Free energy of a folded polymer under cylindrical confinement

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Abstract

Monte Carlo computer simulations are used to study the conformational free energy of a folded polymer confined to a long cylindrical tube. The polymer is modeled as a hard-sphere chain. Its conformational free energy \( F \) is measured as a function of \( \lambda \), the end-to-end distance of the polymer. In the case of a flexible linear polymer, \( F(\lambda) \) is a linear function in the folded regime with a gradient that scales as \( f \equiv |dF/d\lambda| \sim N^0 D^{-1.20\pm0.01} \) for a tube of diameter \( D \) and a polymer of length \( N \). This is close to the prediction \( f \sim N^0 D^{-1} \) obtained from simple scaling arguments. The discrepancy is due in part to finite-size effects associated with the de-Gennes blob model. A similar discrepancy was observed for the folding of a single arm of a three-arm star polymer. We also examine backfolding of a semiflexible polymer of persistence length \( P \) in the classic Odijk regime. In the overlap regime, the derivative scales \( f \sim N^0 D^{-1.72\pm0.02} P^{-0.35\pm0.01} \), which is close to the prediction \( f \sim N^0 D^{-5/3} P^{-1/3} \) obtained from a scaling argument that treats interactions between deflection segments at the second virial level. In addition, the measured free energy cost of forming a hairpin turn is quantitatively consistent with a recent theoretical calculation. Finally, we examine the scaling of \( F(\lambda) \) for a confined semiflexible chain in the presence of an S-loop composed of two hairpins. While the predicted scaling of the free energy gradient is the same as that for a single hairpin, we observe a scaling of \( f \sim D^{-1.91\pm0.03} P^{-0.36\pm0.01} \). Thus, the quantitative discrepancy between this measurement and the predicted scaling is somewhat greater for S-loops than for single hairpins.

Introduction

Recent advances in nanofabrication techniques have enabled the systematic study of the physical behavior of single DNA molecules confined to nanochannels. These studies are largely motivated by such diverse applications as DNA sorting, DNA denaturation mapping, and genome mapping, each of which exploits the effects of confinement on the DNA conformational behavior and dynamics. The development of such applications clearly requires a deep understanding of the physical behavior of polymers in nanochannels, and in recent years there has been considerable progress toward this goal. Much of this progress is due to the refinement and application of advanced Monte Carlo simulation techniques such as the Pruned Enriched Rosenbluth Method (PERM), which enables the simulation of very long polymer chains. Such studies have been instrumental in characterizing the various conformational scaling regimes, which are determined by the degree of confinement in relation to the per-
sistence and contour lengths of the polymer. Notable among this work is the confirmation of the existence of the extended de Gennes regime and the backfolded Odijk regime, which each lie between the classic de Gennes and Odijk regimes upon variation in the confining channel width.

In addition to work characterizing the scaling regimes of polymers confined to nanochannels, some effort has been devoted to elucidating the behavior of confined polymers in out-of-equilibrium folded states. For example, using fluorescence imaging techniques Levy et al. examined the behavior of DNA that was electrophoretically driven into a nanochannel in a folded state. They quantified the degree of stretching in the overlapping portion of the molecule and monitored the internal dynamics as the DNA unfolded to its equilibrium linearly ordered state. In a more recent study, Alizadeheidari et al. examined the unfolding dynamics of a circular DNA molecule upon transformation to a linear topology by a light-induced double-strand break, and in addition compared the equilibrium conformational statistics of the linear and circular configurations. These experimental studies have been complemented by molecular dynamics simulation studies of unfolding of flexible polymer chains in cylindrical and square nanochannels, where the unfolding time was determined to scale as $DN^2$, where $D$ is the channel width and $N$ is the polymer length.

The tendency for a nanochannel-confined polymer to unfold arises from the excluded volume interactions between the portions of the molecule that overlap along the channel. These interactions tend to stretch the overlapping regions and significantly reduce the number of configurations available to the polymer, thus decreasing its conformational entropy. The resulting gradient in the free energy with respect to the degree of overlap provides the effective force that drives the unfolding. This effect is closely related to the segregation of two initially overlapping polymers confined to a narrow channel, a process that is also driven by the increase in conformational entropy as polymer overlap decreases. Such entropy-driven polymer separation is thought to be a factor in the process of chromosome segregation of replicating bacteria and has been extensively studied using computer simulation methods. Another related process is the arm retraction and escape transition for channel-confined star polymers.

Theoretical analyses of unfolding or segregation dynamics obtained from simulations typically employ analytical approximations using scaling arguments for the conformational free energy and its variation with the degree of overlap along the channel. However, such approximations are known to suffer from finite-size effects for the system sizes typically employed in these simulations. For this reason, it is of interest to calculate the free energy functions directly and quantify any discrepancy with the theoretical predictions. Recently, we employed Monte Carlo methods to measure the free energy functions for segregating polymers in nanotubes and examined the scaling of the functions with respect to polymer contour length, persistence length, and channel dimensions for both infinite-length and finite-length tubes.

In the present study, we carry out similar calculations for a single folded polymer confined to a cylindrical channel. We consider several different variations of the system. First, we examine a freely jointed polymer chain similar to that employed in previous MD studies of unfolding. We also study a confined three-arm star polymer and examine the effect of folding one of the arms on the conformational free energy. In this case, the results are relevant to the dynamics of arm retraction in star polymers, which was the focus of a recent simulation study by Milchev et al. In addition to fully-flexible polymers, we also examine semiflexible chains with hairpin folds. Such systems have been the subject of much recent study in the context of the backfolded Odijk regime, for which the persistence length $P$ is of the order of the channel width $D$. In the present case, we consider instead the regime where the condition for the classic Odijk regime, $P \gg D$, is marginally satisfied and, thus, where the presence of a hairpin clearly represents an out-of-equilibrium state. We consider both single hairpins and S-loops.
composed of two hairpins. Figure 1 shows simulation snapshots of the various systems that were examined in this study. The focus in all cases is the measurement of the gradient in the free energy with respect to the degree of internal overlap along the channel, which determines the effective force that drives unfolding. The scaling properties of the free energy are compared with the analytical approximations. While this work is clearly relevant to previous studies in which the free energy of nanochannel-confined polymers was calculated, to our knowledge it is the first to measure the variation of the free energy with the degree of internal overlap of the polymer.

This article is organized as follows. First, we briefly describe the model employed in the study, following which we outline the MC method used to calculate the free energy functions. We then present the main results of the study, which are interpreted and discussed in detail. Results for fully flexible linear polymers and star polymers are presented, followed by those for semiflexible chains in the presence of either a single hairpin fold or an S-loop. In the final section, we summarize the key findings of this study.

Model

We employ a minimal model to describe a polymer confined to a cylindrical tube. The polymer is modeled as a chain of hard spheres, each with diameter $\sigma$. The pair potential for non-bonded monomers is thus $u_{\text{nb}}(r) = \infty$ for $r \leq \sigma$ and $u_{\text{nb}}(r) = 0$ for $r > \sigma$, where $r$ is the distance between the centers of the monomers. Pairs of bonded monomers interact with a potential $u_{\text{b}}(r) = 0$ if $0.9\sigma < r < 1.1\sigma$ and $u_{\text{b}}(r) = \infty$, otherwise. Consequently, the bond length fluctuates slightly about its average value. In addition to linear polymers, we also consider a fully flexible three-arm star polymer with arms of equal length, each connected to one core monomer. The interactions are all the same as for the linear polymer case. The polymer is confined to a hard cylindrical tube of diameter $D$. Thus, each monomer interacts with the wall of the tube with a potential $u_w(r) = 0$ for $r < D$ and $u_w(r) = \infty$ for $r > D$, where $r$ is the distance of a monomer from the central axis of the cylinder. Thus, $D$ is defined to be the diameter of the cylindrical volume accessible to the centers of the monomers.

Most of the simulations in this study employ fully flexible polymer chains. However, we also consider the effects of bending stiffness for linear polymers. To do this, we employ a bending potential associated with each consecutive triplet of monomers. The potential has the form, $u_{\text{bend}}(\theta) = \kappa(1 - \cos \theta)$. The angle $\theta$ is defined at monomer $i$ such that $\cos \theta_i = \hat{u}_i \cdot \hat{u}_{i+1}$, where $\hat{u}_i$ is a normalized bond vector pointing from monomer $i-1$ to monomer $i$. The bending constant $\kappa$ determines the stiffness of the polymer and is related to the persistence

![Figure 1: Simulation snapshots illustrating the main systems considered in this study: (a) fully flexible linear polymer; (b) fully flexible three-arm star polymer; (c) semi-flexible linear polymer with a hairpin turn; (d) semi-flexible linear polymer with an S-loop. The images were generated using VMD.](image-url)
length \( P \) by \( \exp(-\langle l_{\text{bond}} \rangle/P) = \coth(\kappa/k_B T) - k_B T/\kappa \). For our model, the mean bond length is \( \langle l_{\text{bond}} \rangle \approx \sigma \). For the large bending stiffness considered in this study (\( \kappa/k_B T \geq 15 \)), this leads to \( P/\sigma \approx \kappa/k_B T - 0.5 \).

**Methods**

For the model systems described above, Monte Carlo simulations were used to calculate the free energy as a function of the end-to-end distance of the polymer, \( \lambda \), as measured along the axis of the confining tube. In the case of the star polymer, \( \lambda \) is defined as the distance of the end of a selected arm to the core monomer. The simulations employed the Metropolis algorithm and the self-consistent histogram (SCH) method.\(^{41}\) The SCH method efficiently calculates the equilibrium probability distribution \( P(\lambda) \), and thus its corresponding free energy function, \( F(\lambda) = -k_B T \ln P(\lambda) \). We have previously used this procedure to measure free energy functions in a study of polymer segregation,\(^{33}\) as well as in simulation studies of polymer translocation.\(^{42-45}\)

To implement the SCH method, we carry out many independent simulations, each of which employs a unique “window potential” of the form:

\[
W_i(\lambda) = \begin{cases} 
\infty, & \lambda < \lambda_i^{\min} \\
0, & \lambda_i^{\min} < \lambda < \lambda_i^{\max} \\
\infty, & \lambda > \lambda_i^{\max}
\end{cases}
\]

(1)

where \( \lambda_i^{\min} \) and \( \lambda_i^{\max} \) are the limits that define the range of \( \lambda \) for the \( i \)-th window. Within each window of \( \lambda \), a probability distribution \( p_i(\lambda) \) is calculated in the simulation. The window potential width, \( \Delta \lambda \equiv \lambda_i^{\max} - \lambda_i^{\min} \), is chosen to be sufficiently small that the variation in \( F \) does not exceed a few \( k_B T \). The windows are chosen to overlap with half of the adjacent window, such that \( \lambda_i^{\max} = \lambda_{i+2}^{\min} \). The window width was typically \( \Delta \lambda = 2\sigma \). The SCH algorithm was employed to reconstruct the unbiased distribution, \( P(\lambda) \) from the \( p_i(\lambda) \) histograms. For further detail of the histogram reconstruction algorithm, see Ref. \(^{41}\).

Polymer configurations were generated by carrying out single-monomer moves using a combination of translational displacements and crankshaft rotations. Trial moves were accepted with a probability \( p_{\text{acc}} = \min(1, e^{-\Delta E/k_B T}) \), where \( \Delta E \) is the energy difference between trial and current states. For simulations of semiflexible chains, reptation moves were also employed. Initial polymer configurations were generated such that \( \lambda \) was within the allowed range for a given window potential. Prior to data sampling, the system was equilibrated. As an illustration, for a \( N=500 \) polymer chain, the system was equilibrated for typically \( \sim 10^7 \) MC cycles, following which a production run of \( \sim 10^6 \) MC cycles was carried out. On average, during each MC cycle a displacement or rotation move for each monomer, as well as one reptation move, is attempted once.

In the results presented below, quantities of length are measured in units of \( \sigma \) and energy in units of \( k_B T \). In addition, the free energy functions obtained from \( F(\lambda) = -k_B T \ln P(\lambda) \) are shifted such that \( F=0 \) at the minimum in all plots of \( F \) vs \( \lambda \).

**Results**

Figure\(^2\) shows free energy functions for a fully-flexible linear polymer of length \( N=500 \) under cylindrical confinement. Results are shown for a variety of tube diameters. By symmetry, the curves all satisfy \( F(-\lambda) = F(\lambda) \), though the figure only shows a narrow range of negative \( \lambda \). Each curve has a single free energy minimum corresponding to the most probable longitudinal end-to-end distance, which is roughly a measure of the average extension length of the polymer along the tube. As expected, the location of the free energy minimum, \( \lambda_{\min} \), shifts to higher \( \lambda \) as \( D \) decreases. For \( \lambda > \lambda_{\min} \), \( F \) rises steeply with increasing \( \lambda \) due to the reduction in conformational entropy associated with stretched conformations. In the regime \( \lambda < \lambda_{\min} \), the increase in \( F \) with decreasing \( \lambda \) gradually becomes linear. This is evident in Fig.\(^2\)b), which shows the variation of the
derivative $dF/d\lambda$ with $\lambda/\lambda_{\text{min}}$, calculated using the functions in Fig. 2(a). As $\lambda$ decreases, $dF/d\lambda$ approaches a constant. The magnitude of $dF/d\lambda$ in this regime increases with decreasing confinement tube diameter, $D$, and consequently the height of the free energy barrier, $\Delta F \equiv F(0) - F(\lambda_{\text{min}})$, does as well. This linear regime corresponds to the case of intramolecular overlap, which is illustrated in Fig. 1(a). As $\lambda$ decreases and the two end-monomers are brought closer together, portions of the polymer are forced to overlap. This may occur with a single backfold, as illustrated in Fig. 1(a), or with two backfolds. The degree of overlap increases as $\lambda$ decreases, leading to a reduction in conformational entropy and a corresponding increase in $F$.

The observed trends in the free energy functions can be interpreted using a scaling theory developed by Milchev et al. In that study, an expression was derived for the free energy function of a single chain in a tube and the predictions were used to interpret results for simulations of a confined three-arm star polymer. The theory employed a free energy function constructed from two terms, one accounting for compression that uses scaling behavior in the semi-dilute regime, and another term of Pincus form that accounts for stretching. Using this functional form in cases with and without a backfold and optimizing the free energy, they predict a transition from a state of uniform compression or expansion about the free energy minimum and a backfolded regime to lie at $\lambda=0.779\lambda_{\text{min}}$ (using the notation of the present study). However, in Fig 2(b), we observe instead a gradual transition between the backfolded regime the uniformly expanded/compressed regime, with the predicted transition point (labeled with a vertical dashed line in the figure) only roughly marking the region of the transition.

Other tests of the accuracy of the theory of Ref. can be made using the simulation results. One such test is the scaling of the entropic force in the backfolded regime. In this regime, it was shown that the variation of the free energy with end-to-end distance is $F(\lambda) = F_0(2^{1/\nu} - p\lambda/\lambda_{\text{min}})$, where $p \approx 0.91$ for a Flory exponent of $\nu \approx 0.588$, and where $F_0 \sim ND^{-1/\nu}$ is the polymer free energy and $\lambda_{\text{min}} \sim ND^{1-1/\nu}$ is the equilibrium extension of the polymer, both obtained from free energy minimization with respect to $\lambda$. (See Eqs. (2), (3) and (8) in Ref. and note the change in notation to match that used here.) Note that the scaling for $F_0$ and $\lambda_{\text{min}}$ is identical to that predicted from the de Gennes blob model for
the free energy energy and extension lengths of a polymer in a tube. From this relation for $F(\lambda)$, it can be easily shown that the entropic force, defined as the magnitude of the derivative of the free energy, $f \equiv |dF/d\lambda|$, is constant with respect to $\lambda$ and scales as $f \sim N^0 D^{-1}$. This prediction also follows from a straightforward application of the de Gennes blob picture in conjunction with an approximation for overlapping confined chains that was suggested in Ref. 24. As illustrated in Fig. 3(a) the non-overlapping region is approximately of length $\lambda$ and is composed of $n_{bl} = \lambda/D$ blobs of $g \approx D^{1/\nu}$ monomers. Likewise, in the overlapping regime, the monomers may be viewed as two strings of blobs of size $D/\sqrt{2}$, since they effectively occupy half the cross-sectional area of the tube. 24 Thus, each blob has $g' \approx (D/\sqrt{2})^{1/\nu}$ monomers, and there are $(N - (\lambda/D)/g)/g'$ of these smaller blobs present. Noting that each blob contributes roughly $k_B T$ to $F$, it follows that $f \equiv |dF/d\lambda| \sim N^0 D^{-1}$.

Figure 4 shows the variation of $f$ with respect to $D$ calculated from linear fits of the free energy functions in the regime $\lambda < 0.5 \lambda_{\text{min}}$. Results for several chain lengths are shown. Consistent with the prediction, there is no significant dependence on $N$, and the data do satisfy a power law scaling. However, a fit to the data yields a scaling of $f \sim N^0 D^{-1.20 \pm 0.01}$. The fitted curve and a curve for the predicted $D^{-1}$ scaling are both overlaid on the data in figure, and the comparison clearly illustrates that the discrepancy of the prediction is significant. A discrepancy of comparable magnitude was observed for the scaling of the free energy gradient in our previous study of entropy-driven polymer segregation. 33 In that case, the scaling of the gradient in the free energy with respect to the center-of-mass separation distance was $f \sim N D^{-1.93 \pm 0.01}$, compared to the predicted scaling of $f \sim N D^{-1.70}$. The physical origins of that discrepancy are likely the same as those for the single folded polymer, which will be discussed below.

In addition to the conformational free energy, we also consider the variation of the mean extension length of the polymer, $L_{\text{ext}}$, as a function of $\lambda$. Figure 5(a) shows results for $N=500$ for various tube diameters, while Fig. 5(b) shows results for various polymer lengths with fixed tube diameter $D=9$. In each graph, the inset shows the derivative $dL_{\text{ext}}/d\lambda$ vs $\lambda$ for each data set. As expected, at sufficiently high $\lambda$, the polymer is stretched relative to typical equilibrium conformations and $L_{\text{ext}} \approx \lambda$. This is evident from the overlapping curves for all $N$ and $D$ and the limiting behavior of $dL_{\text{ext}}/d\lambda \to 1$ at high $\lambda$. In the backfold regime at low $\lambda$, $L_{\text{ext}}$
is dependent on both \( N \) and \( D \). However, independent of \( N \) and \( D \), the curves in this regime are each linear and parallel with one another with a derivative of \( dL_\text{ext}/d\lambda \approx 0.27 \). The transition between the two regimes occurs in the vicinity of \( \lambda \approx \lambda_{\text{min}} \), which lies at lower \( \lambda \) for higher \( D \) (as evident in Fig. 2(a)) and for lower \( N \).

Figure 5: (a) Extension length of the polymer \( L_\text{ext} \) vs end-monomer separation along the cylinder axis \( \lambda \) for a polymer of length \( N = 500 \). Results for several values of the cylinder diameter \( D \) are shown. The inset shows the derivative \( dL_\text{ext}/d\lambda \) vs \( \lambda \). (b) As in (a), except with fixed \( D = 9 \) and for various \( N \).

As for the case of the free energy, the behavior of the extension length curves in the backfold regime can be understood in the context of the scaling theory of Ref. 36. In that case, it was noted that length \( y \) of the overlapping portions of the polymer satisfies \( y = 2^{(1-3\nu)/2\nu}(\lambda_{\text{min}} - \lambda/0.779) \). Approximating \( L_\text{ext} \approx \lambda + y \), it follows that \( dL_\text{ext}/d\lambda \approx 0.18 \), where we use a Flory exponent \( \nu \approx 0.588 \). Thus, the observed independence of \( dL_\text{ext}/d\lambda \) with respect to \( N \) and \( D \) is consistent with the prediction, though the predicted value of the derivative is somewhat of an underestimate.

To summarize, the scaling theory captures the qualitative behavior and some of the quantitative behavior of the free energy and extension length functions. Unsurprisingly for systems of this size, there are quantitative discrepancies. Finite-size effects have been studied and quantified in other studies of polymers confined to narrow channels and suggest that results consistent with blob model predictions emerge only for diameters of \( D > 10 \). In the present case, the extra crowding associated with backfolded polymer decreases the effective size of \( D \) for each overlapping strand, thus potentially amplifying the finite-size effect. This is also likely a cause of the discrepancy between observed and predicted scaling of the free energy gradient with respect to center-of-mass separation for segregating polymers under cylindrical confinement observed in Ref. 33. Another possible problem concerns the validity of the approximation suggested in Ref. 24 that overlapping polymers can each be viewed as strings of blobs of size \( D/\sqrt{2} \), since they effectively occupy half the cross-sectional area of the tube. In recent work, we have carried out simulations to test this assumption and find significant quantitative discrepancies from this prediction. Together with finite-size effects associated with the de Gennes blob model, this factor likely contributes to the observed inconsistency.

There is a third possible source for the discrepancy, for which we now provide evidence to show is not significant. It concerns a point noted in Ref. 36 regarding the possible presence of two backfolds. The calculated free energy of the polymer depends on the end-to-end distance, which determines the degree of polymer overlap. However, configurations with two backfolds with the same \( \lambda \) can produce the same degree of polymer overlap, and it was suggested that these additional configurations lead to logarithmic corrections to the prediction for free energy. It is possible that this effect may contribute to the discrepancies between our mea-
measurements and the predictions. To test this possibility, we have carried out a modified simulation that imposes a single backfold (at most) on the system for any given $\lambda$. To do this, we insert a hard wall cap in the cylinder, at the center of which a single monomer is fixed. During the simulations, we carry out reptation moves in addition to the regular MC trial moves so that the sequence position of the monomer fixed to the cap, $m$, can take a value from 1 to $N$. Employing the SCH method using $m$ as the fluctuating variable, we measure the free energy function $F(m)$. Clearly, there can only be a single backfold, located at the position of monomer $m$. Furthermore, a given value of $m$ corresponds to a unique value of the degree of internal polymer overlap along the tube. If the results obtained using this model are closer to predictions from the scaling theory, then this would provide evidence that the previous discrepancies were partially due to the presence of multiple backfolds. In addition to helping resolve this question, this calculation has the side benefit of clarifying a particular issue in the context of the related process of polymer translocation. Specifically, when a polymer translocates through a nanopore in a barrier, the likelihood of initially capturing the polymer from one of its ends or at another point along the contour in a folded configuration is expected to be affected by the degree of polymer confinement prior to translocation. The present calculation provides a means to quantify the likelihood for a given capture position along the chain i.e. higher $F(m)$ will correspond to a lower likelihood of capture at monomer $m$.\footnote{17}

Figure 6(a) shows the variation in the free energy with the index of the monomer that is tethered to the cap in the semi-infinite cylinder. Results for a polymer length of $N=350$ and several different tube diameters are shown. As expected, $F$ is greatest when $m=N/2$, in which case the polymer subchains on either side of the anchored monomer are of equal contour length, leading to maximum overlap along the tube. (Note that the extension lengths of the subchains along the channel will differ slightly in this case, as noted in Ref.\footnote{36}, and thus a little fewer than $N/2$ monomers of the longer sub-

chain will overlap with those of the other. This leads to a slightly lower free energy maximum at $m=N/2$ than would otherwise be the case.) As the $m$ increases or decreases from this point, the subchain lengths become increasingly unequal, and the shorter subchain overlaps only partially with the longer subchain. In turn, there is less crowding for a greater number of monomers, leading to a decrease in the free energy. For sufficiently small $D$, the variation is linear over most of each half of the curve, though the functions tend to be slightly less linear with increasing $D$.

Figure 6: (a) Free energy vs monomer index $m$ for a flexible polymer confined to a cylinder of diameter $D$ and semi-infinite length. Monomer $m$ is fixed to a hard cap in the cylinder. Results are shown for a polymer of length $N=350$ for several different values of $D$. (b) Derivative $dF/dm$ vs tube diameter $D$. The derivative was calculated from a fit to the free energy functions in (a) in linear regime, as well as data for other polymer lengths. The solid line is a fit of the $N=350$ data to a power-law function, which yielded a scaling of $dF/dm \sim D^{-2.04\pm0.02}$.  

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To understand this effect, the blob model can once again be used. For \( m < N/2 \), the shorter subchain is of length \( m \), while the longer subchain is of length \( N - m \). Clearly, the portion of the subchain that overlaps the short subchain is also of length \( m \), and thus the remaining (i.e. non-overlapping) portion of the longer subchain is of length \( N - 2m \). The confined polymer system then can be viewed as two chains of length \( m \) and one of length \( N - 2m \) with a corresponding free energy of

\[
F_2 \sim 2m (D/\sqrt{2})^{-1/\nu} + (N-2m)D^{-1/\nu}
\]

From which it follows, \( dF/dm \propto N^0D^{-1/\nu} = N^0D^{-1.70} \). By symmetry, the derivative is equal in magnitude and opposite in sign for \( m > N/2 \). Thus, the decrease in the derivative (and, therefore, the free energy barrier height) is qualitatively consistent with this prediction. Figure 6(b) shows the variation in \( dF/dm \) with \( D \) for several different polymer lengths. Consistent with the prediction, there is no dependence on \( N \) except for small finite-size variations at large \( D \). However, a fit to the data for \( N=350 \) yields a scaling of \( dF/dm \propto D^{-2.04 \pm 0.02} \), which represents a significant discrepancy from the predicted scaling. The magnitude of this discrepancy is essentially the same as between the predicted and observed scaling of the data in Fig. 4. We conclude that presence of two backfolds was not the main source of this disagreement.

We now examine the behavior of a confined star polymer. Figure 7 shows free energy functions for a three-arm star polymer of arm-length \( N_{arm}=150 \) for several different values of \( D \). In this case \( \lambda \) is the longitudinal distance between the core monomer and the end-monomer of a single chosen arm. We consider values of \( N_{arm} \) and \( D \) with a sufficiently high free energy barrier that neither of the other arms has an appreciable chance of crossing while the chosen arm is brought to the selected range of \( \lambda \) for each simulation used to calculate \( F(\lambda) \). The curves are qualitatively similar to those of Fig. 2(a). As before, there is a linear regime at sufficiently low \( \lambda \), in which the arm that defines \( \lambda \) is backfolded. One notable difference from the results for the linear polymer is the presence of two free energy minima separated by a small barrier.

This feature has an origin similar to the behavior observed in Ref. 36 for two overlapping polymers confined to a cylinder and both tethered to a cap at one end. In that case, the lowest free energy state corresponds to that of slightly different elongation lengths for the polymers, with a small barrier located at equal extension length separating states where the roles of compressed and elongated polymers are exchanged. In the present case, the two arms of the star polymer connected to the core monomer play the same role, giving rise to the same effect.

![Figure 7: Free energy F vs distance \( \lambda \) between an end-monomer and the core monomer along the cylinder axis \( \lambda \) for a three-arm star polymer.](image)

Results are shown for an arm length \( N_{arm}=150 \) for several values of the cylinder diameter \( D \). The inset shows the derivative \( f \equiv |dF/d\lambda| \) in the linear domain at low \( \lambda \) vs tube diameter \( D \) for systems with \( N_{arm}=150 \) and \( N_{arm}=100 \). The solid lines are power-law fits to each data set, which yielded a scaling of \( f \sim D^{-1.35 \pm 0.01} \) for \( N_{arm}=100 \) and \( f \sim D^{-1.31 \pm 0.01} \) for \( N_{arm}=150 \).

The free energy gradient \( f \) for the linear portion of the free energy function is plotted as a function of \( D \) in the inset of the figure. Results are shown for arm lengths of \( N_{arm}=100 \) and 150. There is a slight dependence of \( f \) on the arm length, with slightly larger values of \( f \) for the greater \( N_{arm} \). Fitting the results to a power law yields a scaling of \( f \sim D^{-1.35 \pm 0.01} \) for \( N_{arm}=100 \) and \( f \sim D^{-1.31 \pm 0.01} \) for \( N_{arm}=150 \).

In principle, these results can be understood using a simple scaling analysis employing the
de Gennes blob model. Consider the case where two arms lie on one side of the core monomer, one of which is the arm selected to define $\lambda$. If this arm has a backfold (while the other does not), then there are three distinct regimes for these two arms along the cylinder, which are labeled in Fig. 3(b). In regime I, the overlapping polymer arms can be viewed as two strings of blobs, each effectively occupying half the cross sectional area of the tube. Following the approach taken in Ref. 24, the blobs can be viewed as being confined to a tube of effective diameter $D/\sqrt{2}$. The span of this regime is approximately $\lambda$. Likewise, in regime II where the backfolded chain lies, the polymer subchains can be viewed as three strings of blobs of effective diameter $D/\sqrt{3}$. Finally, in regime III, there is only one string of blobs for the stretched arm, each of diameter $D$. Varying $\lambda$ will change the number of blobs in each regime, each of which contributes on the order of $k_B T$ to the free energy. Varying $\lambda$ does not affect the number of blobs for the arm on the other side of the core. Following the same approach used earlier to derive the scaling for the backfolded linear polymer, it can easily be shown that the predicted scaling is $f \sim N_{\text{arm}}^0 D^{-1}$. The poor agreement with the measured scaling is not surprising here. In part, it is due to the very small values of $D$ used here, for which the blob model is expected to be inaccurate. In addition and as noted above, the approximation of Ref. 24 employed here has also been shown to suffer quantitative inaccuracies. It is expected that the scaling will improve for larger values of $D$ and $N_{\text{arm}}$. However, such calculations are not computationally feasible for us at present.

Let us now turn to the folding behavior of longitudinally confined semiflexible polymers in the classic Odijk regime. Figure 8 shows free energy functions for semiflexible polymers of length $N=400$ in a cylindrical tube of diameter $D=6$. Results are shown for several values of persistence length $P$ with $P/D \geq 3$, thus marginally satisfying the conditions for the Odijk regime. The functions have a form that is qualitatively different from those for the flexible chains shown in Fig. 2(a). In this case there are two distinct regimes, one corresponding to a deep free energy well at high $\lambda$, and a second with a linear variation of $F$ with $\lambda$ over a broad range of $\lambda$ to the left of the well, with a sharp boundary between the two. Similar results were observed for other values of $N$ (data not shown). As the chains stiffen, the position of the free energy minimum shifts slightly to higher $\lambda$, and its width narrows. Thus, the polymers become more elongated and the fluctuations in the extension length decrease, in accord with established results for confined semiflexible chains in the classic Odijk regime. The increase in $F$ as $\lambda$ decreases from its value at the minimum corresponds physically to the formation of a hairpin turn. The free energy cost of forming the hairpin is dominated by the bending energy of the chain, but there is also a significant entropic contribution as well. The more gradual change in $F$ in the linear regime corresponds to the change in the degree of overlap of the stiff subchains. As $\lambda$ decreases, the overlap increases leading to a reduction in conformational entropy, thus increasing $F$. Figure 8: Free energy vs $\lambda$ for a semiflexible linear polymer of length $N=400$ in a cylindrical tube of diameter $D=6$. Results for several values of the persistence length $P$ are shown.

Figure 9(a) shows $f$ vs $P$ for a fixed tube diameter of $D=6$, while Fig. 9(b) shows $f$ vs $D$ for a fixed persistence length of $P=29.5$. In each case, results for several polymer lengths are shown. No clear dependence on $N$ is evident. The derivatives were calculated from fits to curves such as those in Fig. 8 for the linear regime, where a single hairpin turn was
present and there was partial intrapolymer overlap. Fits to the data suggest a scaling of 
\[ f \sim N^0P^{-\alpha}D^{-\beta}, \]
where \( \alpha = 0.37 \pm 0.01 \) and \( \beta = 1.72 \pm 0.02 \).

A simple explanation for the scaling behavior of \( f \) follows from using arguments similar to those employed by Odijk in a theoretical study of backfolding regimes of DNA in nanochannels.\(^{10}\) Recall that the confinement free energy of a single semiflexible chain in this regime is given by 
\[ F_1(L) \sim k_B T L / l_{\text{def}}, \]
where \( L \) is the contour length of the polymer and the total Odijk deflection length satisfies 
\[ l_{\text{def}} \approx D^{2/3}P^{1/3}. \]
The confinement free energy of a backfolded chain can be approximated 
\[ F_c = F_1(L - h) + F_h + F_{\text{int}}, \]
where the \( h \) is the length of the hairpin fold, \( F_1(L - h) \) is the Odijk free energy for the two subchains outside the fold, \( F_{\text{int}} \) is the interaction between the strands and \( F_h \) is the free energy of the hairpin. In the regime of interest, only \( F_{\text{int}} \) depends on \( \lambda \), so we neglect the other two terms. To estimate \( F_{\text{int}} \), we use an approximation for the free energy of a system of long, hard rigid rods. For that system, the interaction free energy in the 2nd virial approximation is given by 
\[ F_{\text{int}}(L) / k_B T = \langle l^2 \sigma N^2 / V \rangle \sin \gamma \]
for \( N \) rods of length \( l \) and diameter \( \sigma \) confined to a volume \( V \), where \( \gamma \) is the angle between the rods.\(^{59}\) When the rods are highly aligned, 
\[ \langle \sin \gamma \rangle \sim \sqrt{\langle \theta^2 \rangle}, \]
where \( \theta \) is the angle between the rod and the alignment direction. To apply these results to the present system, we treat each deflection length \( l_q \) as a rigid rod and substitute \( l \rightarrow l_q \sim D^{2/3}P^{1/3}. \) In addition, we assume that the alignment arises principally from confinement, such that 
\[ \langle \theta^2 \rangle \sim (D/l_q)^2. \]
Further, we note that \( V \) is the volume over which the intermolecular segments overlap. This is given by 
\[ V \sim l_{\text{ov}}D^2, \]
where the overlap region is \( l_{\text{ov}} \approx (L - h - \lambda) / 2. \) Finally, replacing \( N \) with the number of deflection lengths of the two overlapping segments, \( N \rightarrow 2l_{\text{ov}} / l_{\text{def}}, \) it can be shown that 
\[ F \sim (L - h - \lambda)D^{-5/3}P^{-1/3} \]
plus terms independent of \( \lambda \). Thus, we predict that 
\[ f \sim |dF/d\lambda| \sim N^0D^{-\beta}P^{-\alpha}, \]
where \( \beta = 5/3 \) and \( \alpha = 1/3 \). These predictions are close to the measured values of \( \beta = 1.72 \pm 0.02 \) and \( \alpha = 0.37 \pm 0.01 \). The small quantitative discrepancy may arise from the fact that the system only just marginally satisfies the condition defining the Odijk regime that \( P \gg D \), as well as from other approximations that have been employed. By comparison, in our related previous study of the segregation of overlapping confined semiflexible chains, the scaling exponents for the free energy gradient were measured to be \( \beta \approx 2 \) and \( \alpha \approx 0.37.33\) The larger discrepancy for the scaling with respect to \( D \) in that study is likely due to the narrower tube diameters considered, i.e. \( 2 \leq D \leq 5 \), in contrast to the range here of \( 5 \leq D \leq 7 \). In the present study, the wider tubes correspond to lower packing fraction, for which the second virial approximation is better suited to describe interactions between the chain segments.
We now consider the deep wells in the free energy functions. As noted earlier, the wells are associated with the formation of hairpin turns of confined semiflexible polymers. Figure 10 shows the dependence of the free energy well depth on the polymer persistence length for various polymer lengths. As expected, there is no significant dependence of $\Delta F_w$ on the polymer length, since the well depth is a measure of the free energy required to form a hairpin turn. Over the range of $P$ considered here, we note that $\Delta F_w$ increases roughly linearly with $P$. Overlaid on the data are two theoretical predictions. The first is that for a mechanical model developed by Odijk. In that work, it was noted that the effects of entropic depletion on the hairpin make an appreciable contribution to the hairpin free energy in addition to the bending energy. A more recent and far more rigorous theoretical analysis by Chen used the Green’s function formalism to determine the hairpin free energy and the global persistence length. Results obtained using the latter were found to be consistent with the simulation results of Muralidhar and Dorfman in their study of the backfolded Odijk regime. An analytical representation to Chen’s numerical solution for the hairpin free energy, $F_{hp}$, is shown in the figure. It is qualitatively similar to Odijk’s prediction, though with somewhat lower values of $\Delta F_w$. This arises from the failure of Odijk’s theory to account for the orientational entropy of the hairpin planes. (Note that the calculations for the Odijk predictions were carried out using a correction to an error that was noted by Chen.) As evident in the figure, the predictions of Chen are much closer to the simulation data than that of Odijk. There appears to be a very slight overestimate of $\Delta F_w$ by an amount $\lesssim k_B T$. This effect may be due to discretization of the polymer and may also be associated with the small fluctuating bond length used in our model, neither of which are present in the theoretical model employed in Ref. 49.

We turn finally to a brief look at the behavior of S-loop configurations in nanotubes. The structure of such a formation is illustrated in Fig. 1(d). In order to prevent the formation of single hairpin turns, the polymer ends are tethered perpendicular to the centers of virtual caps. Note that this has the effect of significantly reducing the conformational freedom of the polymer near the ends, which in turn will affect results, especially for short polymers. Figure 11 shows free energy functions for a linear semiflexible polymer chain with such constraints imposed on its ends. Since single hairpins are precluded, the polymer forms an S-loop composed of two hairpin turns as the end-to-end distance $\lambda$ decreases. The functions are qualitatively similar to those for polymers with a single hairpin turn in Fig. 8. In this case, the linear regime at lower $\lambda$ corresponds to end separations where the S-loop is present. As $\lambda$ decreases and the ends approach each other, the overlap region of the loop increases and confor-
mational entropy decreases, leading to an increase in $F$. The depth of the free energy well located at high $\lambda$ corresponds roughly to the free energy cost of forming the S-loop.

Figure 11: Free energy vs $\lambda$ for a semi-flexible cylindrically confined linear polymer with an S-loop. Results are shown for a polymer of length $N=400$, persistence length $P=24.5$ and for several values of the tube diameter $D$.

Figure 12(a) shows the variation of $f$ with $P$ in the linear regime for $D=4$, while Fig. 12(b) shows $f$ with $D$ for $P=24.5$. In each case, results for $N=200$ and $N=400$ are shown. As in the case of the results for a single hairpin turn, there is no significant dependence on the polymer length. A fit to all of the data suggests a scaling of $f \sim D^{-\beta}P^{-\alpha}$, where $\alpha=1.91 \pm 0.03$ and $\beta=0.36 \pm 0.01$.

To interpret these scaling results, we employ the same theoretical approach as that used for the single-hairpin data. In this case, we note that the overlap volume $V \sim l_{ov}D^2$ has an overlap length $l_{ov} \approx (L - 2h - \lambda)/2$, where $L$ is the polymer contour length and $h$ is the length of each hairpin. In addition, since there are three polymer segments in the overlap region rather than two in the case of the single hairpin, there are $3l_{ov}/l_{def}$ deflection lengths in the overlap region, in contrast to $2l_{ov}/l_{def}$ for the single hairpin. Otherwise, the analysis is identical, leading to the same predicted scaling exponents of $\alpha=1/3$ and $\beta=5/3$. In addition, we expect that the ratio of the derivatives for the S-loop and single hairpin is $f_S/f_{hp} = (3/2)^2 = 2.25$.

The predicted scaling exponents are somewhat less accurate for the S-loop that for the single-hairpin. In addition, we find, for example, that $f_S/f_{hp} \approx 3.1$ at $D=6$ and $P=24.5$, which is somewhat larger than the predicted value. The discrepancies likely arise from multiple sources. We note that for fixed $D$, the density of deflection segments is larger for the S-loop, perhaps leading to a reduction in the accuracy of the 2nd virial approximation to describe excluded volume interactions. Further, as noted above, the reduction in orientational freedom of the ends tethered to the virtual end caps likely affect the results. Additional simulations using
Conclusions

In this study, we have used Monte Carlo simulations to study the conformational free energy $F$ of folded polymers in cylindrical channels. Specifically, the simulations were used to measure the variation of $F$ with respect to the end-to-end distance $\lambda$, which determines the degree of internal overlap along the tube. The gradient in the free energy, $f \equiv |dF/d\lambda|$, is the effective force that drives unfolding toward the polymer’s equilibrium state and thus is essential for a meaningful analysis of unfolding kinetics measurements in experiments and simulations. The main goal of this work was to compare the scaling properties of $f$ with predictions from scaling arguments. For fully-flexible chains, we find that the gradient scaling of $f \sim N^0 D^{-1.20 \pm 0.01}$, for chains of up to $N=500$ monomers and cylinder diameters of $D=3-18$, in units of monomer diameter. This differs appreciably from the prediction $f \sim N^0 D^{-1}$, a discrepancy that is likely due to finite-size effects and deficiencies in the approximation used to account for overlap, i.e. that overlapping polymer strands behave as noninteracting polymers in effective tubes of size $D/\sqrt{2}$. A similar and even greater discrepancy was observed for the folding free energy for a single arm of a star polymer. The transition from a uniformly compressed linearly ordered polymer to a backfolded polymer was found to be gradual, in contrast to a recent theoretical prediction. In the case of confined semiflexible polymers in the classic Odijk regime, we find a free energy gradient scaling of $f \sim N^0 D^{-1.72 \pm 0.02} P^{-0.37 \pm 0.01}$, which is close to the prediction $f \sim N^0 D^{-5/3} P^{-1/3}$ obtained by treating interactions between deflection segments at the 2nd virial level. In the case of S-loops, the agreement was somewhat poorer, perhaps due to the higher segment density in the overlap regime and a resulting breakdown in the 2nd virial approximation. Finally, we note that the measured free energy of a hairpin turn was quantitatively consistent with a recent theoretical prediction.

The work carried out in this study can be extended in various directions. One straightforward and useful extension is an examination of other channel shapes. Although the conformational behavior of polymers confined to cylindrical channels has been the subject of a number of theoretical studies, understanding the effects of confinement in rectangular channels tends to be more directly relevant to experimental studies. Recent studies employing square or rectangular channels using computer simulations and scaling theories have highlighted the importance of the channel shape and size on polymer scaling regimes. In the future, it will be useful to employ the methods of the present study to characterize the free energy functions for folded polymers in such channels. Among other points of interest, it is expected that the entropic contributions to the hairpin free energy of semiflexible polymers will differ appreciably from that for cylindrical channels. Another useful extension would be an examination the free energy functions in the case of $P \sim D$. Here, the calculations could be used to quantify the degree of validity of the assumed form of the interaction between overlapping polymer subchains employed in the theory of the backfolded Odijk regime, as well as extend range of verification of Chen’s prediction of the hairpin free energy. Finally, it would be useful to carry out molecular dynamics simulations to examine the ability of employing the entropic force obtained from the free energy functions to accurately describe the kinetics of unfolding.

Acknowledgement This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants Program. We are grateful to the Atlantic Computational Excellence Network (ACEnet) and WestGrid for use of their computational resources.

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