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Combining Classical MD and QM Calculations to Elucidate Complex System Nucleation: A Twisted, Three-Stranded, Parallel β-Sheet Seeds Amyloid Fibril Conception

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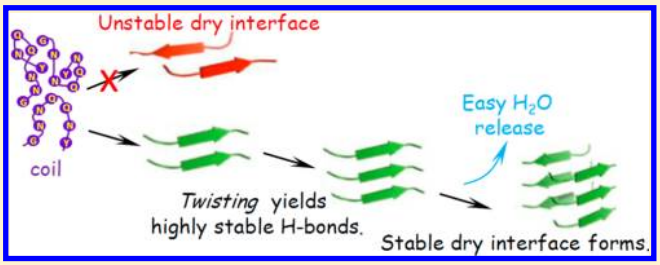
Supporting Information

ABSTRACT: The crystal structure of the Sup35 prion segment, GNNQQNY, revealed precise side chain packing and an extensive H-bond network. However, the conformers and stabilizing interactions involved at nascent amyloid formation are still unclear. Here, long molecular dynamics simulations and quantum mechanical calculations have been utilized to study the conformation and energetics of the initial structure that acts to nucleate further growth. Considering all the plausible intermediates that may act as stepping stones, we find that the initial nucleus is a twisted single-layer, three-stranded parallel β-sheet. H-bonds between β-strands in this twisted sheet, some of which differ from those of the crystal structure's nontwisted β-strands, are key for the nucleus' formation and stability. High level theoretical calculations of these H-bonds' energetics can account for this amyloid-like trimer's remarkable stability. The intermeshing of facing sheets to form the dry interface provides less stability and would occur between two three-stranded β-sheets without metastable water nanowires.



INTRODUCTION

Amyloids are implicated in a score of human illnesses including Alzheimer's and Parkinson's diseases. In the past decade, atomic structures of some amyloid fibrils have been determined by ssNMR or crystallography,[1,2] yet the pathway and energetics for forming the structural kernel which seeds further amyloid growth are unresolved. Many amyloids such as Aβ implicated in Alzheimer's disease are predominately hydrophobic, but others like GNNQQNY are highly polar, which makes this segment from the yeast "prion" Sup35 an interesting puzzle for understanding the physical basis of the formation and stability of complex systems.

On the basis of the exceptionally high packing efficiency of its side chains at the dry interface between two β-sheets, Eisenberg and co-workers proposed that this side chain intermeshing is crucial for amyloid formation and stability.[3,4] Using ballpark estimates of enthalpy and entropy changes, Nelson et al.[3] speculatively proposed that the Sup35 amyloid nucleus would consist of two β-sheets, each composed of two β-strands, whose side chains pack at the dry interface. Somewhat later, on the basis of results obtained using the straight β-sheets of the crystal structure and DFT calculations with plane waves, the main driving force for GNNQQNY amyloidogenesis was proposed to be the cooperative polarization and hyperstability of the intermolecular H-bonds.[5] These results provide the basis for an intuitively attractive model for amyloid formation based on the slow formation of a structural nucleus and then a faster growth step. However, more recent investigations reveal that the GNNQQNY amyloid fibril is moderately twisted in solution and that the experimentally observed straightness of the β-sheets is a consequence of crystal packing forces.[6] The effects of terminal charges, ionic strength, strand configuration, mutation, and temperature on GNNQQNY oligomerization, fibrillization, and crystallization have already been investigated in a score of publications using high resolution experimental[7−9] and computational[10,11] methods (see Table S1 in the Supporting Information for a more complete survey). In addition, studies of GNNQQNY association to form a dimer[12] and to elongate the fibril[13] have provided some details on the nucleation process. Nevertheless, some key questions involving the nature of the first stable intermediate key for oligomer formation, the expulsion of water as the "dry" interface forms, and the possible energy bonus for stabilizing H-bonds in the growing oligomer are still open. These questions are addressed here using a novel combination of independent, time-extensive molecular dynamics simulations and quantum mechanical (ONIOM[14] (DFT:PM6)) calculations to evaluate the structural stability of the key putative structural intermediates, using B3LYP/6-311+G(d,p) for the QM partition. Here, we show that (i) a twisted, three-stranded, parallel β-sheet is the key intermediate for amyloidogenesis, (ii) straight versus twisted β-sheets have different hydrogen bonding networks and the latter are significantly more stable, and (iii) water molecules readily escape as two three-stranded β-sheet intermediates come together to form the amyloid fibril nucleus, without the formation of trapped metastable water nanowires.[15]

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RESULTS AND DISCUSSION

There are two fundamental ways that two Sup35 peptides may combine at the start of amyloid formation. They could stack to form a parallel two-stranded β-sheet. This "sheet" dimer, which we call "**S2**", as it is composed of two strands, is stabilized by multiple side chain and backbone H-bonds.

Alternatively, the two strands could associate laterally to permit the intermeshing of side chains at what will become the "dry interface" in the mature amyloid fibril. We refer to this dimer as "**S1·S1**", where "·" denotes interaction through side chain intermeshing. Tight side chain packing stabilizes **S1·S1**.

We investigated the stability of these dimers by subjecting them to MD simulations. The **S1·S1** dimer dissociates quickly, as evidenced by a large increase in RMSD after 10 ns or less of simulation time (Figures 1 and S1, Supporting Information). Thus, it appears that the stability provided by tight side chain packing is insufficient to hold the two peptides together long enough for further growth. In contrast, the **S2** dimer is more stable; dissociation was never seen before 65 ns (Figures 1 and S2, Supporting Information). A consideration of the bimolecular association kinetics (detailed in the Supporting Information) provides evidence that, when the GNNQQNY concentration is above 3.5 mM, 65 ns would be long enough for a free monomer to encounter another monomer or **S2** dimer. These simulations have been repeated with different initial velocities and conformations, and similar results were always observed (Figures S1 and S2, Supporting Information).

The stacking of an additional peptide on the **S2** dimer could produce a three-stranded, all parallel β-sheet. We call this trimer **S3**. When subjected to MD, **S3** showed a remarkable stability, with no dissociation occurring even after 500 ns. This result is curious considering that parallel β-sheets, due to their suboptimal N−H|||O geometry, are usually thought to be less stable than antiparallel β-sheets. An examination of the structures formed along the simulation revealed the source of **S3**'s remarkable stability. After a few ps, during the equilibration period, the sheet *twists* significantly, which improves the geometry of the H-bonds and changes the pairings of some side chain H-bonds.

In the twisted structure of ***S3***, which we write in *italics* to emphasize its twist, the side chains of the central strand anchor the structure by forming H-bonds with residues of both edge strands (Figure 2, top). Once it twists, ***S3*** remains structurally stable; that is, no significant conformation changes are seen for the remainder of the 500 ns simulation time. In the hexamer consisting of two ***S3*** trimers packed in an antiparallel fashion, the side chain packing between the two sheets is still remarkably tight (the surface complementarity, Sc, is 0.83),[6] almost as tight as the hexamer formed by two nontwisted **S3** trimers (Sc = 0.86),[3] and the percent solvent accessible surface area is only slightly higher in ***S3*** (56%) than **S3** (53%).

We have simulated the behavior of a trimer called **S2·S1**, with the **S2** dimer combined laterally with an additional monomer. This **S2·S1** trimer is structurally unstable, highlighting the necessity of larger systems to form a stable dry interface (Figure S5, Supporting Information).

We have also considered a tetramer called **S2·S2**, which can be regarded as either two **S2** dimers combined laterally through the so-called dry interface or two **S1·S1** dimers stacked and stabilized by H-bond formation. This **S2·S2** system is structurally unstable and evolves toward conformations that resemble ***S3***, or other single β-sheet conformers. This indicates that the side chain packing of four monomers is insufficient to form a stable dry interface. Figure 1 shows the RMSD for the most representative trajectories of **S2**, **S1·S1**, ***S3***, and **S2·S2**, whereas Figures S1−S4 (Supporting Information) contain the set of repetitions to assess the reproducibility of the results.

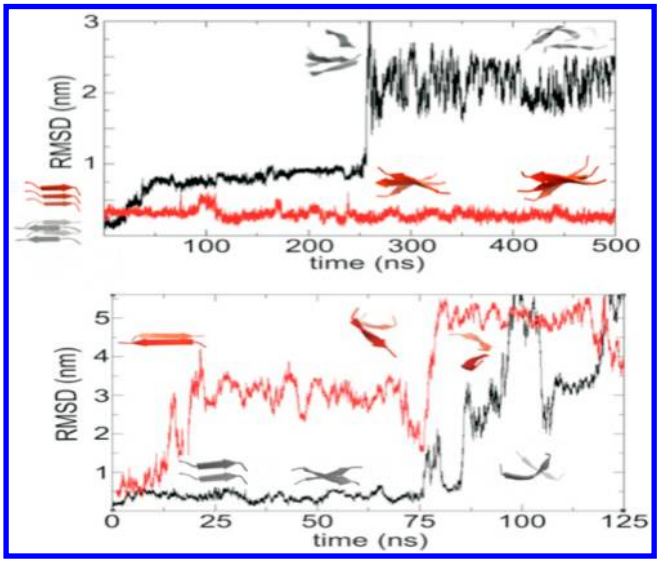

Figure 1. Top: RMSD showing the structural stability of ***S3*** and **S2·S2** systems for a 500 ns run. For the **S2·S2** system, the most representative trajectory from five simulations performed is depicted, in which one strand dissociates and the other three seem to evolve toward an **S3**-like system. For the ***S3*** system, six simulations were performed and all the results showed that this trimer is remarkably stable. The structures are shown at time 0, 250, and 500 ns. Bottom: RMSD for **S2** and **S1·S1** showing selected trajectories. From six runs on each system, the **S2** simulation with the lowest kinetic stability (i.e., quickest dissociation) for **S2** and the **S1·S1** simulation with the highest kinetic stability (i.e., slowest dissociation) are shown, in order to emphasize that the intermeshing only does not provide enough stability (see the Supporting Information for further discussion). The structures shown correspond to snapshots extracted at 0, 50, and 100 ns.

The hydrogen bonding pattern that is present in the crystal structure changes upon twisting, as shown in Figure 2. We have determined that these changes strongly stabilize ***S3*** by −8.92 kcal/mol relative to **S3** (Figure 2).

On the basis of its elevated stability, we conclude that the twisted ***S3*** trimer is the key intermediate for forming the minimal seed of the Sup35 amyloid fibril, which we propose to be a hexamer of two ***S3*** trimers packed in an antiparallel manner (Figure 3, bottom) In ***S3***, residues Q4 and N6 show low mobility, as gauged by their small RMS fluctuations (Figure S6, Supporting Information). We propose that this low dynamic motion of these residues predisposes them to form van der Waals contacts when two trimers come together, thus facilitating the formation of the dry interface of the hexamer ***S3*·*S3***. Moreover, the point substitution of both the central Asn or Gln or both by Gly or Tyr prevents aggregation, as determined theoretically (Figure S7, Supporting Information) and experimentally using NMR (Figure S8, Supporting Information). The NMR characterization of GNNQQNY under solution conditions similar to those employed for crystallography studies demonstrates that the peptide adopts a broad structural ensemble which is dominated by disordered conformers

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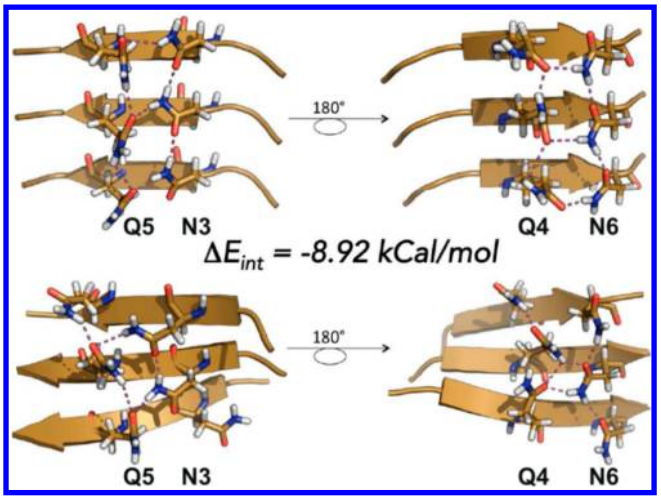

Figure 2. H-bonding pattern for the **S3** structure, resembling the crystallographic data (top); the pattern for the ***S3*** twisted system revealed by the simulations (bottom), each viewed from the perspective of the wet (left) and dry (right) interfaces, respectively. The gain in interaction energy due to twisting is −8.92 kcal/mol, consistent with more compact sheets due to change in the HB pairings; see Methods in the Supporting Information.

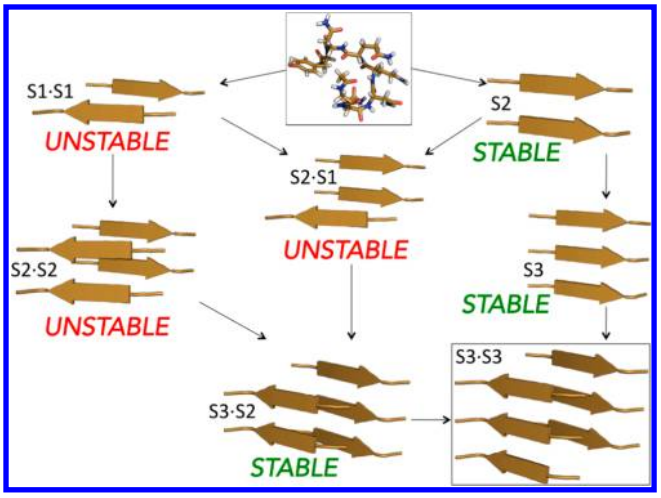

Figure 3. Intermediates considered in the proposed pathway. Systems have been labeled as "stable" or "unstable" based on our simulations. For simplicity, straight β-strands are shown, even though all systems containing ***S2*** or ***S3*** elements are twisted.

(Figure S8, Supporting Information). This is consistent with the behavior of GNNQQNY monomers observed by MD.

In the yeast prion Sup35, the amyloidogenic sequence GNNQQNY occurs as a segment within a large protein containing 685 residues.[16] Considering this, we have performed the simulations of GNNQQNY described above with the N- and C-termini neutralized by acetyl and amide groups, respectively (i.e., $\mathbf{CH_3}-\mathbf{CO}-$HNGly1···Tyr7$-CO-\mathbf{NH_2}$). However, since the crystal structure of GNNQQNY was solved with charged termini, and as this form is often studied experimentally, we have carried out additional simulations to study how charged termini (i.e., $^{+}NH_3-$Gly1···Tyr7$-COO^{-}$) affect the formation of its smallest oligomers. In the dimer consisting of a layer of peptides arranged laterally in an antiparallel manner to permit side chain packing (+**S1·S1**−), the proximity of oppositely charged groups stabilizes the structure (Figure S9, Supporting Information). It resists dissociation and significant structural perturbation, in contrast to the behavior of the uncharged dimer **S1·S1** (Figure 1).

Interestingly, the +**S2**− dimer, despite the close proximity of charged groups of the same sign, is still stable, as was seen in previous studies.[17] Thus, charged termini stabilize the **S1·S1** dimer but do not decisively destabilize the S2 dimer. A discussion on the effect of the electrostatic interactions in the **S2**-like system is given in the Supporting Information.

The direct characterization of the slow formation of a seed for oligomerization and amyloidogenesis, which requires unaffordable computational times, has been tackled here by considering cross-β spine-like structures and testing their stabilities. The instability of **S1·S1**; **S2·S1**, and **S2·S2** arises from the low energetic contribution from dry interface formation. This will usually occur after ***S3*** forms, as ***S3***'s side chains are correctly predisposed for packing, and the interaction energy for two ***S3*** trimers forming the dry interface is highly favorable, as determined by QM methods (−30.46 kcal/mol). This result, and ***S3***'s lack of dissociation during long MD runs, allowed us to propose that ***S3*** is the structural species seeding amyloid conception in GNNQQNY. Whereas a ***S3*** trimer and a **S2** dimer might combine to form an ***S3·S2*** pentamer with a marginally stable dry interface (ΔE_{int} = −8.42 kcal/mol) (Figure S10 and Table S2, Supporting Information), two ***S3*** trimers could combine to form an ***S3·S3*** hexamer with proper side chain packing and a much more stable dry interface (ΔE_{int} = −30.96 kcal/mol) (Figure S11 and Table S2, Supporting Information).

A 100 ns simulation of four of these twisted trimers naturally evolved toward the intermeshed nucleus, in the absence of biasing potentials, as shown in Figure 4. The trimers maintain their stable conformation, as depicted in Figures S11 and S12 (Supporting Information), and form the dry interface. Once formed, the ***S3·S3*** hexamer is stable, as evidenced by low RMSD values observed during the course of a 100 ns simulation (Figure S13, Supporting Information). Previous studies suggested that metastable water molecules may be trapped between two long GNNQQNY β-sheets (made of eight β-strands or more each) as they come together to form the dry interface.[15] These water nanowires persist for ca. 20 ns and retard the formation of the dry interface. Our results strongly suggest that dry interface formation can readily occur between two three-stranded β-sheets. Since water nanowires are much less stable between shorter sheets, water molecules are quickly expelled and the dry interface can form much more quickly. On the basis of these considerations, we conclude that the dry interface formation generally occurs between two β-sheets as small as trimers rather than between long β-sheets.

EXPERIMENTAL METHODS

NMR Spectroscopy. In summary, 1D ^{1}H and 2D TOCSY, ROESY, $^{1}H-^{13}C$ HSQC, and $^{1}H-^{15}N$ HSQC NMR spectra were obtained on the peptides GNNQQNY and GQNNGQNY (negative control) using 600 and 800 MHz Bruker spectrometers equipped with cryoprobes. Both peptides were purchased from Genescript Inc. 1D ^{1}H spectra were used to follow aggregation. After assignment, the ^{1}H, ^{13}C, and ^{15}N chemical shifts were analyzed for tendencies for partial structure formation using the TALOS+ package.[18]

MD Simulations. Briefly, the GROMACS package (version 4.5.5)[19] using the parameters of the amber99sb-ildn force field[20] was used to perform MD calculations. The peptide models for various GNNQQNY peptide oligomers, based on

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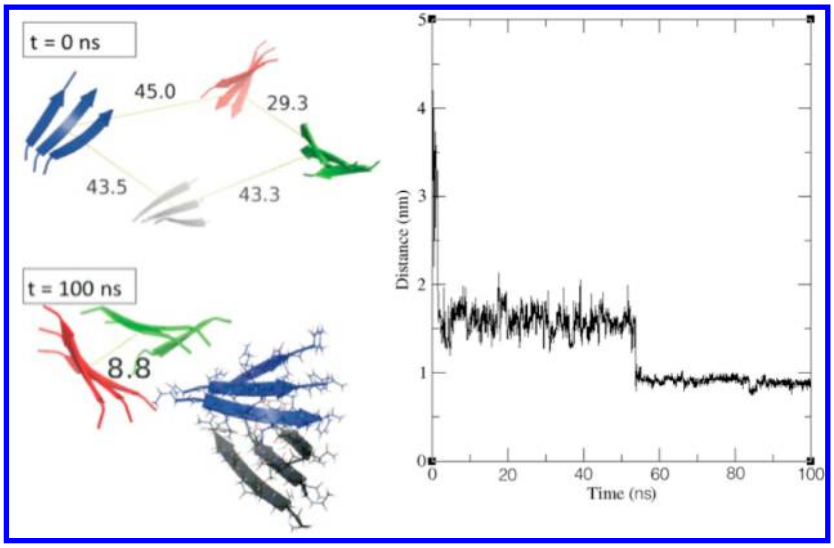

Figure 4. Formation of the dry interface. Four ***S3*** β-sheets were placed far apart in a simulation box following the procedures detailed in the Supporting Information (top left panel). During the course of the simulation, the ***S3*** β-sheets moved closer together, as shown by the decreasing distance separating the Q4 Cα atoms in the middle β-strand of two different ***S3*** trimers (right panel). By 100 ns, the side chains had interdigitated to yield two ***S3·S3*** whose central Q4 Cα's are separated by ~8.8 Å, which corresponds to the distance (represented by red and green arrows in the lower left panel) expected for the nucleus that serves for further growth. The other ***S3·S3*** system (black and blue) is shown as ribbons (backbone) and as sticks (side chains) to highlight the side chain intermeshing. This ***S3·S3*** system resembles the minimal cross-β spine that can form, and it is structurally stable over the course of a 100 ns MD run (see Figure 13, Supporting Information).

the 1YJG PDB structure,[3] were correctly solvated and pre-equilibrated at 1 atm of pressure and 300 K prior to the main 100−500 ns MD runs.

ONIOM Calculations. In a nutshell, the ONIOM approach,[14] which divides the systems into regions of low and high theory, was used to study the energetics of GNNQQNY peptide oligomers. High level DFT/(B3LYP/6-311+G(d,p)) was utilized for the side chain amide atoms, and the PM6 semiempirical method was used for all other atoms. These calculations were carried out using the solvation continuum model (PCM) with the Gaussian 09 package.[21] Due to their high computation cost, these calculations were applied to the most interesting oligomers, namely, *S3*, *S2·S3*, and *S3·S3*. The solvent accessible surface area was calculated using the Lee and Richard's algorithm[22] as implemented in the MOLMOL computer program using a solvent probe radius of 1.4 Å.

For more details on the experimental and computational procedures, please see the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

Detailed descriptions of the computational and NMR spectroscopy methods used, 13 figures, and 5 tables, including Gaussian 09 input files used for the interaction energies and frequency calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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