Twisting Transition between Crystalline and Fibrillar Phases of Aggregated Peptides

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We study two distinctly ordered condensed phases of polypeptide molecules, amyloid fibrils and amyloidlike microcrystals, and the first-order twisting phase transition between these two states. We derive a single free-energy form which connects both phases. Our model identifies relevant degrees of freedom for describing the collective behavior of supramolecular polypeptide structures, reproduces accurately the results from molecular dynamics simulations as well as from experiments, and sheds light on the uniform nature of the dimensions of different peptide fibrils.

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A wide range of chiral biomolecules are found in nature as helical fibrillar assemblies, examples of which include nucleic acids such as DNA and proteinaceous structures such as actin [1], collagen, tubulin, and sickle hemoglobin [2,3]. Many peptide systems can also exist in a condensed form in translationally invariant crystalline phases which have no identifiable phase chirality. In this Letter, we offer a universal phenomenological description of these phases, and the transition between them, by deriving a continuum mechanical model for supramolecular assemblies of chiral elements. This model is essentially parameter free, since we are able to relate all its constants to existing established experimental measurements. We show that a metastability with respect to the untwisting of the helical state into the crystalline one occurs when the filaments reach a critical width. We apply this model to analyze a class of β-sheet rich ordered filamentous and crystalline phases formed through the self-assembly of peptides and proteins, commonly known as amyloid or amyloidlike fibrils [4] and microcrystals [5,6] (Fig. 1), which have become the focus of extensive experimental and theoretical investigation due to their connection with aberrant biological phenomena, e.g., Alzheimer's disease [7].

We first construct the phenomenological free energy of an assembly of stacked polypeptides as a function of the twist angle φ between neighboring strands. Consider very generally a free energy $F(\varphi)$ per peptide strand given as a series expansion with respect to this twist angle φ, limiting ourselves here to the fourth order: $F(\varphi) = F_0 + a\varphi^2 - b\varphi^3 + c\varphi^4$. The additional constant term F_0, describing nontwisting related interactions such as surface effects, is discussed below. This free energy differs fundamentally from that proposed previously for cholesteric liquid crystalline phases [8,9] through the absence of linear terms in φ. For the latter systems, the elements and the chiral interactions between them are assumed to be rigid. By contrast, the system that we consider here possesses internal degrees of freedom and, for instance, the dihedral angles in the polypeptide chains adopt a different value in the microcrystalline state relative to that in the fibrillar state [5,10]. As a result of this, the shape of the polymer molecule is flexible and adjusts to the environment. The free energy $F(\varphi)$ represents the minimization over all other internal degrees of freedom of such a chain. In particular, this flexibility results in the disappearance of the linear term since in the crystalline state the dihedral angles can adjust to accommodate the absence of interstrand twist, as shown by experiment [5] and the full atom molecular dynamics simulations in Fig. 2 and in Ref. [10]; a significant linear

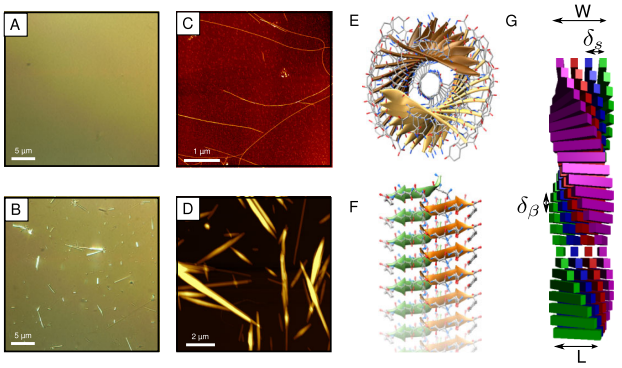

FIG. 1 (color online). (a),(c) Fibrillar and (b),(d) crystalline phases of peptide P [19]. (a) In the fibrillar phase, no structures are visible by optical microscopy, but (c) atomic force microscopy shows uniform protofilaments. (e) The atomic level structure [10] of the protofilaments shows the significant level of interstrand twist. Panel (b) shows an optical and (d) an atomic force microscopy image of the crystalline phase and the atomic level untwisted structure from (f) [6]. Panel (g) shows the coarse-grained model and the definitions of the geometric parameters used in this Letter.

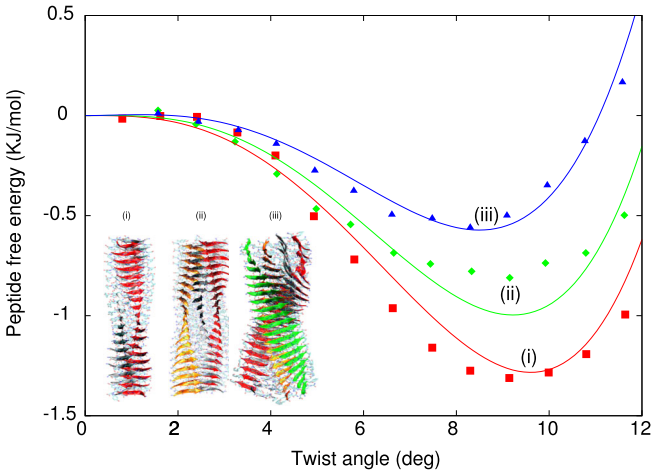

FIG. 2 (color online). The twist-dependent free energy $F(\varphi)$ was computed from molecular dynamics simulations for different numbers of β sheets with corresponding equilibrium atomic coordinates shown in the inset: (i) two, (ii) three, and (iii) four sheets. The lines are a three-parameter global fit of the entire data set to Eq. (1) with $a_T^{\text{bulk}} = 4.1 \times 10^{-2}$ N/m, $k_T^{\text{strand}} = 3.3 \times 10^{-18}$ N $\times$ m, and $\varphi_0 = 9.6°$.

term would manifest itself as a nonvanishing slope of the free energy at the vanishing twist angle, which is not observed in Fig. 2. The building blocks are, however, chiral, a factor which manifests itself in the sign-sensitive cubic term in φ.

This free energy will have extrema $\varphi_{1,2,3}$ for solutions of $dF/d\varphi = 0$ with $\varphi_{1,2} = (3b/8c)[1 \pm \sqrt{1 - 32ac/9b^2}]$ and $\varphi_3 = 0$. Similarly to other first-order phase transitions, the state φ_3 corresponds to the crystal state, whereas φ_1 is the energy minimum for a fibril and φ_2 is the position of the energy barrier separating these two states.

The parameters in the energy function can be defined through the elastic response in the different phases. When the width of the stack of peptides, W, is large, $W \to \infty$, the system is known from experiments to be in a state which has an equilibrium twist angle of 0 and its bending rigidity is driven by the shear between sheets and stretching of the sheets as in a continuous 3D lattice. Hence, in this limit, the mechanical properties are described by a macroscopic shear modulus of a 3D solid. By contrast, at small or vanishing width, the shear between the sheets becomes negligible since the intersheet strain decreases with aggregate width for a constant twist angle, and the mechanical properties are driven by the twisting energy of the individual strands. The first observation requires that only the quadratic term in $F(\varphi)$ survives when $W \to \infty$; furthermore, the W dependence of this term has to be W^2 in this limit due to the geometry of the system (see Supplemental Material [11]). The second observation shows that the φ^2 term has to become small at small widths since otherwise the crystal state would prevail for all widths, contrary to what is observed in experiment (Fig. 1); the W^2 behavior derived at large width satisfies this condition and yields excellent agreement with the molecular dynamics simulations (Fig. 2), and therefore is used for simplicity through the entire width range. The cubic and quartic terms in $F(\varphi)$ may also have a W dependence, but the arguments above show that it is the width dependence of the quadratic term that drives the transition. This situation has analogies to the Landau model of the ferromagnetic transition which is driven by the temperature dependence of the term quadratic in the magnetization.

The curvature of the energy $d^2F/d\varphi^2|_{\varphi=0} = 2a$ in the crystal state is equal to the bulk torsional spring constant $k_T^{\text{bulk}}(W)$. The polar moment of inertia $J \sim W^3$ scales with the cube of the crystal width W for large widths [cf. Fig. 1(e) and the Supplemental Material], and therefore the torsional spring constant scales as $k_T^{\text{bulk}} = a_T^{\text{bulk}} W^2$; a more detailed estimate (see Supplemental Material) yields $k_T^{\text{bulk}} = \delta_s L W^2 G/3\delta_\beta$, the elastic constant as a function of the shear modulus $G \approx 0.3$ GPa [12], and the structural parameters for amyloid related peptide phases determined from x-ray diffraction studies [5,6]: interstrand spacing $\delta_\beta = 0.48$ nm, intersheet spacing $\delta_s = 1$ nm, and interresidue spacing $\delta_r = 0.35$ nm. This ansatz derived at large widths also satisfies the requirement $k_T \sim W^2 \to 0$ for $W \to 0$ dictated by the disappearance of the crystalline state at $W \to 0$; the interstrand twist tends to $\varphi_0 = 3b/4c$, the intrinsic twist which best accommodates the individual strands which have a defined chirality. Then, the curvature of $F(\varphi)$ around $\varphi = \varphi_0$ is given by the interstrand spring constant of a single sheet $d^2F/d\varphi^2|_{\varphi=\varphi_0} = 9b^2/4c = k_T^{\text{strand}}$ which is not a function of W and can be estimated (see Supplemental Material) as $k_T^{\text{strand}} = \kappa_H L^3/(12\delta_r)$, with the hydrogen bond spring constant [13] $\kappa_H \approx 12.5$ N/m.

Thus, within the mean field description developed in this Letter, the parameters a, b, and c in the Landau free-energy expansion are defined uniquely in terms of the mechanical and structural characteristics of the assembly through φ_0, k_T^{strand}, and $k_T^{\text{bulk}}(W)$. Therefore, we can write the free energy in closed form as

$$F = \epsilon \frac{L}{\delta_r} \frac{\delta_s}{W + \delta_s} + \frac{k_T^{\text{bulk}}(W)}{2} \varphi^2 - \frac{k_T^{\text{strand}}}{3\varphi_0} \varphi^3 + \frac{k_T^{\text{strand}}}{4\varphi_0^2} \varphi^4, \tag{1}$$

where $\epsilon = \epsilon_s - \epsilon_v > 0$ is the (unfavorable) free energy per residue associated with an unsatisfied intersheet contact in the first and last sheets of the structure resulting from the loss of a favorable contact ϵ_v and from solvent exposure ϵ_s. The ratio $(W + \delta_s)/\delta_s$ gives the number of sheets in the structure and L/δ_r the number of residues in a single chain where L is the length of a single strand. The equilibrium twist angle can be expressed as a function of the elastic constants as

$$\varphi_1 = \frac{\varphi_0}{2}\left(1 + \sqrt{1 - \frac{4k_T^{\text{bulk}}(W)}{k_T^{\text{strand}}}}\right). \quad (2)$$

Fibril stability is dependent on the root being real in Eq. (2), yielding the critical fibril width W_C for the fibril-crystal transition: $k_T^{\text{bulk}}(W_C) = k_T^{\text{strand}}/4$ or

$$W_C = \frac{1}{4}\sqrt{\frac{\delta_\beta \kappa_H L^2}{G\delta_s\delta_r}}. \quad (3)$$

We now demonstrate that this closed form polynomial energy function $F(\varphi)$ describes accurately the behavior of polypeptide assemblies with thousands of atoms. Molecular dynamics simulations (see Supplemental Material) were performed in order to evaluate the free energy of the system over a range of interstrand twist angles and numbers of sheets. The data are shown in Fig. 2 and reveal that a global fit with fixed values of the elastic constants describes the entire data set. In particular, a striking feature is that, even under conditions where the fibrillar state dominates, $dF(\varphi)/d\varphi|_{\varphi=0} = 0$ and the metastable crystal state is still present in the energy landscape of the system, as predicted by the theory. Remarkably, we can predict both the interstrand k_T^{strand} and the bulk $a_T^{\text{bulk}}W^2$ torsional spring constants to within an order of magnitude from the basic knowledge of system geometry and bond constants alone; their ratio is captured almost exactly ($a_T^{\text{bulk}}/k_T^{\text{strand}} = 1.1 \times 10^{16}$ m^2 vs 1.2×10^{16} m^2 from the fit).

The expression for the elastic energy proposed here has a clear connection with the Landau theory of phase transitions [14], the square of the width of the crystal W^2 playing the role of the control parameter, analogous to the temperature $(T - T_C)$ in classical phase transitions. The phase with the vanishing order parameter (untwisted crystal) occurs at high widths $W > W_C$. However, since in our case the quadratic coefficient $a \sim W^2$ is always positive, we are in a special case where the phase with zero order parameter retains its metastability over the whole width range. The fibril state becomes metastable at a width $W^* < W_C$; indeed, it does so as soon as $F_{W=W^*}(\varphi_1) = F(\varphi_3) = 0$. To evaluate W^*, we solve $F_{W=W^*}(\varphi_1) = 0$, yielding $k_T^{\text{bulk}}(W^*) = (2/9)k_T^{\text{strand}} = (8/9)k_T^{\text{bulk}}(W_C)$ and hence $W^* = \sqrt{8/9}W_C$. There is, therefore, a region of coexistence for $W^* \le W \le W_C$ where the fibrillar minimum is present but is no longer the global minimum of the energy function, indicating the onset of metastability for the fibrillar state. Figure 3(a) presents a summary of model predictions for the fibrillar twist that involves no free parameters, the values of the material constants being taken from the literature.

There have been many important reports in the literature on the mechanical properties, instabilities, and phase transitions of different types of helices and ribbons [3,15–17]. The model presented in this Letter has a clear connection with earlier investigations of the equilibrium twist of composite filaments. In these models, the intrinsic propensity of polypeptide molecules to twist was incorporated into the free energy in the form of an external field which suppresses transitions for finite widths; by contrast, in the present work the energy function is derived as an intrinsic feature of assembled peptides. Both approaches have in common that, in the range where filaments prevail, $W < W_C$ in the present Letter and $W < \infty$ in [3], the interstrand twist decreases with increasing fibril width, an effect driven by the minimization of elastic strain in both cases.

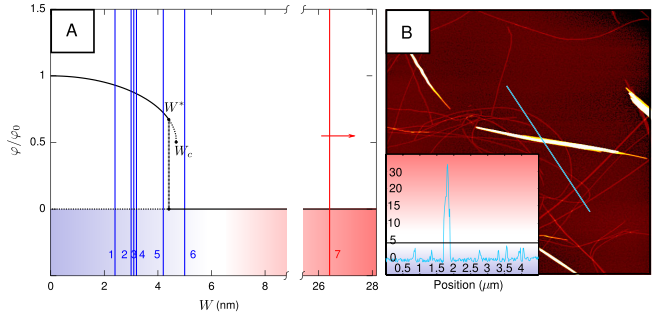

FIG. 3 (color online). (a) The equilibrium twist is shown as a function of filament width. The filamentous state becomes metastable at $W = W^*$. For comparison, experimentally determined dimensions of amyloid protofilaments are shown by vertical lines: (1) PI-SH3 domain [20]; (2) lysozyme (D67H) [21]; (3) insulin [22,23]; (4) apoA1 (L60R) [21]; (5) peptide P [19], present work and Ref. [22]; and (6) TTR (V30M) [21]. The experimentally determined widths of microcrystals [Fig. 1(d)] were in all cases >10 nm. Panel (b) shows an atomic force microscopy height map of a sample containing a mixture of fibrils and microcrystals, and the height profile through the cross section that is highlighted with a line is shown in the inset. The height of the microcrystal in (b) is reported as (7) in (a).

Let us see how our theory links with experimental findings on filamentous and crystalline states of polypeptides. It has recently become apparent that many polypeptide chains possess a propensity towards self-assembly into generic beta-sheet rich amyloid fibrils or amyloidlike microcrystals. Structural [5,6,18] and computational [10] studies confirm that the molecular structure within these two phases, examples of which are shown in Fig. 1, is similar, but that the phases differ in the level of interstrand twist; the three-dimensional translational symmetry within crystals does not allow for twist, whereas in the filamentous state the interstrand twist varies between approximately 10° for short peptides and 1° for longer sequences. Below the width W^*, the energetically most favorable packing of peptides is in fibrillar form, but above this width the elastic energy from twisting penalizes fibrillar structures over crystalline phases. We therefore expect the amyloid protofilaments to prevail only up to a width of $W^* \approx 4.3$ nm. Comparison with experimental data in Fig. 3(a) reveals good agreement with this theoretical limit, providing a physical picture for the origin of the generic width of amyloid protofilaments which emerges as the

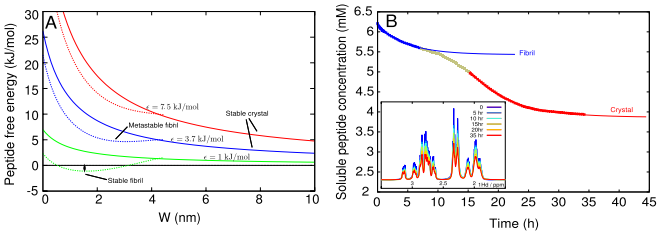

FIG. 4 (color online). (a) The total peptide free energy computed from Eq. (4) as a function of structure width for $\epsilon = 1$, 3.7, and 7.5 kJ/mol using the values of a_T^{bulk}, k_T^{strand}, and φ_0 determined in Fig. 2. Dashed lines represent the fibrillar state, and solid lines represent the microcrystalline state. (b) Experimental observation of the twisting transition with NMR through the measurement of the biphasic incorporation of soluble peptide into supramolecular structures measured from the decrease in intensity of the 1D H spectra acquired between 0 and 35 hours (inset).

result of a competition between twisting propensity and the elastic strain that opposes it. Filaments with a larger width can result from the supercoiling of two or more individual protofilaments.

We now examine under what conditions the twisting transition can be observed. The interstrand twist φ adjusts to a given width, and the minimal energy in the fibrillar phase can be computed from Eq. (1) as a function of filament width W to yield $F_{\text{min}}(W) = F_{\text{twist}}(W) + \epsilon(L/\delta_r)\delta_s/(W + \delta_w)$ with

$$\frac{F_{\text{twist}}(W)}{\varphi_0^2} = \frac{a_T^{\text{bulk}} W^2}{4}\left(1 - \frac{\xi^2}{4}\right) - \frac{k_T^{\text{strand}}}{24}[1 + (1 - \xi^2)^{3/2}], \tag{4}$$

where the dimensionless ratio $\xi^2 = 4a_T^{\text{bulk}} W^2/k_T^{\text{strand}}$ is small everywhere for twisted filaments. The term $\epsilon L\delta_s/[\delta_r(W + \delta_s)]$ gradually promotes an increase in W through the minimization of the interface-to-volume ratio with a consequent maximization of the favorable interpeptide interactions. If this driving force is strong enough for the system to reach the critical width W_C, the only stable state becomes the crystalline phase with $\varphi = 0$. We illustrate this idea in Fig. 4 for different values of the interaction parameter ϵ. If this interaction is smaller than the energy associated with the twisting transition ($\epsilon = 1$ kJ/mol, Fig. 4), the fibrillar state represents the global energy minimum of the system, and untwisting is not observed. In other words, the gain from growing the filament width (and eventually forming a microcrystal) is too small to outweigh the twisting power of individual strands, so fibrils are the only outcome. For intermediate values of ϵ (3.7 kJ/mol), there is a metastable energy minimum for a finite value of W and the fibrillar phase is transiently populated before the system falls into the crystalline ground state. In such a case, the metastability of the fibrillar state of the peptide can be monitored directly, as shown in Fig. 4(b). To this effect, we have carried out experiments to measure the concentration of free peptide remaining in solution as a function of time after the start of the self-assembly process. In the first instance, the fibrillar state is populated, and, in a second step, the conversion into microcrystals occurs; the equilibrium between the peptide in solution and in microcrystals is shifted further away from the soluble state than in the corresponding monomer or fibril equilibrium, an indication of the greater thermodynamic stability of the microcrystalline state. Finally, for values of ϵ much larger than the twist energy, the fibrillar phase does not exist as a well defined energy minimum, and the system proceeds straight to the crystalline state. An extreme case of this behavior is given by crystals of nonchiral monomers such as inorganic ions where the intrinsic twisting energy $\sim k_T^{\text{strand}}$ is 0 and no fibrillar phases are formed.

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