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Published in:

The Journal of Physical Chemistry. B: Materials, Surfaces, Interfaces, & Biophysical

DOI:

[10.1021/acs.jpcb.7b10233](https://doi.org/10.1021/acs.jpcb.7b10233)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2018

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Periole, X., Huber, T., Bonito-Oliva, A., Aberg, K. C., van der Wel, P. C. A., Sakmar, T. P., & Marrink, S. J. (2018). Energetics Underlying Twist Polymorphisms in Amyloid Fibrils. *The Journal of Physical Chemistry. B: Materials, Surfaces, Interfaces, & Biophysical*, 122(3), 1081–1091. <https://doi.org/10.1021/acs.jpcb.7b10233>

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Energetics Underlying Twist Polymorphisms in Amyloid Fibrils

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Supporting Information

ABSTRACT: Amyloid fibrils are highly ordered protein aggregates associated with more than 40 human diseases. The exact conditions under which the fibrils are grown determine many types of reported fibril polymorphism, including different twist patterns. Twist-based polymorphs display unique mechanical properties in vitro, and the relevance of twist polymorphism in amyloid diseases has been suggested. We present transmission electron microscopy images of A β 42-derived (amyloid β) fibrils, which are associated with Alzheimer's disease, demonstrating the presence of twist variability even within a single long fibril. To better understand the molecular underpinnings of twist polymorphism, we present a structural and thermodynamics analysis of molecular dynamics simulations of the twisting of β -sheet protofilaments of a well-characterized cross- β model: the GNNQQNY peptide from the yeast prion Sup35. The results show that a protofilament model of GNNQQNY is able to adopt twist angles from -11° on the left-hand side to $+8^\circ$ on the right-hand side in response to various external conditions, keeping an unchanged peptide structure. The potential of mean force (PMF) of this cross- β structure upon twisting revealed that only $\sim 2k_B T$ per peptide are needed to stabilize a straight conformation with respect to the left-handed free-energy minimum. The PMF also shows that the canonical structural core of β -sheets, i.e., the hydrogen-bonded backbone β -strands, favors the straight conformation. However, the concerted effects of the side chains contribute to twisting, which provides a rationale to correlate polypeptide sequence, environmental growth conditions and number of protofilaments in a fibril with twist polymorphisms.

INTRODUCTION

Amyloid fibrils are elongated protein aggregates characterized by a cross- β sheet structure. Amyloid fibrils can originate from more than 40 different peptides or proteins,¹ which share no primary structure homology. Amyloids are associated with up to 40 human disease syndromes, ranging from common neurodegenerative disorders, such as Alzheimer's disease (AD), to rare disease syndromes like familial amyloid polyneuropathy.^{2,3} Amyloid fibrils can also be functional^{4,5} and display certain mechanical properties that make them potentially attractive for use as artificial biomaterials.^{6–8}

By formal pathological definition, amyloid deposits are filamentous structures of variable length that deposit in body tissues and fluoresce green-yellow when stained with the dye Congo red and viewed between crossed polarizers.¹ Moreover, the fibrils must display the “cross- β ” diffraction pattern caused by the cross- β -sheet motif when irradiated with X-rays.⁹

The ability to form amyloid-like structures is a property shared by many polypeptide sequences given that the right conditions (pH, T, salt concentration, etc.) are met.¹⁰ This observation strongly suggests the involvement of a feature common to all polypeptides independent of their sequence. The primary building blocks of amyloid fibrils are extended β -sheet structures assembled from numerous peptide monomers, known as protofilaments, which are stabilized primarily by backbone (BB)–backbone hydrogen bond interactions.^{3,6,11–21} A protofilament often consists of two extended β -sheets with interlocked side chains (SCs) forming the so-called steric zippers. The β -sheets, which consist of stacked β -strands, run along the long axis of the filament, and the β -strands run

Received: October 16, 2017

Revised: December 6, 2017

Published: December 19, 2017

perpendicular to the filament. This arrangement results in the characteristic cross- β signature of amyloid-like structures shown by X-ray diffraction.^{22–25}

Mature amyloid fibrils, which generally consist of several filaments twisted into a cablelike structure, typically show a high degree of heterogeneity or polymorphism that determines not only their mechanical properties but also their pathology.^{9,26–35} While the backbone provides the structural framework for the cross- β structure,^{14,36–38} side chain properties and their interactions will dictate the peptide conformation within the protofilament as well as the interactions between them.^{39–41} Amyloid fibrils derived from peptides or proteins of the same sequence display profound polymorphic variations. In these cases, the conditions of fibril growth define the structure of the mature fibril.^{40,42–44} Certain conditions even lead to the coexistence of different fibril polymorphs, suggesting that these polymorphs can be very close in energy.^{45,46} One key type of polymorphism (sometimes known as supramolecular polymorphism) relates specifically to differences in the arrangement of protofilaments in the mature fibril, even while the individual protofilaments remain virtually identical in their internal structure.^{47–50}

Protofilament twisting (pitch angle), which can occur without significant peptide conformational changes, defines the appearance of protofilaments and mature amyloid fibrils. Although atomic force microscopy (AFM) experiments and modeling approaches allow one to assess some mechanical properties of fibrils, such as shear and bending,⁵¹ only a few theoretical studies have derived phenomenological models that link the pitch of fibril twists observed in experimental studies to some other structural properties, such as the width of the fibril and some elastic term describing the shear and interaction between protofilaments often extracted from experiments.^{52,53}

Here, we focus on amyloid fibril twist polymorphism. We first analyze transmission electron microscopy (TEM) images to show that variable pitch may be observed in vitro on a single fibril of A β 42. Next, we use the Sup35 peptide GNNQQNY from the prion protein as a model system to explore this phenomenon in more detail. High-resolution structures of this peptide in an amyloid-like but microcrystalline assembly^{11,54} and structural data of actual amyloid-like fibrils^{55–57} showed that GNNQQNY and derivatives form structurally related fibrils and microcrystals. We studied the properties of this amyloid model system and its cross- β structure to rationalize the existence of twisting polymorphisms. We performed molecular dynamics (MD) simulations of full atomistic models and developed an innovative protocol to measure the free-energy associated with protofilament twisting. Our results provide structural and thermodynamic insights into the origin and extent of fibril twist polymorphism.

METHODS

Amyloid A β 42 Fibril Preparation. Human amyloid β peptide (A β 42), which is a proteolysis product of amyloid precursor polypeptide (APP) was obtained as a synthetic peptide (American Peptide) and solubilized in hexafluoro-2-propanol at 1 mg/mL, dried, and stored at -80°C . On the day of the experiment, the peptide was reconstituted in 2 mM NaOH to 1 mg/mL, dried, and diluted in 10 mM sodium borate phosphate buffer at pH 8.6. A β 42 (10 μM) was incubated at 37°C for 24 h and successively placed in a volume of 5 μL onto carbon film 200-mesh copper TEM grids, rinsed with ddH $_2\text{O}$, and counterstained with 1% aqueous uranyl

acetate solution. The samples were viewed with a JEOL 1400 Plus transmission electron microscope and the images were acquired with a Gatan $2k \times 2k$ digital camera.

Models of the GNNQQNY Cross- β Structure. The structure of the peptide GNNQQNY was taken from the Protein Data Bank (entry 1YJP¹¹). The various systems simulated were constructed, as in our previous study,⁵⁸ respecting the lattice parameters and space group reported for the microcrystal.¹¹ A basic unit containing a pair of peptides related by a 2_1 screw axis was built and replicated 20 times along the axis of the fibril to form a two-stranded β -sheet structural model of a cross- β protofilament. To generate multilayered starting structures, this system was replicated along the a and/or c axes.

Simulation Details. Each system simulated was placed in a periodic rectangular box. The minimal distance between the solute and the box edges was at least 1 nm. The systems were solvated in water and energy-minimized. The protonation state of titratable groups in the peptide was as expected at pH 7.0 except for the simulations performed with neutralized termini, in which case, the NH_3^+ and COO^- groups at the termini were replaced by NH_2 and COOH , respectively. We note that none of the side chains are actually titratable. The range of pH in which most experiments on the GNNQQNY peptide are performed, pH 2–3,^{11,54–56} would correspond to a situation where the N-terminus is positively charged and is near the pK_a of the C-terminus.⁵⁹ Referencing published NMR studies of pH-dependent protonation,^{60,61} we interpret published magic-angle spinning solid-state NMR (ssNMR) chemical shifts⁵⁵ of the microcrystals to indicate a positively charged N-terminus and a negatively charged C-terminus. In the calculation of the potential of mean force (PMF), we used a peptide with both termini neutral. The charge configuration of the termini is noted $+/-$, $0/-$, $+/0$, and $0/0$, in which $+/0/-$ indicate the charge carried by the N/ and /C-termini, respectively.

All simulations were performed using the GROMACS simulation package.⁶² The peptides were modeled using the GROMOS 43a1 force field⁶³ and the water molecules using the simple point-charge model.⁶⁴ The solute and solvent were weakly coupled⁶⁵ independently to an external temperature bath at 300 K using a Berendsen thermostat with relaxation time of 0.1 ps. The pressure was weakly coupled⁶⁵ to an external bath at 1 bar using a relaxation time of 1.0 ps. Covalent bonds within the peptide were constrained using the LINCS algorithm.⁶⁶ The SETTLE algorithm⁶⁷ was used to constrain the solvent. Nonbonded interactions were calculated using a twin-range cutoff of 0.8/1.4 nm. The charge-group pair list was updated every five integration steps ($\Delta t = 2$ fs). To adjust for the truncation of electrostatic interactions beyond the long-range cutoff, a reaction-field correction⁶⁸ was applied ($\epsilon_{\text{RF}} = 78$).

Potential of Mean Force (PMF) of the Cross- β Structure upon Twisting. PMF values were calculated using an umbrella sampling approach,⁶⁹ in which the relative orientation of consecutive peptides was controlled by an additional dihedral angle harmonic potential (Figure 2A). This extra potential was applied to the 19 pairs of peptides along each β -sheet, resulting in 38 biasing potentials used simultaneously. The angles were constrained to values from -20 to $+20^\circ$ with 0.5° increments, resulting in 81 umbrella windows; k_θ was set to 10^4 kJ/mol $^{-1}$ rad $^{-2}$ (3.05 kJ/mol $^{-1}$ deg $^{-2}$). A small angle increment was used to assure a sufficient overlap between windows, which is challenging due to the

effective 38 dimensions of the restraints. Each umbrella window was simulated for 5 ns, starting from a conformation of the cross- β structure close to the target value. The last 4 ns of simulation for each window were used to calculate the PMF. The error estimates on the final PMF were obtained through a bootstrap procedure, in which each umbrella simulation was decomposed into four 1 ns windows. A total of 100 bootstrapping cycles were performed.

Once the PMF was resolved in the 38-dimensional dihedral space, the probability distribution was projected onto a reduced two-dimensional (2D) space, x_0 and x_1 , where x_0 is the average dihedral angle (twist angle as defined below) of the 38 individual angles and x_1 is the root-mean-square deviation of the individual dihedral angles from the average dihedral angle of a conformation. The final PMF, $w(x_0)$, was obtained by integrating the 2D-PMF on the root-mean-square deviation dimension from 0 to 1° . More details for the unbiasing of the umbrella simulations are provided in our earlier implementation⁷⁰ of Kumar^{71,72} and Souaille and Roux⁷³ algorithms and in the Supporting Information (SI).

Twist Angle. The twist angle of a cross- β structure was defined as the average of the dihedral angle between the vectors $\text{Ca}/\text{Asn2}-\text{Ca}/\text{Tyr7}$ of neighboring peptides within the same β -sheet. Left-handed angles were given a negative sign (Figure 2).

Energetic Analysis. The enthalpy of the different systems was monitored and decomposed into a series of terms as defined in the GROMOS 43a1 force field.⁶³ To account for the contribution of the N- and C-termini, interactions involving the atoms of the terminal $-\text{NH}_3^+$ ($-\text{NH}_2$) and $-\text{COO}^-$ ($-\text{COOH}$) groups were considered separately. Although the approach used to determine the enthalpy and entropy of the system is not rigorously precise, it has been previously shown to provide good qualitative and quantitative estimations of their contributions.⁷⁴ The different energy terms were shifted along the z axis to assure that the relevant part of all of the curves shows in the plot so that to allow their comparison. Therefore, the energies reported in Figures 5–7 in the main manuscript and Figures S2 and S3 in the SI should be seen as depicting variations in energy contributions, but not absolute values.

RESULTS

Twisting Structure in Alzheimer's Disease A β 42 Fibril.

In vitro structural studies have shown that environmental conditions, such as temperature, growth conditions (i.e., quiescent vs shaking or stirring), and pH, have a dramatic impact on the twisting structure of A β fibrils.^{30,75} To demonstrate the twisting of A β 42 fibrils, we incubated A β 42 peptide under quiescent conditions in sodium borate phosphate at pH 8.6. TEM images revealed a flexible and flat, ribbonlike fibril with 12 nm width and twisting period between 50 and 125 nm with a mean of 77.7 nm and a standard deviation (SD) of 11.5 nm (Figure 1). This image suggests that the degree of fibril twist may vary within a single fibril. A similar observation was reported for an 11-residue fragment of the protein transthyretin.⁴⁸ We note that different incubation conditions resulted in rigid and ropelike A β 42 fibrils characterized by different twisting structures and abundant branching (data not shown).

At present, the A β 42 protofilament, like other clinically relevant amyloidogenic peptides and proteins, is too intricate to study extensively using all-atom MD simulations. However, an amyloid-forming heptapeptide derived from the yeast prion

Figure 1. Twisting structure of an Alzheimer's disease A β 42 fibril prepared in vitro. (A) Representative negative staining transmission electron microscopy images of a long A β 42 amyloid fibril. The bigger image is a composite reconstruction obtained by arranging high-magnification (scale bar = 100 nm) images. A lower magnification of the same fibril is shown in the lower right panel (scale bar = 500 nm). The upper left panel shows a fibril segment (scale bar = 100 nm), where the twisting is visible. (B) Representative negative staining transmission electron microscopy image of an A β 42 fibril. The arrows indicate the twist points. (C) Normal distribution of the distance between twists in an A β 42 fibril (arrows in B)). Mean = 77.7 nm and SD = 11.5 nm. Data are expressed as number of values (total $n = 1012$) per bin (bin size = 6 nm). The twist per peptide for a given fibril pitch was obtained as $\text{twist} = 180^\circ / (\text{pitch} / 0.5 \text{ nm})$, where 0.5 nm is the expected distance between strands in a sheet. See Figure S1 for more details.

Sup35 provides a satisfactory model system to address, for example, the factors that contribute to protofilament twisting polymorphisms.

Twist Polymorphism of the GNNQQNY Cross- β Structure. To explore twist polymorphism of the cross- β structure of the GNNQQNY peptide, a model consisting of two β -sheets with 20 peptides each (Figure 2) was simulated in monomeric, dimeric, and tetrameric forms. Here and elsewhere in the manuscript, we will reserve the terms mono-, di-, and tetramer(ic) for multiples of a cross- β filament that is itself assembled from two interacting β -sheets (Figure 1). The peptide's termini charges and the mode of interaction between the cross- β filament building blocks in the polymeric structures

Figure 2. Twist polymorphism in isolated cross- β filament structures. (A) Definition of the individual twist angle of our model structure shown on a straight conformation of the cross- β . The angles between the vectors Asn2-Tyr7 ($C\alpha$) of two consecutive peptides, i and $i + 1$, are used; in other words, the torsion angle around Tyr7 $_i$ -Tyr7 $_{i+1}$ ($C\alpha$). (B) Equilibrated conformations of the cross- β structure free in solution obtained by a 15 ns MD simulation started from the straight conformation found in a microcrystal environment (shown in (A)). Four configurations of the termini charges were studied (+/–, 0/–, +/0, and 0/0), where the charges for the N- and C-termini, respectively, are indicated. Red spheres are placed on the C-terminus of each peptide to guide the eye in discerning the cross- β structure twist. (C) Normalized distributions of the fibril twist angle (color code indicated in (B)).

were varied. Initial conformations obey the lattice parameters and space group reported for the crystal.¹¹ The twist angle of the cross- β structures, as defined in Figure 2, is used to follow the evolution of the structure.

Monomeric Cross- β Structures. Starting from the straight conformation, which is stable in a microcrystal environment,^{11,58} the cross- β model structure readily adopts a steady left-handed twist.^{45,58,76} Our simulations show that this is true regardless of the charge states of the peptide's termini (Figure 2). The termini's charge configuration, however, defines the extent of the twist of the structure. While the presence of positive charges on the N-terminus does not affect the twist of the structure, the presence of negative charges on the C-terminus increases the twist angle by $\sim 20\%$ as compared to the both-neutral (0/0) and charged N-termini (+/0) structures (from -8 to -10°). Most informative, these effects of the N- and C-terminus charges are independent of each other. Because

strands are parallel within a sheet and the sheets are antiparallel to each other, these charge effects are dominated by repulsive intrasheet electrostatic interactions as opposed to attractive intersheet interactions. As noted in Methods, the experimental ssNMR studies of the microcrystal structures^{11,54–56} indicate that the N-termini are protonated and positively charged, whereas the C-termini are negatively charged (likely reflecting a shift in the C-terminal pK_a in the microcrystalline environment due to the proximity of the oppositely charged N-terminus in comparison to a situation in solution).

Polymeric Cross- β Structures. The possible effects of interactions between cross- β structures on the structure of the monomer were addressed by studying systems in which dimers of cross- β structures interact in two distinct manners (Figure 3). In one case, the cross- β structures interact through their termini (termini-dimer), and in the other through the so-called “wet” interface (wet-dimer), defined as the cross- β structures interacting through their face exposed to the solvent when isolated.¹¹ A tetramer was also built (Figure 4). In that case, both termini-dimer and wet-dimer interfaces are present in the structure, partially recovering the supramolecular interactions present in the crystal lattice. Starting from these interacting straight cross- β structures, placed at distances as found in the microcrystalline environment,¹¹ the systems were relaxed until they reached a stable and long-lived conformation. The final structures of the polymeric assemblies differ systematically, as shown in Figures 3 and 4.

In the case of the termini-dimers, three configurations of the termini charges were probed: both charged (+/–), both neutral (0/0), and with the N-terminus of the peptide (only) charged (+/0). The three configurations relaxed to a different conformation of the cross- β monomers and of their assembly (Figure 3). Most notably, in the +/– configuration, the cross- β structures maintained a straight conformation (twist angle = 0). The formation of a twist in the two monomers (each a cross- β pair of β -sheets) would impair the energetically favorable charge–charge interactions between the termini of the two protofilaments. Although it cannot be completely excluded that the system is kinetically trapped in a local minimum, these interactions are strong enough to counterbalance the intrinsic propensity of the monomers to twist (see above). This system was simulated 6 times longer than the time needed by the other systems to reach a stable twist. These charge–charge interactions have been reported previously and were suggested to stabilize the straight conformation observed in the crystalline environment.⁵⁸

In the 0/0 configuration of the termini-dimer, the water molecules initially found at the termini interface were rapidly expelled, leading to more intricate contacts between the termini of the two protofilaments compared to the +/– configuration (Figure 3A). The interacting protofilaments adopted a -5° twist angle, indicating that the contacts between neutral termini restrain the twisting compared to the similarly charged isolated monomer that adopted a -8° twist angle (Figure 2). In the case +/0, the monomers in the complex adopt the same left-handed twist as found in the isolated filament. In all cases, the two interacting cross- β structures adopted similar conformations (see Figure 3A).

The wet-dimer relaxed into three symmetrical but distinct and stable conformations. The three conformations appeared despite the use of identical starting conformations and termini configuration (+/–). In one case, the cross- β structures adopt a fully left-handed twist; in a second one, a fully right-handed

Figure 3. Twist polymorphism in dimers of cross- β structures. Equilibrated conformations of cross- β structure dimers interacting through their termini (A, termini-dimer) or through their wet interface (B, wet-dimer) are shown. The wet interface is the surface of the sheets facing the solvent in the monomer of the cross- β structure.¹¹ Structures were obtained from at least 15 ns MD simulations free in solution. All simulations were started from the straight conformation of the crystal structure (Figure 2A), and intermonomer distances were taken from the crystal parameters.¹¹ Three different charge configurations of the termini are shown for the termini-dimers (A), and three different conformations are obtained for the wet-dimers under the same termini conditions (B). In each case, a side view (top panel) and a top view (middle panel) are shown together with the normalized distributions of twist angles (bottom panel). The color code of the normalized distributions is indicated by the gray areas. Colored spheres are placed on the C-termini to guide the eye in following the twist of the cross- β structures.

twist; and in a third, both right- and left-handed sections are present and divide the dimer into two segments of equal size (Figure 3B). At this point, it is not clear precisely which energetic factors guided the system to its final conformation, but we observed a strong sensitivity of the conformation to the care given to the preparation of the interface and in particular to the water. The fully left-handed conformation, more likely candidate in view of the monomeric and termini-dimer simulations, is obtained only if the interfacial water molecules that solvate the wet interface of the experimental structure are included in the starting structure. Half and full right-handed sections were obtained when the wet interface lacked the experimental water molecules. Although no further attempt was made to elucidate these observations, they underline the sensitivity of the structure to the protofilament–protofilament interactions and the presence of solvent.

In the case of the tetramer with interfacial water included, the straight conformation (as found in a microcrystal¹¹) relaxed into a stable structure, in which each monomer adopted a small, but nonzero, left-handed twist angle of -2.5° (Figure 4). This value of the twist angle compared to the ones described above indicates that there is a compensation of effect due to the presence of both termini and wet interfaces in the structure. Although the termini interface stabilizes the straight conformation, the wet interface favors the formation of a small twist.

The larger twist observed in the monomeric structure (-10° , Figure 2) compared to the wet dimer (-8° , Figure 3B) is in line with the changes reported by Berryman et al.⁴⁵ and Knowles et al.,⁵³ and predicted by Aggeli et al.⁹³ for systems with increasing numbers of interacting protofilaments. The termini-dimer (0° , Figure 3A) compared to the tetramer (-2.5° , Figure 4) does not follow the trend. The absence of twist in the termini-dimer suggests that interactions among protofilaments mimic an increase of the peptide length through the termini interaction, thereby favoring a straight conformation.³⁸ In contrast, the side chain interactions at the wet interface of the tetramer push the system to a slightly twisted conformation.

Thermodynamics of Twisting a Cross- β Structure. Cross- β Structure Free-Energy Minimum Is Left-Handed. The PMF of the isolated cross- β structure with neutralized termini as a function of its twist angle (Figure 5) undeniably shows that the free-energy minimum of the solvated cross- β spine has a left-handed twist angle of about 8° . This is in agreement with previous simulation results from us⁵⁸ and others groups^{45,46,53,76} and contrasts with the straight conformation observed in a microcrystal environment.^{11,54} The free-energy profile has a v shape with a higher slope (2 kJ/mol/peptide per degree) toward larger left-handed twist values than when reducing the twist and going to a right-handed twist structure (1 kJ/mol/peptide per degree). The profile indicates the presence of a metastable state for right-handed twist in the

Figure 4. Morphology of a tetramer of cross- β structures. (A) Cartoon representation of the resulting conformation of the tetramer after a 30 ns MD simulation free in solution and started from the straight conformation found in the crystal.¹¹ Both termini were charged (+/−). To guide the eye of the reader, red spheres are placed on the C-termini and smaller blue spheres on the N-termini. Each cross- β unit has a different color. (B) Normalized distribution of the twist angle of each monomer obtained with the last 20 ns of the simulation. The color code is in accordance with panel (A).

Figure 5. Thermodynamics of twisting a cross- β structure. Relative free-energy ($\Delta G = \text{PMF}$), enthalpy ($\Delta H = \Delta U$), and entropy ($-T\Delta S = \Delta G - \Delta H$) of the cross- β structure in solution as a function of its twist angle. The twist angle is defined by the average of the 38 individual dihedrals (Figure 2A). The gray areas indicate the limit of the cross- β structural integrity. For convenience, the energies are expressed per peptide.

range of 5–9°. In the following, we focus our interpretation on the values of the twist angle of the cross- β structure within the range −13 to +12°. Beyond these limits, the cross- β structure showed clear signs of irregularity and loses of integrity (see Movie in SI).

Cross- β Structure Twist Is Enthalpy-Driven. The enthalpy of the system (ΔH) was determined from the total potential energy of the system (ΔU) (Figure 5). The strong similarity of ΔH to ΔG around the ΔG minimum demonstrates that the twist of the cross- β structure is enthalpy-driven. The v shape of

ΔH , however, breaks down as the twist angle of the cross- β structure exceeds +3°, at which point, ΔH decreases again until the twist angle reaches +12°, where ΔH starts increasing again. This change occurs close to the plateau in the free-energy profile and suggests that the metastable state is also of enthalpic origin, i.e., both left- and right-handed structures are at a minimum of ΔH .

The entropy contribution ($-T\Delta S$) to the system's free-energy (ΔG) was estimated from the difference between ΔG and the enthalpy of the system (ΔH) (Figure 5). It shows that $-T\Delta S$ has only a limited contribution to structures with twist angles more negative than −3°. In contrast, it contributes significantly to stabilizing the cross- β structure in the range of twist angles between −3 and +7/8°. The gain in entropy compensates for the increase of enthalpy with a maximum contribution at +3°, where the enthalpy of the system is also at its maximum.

Contributions to the Enthalpy of the System. The terms in the system's enthalpy relevant to the twist of the cross- β structure were identified. Bonded terms might be discarded because they do not contribute significantly to the system's enthalpy in the range of twist angle (−13 to +12°) relevant to our analysis. It is only for large values of the twist angle (<−10 and >11°) that they become relevant and then mainly reveal that the peptide is under stress under these extreme twist angles (Figure S2).

Protein–Protein Nonbonded Interactions Shape the Systems' Enthalpy. The nonbonded interactions (sum of electrostatic and van der Waals terms) clearly shape the enthalpy of the system upon moderate twisting (Figure 6), with the protein–protein interactions being the major contribution for twist angles between −10 and +10°. They stabilize both left- and right-handed twist conformations. Outside that region, large contributions from protein–protein, protein–solvent, and solvent–solvent interactions compensate each other. Although they are difficult to interpret in detail, the increasing role of solvent interactions reflects the loss of integrity of the structure.

Peptide Backbone and Side Chain Contributions. A further decomposition of the protein–protein nonbonded interactions reveals that backbone (BB)/BB nonbonded interactions have a v shape and stabilize an almost straight conformation of the cross- β structure. In contrast, BB/side chain (SC) interactions favor twisted forms (Figure 7). Importantly, SC/SC interactions stabilize both left- (−4°) and right-handed (+8°) twists of the cross- β structure with a preference for the left-handed form, thereby shaping up the double well of the enthalpy profile as defined by the sum of BB and SC contributions. The termini contribution becomes significant only for large twist angle and tends to stabilize higher twisted forms.

Inter- and Intrasheet Interactions. The determination of the intra- and intersheet contributions of BB and SC interactions indicates that intrasheet terms govern the potential energy of the system as indicated by the presence of both left- and right-handed twist minima (Figure 7). The intersheet interactions in the isolated cross- β structure tend to stabilize larger twists and left-handed ones in the range of stable protofilament. The double well shape of intrasheet interactions reflects SC/SC interactions at the dry interface (Figure S3).

Structural Changes of the Cross- β Structure Accompanying the Twist. Although significant changes of the morphology of the cross- β structure are observed in the range of twist angles studied (Figures 2 and 3), the peptide itself undergoes only very little conformational change. Thus, we

Figure 6. Decomposition of the protein and solvent nonbonded contributions to the enthalpy of the cross- β filament system shown in Figure 5A, ΔH (red in Figure 5). ΔH = protein–protein (A) + protein–solvent (B) + solvent–solvent (C) + bonded terms. Bonded terms are shown in Figure S2. For convenience, the energies are expressed per peptide.

observe a preservation of the basic conformation of the peptide as found in the microcrystalline environment^{11,54} within the range of twist for which the cross- β structure is stable. The φ and ψ angles of the peptide backbone show monotonous evolution upon twisting of the protofilament (Figure S4), resulting in relatively small changes of each residue. These changes, however, add up to an overall backbone twist of 50° (from +20 to -30°) over the length of the peptide in the range of twist angle, keeping the integrity of the cross- β structure (Figure S5A). The cross-angle between the two sheets in the cross- β structure shows that the two sheets stay parallel for twist angle of the structure around a straight conformation (Figure S5B). It is only when the twist angle exceeds 5° on both right- and left-handed directions that the sheets twist one relative to the other. The solvent accessible surface area (SASA, Figure S5C) of the cross- β structure decreases slightly with its twist, in line with an analysis of protein structures, showing that the twist of β -sheets in proteins has a tendency to minimize SASA.⁷⁷

Figure 7. Decomposition of the backbone (BB), side chain (SC), and termini contributions to the protein–protein nonbonded interactions shown in Figure 6. Protein–protein = all = termini + BB and SC. BB and SC = BB/BB + BB/SC + SC/SC = intersheet + intrasheet. For convenience, the energies are expressed per peptide.

DISCUSSION

The pathophysiological hallmark of AD is the appearance of AD plaques in brain tissue. The plaques consist of A β 42 (and other related peptides, including A β 40) amyloid fibrils. Biophysical investigations of A β -derived amyloids often indicate the presence of several polymorphs, whose internal structure (β -sheet formation and side chain packing),⁴⁰ fibril diameter and shape (e.g., ribbonlike or ropelike), branching, and amount and frequency of twisting events^{9,78} contribute to fibril polymorphism. Importantly, a close correlation between structural polymorphisms and disease phenotype has been suggested.^{33,79}

Twist polymorphism can be demonstrated in filaments and fibrils prepared in vitro under the same conditions. Here, we confirm and extend the characterization of the twisting structure of A β 42 fibrils by showing that the degree of fibril

twist may vary even within a single fibril. Fibrils with the same morphology experience varying pitches falling into a quasi-normal distribution. Although these data are qualitatively valuable and provide insight into the protein morphology involved in pathology, our aim was to understand the energetics that underlies fibril-twisting polymorphism. Many studies have modeled A β 42 using all-atom MD simulations;^{80–86} however, for the purpose of investigating the twist of fibrils, we choose a more simple model system. The yeast prion Sup35 is amenable for our study because the relevant model heptapeptide GNNQQNY forms canonical filaments and fibrils in vitro and is small enough for comprehensive MD studies. Furthermore, relevant structural data are available to establish initial conditions for MD simulations (Figures 2–4), and extensive experimental studies have been performed on crystal and fibril assemblies.

The free-energy profile of twisting the cross- β structure of GNNQQNY (a pair of β -sheets) confirmed that its free-energy minimum in solution is a left-handed twist (-8°) conformation^{45,46,53,58,76} and showed that it is enthalpy-driven (Figure 5). The decomposition of the potential energy of the system further revealed that protein–protein interactions shape up the enthalpy, that the polypeptide backbone favors the straight conformation, and that side chains are determinant in stabilizing the twist of the cross- β structure (Figures 5–7). These observations are in line with the consensus views of β -sheet structures.^{14,36,38,87–89} For the A β , it was suggested that the twist improved side chain packing between neighboring β -sheets.⁸⁰

The PMF also revealed that protofilaments with a -13 to $+10^\circ$ twist angle are accessible within 2–4 kT/peptide (Figure 5), making the protofilament quite flexible. Interestingly, the straight conformation found in the microcrystalline environment^{11,54} is not more than 2 kT/peptide away from the twisted free-energy minimum (Figure 5). Our simulations show that the necessary stabilization energy may be provided by the interaction between two protofilaments through their termini (Figure 3A) as found in the microcrystalline environment.^{11,54}

Although the GNNQQNY protofilament is quite flexible when considered per peptide, collectively, it sits in a deep free-energy minimum and is therefore a very stable structure. The systems started from a straight conformation indeed equilibrate very fast (<100 ps) to their respective final form.⁵⁸ Our ability to twist a protofilament in contrast to breaking it relied on the application of forces homogeneously along the structure, on each peptide. In the determination of the PMF presented in Figure 5, we applied a restraint per peptide pair to mimic what we assume occurs naturally when protofilaments interact. In experiments, forces may not be homogeneously distributed, resulting in local accumulation of strain, leading to defects, breakage, and cracks as often seen in AFM and EM images. It may also occur that a single fibril feels different strains (of any type) along its length, thereby twisting to different extents within its own length without affecting the overall morphology of the fibril (Figure 1).

In terms of the typically multimeric protofilament structures, our simulations show that the systems composed of several copies of a cross- β structure all adopted a different twist angle from that observed for the isolated cross- β structure. This observation strongly supports the notion that the mode of interaction between the cross- β structures is determinant for the final conformation of a multimeric system (Figures 2–4). Protofilaments will easily adapt to external forces, such as the

ones resulting from interactions with other protofilaments in fibrils as well as changes of termini charges, a mimic of pH change. The idea that a conserved cross- β filament structure can self-assemble in different ways (e.g., with different extents of twisting) is reminiscent of experimentally observed supramolecular polymorphism. In this case, amyloidogenic proteins, such as tau or huntingtin exon 1, form different fiber polymorphs, which upon closer inspection appear to be based on a different way of assembling essentially the same cross- β filament structure.^{47–50}

It is, however, not clear at this point if and how the changes in the twist of the cross- β structure reported here may account for the reported polymorphic aggregation by GNNQQNY in an experimental setting. This one peptide forms both monoclinic and orthorhombic crystals, in which the cross- β spines are straight.^{11,54} At higher concentrations, but under otherwise identical conditions, amyloid-like fibrils form, which are characteristically assembled from three distinct but reproducible peptide conformers.^{55,56} The GNNQQNY fibrils are ribbonlike in appearance, with striations thought to reflect cross- β filaments. Presumably, it is twisting of the filaments that prevents their growth into a three-dimensional crystalline state. Although we show the possibility of variable twists in a single long fibril in the case of A β 42 (Figure 1), our computational experiments suggest that the conformation of the peptide is conserved along a protofilament, at least within the range of protofilament twists explored.

To perform our simulations of GNNQQNY peptides, we have built protofilament models starting from the straight arrangement as found in the untwisted microcrystal structure and interpreted the degree of twist they acquired due to the combination of their intrinsic propensity to twist and external factors (mimic of pH change and interaction with other protofilaments). The effects are clear, namely, the interactions between protofilaments determine the twist observed in individual protofilaments, without requiring a significant change in the local monomer structures within the fibrils. Thus, once formed, a particular filament structure appears to be quite robust even when submitted to varying external forces or interactions.

This raises an interesting question that relates to the factors that dictate the fundamental structure of the filaments: the nucleation of amyloid structure. The structure of the initial nucleus encodes a certain degree of spontaneous twist that will be reflected in the final assembly. External conditions that favor the formation of twisted nucleating structures would trigger the formation of long twisted fibrils with a couple of interacting protofilaments. Conditions favoring the formation of untwisted β -sheets (e.g., termini-dimer in Figure 3) would allow the three-dimensional stacking of sheets into large straight assemblies as observed in microcrystals. In other words, the nucleation of twisted protofilaments would prevent the formation of microcrystals by disfavoring protofilament interactions. Both paths are likely to be close in energy, enabling them to occur simultaneously or to compete under specific growth conditions.^{57,90}

In prior ssNMR studies,^{55,56} GNNQQNY fibrils contain three different conformers that are closely interacting (<1 nm of each other) within a coassembled and complex fibril structure that is distinct from that in the microcrystalline state.^{11,54,58,76} Each protofilament featured an internal polymorphism that is distinct from the type of supramolecular polymorphism examined here, but was for instance also

reported for the SNNFGAILSS fragment from hIAPP.¹³ The threefold ssNMR signature of the GNNQQNY filaments was found to be reproduced faithfully even when different types of fibril polymorphs were seen by EM.⁹⁰ This is consistent with our current finding that an identical filament base structure is flexible enough to partake in different types of supramolecular architectures, potentially enabled by variations in the protofilament twists, without sufficient local structural change to see an observable effect by ssNMR. This is reminiscent of a recent study of differently curved HIV capsid assemblies, which examined by ssNMR the local structural changes associated with the obvious difference in capsid curvature.⁹¹ Thus, supramolecular structure and local monomer conformations are clearly related, but their interplay appears to be subject to various subtleties that may not be immediately apparent. It appears to be a combination of both the nucleating structure and the interfibril interactions that are needed to fully explain the final fibril architecture, the protofilament twists, fibril stability, and its functional characteristics.

CONCLUSIONS

We have demonstrated the twist flexibility of a model of a cross- β structure of the GNNQQNY peptide from the yeast prion Sup35 and its adaptation to the external forces resulting from the environment (pH or quaternary interactions). This feature of cross- β filaments should help characterize further the mechanical properties of amyloid-like fibrils and stimulate new experiments to estimate the extent of its contribution to different systems. Notably, the degree of twist of a mature fibril must affect its mechanical properties and could be considered as a tuning feature in the development of functionalized biomaterials,⁹² assuming one could develop a strategy to control the fibril twist. Given the apparent role of the side chains in dictating the twisting, our data would point to compounds interacting with the side chains or termini of amyloidogenic peptides to be a good starting point. It would also be important to determine the impact of fibrils' twist in their pathology,³³ in which case, the development of drugs affecting the twist could be pharmacologically relevant.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.7b10233.

Formalism used in the derivation of the weighted histogram analysis method equations adapted to the problem described in the manuscript and additional figures (PDF)

Cross- β structure twisting (AVI)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by The Netherlands Organisation for Scientific Research (NWO) (X.P. and S.J.M., ECHO.08.BM.041). P.C.A.v.d.W. acknowledges grant support from the National Institutes of Health (R01 GM112678). Computer time was allocated by The Netherlands National Computing Facilities Foundation (NCF grant SH-113-V-08). The authors acknowledge the Electron Microscopy Resource Facility at Rockefeller University.

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