Free energy of a folded semiflexible polymer confined to a nanochannel of various geometries

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Abstract

Monte Carlo simulations are used to study the conformational properties of a folded semiflexible polymer confined to a long channel. We measure the variation in the conformational free energy with respect to the end-to-end distance of the polymer, and from these functions we extract the free energy of the hairpin fold, as well as the entropic force arising from interactions between the portions of the polymer that overlap along the channel. We consider the scaling of the free energies with respect to varying the persistence length of the polymer, as well as the channel dimensions for confinement in cylindrical, rectangular and triangular channels. We focus on polymer behaviour in both the classic Odijk and backfolded Odijk regimes. We find the scaling of the entropic force to be close to that predicted from a scaling argument that treats interactions between deflection segments at the second virial level. In addition, the measured hairpin fold free energy is consistent with that obtained directly from a recent theoretical calculation for cylindrical channels. It is also consistent with values determined from measurements of the global persistence length of a polymer in the backfolded Odijk regime in recent simulation studies.

Introduction

Polymers confined to channels of a width smaller than the radius of gyration stretch along the channel. In recent years, there has been considerable interest in elucidating the conformational behavior arising from such confinement on polymers such as DNA. A thorough understanding of this behavior is expected to be of value in the development of a variety of applications that exploit the effects of confinement on polymers, including DNA sorting, DNA denaturation mapping, and genome mapping.

The conformational behavior of a polymer in a channel is determined by the effective width, \( w \), the persistence length, \( P \), and the contour length, \( L \) of the polymer, as well as the lateral dimension(s) of the channel, \( D \). Several distinct conformational regimes have been identified, each with its own scaling properties for the extension length and its variance, as well as the free energy. For sufficiently narrow channels where \( P/D \gg 1 \), the polymer is highly extended and lies in the Odijk regime with a Gaussian distribution of extension lengths for sufficiently large \( L \). At the opposite extreme of wide channels such that \( D \gg P^2/w \) lies the classic de Gennes regime. Between these limits lie the extended de-Gennes regime and the backfolded Odijk regime. The latter regime was predicted by Odijk and was later fully characterized using computer simulations. For rectangular channels with two independent lateral dimensions, there are additional scaling regimes. The scaling theories used in these studies employ concepts such as deflection segments, hairpins and blobs to de-
scribe local structures that define the separate scaling regimes. Recently, a theory has been developed that avoids such distinctions between microscopic models and accurately describes the statistics of a channel-confined polymer in the range $D \lesssim P^2/w$.\textsuperscript{19} The scaling predictions for the Odijk, backfolded Odijk and extended de Gennes regimes emerge as limiting cases of the more general theory.

Polymer folding in nanochannels results in internal overlap of the polymer along the channel axis. Folding is an essential feature in the backfolded Odijk regime, in which elongated sections of polymer are connected by hairpin turns. Hairpin formation for $D \lesssim P$ has recently been observed in experiments of DNA coated by the protein RecA and confined to rectangular channels.\textsuperscript{20} In addition to folding in equilibrium states, a polymer can be inserted into a nanochannel in a nonequilibrium folded state, whereupon it proceeds to unfold and thereby reduce its internal overlap until it reaches its equilibrium extension. Such conformational relaxation has been observed in various experimental studies of DNA. For example, Levy \textit{et al.} examined the unfolding of DNA that was electrophoretically driven into a nanochannel in a folded state.\textsuperscript{21} Alizadeheidari \textit{et al.} studied the unfolding of an initially circular DNA molecule that was subjected to a light-induced double-strand break.\textsuperscript{22} They observed an increase in the unfolding rate with increasing extension induced by decreasing the buffer concentration and thus increasing the confinement. More recently, Reifenberger \textit{et al.} studied the unfolding dynamics of DNA confined in square channels of width $D=45$ nm such that $P \approx D$.\textsuperscript{23} The unfolding rates are consistent with predictions using a deterministic model that combined the Odijk excluded volume\textsuperscript{14} with numerical calculations of the Kirkwood diffusivity for confined DNA.

DNA unfolding is driven by excluded volume interactions between the portions of the molecule that overlap along the channel axis. These interactions tend to reduce the conformational entropy by an amount that increases as the degree of overlap increases. Computer simulations of unfolding of flexible hard-sphere chains in channels have corroborated this general picture.\textsuperscript{24,25} A steeper gradient in the overlap free energy is expected to increase the unfolding rate. In addition, the scaling properties of the overlap free energy, as well as the free energy of hairpin formation, are integral to theories of the backfolded Odijk regime.\textsuperscript{13,14,26} For this reason, it is of interest to characterize the variation of the conformational free energy with the degree of internal polymer overlap. Recently, we employed Monte Carlo simulations to measure such free energy functions for polymers confined to cylindrical channels.\textsuperscript{27} For the case where $P \gtrsim D$, we found that the scaling of the overlap free energy gradient with respect to $D$ and $P$ was quantitatively consistent with the predicted scaling. In addition, the hairpin free energy was appreciably lower than that predicted by Odijk,\textsuperscript{13,14} a result also observed by Muralidhar \textit{et al.}\textsuperscript{15,16} and was closer to the value predicted by Chen.\textsuperscript{26}

In the present study, we continue the characterization of free energy functions for polymers in the regime $D \lesssim P$, focusing mainly on the effects of varying the channel geometry. In addition to cylindrical channels, we now consider the cases of rectangular and triangular channels. While rectangular channels are the most commonly used in DNA extension experiments, triangular channels are also widely employed.\textsuperscript{28–32} In addition, we consider the effects of varying the asymmetry of the channel cross section, which is known to affect the extension behavior and scaling regimes of rectangular\textsuperscript{18} and triangular\textsuperscript{33} channels. Generally, we find the overlap free energy scales in a manner consistent with the theoretical predictions, with small deviations in the predicted scaling exponents, as observed previously for cylindrical channels.\textsuperscript{27} The asymmetry in the channel shape has negligible effect on the overlap free energy gradient, in contrast to an appreciable decrease in the hairpin free energy with increasing asymmetry. We also find the measured hairpin free energy in rectangular channels is quantitatively consistent with the values obtained from the simulations of Muralidhar \textit{et al.}\textsuperscript{15,16} and significantly less than those predicted by Odijk.\textsuperscript{13,14} Finally, we examine the fluctuations
in the shape and orientation of the hairpin turns that underly this discrepancy. For cylindrical channels, the observed behavior is consistent with that predicted by Chen.\textsuperscript{26}

Model

We employ a minimal model to describe a semiflexible polymer confined to an infinitely long channel. The polymer is modeled as a chain of hard spheres, each with diameter $\sigma$. The pair potential for non-bonded monomers is thus $u_{nb}(r) = \infty$ for $r \leq \sigma$ and $u_{nb}(r) = 0$ for $r > \sigma$, where $r$ is the distance between the centers of the monomers. Pairs of bonded monomers interact with a potential $u_b(r) = 0$ if $0.9\sigma < r < 1.1\sigma$, and $u_b(r) = \infty$, otherwise. The stiffness of the polymer chain is modeled using a bending potential associated with each consecutive triplet of monomers. The potential has the form, $u_{bend}(\theta) = \kappa(1 - \cos \theta)$. The angle $\theta$ is defined at monomer $i$ such that $\cos \theta_i \equiv \hat{u}_i \cdot \hat{u}_{i+1}$, 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 length $P$ by $34 \exp(-\langle l_{bonda}\rangle/P) = \coth(\kappa/k_B T) - k_B T/\kappa$. For our model, the mean bond length is $\langle l_{bonda}\rangle \approx \sigma$.

The channel has constant cross-sectional area and is constructed with hard walls, such that the monomer-wall interaction energy is $u_w(r) = 0$ if monomers do not overlap with the wall and $u_w(r) = \infty$ if there is overlap. We consider walls with cross-sections that are circular (i.e. cylindrical channels), rectangular, and triangular. We define the channel cross-sectional area $A$ to be that for the space within the channel that is accessible to the centers of the monomers. Further, we define the effective channel width to be $D = \sqrt{4A/\pi}$. Thus, in the case of cylindrical channels, the effective channel width is related to its true diameter according to $D = D_{\text{true}} - \sigma$. For rectangular channels, we define the cross-section aspect ratio $r$ as the ratio of the lateral dimensions of the space in the channel accessible to the monomer centers. As an illustration, if the true lateral dimensions are $7\sigma \times 5\sigma$, then the aspect ratio is $r = (6\sigma/4\sigma) = 1.5$ and $D = \sqrt{4(6\sigma \times 4\sigma)/\pi} = 5.53\sigma$. Note that the special case of square channels corresponds to $r=1$. For triangular channels, we consider only channels with cross-sections that are isosceles triangles. We denote the angle of the apex of the triangle as $\theta_{ap}$; thus, the angles of the other two vertices are each $(\pi - \theta_{ap})/2$.

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 channel. The simulations employed the Metropolis algorithm and the self-consistent histogram (SCH) method.\textsuperscript{35} 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 our recent study of polymer folding in cylinders,\textsuperscript{32} as well as in simulation studies of polymer segregation\textsuperscript{36,37} and polymer translocation.\textsuperscript{38-41}

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

$$W_i(\lambda) = \begin{cases} \infty, & \lambda < \lambda_i^{\min} \\ 0, & \lambda_i^{\min} < \lambda < \lambda_i^{\max} \\ \infty, & \lambda > \lambda_i^{\max} \end{cases} \quad (1)$$

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 2–3 $k_B T$. 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,
Polymer configurations were generated by carrying out single-monomer moves using a combination of translational displacements and crankshaft rotations, as well as reptation moves. 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. Most simulations employed a polymer of length $N=200$ monomers, which is sufficient to obtain reliable values of the free energy gradient in the overlap regime and the hairpin free energy. The system was equilibrated for typically $10^7$ MC cycles, following which a production run of $4 \times 10^8$ MC cycles was carried out. On average, during each MC cycle a displacement or rotation move for each monomer and a reptation move is attempted once.

In the results presented below, quantities of length are measured in units of $\sigma$ and energy in units of $k_B T$.

The origins of the qualitative trends observed in Fig. 1 are straightforward and were mostly explained in Ref. [27]. The steep rise in $F$ as $\lambda$ decreases from $\lambda_{\text{min}}$ is associated with the formation of a hairpin turn in the polymer. At the value of $\lambda$ where $F$ is at the top of the well, the polymer has a “candy-cane” shape with a fully formed hairpin. Thus, $\Delta F_W$ is a measure of the free energy associated with hairpin formation. As the polymer stiffens, the energy required to bend the polymer backwards also increases, and this contributes to the increase in $\Delta F_W$ with $P$. Note that there are also important contributions of the entropy of the hairpin to $\Delta F_W$, as will be discussed further below. As $\lambda$ further decreases below the point where the hairpin has formed, portions of the polymer begin to overlap along the channel. As $\lambda$ increases linearly with decreasing $\lambda$ down to $\lambda=0$. At sufficiently high $P$, an intermediate regime is present in which $F$ is approximately constant. This regime occurs over a narrow range of $\lambda$ that increases slightly with increasing $P$.

Figure 1: Free energy functions for a polymer of length $N=200$ in a square channel with $D=6$, where the effective diameter is defined $D \equiv \sqrt{4A/\pi}$ for a channel of effective cross-sectional area $A$, as defined in the text. Results for various persistence lengths are shown. The functions are each shifted such that $F=0$ at the minimum.
decreases the degree of overlap increases, and the excluded volume interactions between these strands reduce the conformational entropy and cause $F$ to increase. The intermediate regime of constant $F$ occurs when one of the two strands is sufficiently short that the probability of a collision with the longer strand is negligible. This arises because the strands on either side of the hairpin initially track along the channel walls on opposite sides of the channel. Only when both strands are sufficiently long will a collision be likely. This avoidance persists longer for stiffer chains, leading to an increase in the width of the nearly-constant-$F$ regime with increasing $P$.

As noted earlier, it is of interest to investigate the dependence of both the free energy gradient in the overlap regime, $f$, and the free energy of the hairpin, $\Delta F_w$, on the key system parameters. These include the polymer persistence and contour lengths, the chain width, $w$, and the channel dimensions. In addition, it is useful to compare the trends for channels of different geometries. To make such a comparison meaningful, we consider results for equal values of $A$, the cross-sectional area of the channel accessible to the monomer centers and, thus, for the same effective channel diameter, $D \equiv \sqrt{4A/\pi}$.

Figure 2(a) shows the variation of $f$ with $P$ for fixed $D=6$, while Fig. 2(b) shows the variation with $D$ for fixed $P=29.5$. In each case, results are shown for cylindrical, square and triangular channels, as well as a rectangular channel with an asymmetry factor of $r=1.5$.

The free energy gradient scales with persistence length as $f \sim P^{-\alpha}$, where $\alpha$ lies in the range $0.37–0.39$ for the different channel geometries. Likewise, the results in Fig. 2(b) show that $f \sim D^{-\beta}$, where $\beta=1.69–1.80$ for the different geometries. Figure 3 shows the variation of $f$ with the polymer width, $w$ at fixed $P/D=4.08$, where $w$ is defined such that $D/w=6$ and $P/w=24.5$ for $w=1$. The scaling is approximately linear, such that $f \sim w^{\gamma}$, where $\gamma=1.05–1.08$ for the different geometries. Note that the results for the scaling with $P$ and $D$ for cylindrical channels were reported earlier.  

The observed scaling of the free energy gradient with $D$, $P$ and $w$ can be understood using the model originally employed by Odijk to describe the backfolding regimes of DNA confined to a channel. This approach was also used recently to analyze folding in simulations of semiflexible polymers in cylindrical channels. The expression for the free energy gradient in the folding regime is derived as follows. The overlapping polymer strands are modeled as rigid rods with a length equal to the Odijk deflection length $l_d \sim (D^2P)^{1/3}$. At the 2nd virial level, the interaction free energy is given by $F^{(int)}/k_B T = (l_d^3 w N^2 / V) \langle |\sin \delta| \rangle$ for $N$ rods of width $w$ confined to a volume $V$, where $\delta$ is the angle between the rods. When the rods are highly aligned, $\langle |\sin \delta| \rangle \sim \sqrt{\langle \theta^2 \rangle}$, where $\theta$ is the angle between the rod and the align-
Figure 3: Free energy gradient $f \equiv |dF/d\lambda|$ vs the polymer width $w$. Results are shown for cylindrical, square and (equilateral) triangular channels for $P/D=4.08$, such that $D/w=6$ and $P/w=24.5$ for $w=1$. The solid lines show fits to the power law $f \sim w^{\gamma}$. The inset shows the free energy well depth vs $w$ for the same systems.

ment direction. Assuming that the alignment arises principally from confinement, it follows $\langle \theta^2 \rangle \sim (D/l_\lambda)^2$. Further, we note that $V$ is the volume over which the intermolecular segments overlap. This is given by $V \sim l_{ov} A$, where $A$ is the cross-sectional area and the overlap region length is $l_{ov} \approx (L-h-\lambda)/2$, where $L$ is the contour length of the polymer and $h$ is the length of the hairpin turn. Using the effective dimension of the channel $D$ such that $A \propto D^2$, and noting that the number of deflection lengths of the two overlapping strands is $N = 2l_{ov}/l_d$, it can be shown that $F \sim (L-h-\lambda) w D^{-5/3} P^{-1/3} + \text{terms independent of } \lambda$. Consequently, the free energy gradient is predicted to scale as

$$f \sim w D^{-5/3} P^{-1/3}. \quad (2)$$

Note that this model predicts that the scaling properties of $f$ are independent of channel geometry. The predicted scaling is close to that observed for each geometry examined. As noted previously, the discrepancy may be due to the fact that the system only marginally satisfies the condition for the Odijk regime that $P \gg D$, the assumption that the deflection segments are sufficiently aligned, modeling the deflection segments as rigid rods, as well as the inadequacy of the 2nd virial approximation for modeling interactions between deflection segments.

Note that the magnitude of the free energy gradient in Fig. 2 tends to be slightly greater for triangular channels compared to that for square or cylindrical channels. This likely arises from entropic depletion near the corners of the triangle, an effect that has been noted previously. This depletion reduces the effective cross-section area, leading to an increase in the likelihood of collisions between overlapping portions of the polymer and thus a greater rate of increase in the free energy with overlap. In Ref. 31 it was observed that the extension length for DNA in triangular channels was greater than that for square channels of the same cross-section. The increase in extension also arises from the same reduction in the effective area. The magnitude of that increase was very small, consistent with the small increase in $f$ observed for triangular channels in the present work.

Figure 4(a) shows the variation of the free energy well depth $\Delta F_w$ with $P$ for fixed $D=6$, and Fig. 4(b) shows the variation with respect to $D$ for fixed $P=29.5$. The well depth monotonically increases with increasing $P$ and decreases monotonically with increasing $D$ for all channel geometries. At any given $D$ and $P$ the values of $\Delta F_w$ for different geometries are very close, though the triangular and rectangular geometries generally have somewhat lower values. Finally, the inset of Fig. 3 shows that $\Delta F_w$ is independent of the polymer width.

As noted earlier, the well depth is a measure of the free energy cost for the formation of a hairpin turn. Two different theoretical studies thus far have examined the behavior of this quantity. In one study, Odijk developed a mechanical model that considered a hairpin turn whose plane is aligned with channel axis. 13 Entropic depletion of the hairpin plane in relation to its transverse motion and azimuthal orientation was used to minimize the free energy and find the associated hairpin turn size for a given channel width and persistence length. Since the model neglects fluctuations in both the size and orientation of the hairpin turn with respect to the channel axis, it is not expected to be of high accuracy, especially in the regime where $P \approx D$. This was borne out in the simula-
Figure 4: (a) Hairpin free energy, $\Delta F_w$, vs $P$ for a polymer in channels of circular, square, rectangular and (equilateral) triangular cross-section. Simulation results are shown for polymers of length $N=200$ and $D=6$. Also shown are the predictions from the theory of Odijk\textsuperscript{13} for cylindrical and square channels, as well as the predictions of Chen\textsuperscript{26} for cylindrical channels. (b) As in (a) except $\Delta F_w$ vs $D$ for polymers of persistence length $P=29.5$. The data for cylindrical channels are taken from Ref.\textsuperscript{27}.

Theoretical predictions of Odijk’s model for cylindrical and square channels, as well as a plot of an analytical representation to Chen’s numerical solution for cylindrical channels are overlaid on the data in Fig. 4. Both predictions capture the same general trends, i.e. $\Delta F_w$ increases with $P$ and decreases with $D$ in roughly the same manner as the simulation data. However, the degree of quantitative agreement with the simulation results differs. In the case of cylindrical tubes (as reported earlier in Ref.\textsuperscript{27}) the predictions of Chen are within 1 $k_B T$ of the measured results, while Odijk’s theory predicts a hairpin free energy that is consistently overestimated by approximately 4 $k_B T$. In the case of square channels, Odijk’s predictions yield a comparable overestimate of the hairpin free energy, except for very stiff chains. Note that Chen’s study does not report results for square or rectangular channels, and no predictions are available for triangular channels. It is noteworthy that Odijk’s model yields values for cylindrical and square channels that differ significantly from one another (except at low $P$), in contrast to the simulation results. Finally, neither theory predicts any change in $\Delta F_w$ with the chain width $w$, in agreement with the trend in the data shown in the inset of Fig. 3. This is to be expected, since $w$ is relevant only to excluded volume interactions, which are not significant in a hairpin turn. The observed invariance of $\Delta F_w$ with respect to $w$ also suggests that the discretization of the chain model used in the simulations (in contrast with the continuum polymer model used in the theoretical studies) is not a likely cause of the discrepancy.

The simulation results presented above correspond to the case where the conditions required for the Odijk regime, i.e. $P \gg D$, are marginally satisfied. However, there has recently been considerable interest in the case where $P \approx D$, in which regime the backfolded Odijk regimes are present.\textsuperscript{15–17} PERM simulations of a channel-confined semiflexible polymer in this regime were used to extract the global persistence length and thus the hairpin free energy. As noted earlier, these calculations yield values that are approximately 5 $k_B T$ lower than Odijk’s prediction.\textsuperscript{16} Figure 5 shows the variation of the hairpin free energy values for square channels with respect to the ratio $D/P$. Also shown are Odijk’s prediction and the difference between the theory and simulation. We ob-
serve a difference of approximately $5 \ k_BT$ in the regime where $P \approx D$, in agreement with Ref. 16.

Figure 5: Hairpin free energy vs $D/P$ for a polymer in a square channel. Simulation results are shown for polymers of length $N=100$ and persistence length $P=24.5$. Also shown are the predictions from the theory of Odijk in Ref. 13 and the difference between these predictions and the simulation results.

As noted in Ref. 26, the poorer predictions of Odijk’s theory in relation to those of Chen arises from the neglect in the former theory to explicitly account for fluctuations in the hairpin size and the orientation of the hairpin plane with respect to channel axis. Figure 5(a) shows the probability distributions of the position of the hairpin tip, $H(2\rho/D)$, for semiflexible polymers in a cylindrical tube. Here, $\rho$ is the transverse distance of the hairpin tip away from the central channel axis. Results are shown for different values of $D/P$. In each case, the distribution is peaked at $\rho=0$, though there is an appreciable probability that the hairpin tip is located away from the center of the channel. The distributions widen with decreasing relative chain stiffness. The results are in agreement with those reported by Chen. Figure 5(b) shows the variation of the quantity $\sigma$ with $D/P$, where $\sigma \equiv \langle \cos 2\phi \rangle$, and where $\phi$ is the angle between the tangent vector $\mathbf{u}$ at the hairpin tip and the radial line segment connecting the tip to the central axis of the channel (see Fig. 4 of Ref. 26). This quantity is a measure of the degree of anisotropy of the projections of $\mathbf{u}$ relative to the radial direction. For hairpins with tips close to the central axis the projections are isotropic and $\sigma=0$, while strong directional alignment is observed when the tip is close to the wall, where $\phi$ tends to $\pi/2$ and $\sigma \to 1$. The curves show a transition between these two limits. As the polymer stiffens and $D/P$ decreases, the entire hairpin becomes more coplanar while maximizing the hairpin size. Hairpins whose tips lie close to the wall become rarer but even more ordered, leading to $\sigma$ decrease closer to the limit of $-1$. The result is the qualitative trend of a reduction of $\sigma$ with increasing $D/P$, while maintaining the boundary conditions of $\sigma = 0$ at $\rho=0$ and $\sigma = -1$ at $\rho=D/2$. The curves are consistent with those presented in Ref. 26.

Figure 6: (a) Hairpin-tip radial distributions for a polymer of length $N=100$ in a cylindrical channel of diameter $D=6$. Results for various persistence lengths are shown. (b) $\sigma$ vs scaled radial distance in a cylindrical channel, where $\sigma$ is a parameter measuring the directional ordering of the tangent vector at the hairpin tip in the transverse plane, as defined in the text and in Ref. 26.
Figure 7 shows the mean radial displacement of the tip, $\langle \rho \rangle$, as a function of $D/P$. Overlaid on the same graph is the orientational order parameter of the hairpin plain at the location of the tip, $\langle P_2 \rangle \equiv \langle P_2 (\cos \theta) \rangle$, where $\theta$ is the angle of the plane relative to the channel axis. Results are shown for both cylindrical and square channels. The increase in $\langle \rho \rangle$ with $D/P$ reflects the narrowing of the distributions with $D/P$ observed in the case of cylindrical channels in Fig. 6. The order parameter $\langle P_2 \rangle$ decreases as the chains stiffen. Note that over the entire range of $D/P$ considered here, $\langle P_2 \rangle$ is appreciably lower than the value $\langle P_2 \rangle = 1$, the value corresponding to perfect alignment of the plane with the channel assumed in Odijk’s model. As noted by Chen, the neglect of the hairpin plane orientational fluctuations that lead to $\langle P_2 \rangle < 1$ is the main factor in the underestimate of the hairpin entropy and the corresponding overestimate of the free energy.

Thus far, most of the results presented have been for maximally symmetric cases of a given channel geometry, i.e., channels with circular, square and equilateral triangular cross sections. Given the importance of asymmetric geometries employed in experiments for both rectangular and triangular channels it is of interest to examine the effects of such asymmetry on the free energy gradient and hairpin free energy. In Figs. 2 and 4 it was shown that $f$ and $\Delta F_w$ for polymers in rectangular channels with an asymmetric ratio of $r=1.5$ scale with $D$ and $P$ in manner consistent with that for square channels. Figure 8(a) shows the variation of $\Delta F_w$ with respect to $r$, while Fig. 8(b) shows the corresponding variation of $f$. Results are shown for two different values of $P$ and two values of $D$. In each case, $\Delta F_w$ decreases approximately linearly with increasing asymmetry, while $f$ is almost constant, though it does decrease very slightly with $r$. The dashed curves overlaid on the data in Fig. 8(a) show the predictions for the hairpin free energy using Odijk’s model for rectangular channels. Generally, the predictions are qualitatively accurate in predicting a monotonic decrease in $\Delta F_w$ with $r$, though they consistently overestimate the value by $2–3 k_B T$ over the range examined. Note that the theoretical predictions for rectangular channels use the approximation of very large asymmetry in the calculation of the entropic contribution to the free energy. Consequently, the results cannot be extrapolated to $r=1$.

How can we understand the fact that $f$ is nearly constant with respect to $r$ at fixed $D$ and $P$? Recall that Eq. (2) was derived for symmetric channels characterized by a single lateral dimension. However, for asymmetric rectangular channels there are two independent channel widths, $D_W$ and $D_H$, which define the asymmetry, $r \equiv D_W/D_H > 1$. To determine the scaling for $f$ with respect to $r$, we first consider the limiting case where $D_W \gg D_H$. As noted by Odijk, the angle $\delta$ describing the relative orientation between interacting deflection segments satisfies $|\langle \sin \delta \rangle| \sim (D_W/l_d)^2$ for this case. Otherwise, the arguments used to derive Eq. (2) are also applicable for rectangular channels. Noting that the cross-sectional area is $A = D_W D_H \propto D^2$, it can easily be shown that:

$$f \sim wD^{-5/3}P^{-1/3}r^{1/6}$$  \hspace{1cm} (3)

Thus, $f$ is predicted to scale with $P$, $w$ and $D$ (for fixed asymmetry $r$) in the same manner as for symmetric channels. As shown in Fig. 2, the measured scaling for $P$ and $D$ for the case of rectangular channels with $r=1.5$ is...
Figure 8: (a) Hairpin free energy, $\Delta F_w$, vs aspect ratio $r$ of a rectangular nanochannel. Results are shown for a polymer with $N=200$ in channels of different cross-sectional areas and for polymers of different $P$. The solid lines of the same color are guides for the eye, while the dashed lines of the same color are predictions from Odijk. (b) The entropic force $f$ vs channel aspect ratio $r$. The solid lines are guides for the eye. The dashed lines are predictions calculated using an approximation that employs Eq. (4) to estimate the scaling of the angle $\delta$ between interacting deflection segments in the calculation of the excluded volume.

Close to this prediction. However, Eq. (3) also predicts that $f$ monotonically increases with $r$ by $\approx 20\%$ over the range $r = 1 - 3$ considered. This constitutes a significant quantitative discrepancy with respect to the simulation result and no doubt arises from imposing the condition $D_W/D_H \gg 1$ in the derivation. To derive a better prediction, we require a more accurate relation for the dependence of the angle $\delta$ on the channel dimensions and persistence length that is valid for smaller values of $D_W/D_H$. This provided by Eq. (B3) of Ref. [14]:

$$\langle |\sin \delta| \rangle \approx \sqrt{\frac{G_D + G_A + 1}{(G_D + 1)(G_A + 1)}},$$

where $G_A \equiv P/D_W$ and $G_D \equiv P/D_H$. As is evident from Fig. 8(b), the predicted free energy gradient is nearly constant with respect to variation in the asymmetry over the range $r = 1 - 3$. While the theoretical prediction fails to reproduce the observed decrease in $f$ with increasing $r$, it is much more accurate than that obtained using Eq. (3), as expected. The remaining discrepancy is likely due to remaining inadequacies in the theoretical model, such as treating the deflection segments as rigid rods and describing the interactions between them using the 2nd virial approximation.

Let us now consider the effects of asymmetry of triangular channels. Specifically, we examine channels whose cross sections are isosceles triangles with an apex angle of $\theta_{ap}$ and the two other angles of $(\pi - \theta_{ap})/2$ each. Figure 9(a) and (b) show the variation of $\Delta F_w$ and $f$ with respect to $\theta_{ap}$, respectively. Results are shown for different values of $P$ and $D$. Again we note the general result that $\Delta F_w$ increases and $f$ decreases with $D$ and $P$ for all values of $\theta_{ap}$, again consistent with the results for symmetric channels in Figs. 2 and 4.

In each case, the hairpin free energy peaks at $\theta_{ap} = 60^\circ$. This is consistent with the expectation that the hairpin plane aligns roughly along the wall between two of the corners of the triangle. For a triangle with different side lengths, the plane is expected to align along the channel wall(s) with the largest side length in order to maximize the hairpin size and thus minimize the energy. When $\theta_{ap}$ is varied for fixed cross-sectional area, the minimum value of this distance between any two corners occurs for an equilateral triangle, i.e. all angles of 60$^\circ$. As $\theta_{ap}$ is increased or decreased from this value, the maximum side length of a channel wall increases, and so the energy decreases. Obviously, the variation in the entropy associated with the azimuthal and longitudinal orientational freedom also contributes to $\Delta F_w$, but the hairpin energy appears to be the dominant factor af-
Figure 9: (a) Hairpin free energy, $\Delta F_w$, vs apex angle $\theta_{ap}$ for a polymer in a triangular nanochannel. Results are shown for a polymer with $N=200$ in channels of different cross-sectional areas and for polymers of different $P$. The solid lines of the same color are guides for the eye. (b) The entropic force $f$ vs apex angle $\theta_{ap}$ obtained from the same free energy functions used in (a). The solid lines are guides to the eye. The inset shows $D_{eff}^{-1,69}$ vs $\theta_{ap}$, where the exponent value is chosen to match the scaling of $f$ with respect to $D$ in Fig. 2(b) for equilateral triangular channels (using an exponent of 5/3 does not appreciably change the result). For each case of $P$ and $D$ shown, the quantity $D_{eff}^{-1,69}$ increases with $\theta_{ap}$ roughly by a factor 2 over the range of apex angles considered. This suggests that $f$ should increase by roughly this factor as well, in contrast to the measured invariance of $f$ with respect to $r$. Thus, this approach fails to account for the trends observed in the present case, in contrast with the case in Ref. 33. This may arise from the fact that we consider much larger $P/D$ ratios in the present study that were used in Ref. 33.

Finally, it is of interest to investigate the effects of channel asymmetry on the orientational behavior of the hairpin plane, which is an important part of the entropic contribution to $\Delta F_w$. Figure 10 shows the probability distribution $p(\phi)$ for (a) square, (b) rectangular, (c) equilateral triangular, and (d) asymmetric triangular channels. Here, $\phi$ is the azimuthal angle describing the hairpin plane orientation around the channel axis. All the results are shown for a fixed channel dimension of $D=6$. In each case distributions are shown for four different persistence lengths. As expected, Fig. 10(a) shows that there are four preferred orientations of the hairpin plane, each located along the diagonal of the square, where the hairpin size is largest (i.e. there are two diagonal directions, each of which corresponds to two hairpin orientations $180^\circ$ apart). The degree of alignment of the plane along the diagonal decreases as the polymer becomes more flexible and the distribution becomes more uniform. The distributions for asymmetric (i.e. rectangular) channels

fecting the qualitative trend for $\Delta F_w$.

Figure 9(b) shows that the free energy gradient is independent of the channel asymmetry. This is a somewhat surprising result for triangular channels, where the effects of entropic depletion near the corners have been shown previously to be appreciable relative to the case of rectangular channels. As noted earlier, entropic depletion leads to a reduction in the effective cross-sectional area of the channel, which in turn should effectively increase the channel dimension $D$. Reinhart et al. developed a measure of the effective area that uses the monomer probability distribution in the transverse plane of the channel. They took the effective channel width to be the geometric average of the full width at half-maximum of the projections of the distributions onto the two axes defined by the eigenvectors of the 2D distribution. We have calculated the same quantity, which we call $D_{eff}$, as a function of apex angle $\theta_{ap}$ for channels of a given dimension $D$ and for polymers of a given $P$. Naively using $D_{eff}$ in place of $D$ in Eq. (2) or (3), one expects $f \sim D_{eff}^{-5/3}$. The inset of Fig. 9 shows $D_{eff}^{-1.69}$ vs $\theta_{ap}$, where the exponent value is chosen to match the scaling of $f$ with respect to $D$ in Fig. 2(b) for equilateral triangular channels (using an exponent of 5/3 does not appreciably change the result). For each case of $P$ and $D$ shown, the quantity $D_{eff}^{-1.69}$ increases with $\theta_{ap}$ roughly by a factor 2 over the range of apex angles considered. This suggests that $f$ should increase by roughly this factor as well, in contrast to the measured invariance of $f$ with respect to $r$. Thus, this approach fails to account for the trends observed in the present case, in contrast with the case in Ref. 33. This may arise from the fact that we consider much larger $P/D$ ratios in the present study that were used in Ref. 33.
in Fig. 10(b) are qualitatively similar to those for the square channels for stiff polymers. As expected, however, the peaks shift to different orientations that correspond to the diagonals of the rectangle. More interestingly, for sufficiently flexible polymers (i.e. \( P \approx D \)) the preferred orientation becomes parallel to the long side of the rectangle. This orientation likely has a slightly higher energy than for an orientation along the diagonal. However, the energy contributes less to the free energy for more flexible chains, and its increase is likely compensated for by an increase in the entropy from the longitudinal orientational fluctuations of the hairpin plane.

Figure 10: Distribution \( p(\phi) \) of the azimuthal angle \( \phi \) for the orientation of the hairpin plane in the plane transverse to the square channel. Results are shown for a polymers of length \( N=100 \) in channels of area \( D=6 \) for various persistence lengths. Results in the separate graphs are for the following cases: (a) square channel; (b) rectangular channel with \( r=1.5 \); (c) equilateral triangular channel (i.e. \( \theta_{ap} = 60^\circ \)); and (d) triangular channel with apex angle of \( \theta_{ap}=70^\circ \).

Figure 10(c) shows the distributions for equilateral triangular channels. The six peaks arise at orientations corresponding to the alignment of the plane close to and along a channel wall between two of the corners (i.e. three identical walls, each associated with two hairpin plane orientations related by a 180° rotation). As for the square channels, the preference for this alignment weakens as the polymers become more flexible and, thus, the distributions become more uniform. Figure 10(d) shows distributions for an isosceles triangle with an apex angle of \( \theta_{ap} = 70^\circ \). For such a seemingly small asymmetry, the effects are pronounced. The preferred orientation are at the two angles where the hairpin plane is parallel to the long side of the triangle, i.e. the side opposite to the apex. The slight preference for orientations parallel to the other walls is evident only in the weak shoulders of the main peaks. Overall, the contrast between the sharp peaks in Fig. 10(d) and the more uniform distributions of if Fig. 10(c) suggest a considerable decrease in orientational entropy with increasing asymmetry. The fact that \( \Delta F_w \) peaks at \( \theta_{ap} = 60^\circ \) suggests that this is more than compensated for by the reduction in energy that comes with larger hairpins.

Conclusions

In this study, we have used Monte Carlo simulations to characterize the conformational free energy of folded polymers confined to long nanochannels. By measuring the free energy \( F \) of a polymer as a function of the end-to-end distance \( \lambda \), we determined the free energy gradient \( f \equiv |dF/d\lambda| \) for polymers in states of internal overlap, as well as the free energy of the hairpin fold. In addition to similar calculations carried out in our previous study using cylindrical channels, we have studied confinement effects for rectangular and triangular channels as well. Generally, we find that \( f \) scales with the channel width \( D \), persistence length \( P \) and effective polymer width \( w \) in a manner consistent with the predictions of Eq. (2). Small deviations in the scaling exponents likely arise from the fact that the conditions for various approximations are only marginally satisfied. Notably, the scaling appears to be independent of the type of channel geometry (i.e. cylindrical, rectangular or triangular). In addition, \( f \) is not appreciably affected by varying the asymmetry of rectangular or triangular channels. The hairpin free energy was not strongly affected by the channel geometry type, though it does exhibit a decrease with increasing channel asymmetry for
both rectangular and triangular channels. The scaling of the hairpin free energy with $P$, $D$ and channel asymmetry $r$ for rectangular channels was qualitatively consistent with predictions from Odijk’s theory,\textsuperscript{13,14} though the quantitative discrepancy was significant. The origin of this discrepancy is the neglect of hairpin shape and orientational fluctuations, as elucidated by Chen\textsuperscript{26} for cylindrical channels and corroborated by analysis of hairpin behavior in this study. Finally, the difference between the predicted and measured hairpin free energy of $\approx 5 k_B T$ from simulations for polymers the backfolded Odijk regime\textsuperscript{15,16} is consistent with the value measured in the present work.

The quantitative characterization of the free energy functions for nanochannel-confined polymers is useful for validating the accuracy of theoretical models used to analyze and interpret experimental results of confined DNA. For example, the expression for the free energy gradient in Eq. (2) has been employed recently in the analysis of both the equilibrium fluctuations in the extension length for singlefold DNA\textsuperscript{20} in rectangular channels and the nonequilibrium unfolding dynamics in square nanochannels.\textsuperscript{23} It is also of value in providing independent confirmation of findings of other simulation studies, such as the observed discrepancy between the predicted and measured hairpin free energy in simulations of the backfolded Odijk regime for rectangular channels. To our knowledge, this is the first direct study of folding properties of polymers in triangular channels, a geometry which has been used extensively in DNA extension experiments. In future work, it will be of value to further examine folding in nanochannels by employing a model that better accounts for the effects of electrostatic interactions present in DNA experiments on the polymer-channel interactions, such as those used in Refs. 44 and 32.

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