *inter-chain* hydrogen bonds, connecting the β -strands of one chain with the corresponding strands of the next chain, to *intra-chain* hydrogen bonds, and connecting the two strands into a β -bend will dissolve a fibril built out of U-shaped A β 40 or A β 42 chains. This is different for the triple-stranded S-shaped A β 42 fibrils. If the chain structures are rearranged so that the *inter-chain* hydrogen bonds, connecting the β 2 and β 3 strands of one chain with the corresponding strands in the next chain, are dissolved and *intra-chain* hydrogen bonds are formed between the two strands of each individual chain, the fibril will be still held together by *inter-chain* hydrogen bonds connecting the β 1 strands. Hence, it is not a priori necessary that our ORF model is unstable and must decay. For this reason, we start our investigation first by analyzing the actual stability of our out-of-register fibril ORF model. For this purpose, we have followed the time evolution of this fibril fragment in two independent molecular dynamics simulations of 200ns, with the simulation protocol described in the method section.

Visual inspection of the trajectories shows that during the simulations, the ORF hexamer remains stable; however, not to the extent the IRF hexamers (PDB id: 2MXU) are preserved in corresponding simulations. This observation can be quantified in both cases by measuring the root-mean-square-deviation (RMSD) to the respective start configurations along the two trajectories for each system. In Figure 2(a) we show this quantity as function of time for both ORF and IRF fibril fragments. For each system, the values from the trajectory where the final RMSD value was largest are displayed. In both models, neither the β 1-strand nor the β 2– β 3 bend are dissolved at the end of 200ns simulations, and while the RMSD values are higher in the ORF case than for the IRF model, measurements of the root-mean-square fluctuations (RMSF), see Figure 2(b), differ for the β 2– β 3 bend little between the out-of-register fibril than in the in-register fibril. Note that the β 2-strand and the turn region itself are even less flexible in the ORF model than in the IRF fibril.

In our out-of-register fibril model ORF, the first intra-chain hydrogen bond connecting the β 2 and β 3 strands is formed between the nitrogen atom of residue G29 and the oxygen atom of residue V40, with the other hydrogen bonds formed accordingly (the final one connecting residue 33 and residue 37). Although the hydrogen bonds connect β 2 and β 3 regions that are arranged perpendicular to the direction of fibril elongation in the initial configuration, after 200ns the hydrogen bonds have rotated in the final configuration by about 20-degrees, leading to a looser side-chain packing in the β 2 region. On the other hand, the β 1-strands

stay unchanged in both models. However, the transition from inter-chain into intra-chain hydrogen bonds leads to a larger average distance between $\beta 2$ and $\beta 3$ regions of adjacent chains than is seen between the N-terminal $\beta 1$ -region, see Figure 2(c). Hence, the ORF fibril is not straight, but bent by an angle of about 15.8° per chain after thermalizing. This bending angle would lead to a tire-shaped fibril with an outer radius of ~ 5.1 nm, consisting of 22–23 chains. In the out-of-register section (the $\beta 2$ and $\beta 3$ hairpin), inter-chain contacts are not formed by hydrogen bonds between main chain atoms but are between side-chains. Hence, the residues in this section, for example, Leu or Val, are not as tightly packed than in the in-register form, which consequently leads to the above mentioned larger average inter-chain distances in this part but not seen between the $\beta 1$ -strands. Measuring the distance between residues V17 and V40 (shown as red balls in Figure 2c), we estimate the radius of curvature to be about 3.4 nm. In a hybrid fibril where ORF and IRF assemblies would alternate, such twisting of the fibril could lead to the “sharp turns” widely observed in Transmission Electron Microscopy or Atomic Force Microscopy images of A β fibrils. Note that in pure in-register-fibrils, such sharp turns or twists, while in principle possible, require breaking of hydrogen bonds and are therefore energetically costly.

The in-register fibril model IRF has been derived from ss-NMR (solid-state Nuclear Magnetic Resonance) measurements.²⁰ However, the relation between NMR signal and molecule structure is not straightforward. Different structures can be within the error bars compatible with a given set of measurements. For this reason, we have compared our out-of-register model ORF with the NMR signals that led to the in-register fibril structure as deposited in the PDB under identifier 2MXU. For this purpose, we have predicted the ^{13}C and ^{15}N chemical shifts that would result from our structure, using the SHIFTX2 software⁴⁰ with the coordinates of our model as input. The so-calculated shifts are shown in Table 2. Note that the experimental chemical shift of some residues, such as E22, N27 and M35, are not given in Ref.²⁰, and therefore are also not listed in Table 2. For comparison, the chemical shift of first and tenth model in IRF structures are also predicted by SHIFTX2 and shown in Table S1. The tenth model is chosen because it has the largest conformational difference to the first model, i.e., the two models span the variation of structures in the NMR ensemble. The root-mean-squared deviations (RMSD) between experimental value and predicted chemical shift of first model of IRF are 1.40 ppm, 0.75 ppm, 0.81 ppm and 3.51 ppm for CA, CB, CO and NH atoms, and 1.37 ppm, 0.81 ppm, 1.02 ppm and 3.45 ppm for the tenth model. These values describe the quality of chemical shift prediction by SHIFTX2, and are comparable to the RMSD values between the predicted shifts for our ORF model and the measured values: 1.32 ppm, 0.96 ppm, 0.52 ppm and 2.5 ppm for CA, CB, CO and NH atoms. The small RMSD differences between calculated and measured chemical shifts, comparable to that found for two models of the NMR ensemble, suggest that our out-of-register fibril model ORF is consistent with the experimental measured chemical shifts. This is even the case for the out-of-register $\beta 2$ and $\beta 3$ regions, where the two fibril models differ most. Hence, the NMR data do not exclude our above-mentioned hypothesis of A β 42 fibrils having a hybrid organization, with most parts being in-register assemblies, but parts of it having out-of-register hydrogen bonding that leads to the experimentally observed twists in the fibril architecture.

Transitions between in-register and out-of-register fibril organizations

Our above conjecture assumes that at least locally S-shaped A β ₁₋₄₂-chains can alter their hydrogen bonding from an in-register fibril organization to an out-of-register fibril, and vice versa. As the free-energy barrier between the two fibril organizations could be high, such transitions may be difficult to observe in regular all-atom molecular dynamics. For this reason, we use Replica-Exchange with Tunneling (RET), a sampling technique developed specifically to cross such high energy barriers, to study the transition between IRF and ORF fragments. Our simulation protocol is described in the Method Section and the resulting simulations realize a random walk between replicas where the physical model is biased strongly toward the in-register-fibril organization of the IRF model, and, on the other side, replicas where the physical model is biased toward the out-of-register organization of the ORF model. In order to test for convergence of the RET simulation we compare the free energy landscape projected on the number of inter-chain and intra-chain hydrogen bonds for two time intervals: 25–62.5 ns and 62.5–100 ns, discarding the first 25 ns for equilibration. Both landscapes show little differences (data not shown). In Figure 3 we show three representative configurations sampled along one such walk that started at the IRF model and led to the out-of-register ORF model. For this walk we also show in Figure 3(b) and (c) the change in hydrogen bonding as observed between the third and fourth chain. As the A β ₁₋₄₂ hexamer converts from an in-register fibril organization to the out-of-register organization, the inter-chain hydrogen bonds in the β 2-turn- β 3 region gradually dissolve, starting in the turn region, and at a later stage are replaced by intra-chain hydrogen bonds, again initially formed close to the turn. This transition starts from surface chains and propagates inside the fibril fragment. The smoothness of this transition implies that the out-of-register fibril could be an on-pathway intermediate for A β ₄₂ fibrils.

Note, however, that because of the artificial dynamics in RET, the above described walk is not necessarily the most likely one. For this to be the case, the walk has to be consistent with the free-energy landscape of the system. The free-energy landscape is obtained by potential of mean force (PMF) which is widely used in MD studies of amyloid peptides^{45–46}. We show this landscape in Figure 4 projected on the number of inter-chain and intra-chain hydrogen bonds involving residues in the β 2-turn- β 3 turn. The IRF model here has only inter-chain hydrogen bonds, and the ORF fibril only intra-chain hydrogen bonds. The free energies are obtained by re-weighting data from all 32 replicas onto the case of $\lambda=0$, i.e., for the case where the physical model is not biased toward either the IRF or the ORF model. The local minimum corresponding to the IRF-fibril (marked as (a) in the figure) is chosen as zero in the free-energy landscape. Going from the IRF form to the ORF form requires the crossing of a free energy barrier of 3.1 RT, where the transition state (d) is characterized by configurations in which the inter-chain hydrogen bonds connecting β 3-strands between neighboring chains are dissolved but not the hydrogen bonds that connect the β 2-strands. Disintegration of these hydrogen bonds lowers again the free energy and leads to the intermediate state (c) which has a relative free energy of 1.7 RT where only about 10% of the segments made of residue 27 to 32 (the β 2-strand region) maintain inter-chain hydrogen bonds, and most of the residues in turn (residue 33–36) and β 3 section (residues 37–42) have a random coil or extended structure. The rearrangements needed for formation of intra-chain hydrogen bonds between the β 2 and β 3 strands of the various chains initially raises the free

energy by about 1 RT before the gain in energy by forming the hydrogen bonding of the ORF fibril (marked (b) in the figure) lowers the free energy again to a value only 0.7 RT higher than the one of the IRF-fibril.

Given the relative small free energy differences, we conjecture that the ORF fibril organization, while maybe not stable as an assembly with hundreds of peptides, could be a nucleus for growth of fibrils that later fully or partially convert into the more stable in-register form. Such transitions are likely as the barrier between the two forms is only of order a few RT, consistent with the gradual changes observed on the walk shown in Figure 3. Note that the radius of gyration changes little in the landscape if one walks from on fibril arrangement to the other, changing monotonically from 15.8nm to 16.4nm. This suggests that the transition between the two forms does not involve separation of monomers from a fibril that would otherwise change their configuration before re-attaching. Note also that the transition starts from the surface of the fibrils, with the upper layer changing first before deeper layer follow. We remark that this observation is consistent with the “dock-lock” process of fibril elongations seen in experimental^{47–48} and computational studies.^{49–52} The rate-limiting lock step of establishing correct hydrogen resembles that of the hydrogen bond reforming from ORF to IRF fibrils seen by us, and leads to similar free energy barriers.

An out-of-register β -barrel model of A β 42

The Eisenberg group¹⁷ has proposed that the toxic oligomers in amyloid diseases share a certain (‘cylindrin’) motif made by out-of-register β -strands forming a β -barrel. While it has been shown that eleven-residue long fragments of A β peptides (covering residues 25–35) can assemble into this cylindrin form,¹⁸ steric constraints forbid assemblies of full-sized A β chains into this motif if the peptides are in the U-shaped configurations seen in A β 40 fibrils. This was the motivation for us to assemble trimers and tetramers (constructed with the protocol described in the method section) where the steric constraints are avoided by the S-shaped structure of the individual A β _{1–42} peptides. If stable, our constructs would support the Eisenberg hypothesis that the out-of-register barrel motif is a general architecture for toxic amyloid oligomer.

In Figure 5(a) and 5(b) we show such trimers and tetramers, build from residues 27–42, i.e., the out-of-register β 2-turn- β 3 region of A β _{1–42}. Adjacent peptides are distinguished by color. For the tetramer, all backbone hydrogen bonds are in anti-parallel β -sheets formed by the β 2-strand and β 3-strand of one chain with the corresponding strands of the neighboring chains. The size of the trimer system is comparable with the cylindrin structure developed by Thanh. D et al.¹⁸ that is made of three chains, each built of two eleven-residue A β fragments connected by two glycine (a total of 24 residues), while in our model the relevant part is made of three β -bend fragments consisting of 16 residues (27–42). Note that the size is also similar to that of the cylindrin barrel build from three tandem-repeats of the eleven-residue peptide K11V that also has 24 residues. The cross section of our oligomers is nearly a circle for the trimer, leading to a compact side chain packing of the six strands. For the tetramer, the packing is less dense, leading to an ellipsoid shape as shown in Figure 5. Both trimer and tetramer have similar height.

During our molecular dynamic simulations of the A β ₂₇₋₄₂ assemblies, the tetramer was more stable than the trimer. The tetramer, shown in Figure 5(b), preserved its β -barrel structure over at least 200ns and changed by only ~ 2.5 Å, while the trimer changed by 2.9 Å. For this reason, we decided to consider only tetramers when building our out-of-register β -barrel assemblies from *full-sized* A β ₁₋₄₂ peptides. In the resulting model, the extended β 1 sections of adjacent chains form an anti-parallel β -sheet as shown in Figure 6(a), held together by in-register inter-chain hydrogen bonds. We tried different geometries for the N-terminal residues 1–10, however, in all cases they quickly assumed a random configuration when following the tetramer in molecular dynamic simulations. On the other hand, the tetramer itself is surprisingly stable and preserved its structure over at least 200ns (Figure 6b). This can be seen from Figure 6(c) where we show as function of time the root-mean-square-deviation to the start configuration, evaluated either for the core segment of residues 27–42, for residues 11–42 (i.e., excluding the flexible N-terminal residues), or for the full-size chain. Especially stable is the core region of residue 27–42. The residues 11–26 are constructed as two pairs of anti-parallel sheets, which during the molecular dynamics simulation shrink to residues 12–16, leading to the small root-mean-square deviation seen in Figure 6(d). Overall, the stability of the barrel-shaped tetramer is comparable to the out-of-register fibril model ORF, likely due to the six hydrogen bonds on the in-register side (between β 1 sections of adjacent chains) and four hydrogen bonds in out-of-register side (connecting β 2 or β 3-regions of one chain with the corresponding strand of a neighboring chain). While most of the hydrophobic residues are solvent exposed (see Table 3), the side-chains of residues I31, L34 and V40 point inside and form a hydrophobic core for the β -barrel as shown in Figure 6(f). The corresponding binding energies of the core-forming residues 27–42, as approximated by *g_mmpbsa*, are shown in Figure 6(e). Note that the side chain of residue I41 does not point inside of the barrel but also contributes to the stability of the barrel by interacting with adjacent chains. In order to prove that the stability of BB4 model depends indeed on this hydrophobic core, and not only on the inter-chain hydrogen bonds, we have also looked into the stability of dimers made of one half of the BB4 tetramer. These dimers, having no hydrophobic core decayed in less than 100 ns into monomers (data not shown here). Hence, as we already see in an earlier study of cylindrin assemblies⁴², the barrel motif relies on the hydrophobic packing of the side chains of residues inside the core, and not on hydrogen bonds between backbone atoms. In our model, the barrel-shaped core is formed by residues 27–42 of the individual chains, i.e., by 16 residues. On the other hand, in earlier work studying the K11V-linker¹⁶ or A β ₂₅₋₃₅-linker¹⁸ models, the cylindrin-like barrel is formed by a trimer where each chain has 24 residues. Since the cylindrin barrels are stabilized by the hydrophobic packing of side chains inside the barrel, the smaller number of residues leads to a lower stability of the trimer than seen in the larger-sized chains of the K11V-linker¹⁶ and A β ₂₅₋₃₅-linker¹⁸ models, and a tetramer is required to provide for sufficient number of stabilizing contacts.

The surprising stability of our tetramer model implies that A β ₁₋₄₂ peptides can indeed assemble into cylindrin-like barrels with an out-of-register β 2– β 3 motif. Note that the side chains inside of the barrel are too tightly packed for the barrel to serve as a water channel, which is consistent with previous work simulating barrel-like assemblies of A β ₂₅₋₃₅ fragments¹⁸ and with our measurements. Using an in-house developed plug-in described in

our previous work,⁵³ we measured an average number of 5.6 water molecules number inside the core region of barrel-like tetramer and 3.3 in the trimer, but did not observe flow of water through the barrel. Hence, while we expect that similar to cylindrin assemblies, our A β ₁₋₄₂ - barrel are cytotoxic, the toxicity mechanism will (again similar to cylindrin, see Ref. ¹⁶) likely not be water and ion leakage through cell membranes. From experiments it is known that the toxicity of A β ₁₋₄₂ oligomers is related to the exposed hydrophobic surface.⁵⁴ For this reason, we have calculated the solvent accessible surface area of the two fibril models and the β -barrel tetramer. Since the first ten residues were omitted in the fibril models but not in the β -barrel, the calculation is done only for residues 11–42. The so-calculated values in Table 3 are given per residues as our fibril models are hexamers and the β -barrel a tetramer. These values show that the BB4 tetramer has both a larger total exposed surface area and a higher hydrophobic exposed surface area than the two fibril models which implies a higher toxicity.

Out-of-register pathways to A β ₁₋₄₂ amyloid formation

In previous work^{5, 55–56} an in-register pathway of A β fibril formation was proposed where monomers assemble into oligomers with in-register U-shaped β -sheets held together by inter-chain hydrogen bonds. These oligomers serve as nuclei for the growth of mature fibrils, i.e., are on-pathway to fibril formation. However, structural data on these meta-stable oligomers, ranging in size from dimers to more than 50 chains, are sparse. Specifically, there is no direct experimental evidence for the in-register arrangements. On the other hand, our molecular dynamics simulations show that the out-of-register β_2 -turn- β_3 motif is stable in oligomers and fibrils, which opens the possibility of pathways to fibril formation that utilize the additional degrees of freedom offered by the out-of-register organization.

Thus, while in-register fibrils may form directly by the pathway described above if inter-chain hydrogen bonding between the β_2 and β_3 strands of spatially close chains leads to in-register oligomers, we conjecture that these fibrils can also form by another pathway, where monomers assemble into small nuclei of out-of-register ORF-like fibril fragments with intra-chain hydrogen bonds between β_2 and β_3 strands. These fragments are metastable and eventually convert into in-register IRF-like assemblies from which the fibril further grows.

Yet another possibility is that A β ₁₋₄₂ chains with the characteristic out-of-register β_2 -turn- β_3 motif assemble into cylindrin-like β -barrel-shaped oligomers. Such cylindrin-like oligomers have been considered toxic species in previous work,¹⁸ and we would expect that our constructs are also cytotoxic. Because of their different hydrogen bond organization, these oligomers are separated from the out-of-register fibril ORF-like assemblies by potentially high energetic barriers. However, as both models share the same out-of-register β_2 -turn- β_3 motif, a transition between both organizations may be possible by first dissolving into free monomers that preserve the β_2 -turn- β_3 motif until the chains re-assemble into the other form. In a second step, ORF-like fibrils would then further interconvert into in-register fibrils.

Given the time scale of amyloid formation (hours to days) it is not yet possible to probe the three pathways directly by molecular dynamics. However, both out-of-register pathways rely on the stability of the β_2 -turn- β_3 motif in *monomers*, which can be tested in simulations. In

Figure 7, we show results from a 200ns-long molecular dynamics simulation of the monomer that starts with the peptide in the structure (Figure 7a) it takes in our out-of-register fibril ORF. While the peptide itself is flexible and its final configuration after 200ns differs by about 30 Å from the start configuration, the β_2 -turn- β_3 motif is preserved in one trajectory for 127 ns, and in the other for 157 ns. Note that this is different for S-shaped monomers taken from the in-register fibril model IRF, which decay within 10 ns (data not shown) as they are missing the stabilizing hydrogen bonds between the β_2 and β_3 strand. Note also that our work is consistent with previous simulations of C-terminal fragments $A\beta_{(x-42)}$ ($x=29-31,39$) that also report a preference for β -hairpin conformation with intra-chain hydrogen bonding.⁵⁷ Interestingly, if residues 41 and 42 are truncated from fragment $A\beta_{30-42}$, the preference shifts to turn-coil states.

The stability of the β_2 -turn- β_3 motif in $A\beta_{1-42}$ peptides suggests that the out-of-register pathway leads to higher rate of aggregation, as the formation of the critical nucleus will be faster. Once a monomer forms the β_2 -turn- β_3 motif, it will keep it for considerable time, during which it can interact with others and form complexes (fibril or barrel-like) that further stabilize it. Formation of such stable structures is much more difficult without the hydrogen-bonding in the β_2 -turn- β_3 motif.

We remark that while such out-of-register pathways have not yet been detected, their existence could be deduced from signals pointing to a high probability of meta-stable intermediate states of monomers with beta-turn motif in residues 27 to 42, or of the K28-A42 salt-bridge, which is different from the salt bridge between residues E22 (or D23) and K28, seen in fibrils with U-shaped chains.

Conclusion

Our guiding assumption is that the higher toxicity of $A\beta_{1-42}$ amyloids in relation to $A\beta_{1-40}$ is due to the ability of $A\beta_{1-42}$ chains to assume three-stranded S-shaped configurations. We have shown that, unlike the U-shaped configurations seen for $A\beta_{1-40}$ peptides, S-shaped $A\beta_{1-42}$ chains can form out-of-register antiparallel β -strands arranged as a cylindrical barrel. We have shown that such barrel-shaped tetramers are stable over at least 200ns. These oligomers are likely cytotoxic, as when inserted into cell membranes, they would cause membrane disruption. This, by itself, may explain the higher toxicity of $A\beta_{1-42}$ peptides over that of the more common $A\beta_{1-40}$ species.

However, there may be a second reason. We could show that the out-of-register β_2 -turn- β_3 motif with its characteristic hydrogen bonding and salt bridge between residues K28 and A42 is surprisingly stable even in $A\beta_{1-42}$ monomers. Monomers with this motif therefore may act as meta-stable intermediates that enhance the chance to form stable oligomers (such as the supposedly cytotoxic barrel structures) and lower the nucleation threshold, which in turn could lead to a higher frequency of toxic species.

The above observation is especially interesting because $A\beta_{1-42}$ peptides can also form fibrils with either in-register or out-of-register hydrogen bonding that are difficult to be distinguished by their chemical shifts in an NMR-experiment. Hybrids of these two fibril

arrangements lead to kinks which can explain the “sharp turns” observed in Transmission Electron Microscopy or Atomic Force Microscopy images of A β fibrils. We have shown that both arrangements have comparable stability and do interconvert. As the out-of-register fibril arrangement and the barrel-shaped oligomers share the same out-of-register β 2-turn- β 3 motif, they likely also interconvert as we saw in earlier work on simulations of cylindrin assemblies.⁴² We plan to test this hypothesis in future work.

Our results suggest a mechanism where S-shaped monomers, β -barrel oligomers, out-of-register fibrils and in-register fibrils exist in an equilibrium. The key point is the stability of the out-of-register β 2-turn- β 3 motif in A β ₁₋₄₂ monomers. Such monomers can be formed directly, but can also appear when the S-shaped chains at the end of an in-register fibril convert into out-of-register fibril forms. These out-of-register fibril fragments are less stable and decay to monomers. Once formed, A β ₁₋₄₂ monomers with the β 2-turn- β 3 motif are sufficiently long-lived to either nucleate formation of out-of-register fibrils that, in turn, may later rearrange into in-register fibrils, or to assemble into the potentially toxic cylindrin-like β -barrel-shaped oligomers. The net-effect of the various transitions and pathways, sketched in Figure 8, is a higher concentration of toxic oligomers than seen for A β ₁₋₄₀, and a lower effective nucleation threshold.

Probably the most exciting aspect of the proposed mechanism is the possibility that the β 2-turn- β 3 motif is “infectious”, i.e., that solvable and therefore mobile monomers (or dimers) with this motif could seed formation of fibril and barrel assemblies by impressing their structure on other monomers. Such self-replicating amyloids are widely discussed^{50, 58–64} and have been observed for the Osaka-mutant^{65–66}. In another ongoing study, we are exploring the possibility of self-replication for S-shaped A β ₁₋₄₂ monomers and oligomers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) Our start-configuration for the in-register (IRF) fibril model as derived from the experimentally determined structure (PDB-Id: 2MXU). (b) Representative conformation of our out-of-register (ORF) fibril model. The in-register section (residue 11–26) is drawn in blue, and the out-of-register parts in yellow. (c) Representative structure for our proposed β -barrel tetramer model. Neighboring chains are drawn alternating in yellow and purple. For each chain, either the β 2-strand or the β 3-strand forms in-register inter-chain hydrogen-bonds with one of its neighbor, while the other will form out-of-register hydrogen-bonds with the other neighbor.

Figure 2.

(a) The root-mean-square-deviation (RMSD) with respect to the start configurations as function of time measured in our simulations of Aβ₁₁₋₄₂ hexamer, either assembled as in-register IRF fibril model (red) or as the out-of-register ORF fibril model (blue). (b) The root-mean-square-fluctuations (RMSF) of each residue measured for both assemblies. (c) Radius of curvature and bend angle per chain in the out-of-register fibril ORF after a molecular dynamics run of 200 ns. The Cα-atoms of residues V17 and V40 are drawn in red.

Figure 3.

Representative configurations of a Aβ₁₋₄₂ hexamer walking between a replica with strong bias toward the in-register fibril model IRF and a replica where the physical model is biased toward the out-of-register model ORF. Note that the inter-chain hydrogen bonds in the β2-turn-β3 region gradually dissolve and are replaced by intra-chain hydrogen bonds. This is quantified in (b) where we show along this tunneling event the number of inter-chain and intra-chain hydrogen bonds involving residues in the C-terminal β2-turn-β3 motif and in (c) where this time evolution is shown in more detail for the four pairs of inter-chain hydrogen bonds seen in the IRF fibril (A and B connecting β2-strands, C and D β3-strands) and the two pairs of intra-chain hydrogen bonds seen in the ORF fibril (E connecting residues 29 and 40, F residues 33 and 37).

Figure 4.

Free energy landscape of the fibril-like hexamer projected on the number of inter-chain (x-axis) and intra-chain (y-axis) hydrogen bonds involving residues in the C-terminal $\beta 2$ -turn- $\beta 3$ motif. Data from all 32 replicas are used and reweighted to the case of zero-coupling parameter λ between physical model and biasing Go-model, i.e., the reweighting corrects for the bias introduced by the Go-term. Representative structures are shown for the main regions in the landscape: in-register fibril IRF (a), out-of-register fibril ORF (b), intermediate state (c) and main barrier, the transition state (d).

Figure 5. Trimer(a) and tetramer(b) assemblies of Aβ₂₇₋₄₂ fragments forming an out-of-register β-barrel. The adjacent peptides are shown in different color. The N-terminal atoms are represented by blue balls, and the C-terminal atoms by red balls.

Figure 6.

The initial (a) and final (b) configuration of the β -barrel $A\beta_{1-42}$ -tetramer. Its root-mean-square deviation (RMSD) to the start configuration as function of time (c) and the root-mean-square fluctuation (RMSF) of all residues (d). Binding energy between chains (e). Only the contributions of residues in the hydrophobic core formed by residues 27–42 are shown. (f) Side chain packing inside of the cylindrin-like barrel.

Figure 7.

A 200 ns long molecular dynamics trajectory of an Aβ₁₋₄₂ monomer. (a) The start configuration (t=0) has an out-of-register β₂-turn-β₃ motif held together by hydrogen bonds between the β₂ and β₃ strands. This structure is the same as the peptides assume in the ORF fibril. The out-of-register motif is stable for more than 140ns (conformation at t=100ns shown here). Then the motif gradually breaks up as shown in the final configuration of the 200-ns trajectory. The root-mean-square-deviation to the start configuration as function of time is displayed in (b), with the root-mean-square-fluctuations of all residues plotted in (c).

Figure 8. Proposed mechanisms of $A\beta_{1-42}$ amyloid formation relying on the ability of $A\beta_{1-42}$ peptides to assume S-shaped motif.

Hydrogen-bonding between backbone atoms in the core region (residues 27 to 42) of the out-of-register fibril (ORF) and the out-of-register β -barrel models BB3 and BB4. All models share the same intra-chain hydrogen-bonds, but inter-chain hydrogen bonds exist in this region only in the barrels BB3 and BB4.

Table 1

Intra-chain hydrogen-bonds		Inter-chain hydrogen-bonds (Only in BB3 and BB4)			
		In-register		Out-of-register	
β 2 strand	β 3 strand	Chain A	Chain B	Chain A	Chain B
40VAL:O	29GLY:N	28LYS:O	34LEU:N	31ILE:O	39VAL:N
40VAL:N	29GLY:O	30ALA:N	32ILE:O	31ILE:N	39VAL:O
38GLY:O	31ILE:N	30ALA:O	32ILE:N	37GLY:O	41ILE:N
38GLY:N	31ILE:O	32ILE:N	30ALA:O	37GLY:N	41ILE:O
37GLY:N	33GLY:O	32ILE:O	30ALA:N		
		34LEU:O	28LYS:O		

Table 2

Comparison of ^{13}C and ^{15}N chemical shifts as predicted for our out-of-register fibril model ORF with the experimental results in previous work by Y. Xiao et al.²⁰ The root-mean-squared deviations (RMSD) between the two models are calculated for CA, CB, CO and NH which (together with that of the weighted average of the RMSD) have the same definition as Y. Xiao's work.²⁰

Residue	ORF – Experimental (ppm)			
	CA	CB	CO	NH
V12	4.08	0.25	3.88	-2.27
L17	3.46	0.41	3.61	-7.4
V18	4.42	-0.12	3.3	-6.58
F19	2.91	0.58	2.96	-7.59
F20	1.38	0.8	3.48	-1.91
A21	4.23	1.01	3.32	-5.47
D23	2.24	2.76	4.11	0
V24	4.49	-0.57	2.79	-4.71
G25	1.03		2.98	-6.07
S26	3.60	-0.04	4.05	0.16
K28	4.16	-0.12	3.81	-6.88
G29	1.57		4.02	-5.11
A30	4.22	0.45	3.56	-3.62
I31	5.50	-1.3	4.23	-5.26
I32	4.49	-0.28	2.78	-5.55
G33	2.49		3.52	-5.83
L34	0.14	1.99	3.06	-1.31
V36	3.36	1.58	3.49	-5.79
G37	2.42		4.57	-5.53
G38	2.74		4.41	-0.11
V39	3.89	-0.32	4.11	-1.46
V40	4.25	0.61	3.64	-6.09
I41	3.30	0.06	3.07	-6.67
Weighted Average	RMSD shifts			
1.06	1.32	0.96	0.52	2.50

Table 3

Solvent accessible surface area (SASA) for the in-register fibril IRF, the out-of-register fibril ORF and the β -barrel tetramer BB4. Values are in nm² and per residue. Only residues 11–42 are considered.

SASA	Hydrophobic (nm ²)	Hydrophilic (nm ²)	Total (nm ²)
IRF	0.37 (0.01)	0.34 (0.01)	0.71 (0.01)
ORF	0.41 (0.02)	0.37 (0.01)	0.78 (0.01)
BB4	0.47 (0.02)	0.42 (0.01)	0.89 (0.02)