Historical perspective

The polymer physics of single DNA confined in nanochannels

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Abstract

In recent years, applications and experimental studies of DNA in nanochannels have stimulated the investigation of the polymer physics of DNA in confinement. Recent advances in the physics of confined polymers, using DNA as a model polymer, have moved beyond the classic Odijk theory for the strong confinement, and the classic blob theory for the weak confinement. In this review, we present the current understanding of the behaviors of confined polymers while briefly reviewing classic theories. Three aspects of confined DNA are presented: static, dynamic, and topological properties. The relevant simulation methods are also summarized. In addition, comparisons of confined DNA with DNA under tension and DNA in semidilute solution are made to emphasize universal behaviors. Finally, an outlook of the possible future research for confined DNA is given.

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Keywords: Polymer physics DNA Nanochannel Confinement Monte Carlo simulation

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http://dx.doi.org/10.1016/j.cis.2015.12.002
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1. Introduction

In recent years, experiments on DNA in nanochannels [1–7] and their relevant applications [8–14] have stimulated the systematic study of the physics of confined DNA molecules. These experiments have been made possible due to concurrent advances in nanofabrication techniques [15–27]. Beyond practical applications, DNA has often been used as a model semiflexible polymer for single-molecule experiments for the purpose of exploring general polymer physics under confinement [28]. Single DNA molecules with well-defined length can be prepared by the molecular biology techniques, and visualization of single DNA molecules is convenient with the aid of various fluorescence dyes. Two examples [2,29] of the visualization of single DNA molecules in two types of confinement: tube-like channels [2], and the slit-like channels [29] are shown in Fig. 1.

One promising application of confining DNA in nanochannels is genome mapping [9,30]. The basic premise, shown in Fig 2, involves labeling specific sequence motifs in DNA molecules by fluorescence dyes, confining and stretching these molecules in nanochannels, and then inferring the number of base pairs between sequence motifs by measuring the distances or the fluorescence intensities between motifs. The sequence motif map can be used directly or facilitate the assembly of short DNA sequences [30]. Many experiments [14,24,31] and simulations [32] have been performed towards the development of this technological platform. Confining DNA in nanochannels has been also applied for other applications, such as DNA sorting [26,33,34], DNA denaturation mapping [11,35], recognizing barcoded DNA [12], and studying DNA-protein interactions [36].

From the viewpoint of polymer physics, confinement is a type of perturbation to polymer systems, providing many fundamental questions to be answered. Intuitively, confining a DNA molecule within a nanochannel will elongate the DNA and slow down its dynamics. Theoretical studies are motivated to obtain more quantitative relationships between the size of channels and resultant DNA physical properties, often expressed as scaling relationships. The dependence of DNA

![Figure 1](image1.png)  
**Fig. 1.** Schematic illustration of single DNA molecules in square/rectangular channels and slit-like channels. The left-bottom image shows experimental results [2] of the averaged intensity of λ-DNA in the 30 nm × 40 nm, 60 nm × 80 nm, 80 nm × 80 nm, 140 nm × 130 nm, 230 nm × 150 nm, 300 nm × 440 nm, 440 nm × 440 nm channels (left to right). The right-bottom image shows experimental results of 2λ-DNA in a 545 nm tall slit-like channel [29].

![Figure 2](image2.png)  
**Fig. 2.** (Top) Illustration of the steps in genome mapping. Adapted from Wang et al. [32] with permission. (Middle) examples of confined λ-DNA with sites labelled. The DNA molecules are coated with cationic-neutral diblock polypeptides to increase stretching and are confined in 150 × 250 nm² channels. The scale bar is 5 μm. Adapted from Zhang et al. [91] with permission. (Bottom) more examples of confined DNA with sites labelled. Image of a single field of view 73 × 73 μm [2]. Adapted from Lam et al. [9] with permission.
extension on channel size has been measured in experiments [2,37–41] and simulations [42–47], and compared with the predictions by Odijk theory [48] and de Gennes theory [49]. These studies have led to the development of theories beyond the classic Odijk and de Gennes theories, including the theory for the extended De Gennes regime [50] and the backfolded Odijk regime [51–53]. The ionic strength dependence of DNA extension in nanochannels has also been investigated by experiments [46,59]. The effects of crowding agents on the conformations of DNA were found to be altered by nanochannels using experiments [54,55] and simulations [56,57]. The force–extension relationship [58–61] and fluctuation in extensions [62–65] in confinement have also been explored. While most experiments involve linear DNA, circular DNA molecules have been used in experiments [66,67] and simulations [68,69] as well. In addition to static properties, the dynamic properties of confined DNA, such as diffusion [29,38,40,70,71], rotation [72], and relaxation [70,73–75], have been extensively studied by experiments and simulations and compared to the predictions by theories. The discrepancy between experimental results and theoretical predictions leads to a modified blob theory for DNA diffusivity in confinement. Confinement within nanochannels has been shown to collapse DNA [76,77], lead to segregation of DNA molecules [78–81], and affect the topological properties of DNA, such as knotting probability, knot size, and knot lifetimes. [82–90]

Several review papers about DNA in nanochannels have been published in recent years, covering the polymer physics of confined DNA [8,92–94], experimental aspects [8,13,92,94,95], and relevant applications [8,13,92,95]. However, recent advances beyond the classic Odijk and de Gennes have yet to be similarly summarized. In this review, we intend to combine these recent advances with the classic theories and present a complete theoretical understanding of the behaviour of DNA under all degrees of confinement. Furthermore, in this review, we draw striking comparisons between polymers in confinement and polymers in other situations, including polymers under tension, in semidilute solutions of polymers, and polymers with topological constraints (knots). Such similarities are sometimes underappreciated in the polymer community, yet the similar physics which are present can be quite instructive. In particular, the knotting problem was found to be essentially similar to the confinement problem [96].

This review starts with simulation methods and techniques frequently applied in the studies of confined DNA, and then moves to the static, dynamic and topological properties of confined DNA. While we focus on presenting recent advances in this topic, we also briefly describe classic theories for completeness. Finally, the similarities between confined DNA and DNA in other situations are discussed.

2. DNA model and simulation method

While a myriad of polymer models and the simulation algorithms exist, we will focus our attention on those which have been used to investigate confined DNA.

To simplify the problem of DNA in nanochannels while retaining the essential physics of the system, theoretical modelling often treats DNA as a homogenous semiflexible chain. Furthermore, the interactions between DNA segments, including the electrostatic interactions, are frequently expressed as purely hard-core repulsions [47] with an effective chain width [97] or other similar repulsive potentials [42,45,75]. The continuous chain needs to be discretized for simulations, and different discretizations result in different levels of coarse-graining.

Using the simplified model for a DNA chain in a nanochannel, the system can be completely described by four parameters:

(i) the persistence length $L_p$;
(ii) the effective chain width $w$;
(iii) the contour length $L$;
(iv) the channel size $D$ for a tube-like channel or the slit height $H$ for a slit-like channel.

There is a good description of these four length parameters for confined DNA in a review paper by Reisner et al. [92], and so here we only briefly introduce these parameters. Realistic values for these four parameters are as follows. The persistence length depends on the ionic strength of the experimental buffer and other factors, while the typical value is 50 nm [98,99]. The definition of persistence length and how persistence length is related to the bending stiffness and orientational correlation can be found in standard polymer physics textbooks [100,101]. An effective chain width, $w$, is used to take account of the hardcore and the electrostatic repulsions between DNA segments, ranging from 10 nm to 3 nm for ionic strengths from 10 mM to 1 M [97]. The DNA molecules used in experiments [2,4,38] are usually λ-DNA or T4-DNA, with contour lengths of approximately 16 μm and 56 μm, respectively. These contour lengths will be increased by florescence labeling [99]. In experiments, the confining dimension of the tube-like channel or slit-like channel typically ranges from 30 to 500 nm [2,4,38]. From the view point of theory, the effective channel size

$$D = D_{\text{real}} - \delta$$  \hspace{1cm} (1)

is more relevant than the real channel size $D_{\text{real}}$ because the centers of monomers are allowed to move in a cross-section of size $(D_{\text{real}}-\delta)$ rather than $D_{\text{real}}$. The DNA–wall depletion width $\delta$ is due to the hardcore repulsion between DNA and walls, and also due to the electrostatic repulsion between DNA and walls in the case the wall is negatively charged. Reisner et al. [92] discussed this depletion width $\delta$ in their review paper. In the simulations of a chain with only hardcore repulsions, the depletion width is simply the chain width, $\delta = w$. Similarly for the slit-like channel, the effective slit height is $H = H_{\text{real}} - w$.

It bears mentioning that obtaining converged simulation results is not an easy task. Very long chains are required so that end effects are negligible and the simulation results can be compared with the theoretical predictions for infinitely long chains. In order to obtain simulation results for these long chains, it is critical to employ efficient algorithms and select or develop polymer models that are coarse-grained at a level that balances precision with computational expense. After briefly describing such models and algorithms, we also discuss their advantages and shortcomings.

2.1. Touching-bead model

The simplest model to capture bending and excluded volume (EV) interactions is the touching-bead model [45,47]. This model can be considered as the continuous version of the lattice model for polymers because the bond length is fixed, yet the angles between successive bonds sample a continuous distribution. In this model (Fig. 3), the chain is represented as a string of $N_{\text{bead}}$ beads connected by $(N_{\text{bead}} - 1)$ inextensible bonds of length $l_b$, corresponding to a contour length $L = (N_{\text{bead}} - 1)l_b$. The bond length equals is equal to the bead diameter, also called the chain width $w$, and for this reason the touching-bead model is named. Beyond connectivity, only the bending energy and hardcore repulsion are considered. The bending energy between two adjacent bonds is used to reproduce the persistence length:

$$g^\text{bend}_{i,i+1}(\theta_{i,i+1}) = \frac{1}{2} \frac{L_p}{l_b} \theta_{i,i+1}^2$$  \hspace{1cm} (2)

where $\theta_{i,i+1}$ is the bending angle between the bonds $i$ and $i + 1$. Hardcore repulsions are applied for every pair of beads and for every bead and the walls of the confining geometry. Hardcore repulsions are implemented in a way that the interaction energy is infinitely large and the conformation is rejected if the distance between two beads is less than the bead diameter and if the distance from a bead to channel wall is less than the radius of the bead.

It is worth pointing out that discretizing a continuous wormlike chain with a bond length $l_b$ as well as using Eq. (2) to reproduce the
persistence length can precisely describe the wormlike chain only when \( l_B \ll L_p \). As the bond length \( l_B \) increases to be comparable to \( L_p \), the discretization model leads to noticeable errors in representing the contour length and the persistence length, as shown in Fig. 4(a). Strictly speaking, each bond does not represent a bond with a contour length \( l_B \), but with an actual contour length \( l_B^{\text{actual}} \) following the equation [101,102]:

\[
l_B^{\text{actual}} = \sqrt{2L_p^2 l_B / L_p - 1 + \exp(-l_B / L_p)}.
\]

The ratio of the actual bond length to the preset bond length is plotted in Fig. 4(b).

The actual persistence length \( L_p^{\text{actual}} \) determined by the bending energy in Eq. (2) is also different from the preset persistence length \( L_p \). Following the standard definition, the persistence length is determined by the correlation length in the orientational correlation along the chain. Then, the actual persistence length can be calculated through the average bending angle over the separation of \( l_B \):

\[
\langle \cos \theta(l_B) \rangle = \frac{\int \exp(-E_{\text{bend}}/kT) \sin \theta \cos \theta d\theta}{\int \exp(-E_{\text{bend}}/kT) \sin \theta d\theta} = \exp\left(-\frac{l_B}{L_p^{\text{actual}}} \right).
\]

Recall that \( E_{\text{bend}} \) is related to the preset persistence length through \( E_{\text{bend}} = (1/2)(L_p/l_B) \theta^2 \). The ratio of the actual persistence length to the preset persistence length is plotted in Fig. 4(c). In a typical simulation with \( l_B = L_p/10 \), the actual bond length and the actual persistence length deviate from the preset values by 0.84% and 0.85%, respectively.

2.2. Freely-jointed rod model

For very thin chains, i.e. \( w/L_p \ll 1 \), the touching-bead model is not computationally efficient because every persistence length needs to be represented by many beads. In addition to the number of beads, the large bending stiffness \( \kappa_{\text{bend}} = L_p/l_B = L_p/w \) as shown in Eq. (2) leads to a small acceptance ratio when performing reptation or crankshaft moves. To overcome the difficulties of the touching-bead model for thin chains, the freely jointed rod model is often employed, depicted in Fig 5(a). In this model, the chain consists of a series of rods without bending energy. Hardcore repulsions are the only interactions that exist between rods. The problem of identifying rod—rod overlaps is equivalent to satisfying three conditions, illustrated in Fig. 5b. First, the distance between axes of two rods must be less than the chain width. Second, we calculate the vector \( \vec{v}_{12} \) that is normal to both rod axes \( \vec{v}_1 \) and \( \vec{v}_2 \). We then determine the plane where \( \vec{v}_1 \) and \( \vec{v}_{12} \) are lying, as shown in Fig. 5b. Both ends of the blue rod (with axis \( \vec{v}_2 \)), A and B, must be located on the different sides of the plane if rods 1 and 2 intersect. Third and finally, both ends of the red rod (with axis \( \vec{v}_1 \)),...
C and D, must be located on the different sides of the plane spanned by $v_2$ and $v_{12}$. For two infinitely long rods, only the first condition is needed to identify overlaps. The second and third conditions are necessary for finite length rods. This algorithm for the excluded volume interaction between rods is fast, but is so at the expense of missing some rare overlap situations.

A key limitation to the freely-jointed rod model is that the bending within a rod (Kuhn length) is ignored. As a result, the freely-jointed rod model is not suitable to study chains in the presence of tight external confinement, $D \leq 4L_k$ since bending within a Kuhn length becomes important in this regime.

2.3. Other polymer models

Polymer models other than the above two models have been used to study confined chains. For example, DNA has been modeled as a string of rods with bending energy [83,104], a hybrid between the touching-bead model and freely-jointed rod model. In these models, the contour length is inextensible. The discretized wormlike chain model [43,44] has also been used to simulate confined DNA, in which the bond connecting two beads is described by a finitely extensible, nonlinear elastic potential (FENE). In the bead-spring model, each bead represents a subchain larger a Kuhn segment, and the spring between beads is used to capture the conformational entropy inside a subchain. Such bead-spring models are more coarse-grained than previously described models, allowing for faster simulation of confined DNA [41,55,73,105–107].

As long as the approximations in the modeling are negligible for a particular parameter set, the simulation results should be independent of the model selected. Usually, simulation results of a newly developed model are benchmarked through comparisons with known theoretical results, such as the distribution of end-to-end distance of ideal chains, the Marko–Siggia equation [108] for the force–extension relationship [105], or through the comparisons with the results from established models. For example, the freely-jointed rod model has been benchmarked [50] against the touching-bead model for chains in a channel with $D > 4L_k \approx 8L_p$.

2.4. Metropolis Monte Carlo simulation

In a typical Metropolis Monte Carlo simulation of a polymer chain, a random conformation is initially generated, and the conformational phase space of the polymer is explored by successive reptation, pivot and crankshaft moves [45,47,109,110]. Fig. 3 illustrates these three types of moves. Both the pivot and crankshaft moves update the polymer conformation globally. These moves are more efficient in sampling conformations than local moves, typically used in Brownian dynamic simulations [111] and some Monte Carlo simulations [42–44]. The reason for the greater sampling efficiency of global moves is straightforward. The computational cost of moving $N$ beads in a step (a global move) is roughly $N$ times that of moving one bead in a step (a local move). Achieving a similar change in the conformation by local moves alone requires much more than $N$ steps. As a result, global moves are efficient in sampling conformations. Owing to simplicity and computational efficiency, these Monte Carlo sampling moves are often used in large computational efforts, such as the precise determination of the Flory exponent [112,113].

2.5. Simplified PERM simulation

Another popular and efficient technique for sampling polymer conformations is known as PERM (Pruned-Enriched Rosenbluth Method). In PERM, polymer conformations are generated by chain growth starting from one bead, and adding beads one by one until the desired length is achieved. The original PERM algorithm was developed by Grassberger [114] for the lattice model and has been applied to study confined polymers [115,116]. To the best of our knowledge, the Dorfman group was the first to apply the PERM simulation with an off-lattice model to study confined polymers [46,53,117,118]. Other groups have also applied PERM simulation with an off-lattice model to study confined polymers [50,84,116]. A recent review of the PERM simulation of polymers can be found in Ref [119].

The basic idea of PERM is that some polymer conformations are duplicated (enriched) or deleted (pruned) during the chain growth in order to increase the sampling efficiency. The statistical weight of each chain is adjusted in order to compensate for the enrichment or deletion of chains. To perform the duplication and deletion in a proper manner, parameter optimization and an initial guess of free energy are needed in the original PERM simulation [114].

Dai et al. [50] simplified the PERM algorithm so that no parameter optimization is required. Only the simplified version is illustrated and described here, while the original PERM algorithm can be found in Ref [114]. Fig. 6 illustrates the simplified PERM algorithm with the touching-bead model. The chains are grown in a batch of $N_C$ chains rather than individually. In the step of adding the $i$-th bead, the bead is added such that the bending angle $\theta$ formed by $(i-2)$-th, $(i-1)$-th, and $i$-th beads follows a Boltzmann distribution $\exp\left(-L_P/L_B^2\right)$. Suppose that $N_D$ chains die after adding $i$-th due to the hardcore repulsion. Then, we randomly select $N_C$ chains from $(N_C-N_D)$ surviving chains and duplicate them. The statistical weight of every chain is reduced by a factor $(N_C-N_D)/N_C$ to account for this duplication. In such a way, the number of chain remains unchanged during the chain growth. Eventually, $N_C$ chain conformations, all with the same weight $W_C$, are used to calculate the quantities of interest.

The simplified version of PERM has some limitations. First, it requires large computer memory. In the simplified version of PERM, many chains must grow simultaneously in a batch, and the number of chains in a batch should be as large as possible to minimize the statistical errors. As a result, the computer memory may be a bottleneck to reduce the statistical error. For example, in our PERM simulation of a chain with 10,000 monomers, the number of chains in a batch is set as 3000,

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**Fig. 6.** Flow chart for the simplified PERM simulation algorithm [50].
which is the maximum number allowed by our computer memory. Second, the simplified version of PERM is only suitable for chains without soft pairwise interactions, i.e., only hardcore repulsions are allowed, because for these chains, every surviving chain conformation (no overlap) has the same Boltzmann weight. Note that the contribution of the bending energy to the Boltzmann weight has already been considered during the biased addition of a monomer to a chain.

2.6. Comparison of simulation methods

Next, we will discuss the relative advantages and disadvantages of the previously described simulation methods. First, PERM simulation gives the free energy directly, while Monte Carlo simulation does not. Note that Monte Carlo simulation is also able to calculate the free energy using techniques such as umbrella sampling [120,121] and thermodynamic integration [122], but these techniques will increase computational cost. As a result, PERM simulation is preferred when the primary simulation objective is the calculation of free energies. Second, Monte Carlo simulation becomes computationally inefficient for thin chains ($w/L_p \ll 1$) with the touching bead model because the acceptance ratio after reptation and crankshaft moves is low due to the large bending energy coefficient $L_p/\theta_0$ as shown in Eq. (2). As a result, in the simulation of thin chains, PERM simulation is preferred (or the freely-jointed rod model). Third, PERM simulation becomes computational inefficient for dense systems because chain growth is more likely to terminate due to hardcore repulsions. Because single chains in good solvent are not typically dense, this issue does not occur for PERM simulation of single chains. However, in crowded systems, such as the mixture of DNA and depletants, PERM simulation is expected to be ineffective.

2.7. Other simulation methods

Dynamic simulations have also been used to investigate confined polymers [41,73]. While dynamic simulations provide information such as diffusion and relaxation, they are usually not as efficient as Monte Carlo simulations in terms of sampling conformations. In addition to sampling conformations in simulations, other numerical methods were also applied to investigate confined polymers. Chen and co-workers applied field-based methods to calculate the segment density (Fig. 7a) and the confinement free energy (Fig. 7b) in a slit [123] or a tube [124]. The basic idea of the field-based methods is as follows. The probability of segments of wormlike chains in space satisfies a partial differential equation [125], analogous to Feynman path integrals in quantum mechanics. Solving the eigenfunctions and eigenvalues of this partial differential equation yields the distribution of segments in space and the confinement free energy. The field-based methods are superior to the method of sampling conformations in the sense that they give exact results for infinitely long chains, ignoring the usually small numerical errors in solving partial differential equations. However, the field-based methods are usually applied to wormlike chains without excluded volume interactions [123,124], due to the difficulty caused by including these interactions.

Another effective numerical method to investigate confined polymers is the randomly-accelerated-particle method developed by Burkhardt, Gompper and coworkers [126–128]. The premise of this method is that each configuration of a strongly confined polymer is interpreted as the position of a randomly accelerated particle in two dimensions. This method yields the prefactors in the scaling behaviors of the free energy, the extension and the fluctuation for $D=0$, and these prefactors are widely adopted in polymer community. This method is only applicable in very strong confinement, because the interpretation of a confined polymer as a randomly accelerated particle in two dimensions assumes the vectors of segments are nearly parallel with the channel walls.

3. Polymer physics of confined DNA

We now turn our attention to the behaviors of DNA in confinement. As we describe the current physical understanding and the theoretical predictions of confined polymers, simulation results are presented to validate theoretical predictions. In addition, the experimental results of extension, fluctuation, diffusion and relaxation of confined DNA are compared to simulations and theory. Bear in mind that experimental systems of DNA in nanochannels involve many unwanted factors that blur the physical picture of confined chains, such as the interaction between DNA and channel walls [129] and the effect of fluorescence dyes on DNA physical properties [99]. Furthermore, DNA molecules in experiments are often too short to follow the long chain behavior [130].

3.1. Static properties of confined DNA

We will begin by first discussing how various scaling regimes for the static properties of confined DNA (Fig. 8) are created by the competition of the three interactions: bending energy, excluded volume (EV) interaction, and confinement. We will note that confinement has two effects. The first (and most obvious) is to restrict the chain to reside within the confining geometry. A secondary effect is that the segments of the chain near the confining walls become aligned with the channel walls.

In the classic Odijk regime, the effect of the alignment of segments by channel walls dominates, and all segments are strongly aligned and are unidirectional. When the bending energy and EV interactions are not strong enough to prohibit backfolding [52,53,131], the chain enters the backfolded Odijk regime. In the backfolded Odijk regime, all segments are also aligned, but the segments are bidirectional due to backfolding. In the partial alignment (transition) regime, the segments close to walls are aligned, while segments further from the walls are randomly orientated. In this regime, the extension is significantly affected by the effect of segment alignment because the fraction of aligned
The confinement reshapess the chain so that one has $V \approx D^3L_p$ in tubes, where $L_p$ is the extension of the chain along the tube, or $V \approx H R$ in slits, where $R$ is the in-plane radius.

For a chain in free space, the Flory-type free energy is built upon Eqs. (5) and (6):

$$F_{\text{bulk}} \approx \frac{R^2}{L_p^2} + \frac{L^2 w}{R^3}. \quad (7)$$

Minimization of $F_{\text{bulk}}$ with respect to $R$ yields the Flory scaling for the polymer size

$$R_{\text{bulk}} \approx L^{5/3} L_p^{1/3} w^{1/5}. \quad (8)$$

As pointed by de Gennes [132], Eq. (8) yields the Flory exponent $3/5$ close to the precise value $0.5876$ but leads to a wrong prediction of the fluctuation around the equilibrium size $\sigma^{2_{\text{fluctuation}}} \approx \left( \frac{\partial F_{\text{bulk}}}{\partial R} - R_{\text{bulk}} \right)^{-1} \approx L_p$.

The proper fluctuation [132] is

$$\sigma^{2_{\text{fluctuation}}} \approx R^2_{\text{blob}} \approx L^{5/3} L_p^{1/3} w^{2/5}. \quad (9)$$

de Gennes [132] explained that while the scalings in $F_{\text{entropy}} \approx R^2/L_p^2$ and $F_{\text{bulk}} \approx L^2 w/R^3$ are not precisely correct, they lead to a scaling of $R_{\text{blob}}$ close to the precise value due to cancellation of errors. The calculation of the fluctuation in size fails to benefit from a cancellation of errors.

A similar situation occurs when the Flory-type free energy is applied to chains in sufficiently wide channels. It predicts the correct scaling in extension but the wrong scaling in fluctuation [133]. The details behind this discrepancy will be presented in the following subsections.

In the following sub-sections, we will describe the physical pictures and predicted scaling behaviors in various regimes, as well as present comparisons of these predicted scalings with simulations and experiments. Note that we only review the results for tube-like channels here, while the case of slit-like channel is similar [47,118]. In addition, we mainly review the results for the infinitely long chains in confinement. For chains of finite lengths, more scaling regimes will appear [53,118].

3.1.1. Classic de Gennes regime

In the classic de Gennes regime, the chain conformation is described by the blob model [49] (Fig. 8). The blob size equals the channel size $D$. Inside each blob, Flory scaling is applied to calculate the contour length inside a blob $L_{\text{blob}} = D^{3/5} L_p^{1/3} w^{1/5}$. Then, the number of blobs is $N_{\text{blob}} = L/L_{\text{blob}} = D^{-3/5} L_p^{1/3} w^{2/3}$. The extension is obtained as:

$$L_{\|} = N_{\text{blob}} D \approx 1.176 L_p^{2/3} w^{1/3}. \quad (10)$$

The prefactor $1.176$ was determined with simulations by Werner and Mehlig [134], while Dai et al. [50] obtained a prefactor $1.05$. The confinement free energy simply equals the number of blobs:

$$F_{\text{conf}} = N_{\text{blob}} \approx 5.04 L_p^{2/3} L_p^{1/3} w^{1/3}. \quad (11)$$

Again, the prefactor $5.04$ was determined by simulations [50]. The fluctuation in extension may be derived by the blob model as well. The fluctuation in size of each blob is proportional to the blob size $\sigma^{2_{\text{conf}}} \approx R^2_{\text{blob}} \approx D^2$. So the total fluctuation is determined by

$$\sigma^{2_{\text{conf}}} = N_{\text{blob}} \sigma^{2_{\text{blob}}} \approx 0.16 L_p^{1/3} w^{1/3}. \quad (12)$$

The prefactor $0.16$ was determined by simulations [103]. The effective spring constant is

$$k^{2_{\text{spr}}} \approx k_T / \sigma^{2_{\text{conf}}} \approx k_T T L^{1/3} D^{-1/3} L_p^{1/3} w^{1/3}. \quad (13)$$
In addition to counting the number of blobs, the extension and confinement free energy has also been derived by another approach. Jun et al. [133] pointed out that the Flory-type free energy by Eq. (4) and Eq. (5) is wrong. They derived the free energy in the classic de Gennes regime by considering a 1-D random walk of blobs [133]:

\[ \left( D - \frac{4}{3} L_p \right)^{2/3} \]

The first term describes the elastic entropy for a 1-D random walk with a step size D, while the second term describes the EV interactions. Eq. (14) yields the same scaling of extension with Eq. (10) and yields the same scaling of free energy with Eq. (11). The effective spring constant in Eq. (13) can be reproduced by \( \frac{1}{2} \pi^2 D^4/3 L_p^2 \).

The scaling of extension \( L_{j}^{ext} \sim D^{-2/3} \) is the classic de Gennes regime was first validated by the ground-breaking Metropolis simulations of Dorfman group [45] as shown in Fig. 9(top). Due to the limitation of the chain length in simulations at that time, the classic de Gennes regime (combined with the extended de Gennes regime) spans a narrow range of the channel width. Good agreement was also observed between the simulation results and Wang et al. [45] and the experimental results by Reisner et al. [2] after adjusting the effective DNA width in experiments as shown in Fig. 9(bottom). Later, PERM simulations [46,50] with an order of magnitude longer chains observed the predicted scaling of extension in wider ranges of the channel size. One example of the simulation results is presented in Fig. 10. The simulation data in Fig. 10 agree with Eq. (10) for \( D \geq 4 L_p \). For \( D \leq 4 L_p \), Eq. (10) underestimates the extension because the blob model ignores the effect of segment alignment close to channel walls, discussed in the beginning of Section 3.1.

The scaling of fluctuation in Eq. (12) was validated by Metropolis simulations [103] and will be shown in Fig. 35, while more precise results could be obtained if the PERM algorithm is applied.

![Fig. 9](image-url)

**Fig. 9.** (Top) Extension of the touching bead model \((w = 4.6 \text{ nm}, L_p = 53 \text{ nm})\) in square nanochannels. \( D_{eff} \) is the effective channel size in Eq. [1]. Symbols: simulations. Thicker solid line: theoretical prediction of Odijk’s regime. Thinner solid line: power-law fit to the data for channel widths ranging from 60 to 120 nm. Dash-dotted line: power-law fit to the data for channel widths ranging from 120 to 200 nm. (Bottom) Comparison of simulation \((w = 4.6 \text{ nm}, L_p = 53 \text{ nm})\) and experimental data [2]. Three widths are used to calculate \( D_{eff} \left( w/L_p \right)\) for the experimental data. Adapted from Wang et al. [45] with permission.

![Fig. 10](image-url)

**Fig. 10.** Extension as a function of the square channel size for three different chain widths. The symbols are from simulations [50] using the touching-bead model with \( 2 \times 10^4 \) beads. The three dashed lines on the right-hand side are plotted based on Eq. (10). The green line on the left-hand side is plotted based on Eq. (23) with prefactor 0.1827.

The scaling of the confinement free energy in Eq. (11) was confirmed by simulations of Dorfman group [46] in Fig. 12. For a relatively thick chain with \( w = 0.2 L_p \) (purple asterisks), de Gennes scaling \( F_D D^{-3/3} \) is reached when \( D \geq 10 L_p \). The de Gennes scaling of confinement free energy was also observed in the simulations by Dai et al. [50] as shown in Fig. 12. The confinement free energy follows Eq. (11) in wide channels (right-hand side of Fig. 12b). Note that the simulation results in Fig. 12 are based on freely-jointed rod model. As a result, the persistence length is replaced by the Kuhn length \( L_k = 2 L_p \).

### 3.1.2. Extended de Gennes regime

There was a debate about the existence of the extended de Gennes regime. This regime was first investigated by Brochard-Wyart and Raphael [135] in 1990, and the confinement free energy was proposed to be \( F \propto L_D^{-3} L_p^{1/3} w^{2/3} \). Later, the same result was reached by Reisner et al. [92] using a Flory-type free energy approach. More recently, Tree et al. found that the prediction \( F \propto L_D^{-3} L_p^{1/3} w^{2/3} \) was inconsistent with the confinement free energy calculated from simulations, which led them to doubt the existence of the extended de Gennes regime [46]. In 2014, Dai et al. [50] pointed out that Tree et al. [46] interpreted the simulation results in an improper manner, and, Dai et al. confirmed the existence of the extended de Gennes regime with their simulation results [50].

The physical picture underpinning the extended de Gennes regime is as follows. One assumption in the classic blob model is that the repulsion between spherical blobs is strong enough to segregate blobs. When the repulsion between spherical blobs is not strong enough to segregate blobs and \( D \geq L_p \), the chain enters the extended de Gennes regime. The boundary between the classic and extended de Gennes regime can be estimated [45,51] by setting the repulsion between two spherical blobs as \( k_B T \), i.e., \( F_{rep} \approx L_b w / D^2 = D^{1/3} L_b^{2/3} w^{1/3} \). The extended de Gennes regime corresponds to \( D > L_b^{1/3} / w \). This regime can be described by replacing the spherical blob with an anisometric blob. In this model [45,51], the shape of a blob is not a sphere but a cylinder, and the length of each blob is increased from \( D \) to

\[ R_{blob}^{ext} \approx D^{2/3} L_p^{2/3} w^{-1/3} \]

such that the EV interaction between two blobs equals \( k_B T [45,51] \). Inside the anisometric blob, the ideal chain scaling is applied: \( L_{blob}^{ext} \approx (R_{blob}^{ext})^3 / L_p = D^{4/3} L_p^{1/3} w^{2/3} \). According to this model, the number of blobs is

\[ N_b^{ext} \approx D^{-4/3} L_p^{1/3} w^{2/3} \]

Then, the extension for the extended de Gennes regime follows [45,51]

\[ L_{j}^{ext} = N_b^{ext} R_{blob}^{ext} \approx 1.176 D^{-2/3} L_p^{1/3} w^{1/3} \]

where \( L_{j}^{ext} \) is the effective length of the bead in the extended de Gennes regime.
The extension in Eq. (17) has the same scaling law with the classic de Gennes regime Eq. (10), and that is the reason why this regime is called as "extended de Gennes regime".

The anisometric model can also be used to derive the confinement free energy. As shown in Fig. 13, the channel walls not only restrict the arrangement of these anisometric blobs, but they also compress each anisometric blob. The compression is due to the fact that an anisometric blob would restore to an isotropic shape in the absence of the channel walls. The free energy contributions from the alignment and compression of blobs are captured by the first and second term in the following equation

\[ F_{\text{conf}}^{\alpha} = 2.4L D^{-4/3} L^{-1} w^{-2/3} + (2\pi^2/3) L_p D^{-2}. \]  

(18)

The first prefactor 2.4 has been determined by the results of simulations [50]. The second prefactor \(2\pi^2/3\) is the theoretical value [123,136]. It can be seen that the second term is the leading term because the ratio of the first term to the second term is \((Dw/L_p^2)^{2/3}\), which is vanishingly small for \(D \ll L_p/w\). The second term, as a leading term, has been neglected in a number of previous studies [46,59,92,135].

The fluctuation in extension in the extended de Gennes regime can be derived by the anisometric blob model through the fluctuation of each anisometric blob [45,51] [103]:

\[ \sigma_{\alpha}^2 \approx \frac{N_{\text{blob}}}{N_{\text{blob}}} F_{\text{conf}}^{\alpha} \approx 0.264L_p. \]  

(19)

The prefactor 0.264 was determined with simulations by Werner and Mehlig [134], while Dai et al. [50] obtained a prefactor 0.28. The independence of fluctuation with respect to the channel size was recently observed in experiments of DNA in nanochannels [65]. Accordingly, the effective spring constant is [45,51]

\[ k_{\alpha} \approx k_{B} T L^{-1} \lambda^{-1}. \]  

(20)

In addition to the anisometric blob model, the Flory-type free energy can be also used to describe the extended de Gennes regime:

\[ F_{\text{conf}}^{\alpha} \approx \frac{L_p}{D} + \frac{L^2}{L_p} + \frac{L^2 w}{D^2 L_p}. \]  

(21)

The application of the above equation in the extended de Gennes regime can be justified in the following manner. The first two terms correspond to the free energy of an ideal chain. If the chain width gradually increases from zero, EV interactions would also gradually increase from zero. When the EV interactions are weak, they can be considered as a weak perturbation to the free energy of an ideal chain and can be captured by the third term in Eq. (21). Therefore, Eq. (21) is valid in the case of weak EV interaction, i.e., the extended de Gennes regime. Eq. (21) can be used to calculate the extension, the fluctuation, and the confinement free energy.

The predictions of extension, fluctuation, and confinement free energy in the extended de Gennes regime have been validated by simulation results [50,103]. Fig. 12 shows that the scaling of extension remains unchanged, while the scaling of confinement free energy gradually varies from \(-D^2\) to \(-D^{5/3}\) during the crossover from the extended de Gennes regime to the classic de Gennes regime \(Dw/L_p^2 < 1\) to the classic de Gennes regime \(Dw/L_p^2 \gg 1\). The boundary between these two Gennes regimes was determined as \(D_{\alpha} \approx 2L_p^2/w\) by the crossing point of two scalings. We highlight that using typical parameters for DNA \(L_p = 50\) nm and \(w = 5\) nm, the critical channel size is \(D_{\alpha} \approx 1000\) nm, which is larger than the most channel sizes in DNA confinement experiments. As a result, the confined DNA in most experiments does not enter the classic de Gennes regime. To specifically validate the first term in Eq. (18), the confinement free energy of the ideal chain, the second term in Eq. (18) is subtracted from the total confinement free energy and the results are plotted in Fig. 12(c).

The independence of fluctuation with respect to the channel size in extended de Gennes regime was observed in simulations [103,137] as well as in experiments as shown in Fig. 14. The simulation results of fluctuation will be shown in Fig. 35.

Recently, the extended de Gennes regime was also studied through the mapping of a confined polymer in this regime to one-dimensional weakly-self-avoided random-walk problem, which has analytic solutions [134]. Using the mapping, Werner and Mehlig successfully derived the scaling of the extension in agree with the ones by Flory theory and the anisometric blob model. Furthermore, such mapping gives the extension and the fluctuation with precise prefactors [134].

3.1.3. Classic Odijk regime

In the classic Odijk regime, the conformation of the DNA molecule is described by the deflection model [48] (Fig. 15), where the chain is frequently deflected by channel walls. The extension and confinement free energy in the Odijk regime are derived as follows [48]. According to the exponential decay of orientation correlation in the semiflexible chain, one has \(\cos \theta = \exp (-\lambda L_p)\), where \(\theta\) is the angle formed by the channel axis and the segment between two deflections, and \(\lambda\) is the average contour length between two deflections (Fig. 15). Because \(\theta\) is a small quantity, the above equation can be approximated as \(1 - \theta^2/2 \approx 1 - \lambda L_p\). Applying \(\theta \approx \sin \theta \approx \lambda L_p\), we obtain the Odijk scaling

\[ \lambda \approx D^2/3 L_p^{1/3}. \]  

(22)

Accordingly, the relative extension follows

\[ L/L = (\cos \theta) \approx 1 - \alpha_{\text{odijk}} (D/L_p)^{2/3}. \]  

(23)

The prefactor \(\alpha_{\text{odijk}}\) has been precisely determined [127,138] for channels with square cross-section \(\alpha_{\text{odijk}} = 0.1827 \pm 0.0001\) and tubes with circular cross-section \(\alpha_{\text{odijk}} = 0.1701 \pm 0.0001\). Recall that these prefactors were obtained from the randomly-accelerated-particle method mentioned in Section 2.7. Since every deflection restricts the chain conformation and contributes a confinement free energy on the order of \(k_{B} T\), the confinement free energy is proportional to the number of deflections:

\[ F_{\text{conf}} \approx L/L \approx \alpha_{\text{odijk}} L D^{-2/3} L_p^{-1/3}. \]  

(24)

The prefactor \(\alpha_{\text{odijk}}\) has been precisely determined [127,138] for channels with square cross-section \(\alpha_{\text{odijk}} = 2.2072\) and tubes with circular cross-section \(\alpha_{\text{odijk}} = 2.3565\).

The fluctuation around the equilibrium extension can be also estimated from the deflection model. It can be considered that the deflection angle fluctuates between 0 and \(\theta \approx D/L\). Then, the extension of each deflection segment fluctuates between \(\lambda\) and \(\lambda \cos \theta\), with the amplitude of fluctuation \(\sigma_\lambda \approx \lambda (1 - \cos \theta) \approx D^2/(2\lambda)\). The fluctuations of \(L/L\) are independent of each other, and hence, the fluctuation of total extension [48,92,138] is

\[ \sigma_\lambda^2 \approx \frac{1}{\lambda} \sigma_\lambda^2 \approx \alpha_{\text{odijk}}^2 LD^2/L_p. \]  

(25)

Again, the prefactor has been precisely determined [127,138] by the randomly-accelerated-particle method for channels with square cross-section \(\alpha_{\text{odijk}} = 0.0096 \pm 0.0002\) and tubes with circular cross-section \(\alpha_{\text{odijk}} = 0.0150 \pm 0.0002\).

The effective spring constant for the force-extension relationship is related to the fluctuation through \(k_{\text{spr}}(6\pi^2) \approx k_{B} T\) because the fluctuation in the free energy is on the order of \(k_{B} T\). As a result, one has

\[ k_{\text{spr}} \approx k_{B} T L^{-1} D^{-2} L_p. \]  

(26)
Validation of the extension in Eq. (23) and the fluctuation in Eq. (25) by simulation results is shown in Fig. 16. The extensions in simulations agree with Eq. (23) for $D/L_p \lesssim 0.4$. The fluctuations in simulations follow Eq. (25) for $D/L_p \lesssim 0.3$. As shown in Fig. 11, the confinement free energy follows Eq. (24) for $D/L_p \lesssim 0.5$.

![Fig. 11. The normalized confinement free energy as a function of the normalized channel size. The dashed line is from an empirical equation proposed by Tree et al. [46] to cover the confinement free energy of a long ideal chain from strong to weak confinement. The symbols are from PERM simulations for $w/L_p = 0.002$ (open upward triangle), 0.005 (open downward triangle), 0.01 (plusses), 0.02 (open diamond), 0.05 (crosses), 0.1 (open pentagons, DNA) and 0.2 (asterisks). The Gauss-de Gennes regime was proposed when the understanding of the extended de Gennes regime was not clear, and it corresponds to the extended de Gennes regime. Adapted from Tree et al. [46] with permission.](image)

The experimental observation of the deflection conformation in the Odijk regime was not easy for DNA because the deflection length is on the order of or less than the persistence length $L_p \approx 50 \, \text{nm}$. As shown in Fig. 17, such deflection conformations can be observed experimentally [139] in the case of F-actin in microchannels, because F-actin has a persistence length on the order of a micron.

### 3.1.4. Backfolded Odijk regime

Due to backfolding, as illustrated in Fig. 18, the extension at $D \approx L_p$ is shorter than the prediction by the deflection model. The simulations of DNA in slits demonstrate that the projected contour length of a long chain on the slit wall follows Eq. [23] for $H \lesssim 2L_p$ [47]. Recall that there is no backfolding for chains in slits. Such a result [47] suggests that if the backfolding does not occur in tube-like channels, the deflection model should be applicable to calculate the extension for $D \lesssim 2L_p$. Hence, efforts have been made to add the effect of backfolding into the deflection models [51–53,140].

Backfolding leads to the formation of S-loop domains. The formation of S-loop domain costs both bending energy and excluded volume interaction energy. The bending energy is mainly due to the two hairpins [140] at both ends of S-loop domain, while the EV interaction is due to high segment density in S-loop domains.

Two approaches have been used to quantify backfolding. The first approach was developed by Odijk [140] and recently examined and refined by simulations [53]. In this approach, the chain in the backfolded Odijk regime is viewed as 1-D random walk in the chain. In analogy to the Flory theory for the 3-D random walk, two parameters are calculated: the step size of random walk and the excluded volume interactions.

The step size of this random walk is defined as the global persistence length $L_g$, which is approximately the average contour length between two occurrences of backfolding in the absence of EV interactions [51]. The global persistence length was followed by $L_g \approx L_{back} \exp (+F_{back})$, where $L_{back}$ is the average contour length inside a backfold, and $F_{back}$ is the free energy cost of backfolding, mainly attributed to bending energy. Odijk [140] derived the expression of $L_g$:

$$L_g = \alpha\pi\exp\left(\frac{\pi}{L_p}\right),$$

$$\tau = \left(\frac{L_p}{6}\right) \left(\sqrt{E_m + 6\sqrt{2}E_m (D/L_p) - E_m}\right),$$

$$T = E_m (L_p/\tau) - 3 \ln \left(\frac{D - r}{D}\right) - \frac{8}{3n},$$

where $\alpha \approx 3.3082$ and $E_m \approx 1.5071$. The global persistence length was numerically calculated from simulations of ideal chains by Muralidhar et al. as shown in Fig. 19. Eq. (27) systematically overestimates the global persistence length, and so Muralidhar et al. [53] modified the expression of $L_g$:

$$L_g = \alpha\pi\exp\left(\frac{T}{4.91}\right).$$

in order to match simulation results.

![Fig. 12. Results from freely-jointed rod simulations of $10^4$ rods, i.e., $L_{back} = 10^4$. (a) Normalized extension as a function of normalized size of a square channel (b) Normalized confinement free energy as a function of normalized channel size. (c) The difference in confinement free energy between a real chain and an ideal chain. The crossover between the extended and classic de Gennes regimes is estimated as $D_{ext} = 0.56L_p^2/\sqrt{w} \approx 2L_p^2/w$. Adapted from Dai et al. [50] with permission.](image)

![Fig. 13. Illustration of the anisometric blob model for the chain in the extended de Gennes regime.](image)
The excluded volume interactions are calculated as follows. For a pair of segments of deflection length $\lambda$, after considering the orientational correlation, the excluded volume interaction is \( \nu \lambda \approx \lambda^2 w D = L_p/C_1/C_2^{1/16}. \) (29)

After knowing the step size $L_g$ and the EV interaction, the free energy of a chain in this regime is \( F = L + \frac{L^2}{L_g} \frac{N^2 \nu \lambda}{L_p D^2}. \) (30)

The first term is the leading-order term as presented in Eq. (24). The second and third terms are the elastic entropy and the EV interaction, respectively. Minimization of $F$ with respect to $L_{||}$ yields \( L_{||} \approx L_g^{1/3}. \) (31)

where $\xi_g$ is a dimensionless parameter that describes the ratio of the volume caused by the excluded volume interactions relative to the volume available for interactions:

\[ \xi_g = \frac{n_{\lambda}^2 \nu \lambda}{L_g D^2} = \frac{L_g w}{D^{1/3} L_p^2}. \] (32)

Here, \( n_{\lambda} = L_g/\lambda \) is the number of deflection lengths in a global persistence length. In the case of $\xi_g > 1$, the EV interactions are strong enough such that backfolding is unlikely to occur, and the chain enters the classic Odijk regime. In the case of $\xi_g < 1$, backfolds can occur, and the extension follows the scaling in Eq (31). Accordingly, the boundary between the classic and the backfolded Odijk regime is specified by $\xi_g = 1$, corresponding to

\[ D_{\text{back}} = L_g^{3/5} w^{3/5} L_p^{-1/5}. \] (33)

The scaling of the extension with respect to the dimensionless parameter $\xi_g$ was confirmed by simulation results [53] in Fig. 20.

The second approach was proposed by Dai et al. [52]. In this approach, the chain is considered as a series of units of contour length $\pi D$, and these units can assume one of two states: the deflection state or the S-loop state, illustrated in Fig. 21. Using this two-state model, analytical calculation becomes feasible based on the Bragg–Zimm cooperativity model [141] or the Ising model. The two input parameters for the cooperativity model are: the excess free energy $F_s$ of a unit in the S-loop state with respect to the deflection state and the free energy cost

![Fig. 14](image1.png)

**Fig. 14.** Semilog plot of the fluctuation in extension $\delta X$ as a function of effective channel size $D_{\text{eff}}$ for rectangular channels with depth $D_1 = 300$ nm and widths $D_2$ ranging from 350 to 750 nm. The experimental mean extension variance (blue □) is the mean of 29 molecules. PERM simulations in the rectangular channels of dimension $D_1 = 300$ nm and $D_2 = 350$ to 750 nm for a contour length $L = 702 \mu$m are included for rectangular channels (black ○) and equivalent square channels (red △) of size $D_{\text{eff}} + w$. The black line corresponds to Eq. (19) with the prefactor 0.264. Adapted from Gupta et al. [65] with permission.

![Fig. 15](image2.png)

**Fig. 15.** Illustration of a semiflexible chain in strong confinement. The average contour length between two deflections is denoted as $\lambda$, and the angle formed by the segment and the channel wall is denoted as $\theta$. The number of deflection lengths in the global persistence length is $n_{\lambda} = L_g/\lambda$. The average contour length between two deflections is denoted as $\lambda$, and the angle formed by the segment and the channel wall is denoted as $\theta$. The number of deflection lengths in the global persistence length is $n_{\lambda} = L_g/\lambda$.

![Fig. 16](image3.png)

**Fig. 16.** (Top) the deviation of the normalized extension from unity as a function of the normalized channel size from simulations. The $L_p/w$ values are 17.2 (green), 35.03 (gold), 71.35 (orange), 145.32 (red), 295.96 (black), 602.74 (gray), 1227.54 (dark-green), and 2500 (brown). The dashed line is Eq. (23). (Bottom) the normalized fluctuation in extension as a function of the channel size from simulations. The dashed line is from Eq. (25) with the prefactor determined by Burkhardt et al. [138] Adapted from Muralidhar et al. [53] with permission.

![Fig. 17](image4.png)

**Fig. 17.** Fluorescence video microscopy images of F-actin fluctuations in microchannels: from left to right, the channels are 20, 10, 5, and 3 $\mu$m wide, respectively, and 1 $\mu$m deep. Top and bottom images were taken in arbitrary time. Scale bar indicates 10 $\mu$m. Adapted from Choi et al. [139] with permission.
2F_u to create an S-loop domain. Here, F_u is the free energy cost of hairpin formation, similar to Eq. (27). The expression of F_u is proposed as

$$F_u = a_1 \frac{6w}{D^2 \xi L_p}$$

(34)

to take account of the excluded volume interactions between partially aligned segments of length nD. Here, a_3 is a prefactor, which is determined as a_3 ≈ 1.08 by simulations for w/L_p = 0.1. The expression of F_u is proposed as

$$F_u = a_1 nL_p/D - a_2,$$

(35)

where the first term captures the bending energy in the hairpin, and the second term is an entropic contribution. The two prefactors a_1 ≈ 1.2 and a_2 ≈ 1.7 are determined by simulations for w/L_p = 0.1. For other chain widths, the values of a_1 and a_2 are slightly varied.

Based on F_s and F_u, the propagation and nucleation parameters are defined as

$$s = \exp(-F_s)$$
$$u = \exp(-2F_u).$$

(36)

In the ground state dominance, the cooperativity model gives the analytic solutions for the average number of S-loop domains per unit contour length f_s and the the average contour length stored in an S-loop domain:

$$f_s = \frac{u}{2nD(1-u)} \left[ \frac{1 + s}{\sqrt{(1-s)^2 + 4su}} - 1 \right]$$

$$L_s = nD \left[ 1 + \frac{1}{2u} (s - 1 + \sqrt{(1-s)^2 + 4su}) \right].$$

(37)

After knowing the frequency and the size of S-loop domains, the extension in the backfolded Odijk regime follows:

$$L_s^{\text{back}} = L_s^{\text{Odijk}} (1 - 2 F_s L_s/3 - nD f_s/3).$$

(38)

In the above equation, the term $-2F_s L_s/3$ is added because the segments in the S-loop state contribute 1/3 of the extension respective to the deflection state. The term $-nD f_s/3$ is added because the segments in hairpin contribute no net extension. Note that the segments in hairpins are considered as the S-loop state (but at junctions) to make the two-state model applicable.

Fig. 20 shows the comparison of Eq. (38) with simulation results [52]. The green line calculated by Eq. (38) overestimates the extension because the formation of C-loops at both ends of the chain is ignored. After considering the reduction of extension by C-loops, the simulation results in the range $D \lesssim 2L_p$ can be explained by theory.

Both approaches are based on the Odijk (reflection) theory and consider the effect of backfolding on the extension. While these two approaches capture some features of backfolding, they have limitations.

In the first approach (Flory-type theory), the chain is considered as a 1-D random walk with a step size $L_g$, based on which the elastic entropy is calculated. The excluded volume interaction (the third term in Eq. (30)) is considered through a mean-field approximation, which may only be valid when the excluded volume interaction can be considered as a weak perturbation, just like the case of the extended de Gennes regime [Eq. (21)]. In this sense, this Flory-type approach should work better as the chain width decreases and work best for $w \rightarrow 0$. On the other hand, it is expected that as the excluded volume interaction becomes stronger, the S-loop domain become less frequent and shorter. Intuitively, a configuration containing rare and short S-loop domains with the domain extension $L_s \ll L_p$ is very different from 1-D random walk with a step size $L_p$ and not suitable to be described by this Flory-type approach. Such configuration should occur when the dimensionless parameter $f_s$ is around one in Fig. 20.

In the second approach, only the occurrence of S-loop is added to the classic deflection model. However, structures more complicated than S-loop can occur. In particular, for thin chains, more than three strands may share the same location in the channel, and such structures are not captured in the S-loop model.

In the backfolded Odijk regime, despite lacking a simple scaling relationship between extension and the channel size, distinct scaling relationships exist as shown in Eq. (31). In this sense, the backfolded Odijk regime is a real scaling regime. On the other hand, the transition zone (the middle part) in Fig. 8 has no clear scaling relationship so far.

Fig. 18. Illustration of backfolding of a chain in confinement. An S-loop domain is created by two occurrences of backfolding.

Fig. 19. The global persistence length as a function of the normalized channel size. The symbols are from PERM simulations. The dashed and solid lines are from Odijk theory without and with rescaling. Adapted from Muralidhar et al. [53] with permission.

Fig. 20. The extension as a function of the dimensionless parameter $\xi$ shown in Eq (32). Adapted from Muralidhar et al. [53] with permission.

State of DNA conformation: DSSSSDSDSDSDD

Fig. 21. A two-state model is applied to the chain in the backfolded Odijk regime. The chain is viewed as a series of units of contour length nD.
and cannot be considered as a scaling regime but just a crossover from the backfolded Odijk regime to the extended de Gennes regime.

3.2. Dynamic properties of confined DNA

3.2.1. Dynamics of a single polymer in bulk

We next move beyond static properties to discuss dynamics of DNA in confinement. We begin by first recalling basic arguments for dynamics of DNA in the absence of confinement. The diffusion of DNA depends on the friction coefficient \( \zeta \) through the Einstein relation

\[
D_{\text{diff}} = \frac{k_B T}{\zeta},
\]

\((39)\)

The relaxation time can be approximated as the time required for the DNA to move a distance of its equilibrium size \( R \) or its fluctuation in size \( \delta R \). Recall that \( \delta R \) is proportional to \( R \) in bulk \[132\].

\[
\tau \approx R^2/D_{\text{diff}} \sim R^2 \zeta. \tag{40}
\]

The relaxation time can also be derived by the dumbbell model, where the chain is simplified as two massless beads connected by a spring. The spring force \( F_{\text{spr}} \approx k_{\text{spr}} R \) is balanced by the drag force \( F_{\text{drag}} \approx \zeta \text{diff} \). Solving the equation \( F_{\text{spr}} + F_{\text{drag}} = 0 \) yields

\[
R(t) \approx R(0) \exp(-t/\tau) \tag{41}
\]

with

\[
\tau \approx \frac{\zeta}{k_{\text{spr}}}. \tag{42}
\]

3.2.2. Hydrodynamic interaction in free space

The Rouse model and Zimm model consider two extreme cases for hydrodynamic interactions within a single DNA molecule. In the more rigorous calculations of dynamic properties, more detailed hydrodynamic interactions (HI) should be considered. Here, HI refers to the force experienced by one particle due to the flow field generated by the motion of another particle. The relationship between the force and the flow field is often calculated based on the Stokeslet approximation.

\[
\zeta_{\text{Rouse}} = N m \zeta_m, \tag{43}
\]

where \( \zeta_m \) is the friction coefficient of a monomer.

In the Zimm model \[143\], the chain is assumed to move like a solid object and the drag force is assumed to proportional to the radius of chain conformation, i.e. the no-draining condition:

\[
\zeta_{\text{Zimm}} \approx \eta R, \tag{44}
\]

where \( \eta \) is the viscosity of solvent.

3.2.2. Hydrodynamic interaction in free space

The Rouse model and Zimm model consider two extreme cases for hydrodynamic interactions within a single DNA molecule. In the more rigorous calculations of dynamic properties, more detailed hydrodynamic interactions (HI) should be considered. Here, HI refers to the force experienced by one particle due to the flow field generated by the motion of another particle. The relationship between the force and the flow field is often calculated based on the Stokeslet approximation.
In this approximation, a point force $F$ generates a flow field $v(r)$ in free space as:

$$v(r) = F / \Omega;$$

where the Oseen tensor is

$$\Omega(r) = \frac{1}{8\pi\eta} \left( \frac{1}{|r|^3} - \frac{r r^T}{|r|^5} \right).$$

This approximation is based on the fact that the far-field flow generated by a force applied on a sphere is independent of the size of sphere. For simplicity, the Oseen tensor is averaged over all orientations:

$$\langle \Omega(r) \rangle = \frac{1}{6\pi\eta r^2}.$$

The pre-averaged Oseen tensor can be applied to calculate the diffusivity using the Kirkwood approximation [144]:

$$D_{\text{diff}} \approx k_B T \sum_{i=1}^{N_m} \sum_{j=1, j \neq i}^{N_m} \left\langle \Omega\left( \frac{r_i - r_j}{|r|^2} \right) \right\rangle,$$

where $r_i$ is the position of $i$-th monomer, and $N_m$ is the number of monomers. The above equation can be understood in the following manner. A force $F$ is uniformly distributed among monomers such that each monomer experiences a force $F/N_m$. Each point force generates a flow field in the space, and these flow fields are linearly superimposed to each other. The velocity of the chain is approximated as the average velocity of monomers. Eq. (48) can be adapted for a continuous chain

$$D_{\text{diff}} \approx \frac{k_B T}{L} \int \frac{\Omega(r) g(r) 4\pi r^2 dr}{L}.$$

where $g(r) = dL/(4\pi r^2 dr)$ is the pair correlation of monomers, and $dL$ corresponds to the contour length of the sub-chain within the spherical shell of thickness $dr$ at distance $r$. The contribution of long-range HI to diffusivity can be inferred from scaling analysis. Considering that $\Omega(r)$ scales as $r^{-1}$, the contribution of long-range HI to diffusivity would be vanishing small if the scaling of $g(r) 4\pi r^2 - r^3$ has an exponent $\beta < 0$. The exponent $\beta$ is 1 for ideal chains and $2/3$ for real chains. Hence, the long-range HI play an important role in the diffusivity of single long polymers. In this sense, the Zimm model is more suitable than the Rouse model to describe the diffusivity of single long polymers. This has been validated by experiments [145].

Note that the Kirkwood approximation is based on a pre-averaged Oseen tensor and will lead to some errors. Recently, Jain and Dorfman found that the Kirkwood diffusivity is always higher than the one calculated by Brownian dynamic simulations with HI, and the maximum error caused by Kirkwood approximation is about 2% for their simulations of confined DNA in the de Gennes regime [146].
3.2.3. Hydrodynamic interaction in confinement

Hydrodynamic interactions are altered in confined geometries due to the presence of the channel walls [147, 148]. A comprehensive review of HI in various confined geometries can be found in Ref. [148]. In square channels of width D, hydrodynamic interactions decay exponentially for \( r > D \) [149]. Accordingly, HI can be considered as screened for \( r \gg D \). In the calculation of the diffusivity, the HI with \( r \geq D \) can be ignored.

In slit-like channels of height H, hydrodynamic interactions decay algebraically as \( \Omega \sim r^{-2} \) for \( r \gg H \) [150, 151]. Whether the scaling for the decay of hydrodynamic interactions results in the screening of long-range HI depends on the spatial distribution of monomers. Rewriting Eq. (49) for slits with \( r \gg H \) (quasi-2D)

\[
D_{\text{diff}} \approx k_B T L Z_\Omega g_2(r) \Omega(r) dr.
\]  

(50)

Here, \( g_2(r) \) is the pair correlation of monomers in two dimensions. Balducci et al. [29] revealed that HI is local (screened) for a real chain considering \( g(r) \sim r^{-1/3} \), and HI is non-local for an ideal chain considering \( g(r) \sim r \). In experiments of confined DNA, DNA usually is seen to follow real-chain behavior. Therefore, the long-range HI of DNA in both tube-like and slit-like channels can be considered as screened.

3.2.4. Diffusivity of confined DNA in slits

In the classic de Gennes regime, the diffusivity can be derived using the blob model. The chain is viewed as a series of blobs, and the friction of each blob is proportional to the blob size. After ignoring the HI between blobs, the diffusivity can be derived as

\[
D_{\text{diff}}^0 \approx \frac{k_B T}{L} \int g_2(r) (1(r)) 2\pi r dr.
\]  

(51)

Here, \( g_2(r) \) is the pair correlation of monomers in two dimensions. Balducci et al. [29] revealed that HI is local (screened) for a real chain considering \( g(r) \sim r^{-1/3} \), and HI is non-local for an ideal chain considering \( g(r) \sim r \). In experiments of confined DNA, DNA usually is seen to follow real-chain behavior. Therefore, the long-range HI of DNA in both tube-like and slit-like channels can be considered as screened.

\[
D_{\text{diff}} \approx k_B T (6 \pi \eta L) h(r)\Omega(r) dr.
\]  

(52)

and \( c_2 \) is a prefactor.

The above result can be reproduced by Eq. (49). Considering that HI is screened, and defining a dimensionless pair correlation function

\[
h(r) \equiv 4\pi r^2 g(r).
\]  

(53)

Eq. (49) becomes

\[
D_{\text{diff}} \approx k_B T (6 \pi \eta L) \int_0^{r_0} (1(r)) h(r) dr.
\]  

(54)

With the dimensionless pair correlation inside a blob derived from the Flory scaling

\[
h(r) \sim c_1 r^{2/3} L_p^{-1/3} w^{-1/3},
\]  

(55)

where

\[
D_{\text{diff}}^0 \approx \frac{k_B T (6 \pi \eta L)}{L}.
\]

3.2.5. Figure captions

- **Fig. 30.** DNA knotting probability as a function of the slit height for DNA with \( L_p = 50 \text{ nm} \) and \( w = 2.5 \text{ nm} \). Reproduced from Micheletti and Orlandini [104] with permission.

- **Fig. 31.** Probability distributions of the sizes of trefoil knots for different confining channel widths. For all curves, the contour length is fixed as \( L = 400 L_p \), and the chain width is fixed at \( w = 0.4 L_p \). The inset shows the total probability of trefoil knots as a function of channel size. The dashed line in the inset shows the probability of trefoil knots in bulk. Adapted from Dai et al. [173] with permission.

- **Fig. 32.** The most probable size of a trefoil knot as a function of the channel size. The solid line corresponds to the minimization of free energy in Eq. (70) with respect to \( L_{\text{ext}} \) using numerical coefficients \( \alpha = 0.1 \) and \( \beta = 0.02 \). The contour length is fixed as \( L = 400 L_p \), and the chain width is fixed as \( w = 0.4 L_p \). Adapted from Dai et al. [84] with permission.

- **Fig. 33.** Sliding motion of a localized knot along a DNA chain confined in a channel. The knot appears a bright spot on fluorescence labelled DNA, and disappears at one end of DNA. Adapted from Metzler et al. [163] with permission.
the diffusivity in Eq. (51) is reproduced. Eq. (54) can be adapted for slit confinement with the replacement of $D$ with $H$:

$$D_{\text{diff}} \approx k_B T \frac{L}{Z H} = 20 \Omega(r) hr(r)$$

(56)

Recently, Dai et al. [152] revisited the blob model for the diffusivity of confined DNA because all experiments of DNA in slits observed a scaling $D_{\text{diff}} \sim H^{\beta}$ with the apparent exponent $\beta$ less than $2/3$ predicted by the blob model. Dai et al. proposed that the pair correlation in Eq. (55) does not capture the rod-like property at the short length scale. Hence, the following pair correlation was proposed:

$$h(r) = \begin{cases} 2 & r < \frac{L_p}{2} \\ \frac{c_1 r^{2/3} L_p^{-1/3} w^{-1/3}}{1} & r \geq \frac{L_p}{2} \end{cases}$$

(57)

With this modified pair correlation, the diffusivity becomes

$$D_{\text{diff}} = D_{\text{deg}} + D_{\text{diff}2} - D_{\text{diff3}}$$

(58)

where

$$D_{\text{diff}2} \approx 2 \ln \left( \frac{L_p}{a} \right) D_{\text{diff}}^0$$

(59)

$$D_{\text{diff}3} = c_2 \left( \frac{L_p}{w} \right)^{1/3} D_{\text{diff}}^0$$

(60)

In Eq. (59), $a$ is the hydrodynamic radius of the chain, which is needed to remove the singularity in the integral of Eq. (56). Eq. (59) approximately corresponds to the diffusivity of a randomly oriented rod with the length $L_p$ and the radius $a$. [153–155] Since $(D_{\text{diff2}} - D_{\text{diff3}})$ is independent of $H$, i.e. scaling as $H^0$, adding it into $D_{\text{diff}} - H^{2/3}$ leads to an apparent exponent less than $2/3$. The apparent exponent approaches $2/3$ with the increasing $H$.

The diffusivity in Eq. (58) has been used to explain simulation [152] (Fig. 23) and experimental results [29] (Fig. 24) of confined DNA. The simulation results correspond to the Kirkwood diffusivities of chain conformations sampled in Monte Carlo simulations [152]. The theoretical predictions agree with simulation and experimental results using the prefactors $c_1 = 2.8$ and $c_2 = 1.68$. Note that the prefactor $c_3$, defined in Eq. (57), is determined from the simulations results of the contour length in blob $L_{\text{blob}}$ versus the slit height. Only the prefactor $c_2$ is used to fit the theoretical prediction of diffusivity with the simulation result.

It is worth mentioning that the modification of the short-scale pair correlation in Eq. (57) should also apply to the static properties, such as the extension as a function of the channel size. The modification has minor effect on statistic properties, but a significant effect on the dynamics properties because the Oseen tensor $\Omega(r) \sim r^{-1}$ makes dynamic properties much more sensitive on the short-scale properties.

Other explanations have been presented regarding the discrepancy between blob theory and experiments in the scaling exponent of DNA diffusivity in slits. Lin et al. suggested this discrepancy is caused by partial hydrodynamic screening of DNA in slits [156]. As shown by the above calculation, the discrepancy is reconciled without considering partial hydrodynamic screening. Jendrejack et al. [107] observed that the scaling exponent of diffusivity is less than 2/3 in their simulation results, and they attributed the discrepancy between their simulation results and the classic blob theory to polymer-wall interactions, which are
not fully represented in the bead-spring model. However, as discussed by Muralidhar and Dorfman [157], the high-resolution touching bead model yields similar results, suggesting that the polymer-wall interactions are unlikely to be the major reason for the discrepancy.

Next, we review the results for the diffusivities in other regimes. The modified blob theory [152] for diffusivity was developed before a clear understanding [50] of the extended de Gennes regime. Later, Muralidhar and Dorfman [157] systematically investigated the diffusivity of polymers in square channels in different regimes by varying the channel size and the relative chain width w/Lp. It was found that the modified blob theory correctly predicts the diffusivity in the extended de Gennes regime using simulation results of thin chains. It was also found that the classic blob theory is sufficient to describe the diffusivity in the classic de Gennes regime using simulation results of thick chains. It is easy to see that for thick chains with w/Lp and a/Lp approaching 1, the correction term (D_{diff} − D_{atm}) in the modified blob theory becomes vanishingly small. For a flexible chain, the correction to the classic blob theory as shown by Eq. (58) becomes unnecessary. It would be interesting to simulate a sufficiently long and thin chain in the classic de Gennes regime and examine whether the correction term (D_{diff} − D_{atm}) is needed.

In the Odijk regime, a deflection segment can be approximated as a rod because it is much shorter than persistence length. For a rod, the friction coefficient for the motion along its axis in the free space [158] is

$$\tau_{\text{rod}} = \frac{2\pi \eta a}{\ln(k T/\eta)}.$$  \hspace{1cm} (61)

where a is the length of rod, and a is the hydrodynamic diameter. The chain is viewed as (L/D) rods with length D, considering that HI for r → D is screened and the HI for r → D is assumed to be unaffected by channels. Then, the diffusivity in the Odijk regime is obtained as [92,159]

$$D_{\text{Diff}}^{\text{Odijk}} \approx k T \frac{\ln(D/a)}{2\pi a}.$$  \hspace{1cm} (62)

The predicted diffusivity for the Odijk regime has been confirmed by simulation results [157,159].

In the backfolded Odijk regime and the transition regime, Tree et al. [159] proposed that the number of segments in the screening volume is not high enough for substantial HI, and hence the diffusivity follows the Rouse-like scaling:

$$D_{\text{Diff}}^{\text{back}} \approx \frac{k T}{\tau_{\text{Rouse}}} L^2.$$  \hspace{1cm} (63)

The predicted diffusivity for the backfolded Odijk regime and the transition regime was also confirmed by simulation results [157,159]. It is intriguing that the extension in the backfolded Odijk regime and transition regime is very sensitive to the channel size, while the diffusivity is insensitive to the channel size [159].

### 3.2.5. Relaxation of confined DNA

As presented in the beginning of Section 3.2, the relaxation time depends on the diffusivity and the spring constant in the force-extension relationship through $\tau \approx k T / (k_{\text{spr}} D_{\text{Diff}})$. The effective spring constants in various regimes have been shown in Eqs. (13), (20), and (26). Combining the diffusivities in Eq. (51), (58), and (62), the relaxation times for polymers confined in tube-like channel are

$$\tau_{\text{odijk}} \approx \frac{2\pi \eta L^2 D^2}{k T L_p \ln(D/a)}.$$  \hspace{1cm} (64)

in the Odijk regime,

$$\tau_{\text{dec}} \approx \frac{6\pi \eta L_p^{1/3} W^{1/3} L^2}{k T D^{1/3}}.$$  \hspace{1cm} (65)

in the classic de Gennes regime, and

$$\tau_{\text{ex}} \approx \frac{6\pi \eta L_p^{1/3} W^{1/3} L^2}{k T L_p^{1/3} D^{1/3} + 2 \ln(L_p/a) - c_2 L_p^{1/3} W^{1/3}}.$$  \hspace{1cm} (66)

in the extended classic de Gennes regime. Eqs. (64) and (65) are identical with the ones in the review paper by Reisner et al. [92], but Eq. (66) is different because the diffusivity is the extended de Gennes regime is modified in Eq. (58).

In the extreme case D → 0, the relaxation time in Eq. (64) approaches zero because the reduction in fluctuation $\sigma_{\text{diff}}^2 \sim D^2$ overwhelms the reduction in the diffusivity $D_{\text{Diff}}^2 \sim \ln(D/\eta)$ in the Odijk regime. In the extreme case D → 0, the relaxation time in Eq. (65) also approaches zero because the increase of diffusivity $D_{\text{Diff}}^2 \sim D^{2/3}$ overwhelms the increases in the fluctuation $\sigma^2 \sim D^{1/3}$. Considering the zero relaxation time in both limits of channel size, the finite relaxation time in the between, it is expected that there is non-monotonic dependence of relaxation time on the channel size, which was observed in experiments [2]. Tree et al. [137] used simulation results of the diffusivity and spring constant of DNA to explain the non-monotonic behavior and the results are in agreement with the experimental data by Reisner et al. [2] as shown in Fig. 25. Because no analytic expression exists for the diffusivity and the fluctuation in the crossover from strong to weak confinement, Tree et al. [137] extracted those data from simulations, and used them in the calculation of relaxation time.

In the above paragraphs, we discuss the relaxation around the equilibrium size. The relaxation of highly-stretched DNA in nanoslit was also investigated by experiments [74] and simulations [72]. Two distinct relaxation regimes were observed with different relaxation times. In the first relaxation regime, highly-stretched DNA is relaxed to a line of blobs. In the second relaxation regime, the blobs are relaxed in two-dimension.

#### 3.3. Topological properties of confined DNA

DNA can form knots in free space [160–162] and confinement [31, 163]. The DNA knots in free space [162,164] and in nanochannels [31, 163] have been directly observed in experiments. Simulation studies have been performed to investigate the effects of confinement on knotting probability [82,89,104,165–167], the knot size [168,169], the knot type [83,88,170] and the knotting/unknotting kinetics [90,171], Orlandini and Micheletti and co-workers performed numerous pioneering simulation studies of knots. Two review papers by Orlandini and Whittington [172], and Micheletti et al. [93] have covered the basic knowledge of knots - polymer physics, relevant simulation techniques and simulation results of knots in free space and confinement. In this section, we focus on describing the recent studies of DNA knots using a theory proposed by Grosberg and Rabin [96] due to two reasons. First, as can be seen below, the topological constraint by knots is fundamentally similar to the spatial confinement, which is the theme of this review. Second, this theory can be extended to understand the size and probability of knots in confined DNA.

Strictly speaking, a knot is only strictly defined on a closed loop (circular chain). However, for linear chains, a tight knot localized in a small portion of a linear chain can be easily identified visually and the application of chain closure schemes allow for it to be located algorithmically. The knots of linear chains are reviewed in the following sub-sections. Recently, free energy analysis has been applied to study the knotting probability. As pointed by Grosberg and Rabin [96], a polymer knot shares a similar free energy to confined DNA because the polymer chain inside the knot core is essentially confined by a virtual tube (Fig. 26). The free energy analysis of Grosberg and Rabin predicts knots on wormlike chains will spontaneously shrink to a tightened size. This prediction was subsequently validated by simulations of Dai et al. [173] Later, Dai et al. extended the Grosberg-Rabin theory to
semiflexible chains with EV interaction [173], flexible chains [174], and chains in confinement [84]. The relevant theories and simulation results are summarized in this section.

3.3.1. DNA knots in free space

Grosberg and Rabin [96] proposed that the sub-chain in the knot core is confined by a virtual tube formed by sub-chain itself (Fig. 26a). The size and shape of the virtual tube can be estimated through the tight knot formed by pulling both ends of a rope with diameter D_{virtual}. The length of virtual tube is related to the diameter of virtual tube by a parameter:

\[ p = \frac{L_{\text{knot}}}{D_{\text{virtual}}} . \]  

(67)

The confinement of the virtual tube leads to a confinement free energy \( F_{\text{conf}} \approx L_{\text{knot}} D_{\text{virtual}} L_p^{-3/2} \), using the Odijk scaling in Eq. (24), where \( L_{\text{knot}} \) is the contour length in the knot core. It will be shown later that the confinement by the virtual tube is strong such that the Odijk scaling is applicable. In addition to the confinement free energy, the sub-chain within the knot has a bending energy contribution scaling as \( F_{\text{bend}} \approx k_3 L_{\text{knot}}^3 \), where \( k_3 \) is the size of knot. For a tight knot, the quantities \( L_{\text{knot}}, R_{\text{knot}}, \) and \( D_{\text{virtual}} \) are proportional to each other, and hence, both \( L_{\text{knot}} \) and \( D_{\text{virtual}} \) can be replaced by \( L_{\text{knot}} \) after ignoring numerical coefficients. Eventually, Grosberg and Rabin [96] obtained the free energy for the knot formation

\[ F_{\text{conf}} = k_1 \left( \frac{L_{\text{knot}}}{L_p} \right)^{1/4} + k_2 \left( \frac{L_{\text{knot}}}{L_p} \right)^{1/3} . \]

(68)

where \( k_1 \) and \( k_2 \) are prefactors that take account of all numerical coefficients ignored in the derivation. The first and second terms in the above equation tend to swell and shrink the knot size, respectively. The competition of these terms leads to a local minimum of free energy, which corresponds to a metastable knot.

The Grosberg-Rabin theory was modified by Dai et al. [173] for real chains with finite thicknesses. For a chain with an effective width \( w \) confined in a tube with a diameter \( D_{\text{virtual}} \), the effective diameter of the confining tube becomes \( (D_{\text{virtual}} - w) \) due to the repulsion between the chain and tube walls. As a result, the confinement free energy becomes \( F_{\text{conf}} \approx k_4 (D_{\text{virtual}} - w)^{-2/3} L_p^{1/3} \). Eventually, Eq. (68) becomes

\[ F_{\text{conf}} = k_1 \left( \frac{L_{\text{knot}}}{L_p} \right)^{1/4} + k_2 \left( L_{\text{knot}} - pw \right)^{-2/3} L_p^{1/3} . \]

(69)

It is easy to see that when \( w = 0 \), Eq. (69) returns to Eq. (68). Fig. 27 shows simulation results [173] for the size distribution of trefoil knots in wormlike chains with \( w = 0 \). The peak value corresponds to a metastable knot with contour length of \( 12 L_p \). The radius of gyration of the metastable knot is only \( 1.3 L_p \), corresponding to a tight knot. The size distribution was converted to the free energy through \( F = - \log (P_{\text{knot}}) \). The fit of Eq. (68) to the free energy yields \( k_1 \approx 17.06 \) and \( k_2 \approx 1.86 \).

Fig. 28 shows simulation results [173] for the metastable knot size as a function of the chain width. Note that the effective chain width of DNA can be controlled by the ionic strength of the buffer [97]. The metastable knot becomes larger with the increasing chain width. The prediction of Eq. (69) was used to fit the simulation results, yielding \( p \approx 16 \).

3.3.2. DNA knots in nanochannels

Dai et al. [84] further extended the Grosberg-Rabin theory to DNA knots in nanochannels, based on the fact that the segments in knots experience the different confinement free energy from the ones in unknotted portions. If the knotted segments experience less confinement free energy, then the knot formation should be favored in confinement in order to reduce the overall free energy. In addition, the knots of different sizes should experience different confinement free energy, and hence, the size distribution is expected to be reshaped by the confinement. Following this idea, Dai et al. [84] wrote the free energy cost of knot formation in a nanochannel as

\[ F_{\text{knot}}(L_{\text{knot}}) = k_{\text{bulk}} L_{\text{knot}}^3 (L_{\text{knot}}) + F_{\text{excess}}. \]

(70)

\[ F_{\text{excess}}(L_{\text{knot}}) = F_{\text{wall}}(L_{\text{knot}}) - F_{\text{wall}}(L_{\text{unknot}}). \]

(71)

where \( F_{\text{wall}}(L_{\text{knot}}) \) corresponds to the free energy cost of confining a knot while the contour length of the knot is maintained as \( L_{\text{knot}} \), and \( F_{\text{wall}}(L_{\text{unknot}}) \) corresponds to the confinement free energy of an unknotted chain with contour length \( L_{\text{unknot}} \). The term \( F_{\text{bulk}}(L_{\text{knot}}) \) follows the expression in Eq. (69).

The excess free energy is calculated as follows. The confinement free energy of unknotted segments \( F_{\text{wall}}(L_{\text{unknot}}) \) was approximated by

\[ F_{\text{wall}}(L_{\text{unknot}}) = 5.0 D_{\text{virtual}}^{-5/3} w_{\text{bulk}}^{2/3} L_p^{1/3} L_{\text{knot}}. \]

(72)

because Dai et al. focused on the confinement strength in the classic de Gennes regime. The confinement free energy of knotted segments \( F_{\text{wall}}(L_{\text{knot}}) \) depends on the size of the knot relative to that of the channel. When the knot size is much smaller than the channel size (“small” knot regime in Fig. 29a), the knot is weakly deformed and can be considered as a ball with an effective diameter \( b_{\text{knot}} \). The ball size may be approximated as \( b_{\text{knot}} = \alpha L_{\text{knot}} \), where \( \alpha \) is a numerical coefficient less than 1. The confinement free energy of this ball was approximated as the one for a bead on a flexible chain of identical balls [133]:

\[ F_{\text{wall}}(L_{\text{knot}}) = \beta L_{\text{knot}}^{5/3} (D - \alpha L_{\text{knot}})^{-5/3}. \]

(73)

When the knot size is much larger than the channel size (“large” knot regime in Fig. 29b), different portions of the knot were considered to be confined in sub-channels with channel size less than \( D \), which was discussed by Nakajima and Sakaue [168]. In the “large” knot regime, knotted segments experience a larger confinement free energy penalty than outside the knot. As a result, “large” knots are rare and are not considered in the calculation of the metastable knot size in channels. Fig. 30 shows the total knotting probability of DNA as a function of the slit height with \( L_p = 50 \) nm and \( w = 2.5 \) nm. The total knotting probability exhibits a non-monotonic dependence on the slit height. Such non-monotonic behavior is due to the competition of the confinement free energy density in the knotted portion Eq. (73) and the unknotted portion Eq. (72).

Fig. 31 shows the size distributions of trefoil knots in channels from simulations. The peak locations, corresponding to the metastable knot sizes, are plotted in Fig. 32. The dependence of the metastable knot size on the channel size can be explained by the theoretical prediction using Eqs. (70–73) with numerical coefficients \( \alpha = 0.1 \) and \( \beta = 0.02 \). The knots in confined DNA have been observed in experiments [163] as shown in Fig. 33. The channel confinement stretches DNA, and hence a knot appears a bright spot diffusing DNA and disappears at one end of DNA.

4. Comparison of confined polymers and polymer under other conditions

Polymers in confinement are similar to polymers under tension and polymers in the semidilute solution because the blob model can be applied in all of these three cases. For polymers in confinement, the separation of classic/extended de Gennes regime occurs because the real-chain scaling will be replaced by the ideal-chain behavior at short length scales when the EV interactions are weaker than the thermal energy. Similar separations of regimes also occur for polymers under tension and polymer in semiflexible solutions, which are presented below.
4.1. Polymers in confinement versus polymers under tension

It is well known that a polymer under tension has the great similarity with the polymer in confinement because the force $f$ applied to the end of a polymer introduces a characteristic length $\xi = kT/f$ that is similar to the channel size $D$ [175]. Fig. 34 shows the comparison of regimes of polymers in confinement versus polymer under tension [103]. In the classic Pincus regime, the scalings of extension $L_0 \sim f^{-2/3}$ and fluctuation $\sigma^2 \sim f^{-1/3}$ are similar with the scaling $L_\parallel \sim D^{-2/3}$ and $\sigma^2 \sim D^{-1/3}$ in the classic de Gennes regime. However, in the extended Pincus regime, the scaling of extension $L_0 \sim f^0$ is different from the scaling $L_\parallel \sim D^{-2/3}$ in the extended de Gennes regime. The fluctuation in the extended Pincus regime $\sigma^2 \sim f$ is still similar with the scaling $\sigma^2 \sim D^0$ in the extended de Gennes regime. These scalings are confirmed by simulation results in Fig 23. The difference in scaling of extension between polymers in confinement and under tension is caused by the driving force to separate the blobs [103]. For polymers in confinement, the blobs are separated by excluded volume interactions. If the blob is smaller than the thermal blob, EV interactions are not strong enough to separate the spherical blobs. For polymers under tension, the blobs are separated by tensile forces. The backfolding of blobs costs energy of $k_BT$ and hence is prohibited. In this case, even if the blob is smaller than a thermal blob, the tensile force can still separate the blobs.

4.2. Polymers in confinement versus polymers in semidilute solution

Polymers in confinement also share great similarities with polymers in semidilute solutions because both cases can be explained by the blob model. Recently, Dai et al. revealed the similarity between the extended de Gennes regime for a confined polymer and the semidilute marginal condition for polymer solutions. The semidilute marginal condition was proposed and studied by Schaefer et al. [176] and Birshtein [177], which is shown in Fig. 36. Note that the $y$-axis in the diagram by Schaefer et al. is the normalized temperature ($T - T_m$)/$T$, where $T_m$ is the $T$ temperature. The effective chain width is determined by the effective excluded volume interaction, which is determined by the temperature. The conceptual picture behind the semidilute marginal condition is as follows. Applying the classic blob model, a chain in the semidilute solution is viewed as a string of blobs. As in the extended de Gennes regime, intra-blob conformations should follow ideal-chain scaling rather than real-chain scaling when the EV interactions inside a blob are weaker than $k_BT$. This situation was investigated and termed as semidilute marginal condition by Schaefer et al. [176] The boundary for the marginal condition was determined as the critical concentration $c^{\text{critical}} = 4\xi/L_k\sim 4\xi$ of Kuhn segments. A new characteristic length $\xi \sim w^{-1/2} \sim L_k^{-1/2}$ was also determined in the monomer–monomer pair-correlation $g(r) - 1/r^2 \exp(-r/\xi)$. The critical concentration in the marginal condition shares the same expression with the one in the extended de Gennes regime $c = (L/L_k)/(L_0/D^2) \approx wL_k^{-4}$. The characteristic length in the marginal condition is similar to the length of anisometric blob.

5. Summary and outlook

The competition of three interactions, bending (elastic entropy), excluded volume, and confinement, leads to distinct chain behaviors in various regimes of a confined polymer molecule. The competitions of three interactions can be also as the competition of three length scales: the persistence length $L_p$, the chain width $w$, and the channel size $D$. In the case of DNA, the chain width $w$ is usually much less than $L_p$ and $D$. Accordingly, the parameter space is often separated to $D < L_p \ll L_\parallel \ll L_p$. For the two extreme cases, $D < L_p$ and $D \gg L_p$, theoretical treatments are relatively simple, and classic models give predictions that are validated by simulations and experiments in the last decade. A notable recent development is a deeper understanding of the extended de Gennes regime and the backfolded Odijk regime.

An important interaction underappreciated in this review is that of electrostatics [178]. Accordingly, the corresponding length scale, the Debye length, is underappreciated. In this review, long-range electrostatic interactions are approximated by a short-range hardcore repulsion with an effective chain width. The introduction of this new length scale, Debye length, should lead to more regimes in the diagram shown in Fig. 8. Experiments of stretching DNA under different ionic strengths have revealed rich phenomena due to the competition of Debye length with other length scales [179,180]. In addition, DNA–DNA interactions can be complex and even attractive in some cases [121,181].

Compared to equilibrium properties of confined DNA, the theories for non-equilibrium properties of confined DNA, for example, the coil-globule transition, are less developed. Several experiments discovered that DNA compaction is much easier and much faster in confinement. For example, the coil-globule transition or the compaction of DNA in channels induced by neutral crowding agents or charged ligands has been investigated by many recent experiments [34,95,182] and simulations [55,183], all of which reveal that compaction is greatly facilitated by confinement. To understand the effect of confinement on the compaction of DNA in confinement, explicit consideration of electrostatic interactions should be required.

Exploring the knots in confined DNA by experiment is feasible from the technical point of view, and much of work can be done to deepen our understanding of knots as well as the effect of confinement on polymer. It is fortunate that the experimental observation of a knot in DNA should be easier in confinement than in bulk, because the channel-confinement restricts DNA and the knot appears as a bright spot and diffuses along DNA until untied at ends [163]. Systematic experiments could be performed to measure the diffusion coefficient of a knot as a function of the channel size, the effective width and persistence length of DNA, and these results can be compared with simulations and theory. The size of knots can be also inferred from the total intensity in the bright spot.

Acknowledgment

This research was supported by the National Research Foundation Singapore through the Singapore MIT Alliance for Research and Technology’s research program in BioSystems and Micromechanics, the National Science Foundation CBET-1335938.

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