

Does Changing the Pulling Direction Give Better Insight into Biomolecules?

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(Received 23 May 2006; published 23 January 2007)

Single-molecule manipulation techniques reveal that the mechanical resistance of a protein depends on the direction of the applied force. Using a lattice model of polymers, we show that changing the pulling direction leads to different phase diagrams. The simple model proposed here indicates that in one case the system undergoes a transition akin to the unzipping of a β sheet, while in the other case the transition is of a shearing (slippage) nature. Our results are qualitatively similar to experimental results. This demonstrates the importance of varying the pulling direction since this may yield enhanced insights into the molecular interactions responsible for the stability of biomolecules.

DOI: [10.1103/PhysRevLett.98.048101](https://doi.org/10.1103/PhysRevLett.98.048101)

PACS numbers: 87.14.-g, 36.20.Ey, 64.90.+b, 82.35.Jk

The last decade has witnessed an intense activity in experiments involving the manipulation of single biomolecules. This interest has been fueled on the one hand by the desire to understand the fundamental mechanisms at play in biological systems, and on the other hand by the development of revolutionary single-molecule force spectroscopy experiments [1–5]. These experiments provide unexpected insights into the strength of the forces driving biological processes and help to determine various biological interactions as well as the mechanical stability of biological structures. In some cases the experimental setup also allows one to locate precisely the positions of the forces occurring within the biomolecule [6].

The theoretical studies (numerical and analytical) which followed the experimental efforts have mostly been confined to modeling the molecule within the context of statistical mechanics. The models use various kinds of simplified interactions and compare the resulting theoretical predictions against the experimental findings. For example, the most widely used models are the freely jointed chain (FJC) and wormlike chain (WLC) models [7,8], which describe the force-extension curves in the intermediate and high-force regimes. However, both these models ignore crucial excluded volume effects [9], and are thus only well suited to modeling the stretching of proteins in a good solvent. Note that solvents relevant in a biological context are usually poor. Therefore, these models and numerical studies (Monte Carlo simulations) are unable to access the low temperature regime relevant in a biological context. Consequently, the study of the emergence of intermediate states stabilized by a force at low temperature is beyond the scope of these models.

Efforts have recently shifted to the experimental study of molecular conformations of biopolymer by changing the pulling direction [10–12]. For example, in a recent experiment, Dietz and Rief [11] showed that by changing the pulling direction (mechanical triangulation) one obtains distinct force-extension curves from which angstrom-precise structural information can be obtained about single proteins in a solution. Neither the FJC or WLC models nor

the self-avoiding walk (SAW) model (which does include excluded volume effects [9,13,14]) show any of the effects related to a change in the pulling direction contrary to the observations of recent experiments [11,12]. This is because the shape of the chain conformations and the interactions seen in these models are isotropic in nature. Notably, all the proteins studied so far are highly anisotropic both in shape and interactions.

In this Letter, we study the force-extension curves of flexible and semiflexible polymers by changing the direction of the pulling force using an inherently anisotropic lattice walk model. We show that a change in the pulling direction [10–12] gives rise to many new intermediate states and that the force-temperature phase diagrams are significantly different. In order to model the anisotropy of the biomolecules, we use a lattice model of partially directed self-avoiding walks (PDSAWs) in which steps with negative projection along the x axis are forbidden [13]. At low temperature and high stiffness, the model mimics the structure of β sheets [14] as seen in molecules like titin [1,2]. The major advantage of the model is that it can be solved exactly in the thermodynamic limit. In all single-molecule experiments, a chain of finite size has been used and hence in principle no “true phase transition” can be observed [15]. In order to study finite-size effects, it is

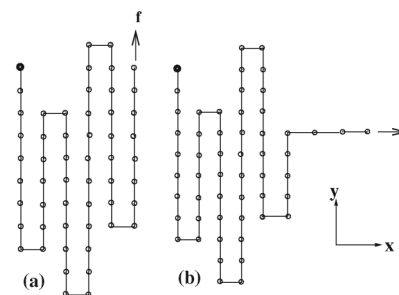


FIG. 1. Schematic illustrations of PDSAWs on the square lattice. One end is fixed and the other end is subjected to a pulling force (a) perpendicular to the preferred direction (y direction). (b) Along the preferred direction (x direction).

essential to study first chains of finite length and then their thermodynamic limit. The PDSAW model can be solved exactly in the canonical ensemble for finite chain lengths N , and using finite-size data, the thermodynamic limit of the model can be extrapolated and compared with values obtained from exact solutions [16,17].

The model of PDSAWs on a two-dimensional square lattice is shown in Fig. 1. The stiffness of the chain is modeled by associating a positive energy (Δ) with each turn or bend of the walk [14]. For a semiflexible polymer chain the extended state may be favored by increasing the stiffness. The stretching energy E_s arising due to the applied force f is taken as $E_s = -f\alpha$, where α is the x component (or y component) of the end-to-end distance $[(|x_1 - x_N|) \text{ or } (|y_1 - y_N|)]$. This distance has been used as the mechanical reaction coordinate that monitors the response of the force [3] and it gives important information about the conformation of the biomolecules.

The complete partition function of the system under consideration can be written as $Z_N(N_b, \sigma, |\alpha|) = \sum_{(N_b, \sigma, |\alpha|)} C_N(N_b, \sigma, |\alpha|) s^{N_b} a^\sigma p^{|\alpha|}$, where $C_N(N_b, \sigma, |\alpha|)$ is the total number of PDSAWs of N steps having N_b turns (bends) and σ nearest neighbor pairs; p is the Boltzmann weight for the force defined as $\exp[\beta(f \cdot \hat{\alpha})]$, where $\hat{\alpha}$ is a unit vector along the x axis (or y axis); $a = \exp[-\beta\epsilon]$ and $s = \exp[-\beta\Delta]$ are the Boltzmann weights associated with nearest neighbor interactions ϵ between nonbonded monomers and bending energy, respectively. We use the exact enumeration technique to find C_N for chains of length up to $N = 30$ and analyze the partition functions. Scaling corrections can be taken into account by suitable extrapolation schemes enabling us to obtain accurate estimates in the thermodynamic (infinite length) limit [13]. The reduced free energy per monomer is found from the relation $G = \lim_{N \rightarrow \infty} (1/N) \log Z_N[(N_b, \sigma, |\alpha|)]$. The limit $N \rightarrow \infty$ is achieved by using the ratio method [13] for extrapolation. The transition point for flexible chains ($\Delta = 0$) at zero force ($p = 1$), i.e., a coil-globule transition, can be obtained either from a plot of G versus a , or from the peak value of $\frac{\partial^2 G}{\partial (\ln a)^2}$. We find $a = 3.336$ at $p = 1$. This is shown (in terms of temperature, $T = 0.83$) in the force-temperature (f - T) phase diagram. The force and temperature are obtained from the expressions for the Boltzmann weights $f = \log(p)/\log(a)$ and $T = 1/\log(a)$, respectively, by setting $\epsilon = -1$. This value is in excellent agreement with the exact value ($T = 0.8205$) [17]. Moreover, this value is also quite close (within error bars of ± 0.02) to the one obtained from the fluctuations in nonbonded nearest neighbors (which can also be calculated exactly for finite $N = 30$). At zero force, the system attains the globule (folded or β sheet) state as shown in Fig. 1 below $T < T_c$. The qualitative behavior of the phase diagram (shown in Fig. 2) is similar to the one reported in [14] for SAWs. It should be noted that for finite N , as well as in the thermodynamic limit, the phase diagrams obtained when the force

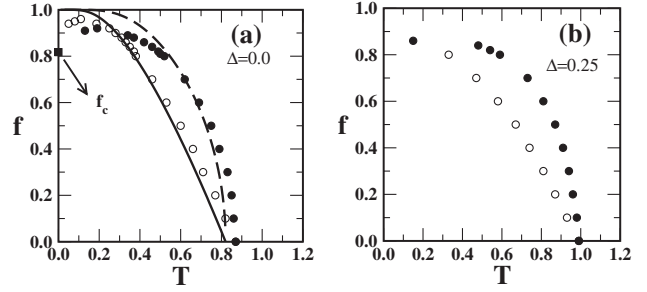


FIG. 2. The globule-coil phase boundary in the force-temperature plane: (a) for flexible polymer chains and (b) for semiflexible polymer chains. The phase diagram corresponding to a force applied along the y direction (closed circle: finite N and dashed line: exact phase boundary [16]) is distinctly different from that of a force along the x direction (open circle: finite N and solid line: exact phase boundary [17]).

is applied along the y direction are distinctly different from the corresponding phase diagrams with the force applied along the x direction. Reduced temperature and force may be expressed in real units by using the following expressions: $T_{\text{exp}} = \epsilon_{\text{exp}} T/k_B$ and $f_{\text{exp}} = \epsilon_{\text{exp}} f$. Here, k_B is the Boltzmann constant and the subscript “exp” corresponds to values in real units. For example, if one chooses $\epsilon_{\text{exp}} = 1$ kcal/mol, then the equivalent force will be of the order of 70 pN nm.

Remarkably, for *finite* N the force-temperature phase diagram shows reentrance for flexible chains, but reentrance is absent for semiflexible chains. However, in the thermodynamic limit, there is no reentrance [17] for flexible chains. The presence of reentrance (at finite N) may be explained by using a phenomenological argument near $T = 0$. The dominant contribution to the free energy,

$$G(\equiv -fN) = N\epsilon - 2\sqrt{N}\epsilon - NTS_c, \quad (1)$$

is from the first term. The second term is due to surface corrections which vanishes in the thermodynamic limit, but plays a very important role for finite N . The last term is a contribution due to the entropy associated with the globule, where S_c is the entropy per monomer. It may be noted that for PDSAWs at $T = 0$, there are only two conformations (Hamiltonian walks) which are the most compact configurations and hence one does not see any reentrance in the thermodynamic limit [17]. For $T > 0$, there is a finite entropy associated with the deformed globule, which along with the surface correction term gives rise to reentrance in the finite chain. The critical force, f_c , for $N = 30$ found from Eq. (1) is equal to 0.8174 at $T = 0$. This value is indicated by a black square on the y axis of Fig. 2. This is less than 1 [17] as obtained in the thermodynamic limit from Eq. (1).

In Figs. 3 and 4, we plot the average scaled extension for flexible and semiflexible polymer chains, respectively, by using the expression: $\langle \alpha \rangle / N = (1/N) \times \sum \alpha C(N_b, \sigma, \alpha) a^\sigma p^\alpha / \sum C(N_b, \sigma, \alpha) a^\sigma p^\alpha$. The

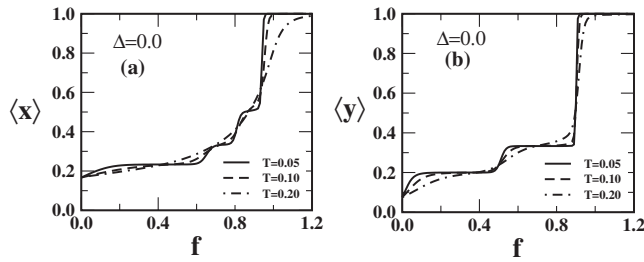


FIG. 3. (a) The average scaled extension of a flexible polymer chain as a function of the pulling force f at different temperatures: (a) along the x direction; (b) along the y direction.

extension-force curves show multistep transitions at low temperature corresponding to intermediate states. In the constant force ensemble, there is an additional contribution to the free energy proportional to the product of the force and the extension (along the direction of the force). This contribution stabilizes the intermediate states of the globule and hence the observed multistep behavior. Multistep transitions have also been observed in recent experiments [18] 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 [18]. This shows that finite-size effects are crucial in all single-molecule experiments [19]. When the temperature is increased the multistep character of the extension-force curve is washed out due to increased contributions from the entropy [20]. This effect can be seen in Fig. 3.

In contrast to the FJC, WLC, or SAWs, in PDSAWs the walk is directed along the x direction and it is inherently anisotropic so that the perpendicular and parallel components scale differently, namely, as \sqrt{N} and N , respectively [13]. Hence the phase boundaries for these two cases remain distinct even in the thermodynamic limit, as shown in Fig. 2. Here, one can also see that in order to unfold the chain at a given temperature, one needs a stronger force along the y axis than along the x axis.

In the case of a semiflexible chain, the response of the force is more pronounced and the emergence of intermediate states by changing the pulling direction can be seen in Fig. 4. From Fig. 2(b), it is evident that a much stronger force is required for the unfolding when the force is applied along the y axis. The physical origin of this may be understood from Fig. 5, where we have plotted schematic diagrams

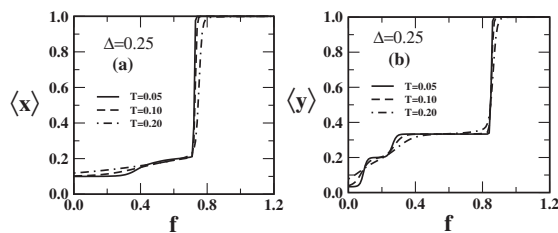


FIG. 4. Same as Fig. 3, but for the semiflexible chain.

grams, keeping $x = 1$ [Fig. 5(a)] and $y = 1$ [Fig. 5(b)], for a fixed extension of say $N/2$. It is easy to see that in both cases, the number of contacts σ is the same ($N/2$), while the number of turns (or bends) in Fig. 5(a) is 2 and in Fig. 5(b) it is $N/2$. As stiffness helps to stabilize the stretched state, the required force is less in the case of a force applied along the x direction as compared to a force along the y direction.

In the constant force ensemble the control parameter α gets averaged; therefore, one does not find any oscillations (sawtooth) in the control parameter in contrast to experiments [1–5]. In a recent paper [15], we have shown that the probability distribution curve of the control parameter gives important information about the conformation of biomolecules in the form of oscillations in the control parameter. Keeping f and T constant near the transition line we plot, in Fig. 6, the probability distribution curves $P(|\alpha|) = (1/Z_N) \sum_{N_b, \sigma} C_N(N_b, \sigma, |\alpha|) s^{N_b} a^\sigma p^{|\alpha|}$ as a function of α . Striking differences are apparent from these plots. When a force is applied either along the x or y axis, the probability distribution curve for flexible and semiflexible chains remains smooth at high temperatures. However, at low temperature, when a force is applied along y axis, the emergence of peaks indicate the structural changes in biomolecules. These features become more apparent for semiflexible chains, where we find peaks at much higher temperature. This may be understood in the following way: If force is applied along the x axis, the loss of one monomer contact gives a unit of extension along the x axis. However, when force is applied along the y axis, there a loss of either one or two contacts always gives two units of extension along the y axis. This clearly shows that by changing the pulling direction one can obtain better semimicroscopic information about the conformation of biomolecules. The features observed here should not be viewed as artifacts of the lattice model, because they appear only when the direction of pulling force is changed.

It is interesting to compare the qualitative features of our results with the ones obtained in experiments [11,12]. First, we note that in one case (Refs. [11,12]), the system undergoes a shearing kind of transition for which the applied force is higher. This is the case [Fig. 1(a)] when a force is

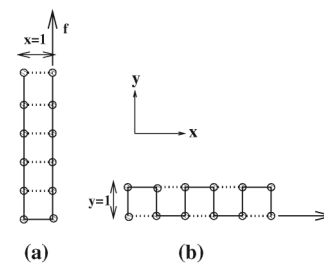


FIG. 5. Schematic of two cases (having $N/2$ contacts and $N/2$ extension) where a force is applied along (a) the y direction; (b) the x direction.

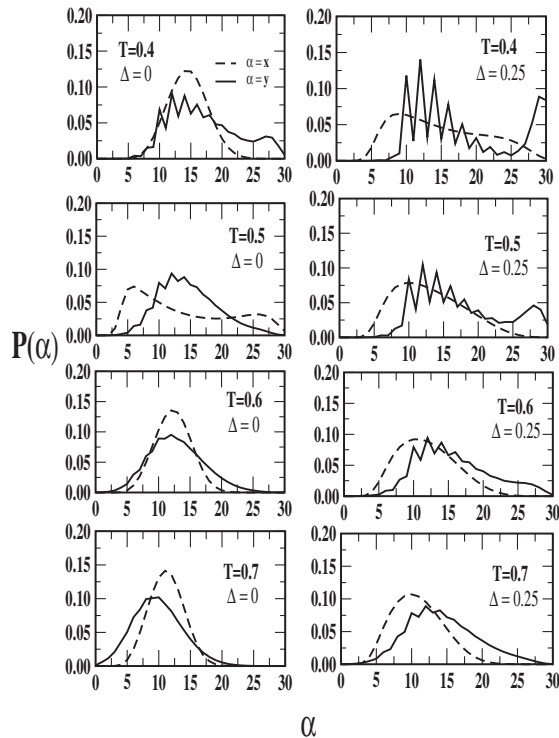


FIG. 6. Probability distribution curve (dashed and solid line correspond to force along x and y , respectively) at different temperature for flexible chain ($\Delta = 0$) and semiflexible chain ($\Delta = 0.25$).

applied along the y axis. In the other case, it undergoes a β -sheet unzipping kind of transition for which the applied force is less. This is reflected in Fig. 1(b), where we find that the critical force is less. Our results provide strong evidence that saw-tooth-like oscillations are enhanced in force-extension curves due to the shearing (slippage) kind of transition. This can also be seen in experiments, where the peaks of sawtooth are much larger for shearing like transitions compared to the unzipping of β -sheets. At this moment additional analytical and computer simulation work is required for a deeper understanding of the role of anisotropy in the stability of biomolecules.

It may be noted that the conformation of biomolecules remain unchanged by changing the direction of the force in the model discussed here. However, in experiments, by fixing one end only, the entire molecule will rotate due to the torque about the fixed end of the chain. In order to see effects predicted by the model, one has to fix not only the end point (as discussed above) but one more point in the chain. This will ensure that rotation will not take place around the fixed end due to the change in direction of the applied force. A force may be applied at the other end of the chain either along the direction of the line connecting these points or perpendicular to this line. By changing the position of the second point, one may get enhanced insight into the molecular interactions.

In conclusion, we have clearly demonstrated that finite-size effects are crucial in understanding the experimental phase diagram and that there are many intermediate states at low temperature. Moreover, by considering a simple model which takes into account the anisotropy of biomolecules, we have shown for the first time that changing the pulling direction gives distinct force-temperature curves even in the thermodynamic limit. When a force is applied along the preferred direction, we observe the unzipping or opening of β -sheets layer by layer. However, when force is applied perpendicular to the preferred direction, we see the effects of slippage (shearing). It is evident from our studies that the mechanical resistance of biomolecules, e.g., proteins, is not dictated solely by the amino acid sequence or unfolding rate constant but depends critically on the topology of the biomolecules and on the direction of the applied force.

We thank Haijun Zhou, Iwan Jensen, and R. Rajesh for fruitful discussions on the subject and UGC, India for financial support.

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