

Sanjay Kumar,<sup>1</sup> Iwan Jensen,<sup>2</sup> Jesper L. Jacobsen,<sup>3</sup> and Anthony J. Guttman<sup>2</sup>

<sup>1</sup>*Department of Physics, Banaras Hindu University, Varanasi 221 005, India*

*Max-Planck Institute for the Physics of Complex Systems, Noethnitzer, 01187 Dresden, Germany*

<sup>2</sup>*ARC Centre of Excellence for Mathematics and Statistics of Complex Systems,  
Department of Mathematics and Statistics, The University of Melbourne, Victoria 3010, Australia*

<sup>3</sup>*Université Paris Sud, UMR8626, LPTMS, F-91405 Orsay Cedex, France*

*Service de Physique Théorique, CEA Saclay, F-91191 Gif-sur-Yvette, France*

A statistical mechanical description of flexible and semi-flexible 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 of pulling and compressing force has been obtained in the constant distance ensemble. We show the signature of a cross-over length which increases linearly with the chain length. Below this cross-over length, the critical force of unfolding decreases with temperature, while above, it increases with temperature. For stiff chains, we report for the first time a “saw-tooth” like behavior in the force-extension curves which has been seen earlier in the case of protein unfolding.

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

## I. INTRODUCTION

During the past decade force has been used as a thermodynamic variable to understand molecular interactions and their role in the structure of bio-molecules [1–3]. By exerting a force in the pN range, most commonly using atomic force microscopes, optical tweezers, glass micro-needles etc., one aims to experimentally study and characterize the elastic, mechanical, structural and functional properties of bio-molecules [4]. The dependence of force on concentration, pH of the solvent, loading rate and temperature provides basic understanding of these interactions [5–7]. The force-temperature phase diagrams show that the unfolding temperature decreases with the strength of the applied force [6].

Many biological reactions involve large conformational changes which provide well defined mechanical reaction co-ordinates, *e.g.* the end-to-end distance of a bio-polymer, that can be used to follow the progress of the reaction. The effects of an applied force give important information about the free energy surface of the reaction. Such processes have been modeled by a simple two state model [4]. The applied force “tilts” the free energy surface along the reaction co-ordinate 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 dsDNA [8, 9] or the ball-string transition of a polymer [10]. From polymer theory we know that a polymer chain will, depending on temperature, be in either a collapsed state or a swollen state [11]. 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  $\nu = 3/(d + 2)$  [11] (actually this formula is exact for  $d = 1, 2$  and  $4$  whereas in  $d = 3$  it is only a good approximation). 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 [10, 12, 13].

The non-equilibrium thermodynamics of small systems have mostly been studied in the “constant force ensemble” where the control parameter is the average extension. In most applications of atomic force microscopy, the force is applied according to a linear ramp protocol  $x(t) = x_0 + vt$ . Here  $x_0$  is the cantilever position at time  $t = 0$  and  $v$  is a constant velocity (typically very small). Hence, such systems may be considered to be in quasi-static equilibrium and the appropriate ensemble will be the “constant distance ensemble”. In the thermodynamic limit, both ensembles are expected to give the same results [14]. However, single molecule experiments study systems of finite size and the results may depend on the ensemble [15]. 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 in the framework of statistical mechanics based on simple models that are amenable to analytical treatment such as freely jointed chain (FJC) or worm like chain (WLC) models [16, 17]. The WLC model has been mostly used to understand the force-extension curves of bio-molecules. However, the WLC model ignores excluded volume effects and attractive interactions between different chain segments and is thus only well suited for modeling the



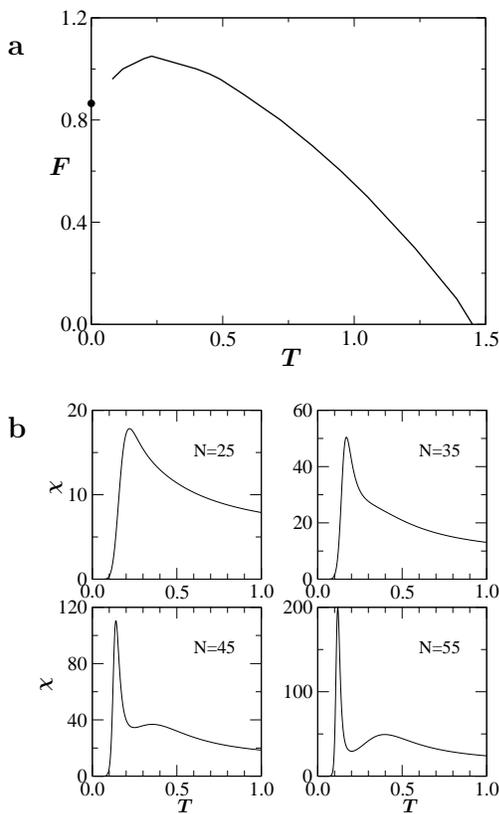


FIG. 2: **a** The force-temperature phase diagram for flexible chains. **b** The fluctuations  $\chi$  in the number of contacts vs. temperature at fixed force  $F = 1.0$  for various values of the chain length  $N$ .

undergoes a phase transition to an extended state. The transition temperature as plotted in Fig. 2a was found (in the thermodynamic limit  $N \rightarrow \infty$ ) by studying the reduced free energy per monomer [24]. The most notable feature of the phase-diagram is the *re-entrant* behaviour. Using phenomenological arguments at  $T = 0$ , the critical force  $F_c = 0.8651$  (indicated by a black circle on the  $y$ -axis) is found from the expression,  $F_c = 1 - 1/\sqrt{N} + TS$  [24]. The positive slope  $dF_c/dT$  at  $T = 0$  confirms the existence of re-entrance in the  $F - T$  phase-diagram. We note that the value of the transition temperature obtained in the thermodynamic limit and the one obtained from the fluctuations in non-bonded nearest neighbors (which can be calculated exactly for finite  $N = 55$ ) gives almost the same value (within error bars of  $\pm 0.01$ ). The fluctuations are defined as  $\chi = \langle m^2 \rangle - \langle m \rangle^2$ , with the  $k$ 'th moment given by

$$\langle m^k \rangle = \frac{\sum_{m,x} m^k C(N, m, x) \omega^m u^x}{\sum_{m,x} C(N, m, x) \omega^m u^x}.$$

In the panels of Fig. 2b we show the emergence of two peaks in the fluctuation curves with increasing  $N$  at fixed force  $F = 1.0$ . The twin-peaks reflect the fact that in the re-entrant region as we increase  $T$  (with  $F$  fixed) the polymer chain undergoes two phase transitions. Note

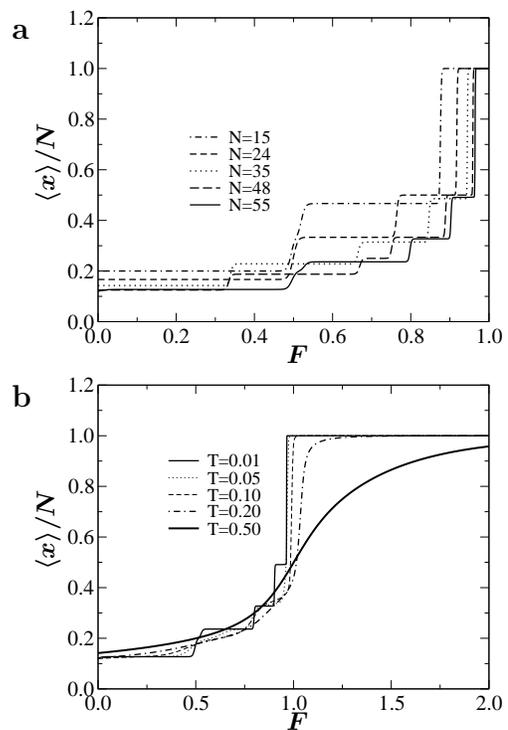


FIG. 3: **a** The average scaled elongation  $\langle x \rangle / N$  as a function of the force  $F$  at fixed low temperature  $T = 0.01$  for different chain lengths. **b** The average elongation at different temperatures for length  $N = 55$ .

that the twin-peaks are not apparent for small values of  $N$ .

#### IV. FORCE-EXTENSION CURVES

First we study the model in the constant force ensemble. In Fig. 3a, we plot the average scaled elongation,

$$\langle x \rangle / N = \frac{1}{N} \frac{\sum_{m,x} x C(N, m, x) \omega^m u^x}{\sum_{m,x} C(N, m, x) \omega^m u^x},$$

as a function of the applied force for different chain lengths at low temperature. Experimentally several transitions were found in the force-extension curves corresponding to many intermediate states [10, 12, 13]. 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 [23]. Evidence to this effect can also be seen in Fig. 3a where we note that the plateaus at an extension around 0.2 tends 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 zig-zag pattern of the chain). Note however, that as we change the chain length from 25 to 55, we find more and more of these intermediate states. This has also been observed

in recent experiments 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 [10]. This shows that finite size effects are crucial in all the single molecule experiments and can be seen even for long chains [13]. 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

$$G = N\epsilon - \sigma(N, F)\epsilon - NTS \quad (3)$$

is the non-bonded 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 constant force ensemble, 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 multi-step character of the force-extension curve is washed out due to increased contributions from entropy. This effect can be clearly seen in Fig. 3b where we have plotted force-extension curves at different  $T$ .

Next we study the model in the constant distance ensemble. The force-extension curve shown in Fig. 4a 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 that observed in experiments [26] and computer simulations [27]. Since most models do not include confinement in their description, such behavior could not be predicted until now. In Fig. 4b we plot the behaviour of the average 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 [10, 12, 13]. From Fig. 4 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 cross-over point. In Fig. 5 we plot the position of the cross-over point  $L_{cross}$  as a function of the length  $N$  of the polymer chain. We see that the cross-over 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),

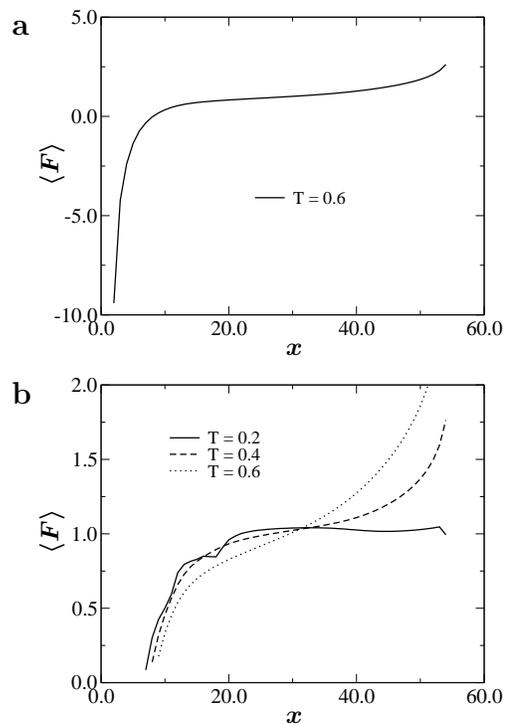


FIG. 4: **a** The average force  $\langle F \rangle$  required to obtain an elongation  $x$  at fixed temperature  $T = 0.6$  for chain length  $N = 55$ . **b** As above but for different temperatures and only in the region where the chain is being extended by the force.

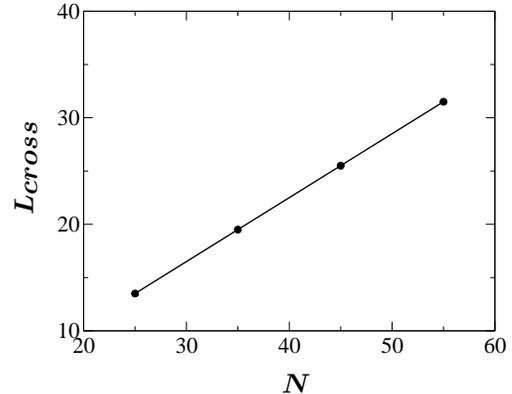


FIG. 5: Plot of the cross-over extension  $L_{cross}$  as a function of the chain length  $N$ .

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 favours 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 semi-flexible polymers by associating a positive energy  $\epsilon_b$  with each turn or bend of the walk [24]. The corresponding Boltzmann weight is  $\omega_b = \exp(-b\epsilon_b)$ , where  $b$  is the number of bends in the ISAW. We again

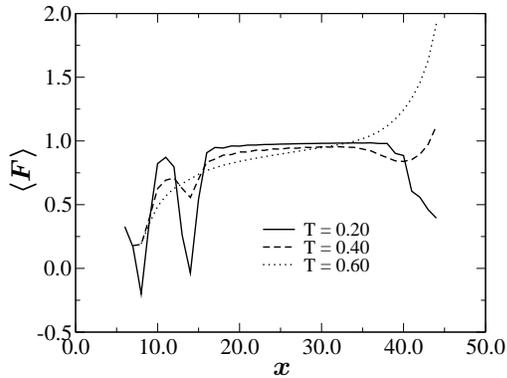


FIG. 6: Plot of average force  $\langle F \rangle$  vs the elongation  $x$  for a semi-flexible chain with bending energy  $\epsilon_b = 0.3$  at different temperatures  $T$  for chain length  $N = 45$

enumerate all walks, but because of the additional parameter  $b$ , we were restricted to 45 steps. For a semi-flexible polymer chain, a stretched state may be favored by increasing the stiffness. The phase diagram for semi-flexible 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 [18, 19, 24]. We note that while the flexible and semi-flexible  $F-T$  phase-diagrams are qualitatively similar [24], the re-entrant behavior is suppressed because of stiffness and becomes less pronounced with increasing bending energy. In the constant force ensemble, the probability distribution of the end-to-end distance has a "saw-tooth" like behavior corresponding to intermediate states during unfolding [24]. Therefore, it is important to study the effect of stiffness on force-extension curves in the constant distance ensemble. The force extension curves shown in Fig. 6 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  $\beta$ -sheets, it takes more force to unfold a layer. When

about half of the layer has been opened, the bending energy favours a complete stretching of the layers and hence the force decreases. Though a somewhat similar effect has been seen experimentally in molecules like Titin [28], a semi-flexible polymer chain with greater stiffness has not been probed so far.

## V. SUMMARY

To summarize, we have extended the chain length to 55 steps and shown the emergence of intermediate states which are stabilized by the applied force. We show for the first time the unified response of pulling and compressing force in the constant distance ensemble. The force-extension curves shown in Fig. 4 give an unequivocal signature of a crossover length. Above and below this crossover length, the role of entropy is established in describing the response of the force at different temperatures. The occurrence of "saw tooth" like oscillations (earlier seen in protein unfolding) in the force extension curves for semi-flexible polymer chains is a pleasant surprise and may be seen for DNA in solutions with multivalent counter ions or heteropolymers. We anticipate that advances in high precession experiments will be able to verify these predictions.

## VI. ACKNOWLEDGMENTS

This research has been supported by the Max Planck Institute for Physics of Complex System (MPIPKS), Germany (SK), the Australian Research Council (IJ,AJG) and the Indo-French Centre for the Promotion of Advanced Research (CEFIPRA) (JLJ). The calculations presented in this paper used the computational resources of the Australian Partnership for Advanced Computing (APAC) and the Victorian Partnership for Advanced Computing (VPAC).

- 
- [1] Rief, M., Gautel, M., Oesterhelt, F., Fernandez, J. M. and Gaub, H. E. Reversible unfolding of individual Titin immunoglobulin domains by AFM. *Science* **276**, 1109–1112 (1997).
  - [2] Kellermayer, M. S. Z., Smith, S. B., Granzier, H. L. and Bustamante, C. Folding-unfolding transitions in single Titin molecules characterized with laser tweezers. *Science* **276**, 1112–1116 (1997).
  - [3] Tskhovrebova, L., Trinick, J., Sleep, J. A., and Simmons, R. M. Elasticity And Unfolding Of Single Molecules Of The Giant Muscle Protein Titin. *Nature* **387**, 308–312 (1997).
  - [4] Bustamante, C. Y., Chemla, Y. R., Forde, N. R. and Izhaky, D. Mechanical processes in biochemistry. *Annu. Rev. Biochem.* **73**, 705–748 (2004).
  - [5] Itzhaki, L. S. and Evans, P. A. Solvent isotope effects on the refolding kinetics of hen egg-white lysozyme. *Protein Sci.* **5**, 140 (1996).
  - [6] Rouzina, I. and Bloomfield, V. A. Force-induced melting of the DNA double helix 1. Thermodynamic analysis. *Biophys. J.* **80**, 882–893 (2001); Force-induced melting of the DNA double helix 2. Effect of solution conditions. *Biophys. J.* **80**, 894–900 (2001).
  - [7] Evans, E. and Ritchie, K., Dynamic strength of molecular adhesion bonds. *Biophys. J.* **72**, 1541–1555 (1997); Strength of a weak bond connecting flexible polymer chains. *Biophys. J.* **76**, 2439–2447 (1999).
  - [8] Bhattacharjee, S. M. Unzipping DNAs: towards the first step of replication. *J. Phys. A* **33**, L423–L428 (2000).
  - [9] Bustamante, C., Bryant, Z. and Smith, S. B. Ten years

- of tension: single-molecule DNA mechanics. *Nature* **421**, 423-427 (2003).
- [10] Haupt, B. J., Senden, T. J. and Sevick, E. M. AFM evidence of Rayleigh instability in single polymer chains. *Langmuir* **18**, 2174-2182 (2002).
- [11] de Gennes, P. G. *Scaling Concepts in Polymer Physics* (Cornell Univ. Press, Ithaca, 1979).
- [12] Mao, H., Arias-Gonzalez, J. R., Smith, S. B., Tinoco Jr., I. and Bustamante, C. Temperature control methods in a laser tweezers system. *Biophys. J.* **89**, 1308-1316 (2005).
- [13] Lemak, A. S., Lepock, J. R. and Chen, J. Z. Y. Unfolding proteins in an external field: Can we always observe the intermediate states? *Phys. Rev. E* **67**, 031910 (2003); Molecular dynamics simulations of a protein model in uniform and elongational flows. *Proteins: Structures, Function and Genetics* **51**, 224-235 (2003).
- [14] Bustamante, C., Liphardt, J. and Ritort, F. The nonequilibrium thermodynamics of small systems *Phys. Today* **58**, 43-48 (July, 2005).
- [15] Zemanova, M. And Bleha, T. Isometric and isotensional force-length profiles in polymethylene chains. *Macromol. Theor. Sim.* **14**, 596-604 (2005).
- [16] Fixman, M. Polymer conformational statistics II. Characteristic function of the rotational-isomeric model. *J. Chem. Phys.* **58**, 1559-1563 (1973).
- [17] Doi, M. and Edwards, S. F. *Theory of Polymer Dynamics* (Oxford Univ. Press, Oxford, 1988).
- [18] Bastolla, U. And Grassberger, P. Phase transitions of single semi-stiff polymer chains. *J. Stat. Phys.* **89**, 1061-1078 (1997).
- [19] Doniach, S., Garel T. and Orland, H. Phase diagram of a semiflexible polymer chain in a theta solvent: Application to protein folding. *J. Chem. Phys.* **105**, 1601 (1996).
- [20] Vanderzande, C. *Lattice Models of Polymers* (Cambridge Univ. Press, Cambridge, 1998).
- [21] Guttman, A. J. *Phase Transitions and Critical Phenomena* vol. 13, edited by Domb, C. and Lebowitz, J. L. (Academic Press, New York, 1989).
- [22] Singh, Y., Giri, D. and Kumar, S. Crossover of a polymer chain from bulk to surface states. *J. Phys. A* **34**, L67-L74 (2001).
- [23] Marenduzzo, D., Maritan, A., Rosa, A. and Seno, F. Stretching of a polymer below the  $\theta$  point. *Phys. Rev. Lett.* **90**, 088301 (2003).
- [24] Kumar, S. and Giri, D. Force-induced conformational transition in a system of interacting stiff polymers: Application to unfolding. *Phys. Rev. E* **72**, 052901 (2005).
- [25] Jensen, I. Enumeration of self-avoiding walks on the square lattice. *J. Phys. A* **37**, 5503-5524 (2004).
- [26] Guffond, M. C., Williams, D. R. M. and Sevick, E. M. End-tethered polymer chains under AFM tips: Compression and escape in theta solvents. *Langmuir* **13**, 5691-5696 (1997).
- [27] Jimenez, J. and Rajagopalan, R. Interaction between a grafted polymer chain and an AFM tip: Scaling laws, forces, and evidence of conformational transition. *Langmuir* **14**, 2598-2601 (1998).
- [28] Rief, M., Oesterhelt, F., Heymann, B. and Gaub, H. E. Single molecule force spectroscopy on polysaccharides by atomic force microscopy. *Science* **275**, 1295-1297 (1997).