Role of Conformational Entropy in Force-Induced Biopolymer Unfolding

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A statistical mechanical description of flexible and semiflexible polymer chains in a poor solvent is developed in the constant force and constant distance ensembles. We predict the existence of many intermediate states at low temperatures stabilized by the force. A unified response to pulling and compressing forces has been obtained in the constant distance ensemble. We show the signature of a crossover length which increases linearly with the chain length. Below this crossover length, the critical force of unfolding decreases with temperature, while above, it increases with temperature. For stiff chains, we report for the first time sawtoothlike behavior in the force-extension curves which has been seen earlier in the case of protein unfolding.

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During the past decade force has been used as a thermodynamic variable to understand molecular interactions and their role in the structure of biomolecules [1,2]. By exerting a force in the pN range one can experimentally study the elastic, mechanical, structural, and functional properties of biomolecules [3]. The dependence of force on concentration, pH of the solvent, loading rate, and temperature provides basic understanding of the interactions [4–6]. Many biological reactions involve large conformational changes which provide well-defined mechanical reaction coordinates, e.g., the end-to-end distance of a polymer, that can be used to follow the progress of the reaction [3]. Such processes have been modeled by a simple two state model [3]. The applied force “tilts” the free energy surface along the reaction coordinate by an amount linearly dependent on the end-to-end distance. The kind of transitions induced by the applied force are the folding-unfolding transition of proteins [1], the stretching and unzipping transition of double-stranded DNA [7,8], or the ball-string transition of a polymer [9]. From polymer theory we know that a polymer chain will, depending on temperature, be in either a collapsed state or a swollen state [10]. The end-to-end distance \( \langle R \rangle \) scales as \( N^{\nu} \), where \( N \) is the chain length and \( \nu \) is the end-to-end distance exponent. In the collapsed state (low temperatures) \( \nu = 1/d \), while at high temperatures \( \nu \) is given quite accurately by the Flory approximation \( \nu = 3/(d + 2) \) [10] (actually this formula is exact for \( d = 1, 2, \) and 4). It should be noted that by varying temperature alone, a polymer cannot acquire the conformation of a stretched state where \( \nu = 1 \). Hence force not only tilts the free energy surface but also induces a new “stretched state” which is otherwise not accessible. Moreover, recent experiments suggest that there are many intermediate states involved which are crucial to the understanding of unfolding experiments and lie beyond the scope of two state models [9,11,12].

The nonequilibrium thermodynamics of small systems has mostly been studied in the “constant force ensemble” (CFE) where the control parameter is the average extension. Most applications of atomic force microscopy apply the force using a linear ramp protocol with a very small velocity. Such systems may be considered to be in quasi-static equilibrium and the appropriate ensemble is the “constant distance ensemble” (CDE). In the thermodynamic limit both ensembles are expected to give similar results [13]. However, single molecule experiments study systems of finite size and the results may depend on the ensemble [14]. Apart from this, the physical constraints imposed by experimental setups have not been fully taken into account. For example, in atomic force microscopes, receptor and ligand molecules are attached to a substrate and a transducer, respectively. The loss of entropy due to the confinement has been ignored in most models.

Theoretically these transitions have been studied using simple models such as freely jointed chain or wormlike chain (WLC) models [15,16]. The WLC model has been used to study the force-extension curves of biomolecules. However, this model ignores excluded volume effects and attractive interactions between chain segments and is thus well suited only for modeling the stretching of polymers in a good solvent [16]. For a polymer in a poor solvent, the force-extension curve shows the existence of a plateau region at a well-defined force [9,11]. Experimental observations of polymers in poor solvents and an improved theoretical understanding of semiflexible polymers have given rise to theories of globules with well-defined internal structure [17,18]. These theories have potential applications in the study and understanding of the basic mechanisms of protein folding.

The purpose of this communication is first to provide a complete phase diagram using exact results of finite chains. However, we focus our studies on the behavior of the force-
extension curves at low temperatures where the usual Monte Carlo approach and the theoretical models discussed above fail. These studies have direct relevance to phenomena seen in experiments with single biomolecules. We report the existence of intermediate states stabilized by the force at low temperatures. We also show the signature of a crossover length, below which the transition temperature decreases with force, while above the crossover length the transition temperature increases with force. When bending rigidity is taken into account, we predict "saw-toothlike" oscillations in the regime of large polymer stiffness, as observed in protein unfolding experiments.

To obtain exact results for small chains we model them as interacting self-avoiding walks (ISAWS) on the square lattice [19] as shown in Fig. 1. Interactions are introduced between nonbonded nearest-neighbor monomers. In our model one end of the polymer is attached to an impenetrable neutral surface (there are no interactions with this surface) while the polymer is being pulled from the other end with a force acting along the x axis to model the experimental setup. We introduce Boltzmann weights \( \omega = \exp(-\epsilon/k_BT) \) and \( u = \exp(-F/k_BT) \) conjugate to the nearest-neighbor interactions and force, respectively, where \( \epsilon \) is the interaction energy, \( k_B \) is Boltzmann’s constant, \( T \) the temperature, and \( F \) the applied force. In the rest of this study we set \( \epsilon = -1 \) and \( k_B = 1 \). We study the finite-length partition functions

\[
Z_N(F, T) = \sum_{\text{all walks}} \omega^m u^x = \sum_{m, x} C(N, m, x) \omega^m u^x, \tag{1}
\]

where \( C(N, m, x) \) is the number of ISAWS of length \( N \) having \( m \) nearest-neighbor contacts and whose end points are a distance \( x = x_N - x_0 \) apart. The partition functions of the CFE, \( Z_N(F, T) \), and CDE, \( Z_N(x, T) = \sum_m C(N, m, x) \omega^m \), are related by \( Z_N(F, T) = \sum_x Z_N(x, T) u^x \). The free energies are evaluated from the partition functions

\[
G(x) = -T \log Z_N(x) \quad \text{and} \quad G(F) = -T \log Z_N(F). \tag{2}
\]

Here \( \langle x \rangle = \frac{\partial G(F)}{\partial F} \) and \( \langle F \rangle = \frac{\partial G(x)}{\partial x} \) are the control parameters of the CFE and CDE, respectively.

We enumerate all possible conformations of the ISAW by exact enumeration techniques. The major advantage of this approach is that the complete finite-length partition functions can be analyzed exactly. Furthermore scaling corrections can be taken into account by suitable extrapolation schemes enabling us to obtain accurate estimates in the thermodynamic (infinite length) limit [20]. To achieve a similar degree of accuracy using Monte Carlo simulations one typically has to use chains at least 2 orders of magnitude longer than in the exact enumerations [21]. The greatest challenge facing exact enumerations is to increase the chain length. Until now most exact results for models of small proteins were confined to chain lengths of 30 or so [22,23]. Here the number of ISAWS was calculated using transfer matrix techniques [24]. Combined with parallel processing, we were able to almost double the chain length. To be precise we calculate the partition functions up to chain length 55.

In Fig. 2, we show the force-temperature phase diagram for flexible chains. The qualitative phase diagram remains largely the same [23]. The observed reentrant behavior can be explained by a ground state with nonzero entropy [23,25]. However, the extension up to length 55 allowed us to obtain a new transition line from the extended state to the stretched state which is solely induced by the applied force. In contrast to the lower phase boundary (collapse transition), where the force decreases with temperature, the upper phase boundary (stretching transition) shows that the force increases with the temperature.

First, we study the model in the CFE. In Fig. 3(a), we plot the average scaled elongation with force for different chain lengths at low temperature. Experimentally several transitions were found in the force-extension curves corresponding to many intermediate states [9,11,12,26]. This phenomenon is clearly confirmed by our study. It has been argued that in the limit of infinite chain length, the intermediate states should vanish and there will be an abrupt transition between a folded and fully extended state [22]. Evidence to this effect can also be seen in Fig. 3(a) where we note that the plateaus at an extension around 0.2 tend to increase with \( N \) while the other plateaus tend to shrink with \( N \) (this is particularly so for the plateau around 0.5 corresponding to a simple zigzag pattern of the chain). Note, however, that as we change the chain length from 25 to 55,

![FIG. 1 (color online). An ISAW on the square lattice with one end attached to a surface and subject to a pulling force on the other end.](image1)

![FIG. 2 (color online). The phase diagram for flexible chains.](image2)
we find more and more of these intermediate states. This has also been observed in recent experiments [9,26] where the globule deforms into an ellipse and then into a cylinder. At a critical extension the polymer undergoes a sharp first-order transition into a “ball string" conformation [9,26].

This shows that finite size effects are crucial in all the single molecule experiments and can be seen even for long chains [12]. A simple theoretical argument for the observed behavior is that at low temperature, where the entropy \( S \) (per monomer) of the chain is quite low, the dominant contribution to the free energy

\[
G = N \epsilon - \sigma(N, F) \epsilon - NTS
\]

is the nonbonded nearest-neighbor interaction \( N \epsilon \). The second term is a surface correction and it vanishes in the thermodynamic limit. However for finite \( N \), the system has many degenerate states depending upon the shape of the globule. This leads to a surface correction term \( \sigma(N, F) \) which is a function of \( N \) and \( F \). If \( F = 0 \) the shape of the globule is like a square and the surface correction term \( \sigma(N, 0) \) will be minimized and equal to \( 2\sqrt{N} \). In the CFE, there is a force-induced additional contribution proportional to the extension of the globule which along with \( \sigma(N, F) \) stabilizes the intermediate states. When the temperature increases the multistep character of the force-extension curve is washed out due to increased contributions from entropy. This effect can be clearly seen in Fig. 3(b) where we have plotted force-extension curves at different \( T \).

Next we study the model in the CDE. The force-extension curve shown in the inset of Fig. 4(a) has interesting features. It shows that when the distance between the first and the last monomer (where force is applied) is less than the average size of the coil (without force), one needs a compressing force instead of a pulling force. The qualitative behavior is similar to one observed in experiments [27] and computer simulations [28]. Since most models do not include confinement in their description, such behavior could not be predicted. In Fig. 4(a), we show the response of the force when the elongation exceeds the average size of the polymer. The flat portion of the curve gives the average force needed to unfold the chain. Such plateaus have been seen in experiments [9,11,12]. From Fig. 4(a) one can also see that the force required to obtain a given extension initially decreases with temperature. But beyond a certain extension (close to 30 in this case) the required force increases with temperature. We note that the curves cross each other at a “critical” extension for any temperature (below the \( \theta \) point). We identify this as a crossover point. In Fig. 4(b) we plot the position of the crossover point \( L_{\text{cross}} \) as a function of the length \( N \) of the polymer chain. We see that the crossover extension increases linearly with the chain length. This shows that above this point the chain acquires the conformation of the stretched state. The increase in force with temperature generates a tension in the chain sufficient to overcome the entropic effect. Since the contribution to the free energy from this term is \( TS \) (\( S \) being the entropy), more force is needed at higher \( T \) as seen in experiments. Our exact analysis for finite chain length shows that applying a force at first favors taking the polymer from the folded state to the unfolded state. However, rupture or second unfolding occurs when the tethered or unfolded chain attains the stretched state, and one requires more force at higher temperature.

We model semiflexible polymers by associating a positive energy \( \epsilon_b \) with each turn or bend of the walk [23]. The corresponding Boltzmann weight is \( \omega_b = \exp(-b\epsilon_b) \), where \( b \) is the number of bends in the ISAW. We again enumerate all walks, but because of the additional parameter \( \omega_b \), we were restricted to 45 steps. For a semiflexible polymer chain, a stretched state may be favored by increasing the stiffness. The phase diagram for semiflexible chains is now well established. It has three states: namely, (i) an open coil state at high temperature, (ii) a molten globule at low temperature and low stiffness, and (iii) a “frozen” or “folded” state at low temperature and large stiffness [17,18,23]. We note that while the flexible and semiflexible \( F-T \) phase diagrams are qualitatively similar [23], the reentrant behavior is suppressed because of stiffness and becomes less pronounced with increasing bending energy. In the CFE, the probability distribution of the end-to-end distance has sawtoothlike behavior corresponding to intermediate states during unfolding [23]. Therefore, it is im-

**FIG. 3.** The average scaled elongation \( \langle \chi \rangle / N \) vs \( F \) at \( T = 0.01 \) for various lengths (a) and temperatures at length \( N = 55 \) (b).

**FIG. 4.** Plot of the average force \( \langle F \rangle \) vs the elongation \( x \) at various temperatures \( T \) for \( N = 55 \) (a) and the crossover length vs \( N \) (b).
important to study the effect of stiffness on force-extension curves in the CDE. The force-extension curves shown in Fig. 5 have striking differences to the flexible ones. At low temperatures we see strong oscillations which vanish as the temperature is increased. Since the polymer chain has “frozen conformations” like β-sheets (the zero-force limit of which describes zigzag configurations inscribed in a square) [29], it takes more force to unfold a layer. When about half a layer has been opened, the bending energy favors a complete stretching of the layers and hence the force decreases. This phenomenon allows us to probe a molecule like Titin which has similar β-sheet structure [30].

To summarize, we have presented the exact solution of a model of long (finite) polymer chains, of direct relevance to recent experiments on the elastic properties of single biomolecules. The model takes into account several constraints imposed by the experimental setups: geometric constraints, excluded volume effects, attraction between chain segments, finite but large chain length (here up to \(N = 55\)). It permits one to choose the thermodynamic ensemble (CFE or CDE) dictated by the experimental protocol. The exact enumeration data permit us to access all parameter values, including biologically relevant low temperatures where previous studies have failed. Our results correctly reproduce several experimentally observed effects: multiple transitions during unfolding, sawtoothlike oscillations in the force-extension curve of semiflexible chains and first-order transition into a ball-string conformation. Finally, we have identified crossover behavior that provides a unified treatment of both pulling and compressing forces in the CDE.

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[29] In 3D [V. A. Ivanov et al., Macromol. Theory Simul. 9, 488 (2000)] a toroidal state dominates for stiff chains at low temperatures with no applied force. In our (2D) model toroidal states are topologically impossible. However the β-sheet conformations are analogous to toroidal states.