Revisiting Blob Theory for DNA Diffusivity in Slitlike Confinement

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Revisiting blob theory for DNA diffusivity in slitlike confinement

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Blob theory has been widely applied to describe polymer conformations and dynamics in nanoconfinement. In slit confinement, blob theory predicts a scaling exponent of 2/3 for polymer diffusivity as a function of slit height, yet a large body of experimental studies using DNA produce a scaling exponent significantly less than 2/3. In this work, we develop a theory that predicts that this discrepancy occurs because the segment correlation function for a semiflexible chain such as DNA does not follow the Flory exponent for length scales smaller than the persistence length. We show that these short length scale effects contribute significantly to the scaling for the DNA diffusivity, but do not appreciably affect the scalings for static properties. Our theory is fully supported by Monte Carlo simulations, quantitative agreement with DNA experiments, and the results reconcile this outstanding problem for confined polymers.

The conformation and dynamics of single DNA molecules in confinement have been extensively studied, facilitated by nanofabrication techniques capable of manufacturing devices with well-defined canonical geometries and direct visualization of single DNA via fluorescence microscopy. Practically, the understanding of DNA physics in confinement is vital for the development of nanodevices for genome analysis [1–4]. Moreover, simulations and experiments of DNA in confinement have been used to critically examine classic and long-existing theories in polymer physics.

Proposed by de Gennes [5], blob theory has been applied to predict the static and dynamic scaling behavior of single polymers when varying the confining dimension, *e.g.* the nanochannel diameter [6, 7] or the nanoslit height [8–11]. In slitlike confinement (two parallel plates), blob theory predicts a scaling of DNA extension with respect to the slit height of $R_{\|} \sim H^{1/4}$, which agrees with experiments [9, 10] and simulations [11]. Despite the success of blob theory in predicting scalings for static properties, significant discrepancies exist between blob theory and the results of experiments and simulations for dynamic scalings: blob theory yields a scaling for diffusivity versus slit height of $D \sim H^{2/3}$ which is substantially larger than the scaling exponent seen in experiments [8, 10, 12–14] and simulations [15].

In this work, we reconcile the predictions of blob theory and experimental data by developing a modified theory that approximately accounts for the pair correlation of DNA segments at length scales smaller than the persistence length. While pair correlations below the persistence length have little effect on static scalings, they dramatically affect the diffusivity. By accounting for the difference between the DNA pair correlations below the persistence length, we obtain the excellent agreement between theory and experiment seen in Fig. 1.

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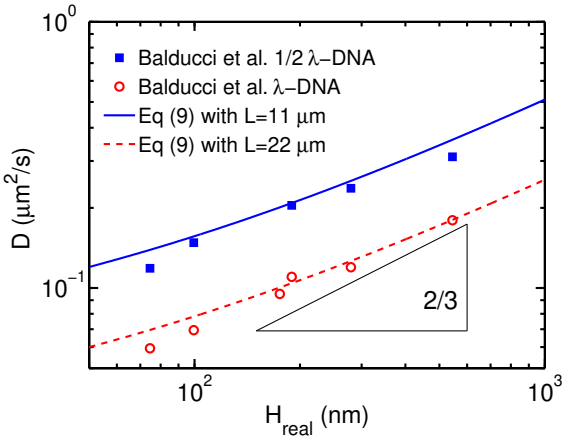

FIG. 1. (Color online) Diffusivity as a function of slit height. The symbols are from previous experiments [8] for both λ-DNA and 1/2 λ-DNA. The two lines are calculated from Eq. (9) using the prefactor $c_2 = 1.68$. The triangle indicates the de Gennes scaling of 2/3.

Let us first recall the classic blob theory arguments for the scaling of DNA diffusivity in slits for the de Gennes regime ($L_p \ll H \ll R_{\mathrm{g,bulk}}$ with persistence length, L_p, and DNA bulk radius of gyration, $R_{\mathrm{g,bulk}}$). Within the slit, DNA is represented by a series of self-avoiding blobs, each with diameter equal to the height H. Using Flory scaling [16], the contour length within a blob is

$$L_{\mathrm{blob}} \sim H^{5/3} L_p^{-1/3} w^{-1/3}, \tag{1}$$

where w is the effective chain width. Here, we use a simple Flory exponent of 3/5. (The precise value [17] is 0.5877 ± 0.0006.) The number of blobs is $N_{\mathrm{blob}} = L/L_{\mathrm{blob}}$, where L is the contour length. In the Zimm model [18], the polymer in each blob is hydrodynamically coupled to its entrained solvent, resulting in a drag force on each blob proportional to H. The resulting scaling of diffusivity is $D \sim 1/(N_{\mathrm{blob}} H) \sim H^{2/3} L_p^{-1/3} w^{-1/3} L^{-1}$.

To understand why the classic blob theory does not capture experimental data, we need to consider a more detailed approach which accounts for correlations at the persistence length scale. The DNA diffusivity in slits is determined by hydrodynamic interaction (HI) between DNA segments. Here, HI refers to the force exerted on a particle due to the flow induced by the movement of another particle. The diffusivity in slits is approximated [19] as

$$D = \frac{k_B T}{L} \int_0^{H/2} h(r)\Omega(r)\mathrm{d}r \tag{2}$$

where $k_B T$ is the thermal energy, $\Omega(r) = 1/(6\pi r\eta)$ is angle-preaveraged Oseen tensor in free solution, η is the viscosity of the solvent, and $h(r) \equiv 4\pi r^2 L_p g(r)$ is a dimensionless form of the pair correlation function, $g(r)$. Based on Eq. (1), the dimensionless pair correlation used in the classic blob theory is

$$h(r) = c_1 r^{2/3} L_p^{-1/3} w^{-1/3}, \tag{3}$$

where c_1 is a prefactor. After substituting this expression into Eq. (2), the resulting diffusivity is

$$D_1 = c_2 H^{2/3} L_p^{-1/3} w^{-1/3} D_0, \tag{4}$$

where

$$D_0 = k_B T/(6\pi\eta L), \tag{5}$$

is the Rouse diffusivity and c_2 is a prefactor that corrects for the approximation of a free solution Oseen tensor in Eq. (2). Note that applying the precise Flory exponent of 0.5877 yields the scaling $D_1 \sim H^{0.7015}$.

As expected, this calculation reproduces the result cited by many authors [8, 10, 12]. However, it fails to explain the experimental data because the Flory pair correlation function is used throughout the entire domain in the integral of Eq. (2). DNA behaves like a stiff rod below the persistence length, so we propose modifying the pair correlation with the approximate form:

$$h(r) = \begin{cases} 2, & r < L_p/2 \\ c_1 r^{2/3} L_p^{-1/3} w^{-1/3}, & r \geqslant L_p/2. \end{cases} \tag{6}$$

This modified pair correlation function minimally affects the static properties of DNA in slits, such as the scaling of DNA extension. Using blob theory and Eq. (1), the in-plane DNA extension is determined as $R_{||} \approx H N_{\mathrm{blob}}^{3/4} = H(L/L_{\mathrm{blob}})^{3/4} \sim H^{-1/4}$. If the modified $h(r)$ is considered, the calculation of L_{blob} is broken up into two integrals.

$$L_{\mathrm{blob}} = \int_0^{L_p/2} h(r)\mathrm{d}r + \int_{L_p/2}^{H/2} h(r)\mathrm{d}r \tag{7}$$

Substituting Eq. (6), above equation becomes

$$L_{\mathrm{blob}} = \begin{cases} H, & H < L_p \\ c_1 \frac{3}{5} \left[\left(\frac{H}{2}\right)^{\frac{5}{3}} - \left(\frac{L_p}{2}\right)^{\frac{5}{3}} \right] (L_p w)^{-\frac{1}{3}} + L_p, & H \geqslant L_p. \end{cases} \tag{8}$$

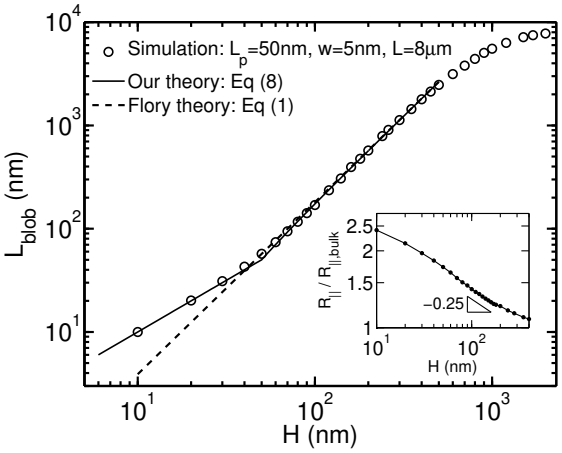

FIG. 2. The contour length inside a blob as a function of slit height. The solid line is calculated from Eq. (8) using L_p = 50 nm, w = 5 nm, and a fit value $c_1 = 2.8$. The dashed line is the result from classic blob theory, Eq. (1). The statistical errors are less than the symbol sizes. The inset plot shows the normalized in-plane radius of gyration as a function of slit height in simulations. The triangle indicates the de Gennes scaling of −0.25.

For the de Gennes regime, where H is always at least a few times L_p, this modification usually causes only a few percent change in L_{blob}. As a result, the scaling exponent of $R_{||}$ versus H will be very close to the value 1/4 predicted by classic blob theory [10, 11].

To confirm this conjecture, we used Monte Carlo simulations DNA in slits [11]. In the simulation, DNA is modeled as a chain of N_b beads connected by $(N_b - 1)$ inextensible bonds of length l_B, corresponding to a contour length $L = (N_b - 1)l_B$. Three types of interactions are considered: hard-core repulsions between DNA beads, hard-core repulsions between DNA beads and slit walls, and bending energies between adjacent bonds. The hard-core diameter of the bead, w, is set to equal the bond length l_B. The bending rigidity is set to reproduce the persistence length L_p of 50 nm. The chain width is 5 nm and the contour length is 8 μm (N_b = 1601 beads). The simulation starts from a random conformation. In each Monte Carlo cycle, we perform either one crankshaft move or one reptation move (randomly picking the type of move). Each chain is allowed to equilibrate for 10^8 steps. After equilibration, we perform more than 10^9 steps, recording one configurations every 10^6 steps for data analysis.

We used the simulation data to estimate the contour length L_{blob} inside a spherical blob whose diameter equals the slit height. Note that the slit heights H in all figures are always the effective slit height, *i.e.*, the real slit height H_{real} minus the chain width w, because this effective slit height is consistent with the slit height used in theoretical predictions. Recall that L_{blob} corresponds to the integral of $h(r)$. For each bead in a given DNA configuration, we counted how many beads are located

within the distance of H/2. Then, we multiply this number with the bond length to obtain $L_{\rm blob}$. Figure 2 shows $L_{\rm blob}$ as a function of slit height. The simulation results with $L_p = 50$ nm are compared to Eq. (8) using a fit value of $c_1 = 2.8$. We note that although a simple piecewise function is used in Eq. (8), good agreement is obtained all through the Odijk and de Gennes regimes. There are minor discrepancies when $H \approx L_p$, where DNA behaves as neither a stiff rod nor a long chain. The modification of $h(r)$ leads to only a few percent change of $L_{\rm blob}$ when $H > 2L_p = 100$ nm. As a result, considering the sub-persistence behavior of $h(r)$ has a negligible effect on $L_{\rm blob}$ as well as the scaling of DNA extension when slit height is a few times the persistence length. Indeed, the best power law fit to the in-plane extension in the de Gennes regime [11] yields an exponent of -0.249 ± 0.010, as shown in the inset of Fig. 2 (also see supplemental material). This exponent is very close to $-1/4$ predicted by the classic blob theory.

While the modified pair correlation function in Eq. (6) has minimal impact on the scaling for the size of the confined chain, it has a much stronger impact on the diffusivity because $\Omega(r) \sim r^{-1}$ in Eq. (2), which dramatically enhances the importance of the short-scale property of $h(r)$. Substituting Eq. (6) into Eq. (2), the diffusivity becomes

$$D_{dg} = D_1 + D_2 - D_3, \tag{9}$$

where

$$D_2 \approx 2\ln(L_p/a) D_0 \tag{10}$$

$$D_3 = c_2 (L_p/w)^{1/3} D_0. \tag{11}$$

The terms D_2 and $(D_1 - D_3)$ correspond to the integral over the intervals $[0,\ L_p/2]$ and $[L_p/2,\ H/2]$ in Eq. (2), respectively. In Eq. (10), a is the hydrodynamic radius of the chain. In computing D_2, we regularized the integral to remove the singularity (see supplemental material). Equation (10) approximately corresponds to the diffusivity of a randomly oriented rod with the length of L_p and the radius of a [20–22]. Owing to the sharp cross-over between forms of $h(r)$ in Eq. (6), it would be inappropriate to regard c_2 as a universal prefactor. Rather, we would expect that c_2 will have a slight dependence on the ratio L_p/w that arises from the details of the crossover from rod-like correlations to real chain correlations over the length scale of the slit.

Our results so far already start to explain the deviation between experiments and classic blob theory. Equation (9) differs from Eq. (4) by two additional terms D_2 and $-D_3$. Note that $(D_2 - D_3)$ is positive and independent of H, *i.e.* the scaling exponent is zero. The mixture of two scaling exponents: $D_1 \sim H^{2/3}$ and $(D_2 - D_3) \sim H^0$ results in an apparent exponent less than 2/3. This finding qualitatively agrees with experimental results [8, 10, 12–14].

To obtain the quantitative results seen in Fig. 1, we need to provide values for the hydrodynamic radius a appearing in Eq. (10) and the prefactor c_2 appearing in Eqs. (4) and (11). We first set $a = 1.25$ nm, which was determined from sedimentation data by Yamakawa and Fujii [21]. To obtain c_2, we fit the prediction of our theory to the diffusion coefficient obtained from Monte Carlo sampling of

$$D_{\rm sim} = \frac{k_B T}{N_b^2} \sum_{i,j}^{N_b} \left[\frac{\delta_{ij}}{6\pi\eta a_{\rm sim}} \mathbf{I} + \mathbf{\Omega}^{\rm slit}(\mathbf{r}_{ij}) \right], \tag{12}$$

following the approach used in previous work [19, 23]. The first term is the Stokes friction on each bead, which includes a parameter $a_{\rm sim}$. Since the simulation model is discrete, the hydrodynamic radius used in the simulations should differ from the one used in a continuous model (see supplemental material). For the touching bead model we used here, the value $a_{\rm sim} = 1.38$ nm leads to simulated DNA diffusivities in free solution that match experimental data over a large range of DNA lengths [19, 24]. The second term is the sum of the hydrodynamic interactions between beads in the presence of slit walls, which is calculated from the analytic solution of the Stokeslet in slits [25] (see supplemental material).

The normalized (dimensionless) diffusivities calculated from simulations are shown in Fig. 3. The best power-law fit to the simulation data points in the region $2L_p < H < R_{g,bulk}$ (blue filled circles) yields an apparent scaling exponent of 0.523. This exponent is close to most experiments [8, 10, 12], but less than the value 2/3 predicted by classic blob theory (dashed black line). The solid (red) line is calculated from Eq. (9) using the best fit to the filled (blue) circles, giving a value of the prefactor $c_2 = 1.68$ and in very good agreement with the simulations.

Our theory is only valid for the de Gennes regime. In

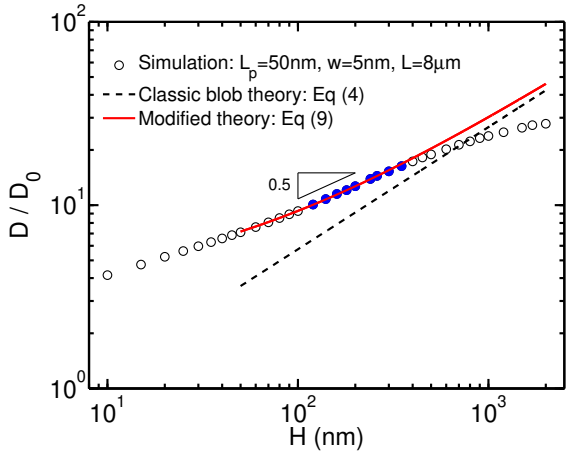

FIG. 3. (Color online) Normalized DNA diffusivity as a function of slit height. The filled circles are located in $2L_p < H < R_{g,bulk}$. The fit value of the prefactor c_2 in Eq. (9) is 1.68. For the parameters used in our simulations, the correlation time for the Kirkwood diffusivity is around 10^6 steps (see supplemental material). The statistical errors are less than the symbol sizes.

thin slits with $H < L_p$, the angled averaged free-solution Oseen tensor is no longer a good approximation in Eq. (2) as hydrodynamics will become partially screened near the channel boundaries. Furthermore, in thin slits, chain segments tend to align with slit walls, and this alignment also affects hydrodynamic interaction. As H increases, Eq. (9) approaches the diffusivity scaling of classic blob theory, indicating a vanishing contribution of sub-persistence length conformations to overall diffusivity for large slit heights. The simulation data for our 8 micron chain, naturally, follow neither our modified theory or blob theory after the slit height passes one micron in size because the chain diffusivity is transitioning to its bulk value. Also, note that the diffusivity is normalized with the Rouse diffusion coefficient, so the dimensionless diffusivity in an infinitely wide slit is not unity.

We are now in a position to compare our theoretical predictions with experimental results by Balducci *et al.* [8], as shown in Fig. 1. The parameters used for the theoretical predictions follow the experimental condition: $T = 22.5°$C, $\eta = 1.1$ cp, and $L = 22$ μm (YOYO-labelled λ-DNA) or $L = 11$ μm (YOYO-labelled 1/2 λ-DNA). Note that the DNA contour length is increased 38% by YOYO labeling at a ratio of 1 dye/ 4 bp [26–29]. We set the effective chain width to $w = 5$ nm, which is estimated by Balducci *et al.* [8]. The persistence length is set to 50 nm [26, 30]. Using these parameters, Eq. (9) agrees with the experimental results in moderate confinement in Fig. 1 with no adjustment of the the prefactor $c_2 = 1.68$, which was obtained from the independent comparison to simulations.

Classic blob theory only gives the asymptotic behavior for the diffusivity scaling, as demonstrated by the dashed and solid lines in Fig. 3. We will now estimate at what slit height and contour length the scaling of diffusivity becomes sufficiently close to the de Gennes scaling of 2/3. We define the apparent scaling exponent as the slope of D-H curve in the log-log plot. The relative deviation of the slope from the de Gennes scaling $\epsilon \equiv (2/3 - slope)/(2/3)$ can be determined from Eq. (9). For a given value of ϵ, the corresponding slit height is the solution of $D_1 = (1 - \epsilon)D$ for H. Picking a 5% error, $\epsilon = 0.05$, yields $H = 4.3$ μm. Using Eq. (8), the value of $L_{\rm blob}$ is approximately 100 μm. If we assume that the application of blob theory requires at least five blobs, then the minimum contour length of DNA is about 500 μm (~ 1100 kbp) to observe an exponent close to the de Gennes scaling for diffusivity. This value is one order of magnitude greater than the contour length of DNA used in previous experiments [8, 10, 12–14]. As a result, interpretation of DNA dynamics in micro-/nano- fluidic devices nearly always requires modification of classic blob theory.

In addition to resolving the longstanding mystery regarding observed scalings of DNA diffusivity in confinement, this work deepens our fundamental understanding of statics and dynamics of confined semiflexible chains. The scaling behaviors in confinement have been traditionally interpreted using de Gennes' blob theory. It is very intriguing that this classic theory works well for statics (thermodynamics), but not for dynamics (hydrodynamics). Our analysis gives a straightforward explanation related to a correction to the pair correlation at short length scales. Dynamics are sensitive to short length scale chain statistics due to the 1/(distance) scaling of the hydrodynamic interaction tensor which weights the interactions. However, statics lack this nonlinear weighting and hence are more forgiving to slight modifications of short length scale pair correlations. Looking forward, we expect that similar arguments may be applied to resolve the difference in diffusivity scaling exponents between blob theory (ν=2/3) and simulation [19, 23, 31] ($\nu < 2/3$) in square-channel ("tubes") confinement. Furthermore, other dynamical properties, *e.g.* the relaxation time, are also expected to deviate from the classic blob theory.

In conclusion, we find that the classic blob theory should be modified to include the short-scale pair correlation when applied to the dynamics of semiflexible polymers in confinement. Via modification of the sub-persistence pair correlation, we have reconciled DNA experiments and simulation results with blob theory of polymers in slitlike confinement. This modification is necessary to interpret confined semiflexible polymer dynamics.

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