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Evaluation of Blob Theory for the Diffusion of DNA in Nanochannels

Damini Gupta, Aditya Bikram Bhandari, and Kevin D. Dorfman*

Department of Chemical Engineering and Materials Science, University of Minnesota – Twin Cities, 421 Washington Ave SE, Minneapolis, Minnesota 55455, USA

Abstract

We have measured the diffusivity of λ -DNA molecules in approximately square nanochannels with effective sizes ranging from 117 nm to 260 nm at moderate ionic strength. The experimental results do not agree with the non-draining scaling predicted by blob theory. Rather, the data are consistent with the predictions of previous simulations of the Kirkwood diffusivity of a discrete wormlike chain model, without the need for any fitting parameters.

Graphical TOC Entry

1 Introduction

The equilibrium response of DNA to nanochannel confinement has received considerable attention as a fundamental problem in polymer physics^{1,2} as well as the basis behind a genome mapping technology.^{3–5} Experimentally, the statistical mechanical properties of DNA in nanochannel confinement (such as its average extension and the variance about that average extension) have been investigated in detail by varying the degree of confinement, the flexibility of the chain, the length of DNA molecules and the amount of bound fluorescent dye.^{6–25} However, the dynamic response of DNA in nanochannel confinement still remains relatively unexplored, particularly with respect to the center-of-mass diffusion coefficient.

The salient open question about DNA dynamics in nanochannel confinement is whether the diffusivity scaling predicted by the blob theory of Brochard and de Gennes²⁶ can be applied to practical situations involving DNA, which typically take place in the so-called extended

*To whom correspondence should be addressed: dorfman@umn.edu.

Supporting Information Available

Mean squared displacement curves, details of the hypothesis test for Fig. 4, and the movies used to generate Fig. 1. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

de Gennes regime.^{27–30} Even though no experimental data for diffusivity in nanochannels are available, there is a substantial body of experimental data on DNA diffusion in nanoslits that contradict the prediction from the classical blob theory.^{31–35} This discrepancy in the nanoslit geometry was reconciled by accounting for local correlations arising in a semiflexible chain, which introduce a correction to the blob theory.³⁶ Recently, Muralidhar and Dorfman³⁷ proposed that a similar correction for local chain correlations would also work in nanochannels. Indeed, their mobility data, obtained from simulations of a wormlike chain model in channel sizes corresponding to the extended de Gennes regime, agree well with a modified blob theory.³⁷ In the present contribution, we confirm that these computational predictions hold true in experiments. In addition to showing that the classical blob theory fails to describe the experimental data, we observe almost quantitative agreement between experimental data and simulation data³⁷ without the need for any fitting parameters.

2 Theory

We are concerned here with the diffusivity of a semiflexible polymer with a persistence length l_p , effective width w and contour length L , confined in a square channel of size D . In the classical de Gennes regime, corresponding to $D \gg l_p^2/w$,²⁷ the chain is comprised of a series of isometric blobs of dimension proportional to D . The chain segments inside a blob comprise a three-dimensional, self-avoiding coil in a good solvent, obeying Flory statistics. The contour length in each blob is then $L_{\text{blob}} = D^{5/3}(wl_p)^{-1/3}$, where we use standard Flory exponent, $\nu = 3/5$.³⁸ The extension is then³⁹

$$X \sim \frac{L}{L_{\text{blob}}} D = L \left(\frac{wl_p}{D^2} \right)^{1/3} \quad (1)$$

where L/L_{blob} is the total number of blobs in the confined chain.

One way to determine the axial diffusion coefficient, D_t , is by estimating first the friction factor of the confined chain, ζ_{chain} , and then using Einstein's relation, $D_t = k_B T / \zeta_{\text{chain}}$, where k_B is the Boltzmann constant and T is the absolute temperature. Blob theory²⁶ assumes that hydrodynamic interactions (HI) are screened over length scales greater than D . Consequently, the total friction factor of the chain is the sum of the individual friction factor of the blobs, $\zeta_{\text{chain}} = (L/L_{\text{blob}})\zeta_{\text{blob}}$. The friction factor of the blob is estimated as $\zeta_{\text{blob}} \simeq 6\pi\eta D$, where η is the solvent viscosity. The diffusion coefficient is then²⁶

$$D_t \simeq D_R \left(\frac{D^2}{wl_p} \right)^{1/3} \quad (2)$$

where $D_R = k_B T / (6\pi\eta L)$ is the (Rouse) diffusivity of a freely-draining chain. Comparing Eq. 1 and Eq. 2 leads to the convenient relationship

$$D_t \simeq D_R \left(\frac{X}{L} \right)^{-1} \quad (3)$$

In other words, the confined chain is a non-draining object (Zimm) whose friction is proportional to its size.

An equivalent way to determine the scaling law in Eq. 2 is by using the pre-averaged Kirkwood approximation,⁴⁰

$$D_t = \frac{k_B T}{L} \int_0^{D/2} h(r) \Omega(r) dr \quad (4)$$

where $\Omega(r) = 1/(6\pi r\eta)$ is the angle-averaged Oseen tensor in free solution, and $h(r) \equiv 4\pi r^2 l_p g(r)$ is a dimensionless form of the pair correlation function $g(r)$. The limited HI outside the length scale D sets the upper bound in the integral in Eq. 4. The pair correlation function inside a de Gennes blob is

$$h(r) \sim \left(\frac{r^2}{l_p w} \right)^{1/3} \quad (5)$$

Equations 4 and 5 produce the same scaling relation as Eq. 2.

For channel sizes $l_p \ll D \ll l_p^2/w$, the conformation of semiflexible chain in confinement takes shape of a series of anisometric blobs of length $(D l_p)^{2/3} w^{-1/3}$ and diameter D in the extended de Gennes regime.^{27,30} The excluded volume interactions within a blob in this regime are marginal. Note that the extended de Gennes regime is similar to the marginal condition of semiflexible polymers in semidilute solution.^{30,38} Both the average extension and variance about that average extension in this regime are well established from both experimental^{21,23} and theoretical^{27–30} perspectives. While early work suggested that the blob theory of Brochard and de Gennes²⁶ for $D \gg l_p^2/w$ would produce the appropriate diffusivity scaling in the extended de Gennes regime,^{1,40} recent simulations of the Kirkwood diffusivity of a wormlike chain model³⁷ show that a modified form of blob theory, inspired by similar work on diffusion in nanoslits,^{36,41} is necessary. The classical blob theory neglects the effect of local stiffness of the chain while computing the pair correlation function in Eq. 5. In its modified form, the theory assumes that the local chain stiffness results in strong correlations along the backbone of the chain at length scales smaller than l_p . Consequently, the pair correlation function is approximately³⁶

$$h(r) \approx \begin{cases} 2 & \text{if } r < l_p/2 \\ \left(\frac{r^2}{l_p w}\right)^{1/3} & \text{if } r \geq l_p/2. \end{cases} \quad (6)$$

Using this modified pair correlation function in Eq. 4 and casting the final result in terms of the chain extension, we obtain³⁷

$$D_t \simeq D_R \left[c_1 + c_2 \left(\frac{X}{L} \right)^{-1} \right] \quad (7)$$

where c_1 and c_2 are functions of l_p/w . As a result of accounting for local chain stiffness, an additional term in the diffusivity scaling appears in Eq. 7 when compared to Eq. 3 for the classical blob theory.

In principle, one should be able to compute the coefficients c_1 and c_2 for given values of l_p and w up to some universal prefactors.^{36,37} However, comparisons of the predictions of the modified blob theory using universal prefactors and simulations of the Kirkwood diffusivity in slits³⁶ and channels³⁷ is not satisfactory. In order to achieve satisfactory agreement between theory and simulation, the prefactors in the theory also need to be weak functions of l_p and w . We suspect that this shortcoming in the modified blob theory arises from the abrupt way that Eq. 6 goes from the rod-like behavior for small r to a self-avoiding random walk at large r . There are subtleties in the crossover from rod-like to coil behavior for wormlike chains^{42,43} that depend on l_p and w . Such effects are not incorporated into Eq. 6 and must also contribute to the diffusivity.

While the modified blob theory is not a complete description of the chain diffusivity, its limitations do not impede our goal of assessing the applicability of blob theory²⁶ to the practical problem of DNA diffusion in nanochannels.

3 Experimental Methods

3.1 Device fabrication

The nanochannel devices were fabricated from 500 μm thick fused silica wafers (University Wafers) using a layer of electron-beam lithography to define the nanochannels (100 μm long) and a layer of contact photolithography to define the microchannels (50 μm wide, 1 μm deep). We used a standard design where two parallel microchannels are connected with an array of approximately square nanochannels.⁶ Each lithography step was followed by a reactive ion etching (RIE: $\text{CF}_4/\text{CHF}_3/\text{Ar}$) step to transfer the pattern from the resist layer to the wafer. We used a cyclic RIE method to improve the etch selectively of fused silica when compared to the PMMA resist used in the electron-beam lithography step.⁴⁴ This led to relatively straight walls when compared to the slanted walls observed in some previous nanochannel studies.^{21,23} The access ports to the microchannels were created using sand-blasting. Finally, the devices were fusion-bonded to 170 μm thick fused-silica coverslips.

The channel depth in the devices was characterized prior to the bonding using atomic force microscopy (AFM). The widths of the channel were measured by scanning electron microscopy (SEM) in a duplicate device. The nanochannel cross-sectional dimensions are listed in Table 1.

3.2 DNA preparation

The λ -DNA molecules (48.5 kilobase pair, New England Biolabs) were dissolved in 0.5 $\times$ TBE buffer and labeled with YOYO-1 fluorescent dye (Invitrogen) at a concentration of 1 dye molecule for every 10 base pairs (bp) of DNA. The DNA solution was kept at room temperature for an hour followed by heating at 50 °C for 12 hours to achieve a more homogenous staining than samples stained at room temperature.¹⁷ Prior to the start of the experiments, the anti-photobleaching agent β -mercaptoethanol (Sigma-Aldrich, 6% v/v) was added to the solution. The ionic strength of this solution has been calculated to be 28 mM using a method developed previously.¹⁹

The reasonably moderate molecular weight of λ -DNA, compared to short alternatives (typically 1/2 λ -DNA) and long alternatives (typically T4-DNA), was critical to the success of our experiments. On one hand, the diffusivity of shorter molecules is higher, making the measurement simpler, but their extension in the channel is comparable to the measurement uncertainty in the largest channel size used here. On the other hand, longer molecules are prone to shear cleavage and photo-nicking. Moreover, longer molecules have smaller diffusion constants, requiring longer exposures for accurate measurements. The moderate molecular weight of λ -DNA is an attractive compromise between these experimental limitations.

3.3 Parameter estimation

In order to draw a comparison between our results and previous studies, we need to estimate three parameters, namely the contour length L , the persistence length l_p , and the effective width w . The bisintercalating dye (YOYO-1) used for visualization of DNA is expected to increase L linearly in proportion to the amount of dye bound. We assume that every bound dye molecule adds 0.51 nm to the bare contour length of 0.34 nm per base pair which gives $L = 19 \mu\text{m}$ for λ -DNA.^{45,46}

Although many studies in the past have investigated the effect of dyes on mechanical properties of DNA,^{17,46,47} the results are contradictory. Moreover, a recent study by Kundukad *et al.*⁴⁵ found that the persistence length of DNA is at best weakly affected by the intercalation for a well-equilibrated system. Therefore, we chose to neglect the effect of dye and use the experimentally validated⁴⁸ theory of Dobrynin⁴⁹ to estimate $l_p = 58 \text{ nm}$ at our moderate ionic strength of 28 mM. We employed Stigter's theory⁵⁰ to obtain the effective width $w = 10 \text{ nm}$.

3.4 Nanochannel experiments

The two parallel microchannels on a device were filled with DNA solution by capillary action. The DNA molecules then were driven to the center of the 100 μm long nanochannel using pressure-driven flow and allowed to relax for 120 s before video acquisition. We

observed laser excited (Coherent OBIS 473 nm), single DNA molecules using an inverted epifluorescence microscope (Olympus IX73) with a 100× (1.4 N.A.) oil-immersed objective. The images were taken with a EMCCD camera (Photometrics, Cascade II:512) at 2 fps with a 200 ms exposure time. A total of 2000 frames were acquired for each molecule. Each filling resulted in acquisition of approximately 30 molecules; we made multiple fillings for each channel. After the experiments, the device was cleaned with a standard RCA-1 step and heated to 1000 °C for 6 hours to allow its reuse. The experiments were conducted at room temperature.

3.5 Data processing

Figure 1 illustrates a typical image at the start of an experiment. As a first screening step, we identify molecules in the movie that (i) are clearly fragmented; (ii) diffuse outside the field of view; or (iii) are too close to another molecule to easily resolve throughout the movie. Examples of all of such molecules are indicated in Figure 1a.

Within a given movie, the centers-of-mass of the selected molecules were tracked using a custom-written Matlab program.⁷ First, the location of two extreme ends of the molecule (x_1 , x_2) were extracted by fitting the molecule's intensity profile, $I(x)$, inside a nanochannel to a linear combination of two error functions, which is a convolution of a Gaussian point-spread function and a box function.⁶ Then, the intensity-weighted center-of-mass of the molecule,³³ $x_{\text{com}}(t)$, for a given frame recorded at time t was computed as

$$x_{\text{com}}(t) = \frac{\int_{x_1}^{x_2} x I(x, t) dx}{\int_{x_1}^{x_2} I(x, t) dx} \quad (8)$$

We use these data to compute the mean-squared displacement (MSD),

$$\text{MSD}(\delta t) = \langle [x_{\text{com}}(t) - x_{\text{com}}(t - \delta t)]^2 \rangle_{n,t}, \quad (9)$$

where δt is the time lag between images. In general, a single molecule does not generate sufficient statistics to compute its diffusivity due to photobleaching. To overcome the effect of the sampling error, we combine the displacement data obtained for all of the analyzed molecules within a given channel to compute the MSD, whereupon the $\langle \dots \rangle_{n,t}$ operation corresponds to an average over both all times t and the ensemble of n molecules listed in Table 1 for that channel size.

To construct the ensemble of trajectories used to compute the MSD, we controlled for two key experimental artifacts. The first is the possibility of either adsorption of DNA molecules on the channel surface or induced motion of DNA molecules due to spurious fluid flow. To filter out the systematic errors that would occur in either case, we computed the exponent β of the $\text{MSD} \sim \delta t^\beta$ for ensemble averaged trajectories of the molecules recorded within a given movie. Any movie whose ensemble of DNA molecules produced an anomalously

diffusive trajectories ($|\beta - 1| > 0.1$) was not included in the final ensemble average used in Eq. 9. Figure 1b provides an example of a movie that was excluded from further analysis; it is clear from the snapshots in Figure 1b that there is a fluid flow in the upward direction in the image. Table 1 lists the number of movies acquired for each data set and the number that were sufficiently Brownian for subsequent analysis.

The second potential artifact is photobleaching, especially with the relatively long exposure time used in the experiments here when compared to our previous experiments in a similar setup.²³ Moreover, photochemical reactions can lead to either shortening of the contour length or fragmentation of the molecule.^{20,52} Photocleaved molecules become systematically shorter in length and thus diffuse faster over time. In order to make sure that the physical properties of the DNA are consistent over the time period of image acquisition, we used the following steps. First, we divided single movies (2000 frames) into five sub-movies of equal length. Then, we compared the extension of molecules among sub-movies. We did not find any statistically significant shortening of molecules over time, indicating a negligible effect of either photobleaching or photocleaving. We also used a method developed by Hsieh *et al.* for DNA studies in nanoslits³² where they compare MSD of short movies as a secondary test. Figure 2 shows one such analysis done inside a nanochannel with cross-sectional dimension as $150 \times 118 \text{ nm}^2$. The MSD curves are very close to each other for $\delta t < 200 \text{ s}$ and no systematic deviation is observed for $\delta t > 200 \text{ s}$ beyond that caused by fluctuations due to limited statistics, thus lending confidence in absence of any substantial effect caused by prolonged light exposure times.

In general, the MSD increased linearly with time, as seen in Figure 2 and Figure S1. However, the dynamic diffusion coefficient, $\text{MSD}/2\delta t$, decays continuously up to a maximum time lag of 150 s in the smallest channel size before reaching a fairly constant value. Similar behavior was observed in nanoslits³² for $\delta t < 0.2 \text{ s}$ and was investigated in detail for colloidal particle tracking experiments.⁵¹ At larger time lags ($\delta t > 400 \text{ s}$), $\text{MSD}/2\delta t$ fluctuates substantially due to limited statistics.

The one-dimensional axial diffusivity, D_t , was obtained by fitting the MSD curves obtained using all 2000 frames in Figure 2 and Figure S1 with a linear function

$$\text{MSD}(\delta t) = 2D_t\delta t + c \quad (10)$$

for $\delta t \in [150 \text{ s}, 400 \text{ s}]$ to take advantage of the stable, well-sampled region of the dynamic diffusion coefficient. The addition of an offset c in the fit is required because the initial region of the MSD plot is non-linear.

4 Results

The first step in our analysis is to check whether the experimental data we are analyzing belong to the extended de Gennes regime. Since the channel sizes used here are almost square, we calculate the effective channel size as $D_{\text{eff}} = \sqrt{(D_1 - \delta)(D_2 - \delta)}$, where D_1 and D_2

are the channel height and width, respectively. The parameter δ is a wall-DNA depletion length that accounts for electrostatic interactions. We estimate $\delta = w$, following previous studies.^{21,23,28} Our effective channel sizes range from 117 nm to 260 nm (Table 1) which indeed spans much of the extended de Gennes regime; the scaling theory by Odijk²⁷ suggests that the range of the extended de Gennes regime for the buffer used here is 116 nm to 336 nm, which is quite narrow. However, simulations by Dai *et al.*³⁰ suggest that the lower bound is 232 nm and upper bound is 754 nm. The possible consequences of this contradiction will be discussed later.

Figure 3a shows the results for the average extension of DNA molecules, X , as a function of effective channel size, D_{eff} . The extension and the channel size are normalized in a manner to produce universal curves in the extended de Gennes regime. Our results agree with previous experimental studies and are very close to the theoretical predictions of Werner and Mehlig,²⁹ $X/L = 1.176(62)(D_{\text{eff}}^2/(l_p w))^{-1/3}$. The deviation between experiments and theory have been explained in the past as a likely result of the inaccuracy in estimation of D_{eff} .^{21,23}

Figure 3b shows the results for the variance about the average extension, σ^2 ; the values are normalized to collapse the experiments done with DNA of different sizes in different ionic strength environments. As expected, σ^2 from our experiments does not depend upon D_{eff} , similar to previous studies in the extended de Gennes regime.^{21,23} When compared to the exact value of variance predicted by theory in this regime,²⁹ $\sigma^2/(Ll_p) = 0.264(99)$, we observe a systematic deviation in experimental value as a function of chain semiflexibility l_p/w ; σ^2/Ll_p increases with increasing l_p/w . This can be attributed to either inaccuracy in the methodology used to determine l_p as a function of ionic strength or the failure of the experimental system to satisfy the strong inequalities assumed to formulate the extended de Gennes theory. Indeed, the lower bound for the regime suggested by Dai *et al.*,³⁰ $D_{\text{eff}} = 4l_p$, is not satisfied by most experimental studies. Overall, the relative insensitivity of the variance to the channel size is the most compelling evidence that the present experiments are conducted in the extended de Gennes regime.²⁸ Moreover, these data allow us to exclude transient adsorption and desorption of the chain as a major factor affecting the diffusivity. If DNA-wall interactions were substantial, we would expect to observe a significant increase in σ^2 relative to the predictions of the theory.

In addition to the deviations between the theory and our experiments, there are also deviations between our experiments and previous work by Iarko *et al.*²¹ and Gupta *et al.*²³ In the context of DNA-nanochannel experiments, the magnitude of the differences between the various experiments here are consistent with a recent survey of the experimental literature.⁵³ In addition to the random experimental error that contributes to the standard error of the mean for a given data set, there are systematic errors in DNA-nanochannel experiments that contribute to the differences between different experimental data sets. These errors include random fragmentation of the DNA due to shear or photocleavage,²³ inhomogeneous staining,¹⁷ and uncertainties in the role of intercalating dyes,²¹ all of which contribute to systematic errors in L . There are also systematic errors in D that arise from the device fabrication and characterization and become increasingly more important as the channel size decreases. Perhaps even more importantly, the uncertainty in how to compute the depletion

length δ leads to systematic errors between experiments performed at different ionic strengths and/or different channel materials, since δ depends on both the channel surface potential and the screening length for DNA-wall interactions required to convert the physical channel size D into the effective channel size D_{eff} .¹ While a small standard error of the mean indicates that a sufficient number of experiments have been performed to reduce the random errors to an acceptable level, the persistence of these systematic errors stymies comparisons between different data sets.²¹ Although there remain discrepancies between our experiments and theory, Figure 3 shows that the thermodynamic data in the present experiments are in good agreement with previous experimental work.

We thus proceeded to investigate the dependence of the axial diffusivity of DNA, D_t , as a function of average extension, X , shown in Figure 4. We chose to plot the data in terms of the extension X because it can be measured directly in the experiments. As a result, we avoid any systematic errors that arise from using D_{eff} , whose accuracy has been questioned in previous nanochannel experiments.^{21,23} In order to test the validity of predictions from the blob theories, the simplest approach would be to compute a power law for $D_t \sim X^\alpha$. We obtain $\alpha = -0.85 \pm 0.15$ at a 95% confidence level in Figure 4a. The apparent exponent is weaker than the $\alpha = -1$ scaling predicted from the classical blob theory. Moreover, the range for X used here is too small to accurately test a power law dependence and the error bars are substantial. Overall, the outcome of the power law analysis is inconclusive.

Instead, in Figure 4b, we directly compare the predictions from the blob theories in Eqs. 3 and 7 to our experimental data. Note that the value of the Rouse diffusivity appearing in both equations, $D_R = 0.0129 \mu\text{m}^2/\text{s}$, is estimated for λ -DNA ($L = 19 \mu\text{m}$) in water ($\eta = 0.89 \text{ cP}$) at room temperature ($T = 25^\circ\text{C}$). Clearly, the modified blob theory expression in Eq. 7 agrees well with our data for the entire range of X used here when compared to the classical blob theory prediction from Eq. 3. A more robust method to compare two theories is to investigate the statistical significance of the additional term c_1 appearing in Eq. 7 via a hypothesis test. The experimental data in Figure 4b were regressed to the model in Eq. 7 to obtain the coefficients $c_1 = 0.86 \pm 0.79$ and $c_2 = 1.2 \pm 0.19$ at a 95% confidence level. For a more thorough analysis we examined the statistical significance of the intercept, c_1 , via a hypothesis test (see Supplementary Information) with the null hypothesis $c_1 = 0$. We obtained a p -value of 0.04, which rejects the null hypothesis and thus experimentally confirms the statistical significance of the additional term c_1 .

We also compared the experimental data to prior simulations of the Kirkwood diffusivity of a discrete wormlike chain model.³⁷ Since simulation results for the l_p/w value corresponding to our experimental conditions are not available, we plot the simulation data for the two closest values of l_p/w . The diffusion coefficients were computed from the dimensionless simulation results using the estimated Rouse diffusivity, $D_R = 0.0129 \mu\text{m}^2/\text{s}$ for $L = 19 \mu\text{m}$. The quantitative agreement between the simulation data at $l_p/w = 4.95$ and the experimental data at $l_p/w = 5.8$ in Figure 4b is excellent considering that this analysis did not require any fitting parameters. Indeed, as an alternate test, we fit Eq. 7 to the simulation data and obtained $c_1 = 1.4 \pm 0.4$ and $c_2 = 1.0 \pm 0.1$ at a 95% confidence level. The reduced error bounds for the simulations relative to experiments are due to the larger number of data

points for the simulations and the smaller sources of systematic errors in the calculations relative to experiments.

5 Discussion

This key results of the present contribution are (i) establishing at 95% confidence that the constant c_1 in Eq. 7 is non-zero and (ii) the close agreement between experiments and simulations without the need for any adjustable parameters. We posit that the data presented here are sufficient to demonstrate that blob theory²⁶ does not describe DNA diffusion in nanochannels for these channel sizes. To increase further the statistical confidence in the modified blob theory, further experiments probing the diffusivity over a range of fractional extensions similar to the simulations³⁷ are necessary. Such experiments are challenging, both in terms of the stringency required to fabricate channels with very small differences in widths and the difficulties we already encountered in experimental stability for the long durations required to measure DNA diffusivity. However, the relatively good quantitative agreement between the simulations and experiments indicates that such experiments are likely to be successful if these technical obstacles are overcome.

It is intriguing to speculate on what might happen during additional experiments in smaller channels. We suspect that such experiments are likely to begin to probe the “Rouse-like” regime identified in previous simulations.^{37,40} Explicitly, simulations³⁷ suggest that the DNA diffusivity for fractional extensions $0.3 \lesssim XL \lesssim 0.7$ should be independent of channel size,

$$\frac{D_t}{D_R} \approx 1.75 \ln \left(\frac{l_p}{a} \right) \quad (11)$$

where a is the hydrodynamic radius of DNA. The data we have obtained in the smallest channels are in the transition between the regime of validity for the modified blob theory and this plateau regime. As such, it is possible that the statistical confidence in Eq. 7 is impacted more by the emergence of the Rouse-like regime in the data in the smaller channels than agreement between our experimental data and blob theory²⁶ for the larger channels. Indeed, even a casual inspection of Figure 4b indicates that the experimental data deviate from the scaling law in Eq. 3. Experiments at even higher fractional extension are extremely challenging due to the even lower tolerance for errors in fabrication and characterization, which would affect the channel size, and the increased time required to measure substantial motion of the center-of-mass, which could make it challenging to control the experiment. While errors in the value of D_{eff} would not impact the test of Eq. 11, since the diffusivity is independent of the channel size, it is very important to keep the aspect ratio close to unity to compare the experiments to theory. It is unknown how the configurational statistics in rectangular channels, which differ substantially from their square counterparts,^{19,27,54,55} are coupled to the hydrodynamics in such channels.

6 Conclusion

We have presented measurements of center-of-mass diffusivity of DNA confined in approximately square nanochannels with effective sizes ranging from 117 nm to 260 nm at moderate ionic strength. We found that it is necessary to modify the blob theory to account for local chain stiffness in order to explain the experimental results in the extended de Gennes regime. This modification is very similar to that done in nanoslits albeit over a wider range of nanoslit height. Our results are in quantitative agreement with previous simulations of the Kirkwood diffusivity of a discrete wormlike chain model without need for fitting parameters, thus lending confidence to the simulation predictions.

From a methodology point of view, our work will serve as a valuable reference for future experimental studies of DNA diffusivity over wider range of channel sizes via careful control of the ionic strength, which can probe the role of local chain stiffness for DNA in confinement. Another area of interest would be to see how the aspect ratio of rectangular channels alters the dynamic response of DNA, as the rectangular channel shape affects the hydrodynamics and polymer configurations differently.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Snapshots of λ -DNA obtained from two movies in the $D_{\text{eff}} = 143$ nm nanochannel. (a) Movie where $|\beta - 1| < 0.1$. The movie contains a sheared molecule (arrow in the $t = 0$ s image), a molecule that diffuses out of the field of view (arrow in the $t = 400$ s image), two molecules that merge in the nanochannel (arrow in the $t = 1000$ s image). These molecules are not included in the analysis. The arrow in the image at $t = 150$ s indicates a typical molecule included in the analysis. (b) Movie with net upward drift of all molecules, leading to $|\beta - 1| > 0.1$ for their ensemble-averaged mean-squared displacement. Such a movie is excluded from the calculation of the diffusion coefficient for this channel. The movies used to generate this figure are available as Supporting Information.

Figure 2.

Mean squared displacement of 57 λ -DNA molecules inside a $137 \times 118 \text{ nm}^2$ nanochannel as a function of time lag from different segments of recorded images. The power law fit to the data from the complete movie (blue $\times$) for $\delta t \in [150 \text{ s}, 400 \text{ s}]$ yields an exponent 0.98, indicating normal diffusive behavior. The inset shows the dynamic diffusion coefficient, $\text{MSD}/2\delta t$, as a function of time lag. After an initial decay till $\delta t \sim 150 \text{ s}$, the $\text{MSD}/2\delta t$ value is reasonably constant. A linear fit to MSD for $\delta t \in [150 \text{ s}, 400 \text{ s}]$ in this nanochannel gives the diffusion coefficient $D_t = 0.0550 \text{ } \mu\text{m}^2/\text{s}$. Similar plots for the other channel sizes are included as Supplementary Information.

Figure 3.

(a) Average fractional extension, X/L and (b) normalized variance in extension, σ^2/Ll_p , from the current contribution (red ■) and previous experimental studies^{21,23} (black ○, ▲, ▼). The experimental results are compared to the extended de Gennes theory²⁹ (black solid line). The standard errors for our experiments are smaller than the size of data points.

Figure 4.

Log-log plot of diffusion constant, D_t , as a function of the average fractional extension, X/L . (a) Power law fit (solid black line) to the experimental data (red ■) yields an exponent -0.85 ± 0.15 at a 95% confidence level. (b) The simulation³⁷ and theoretical predictions (Eqs. 3 and 7) were computed with Rouse diffusivity, $D_R = 0.0129 \mu\text{m}^2/\text{s}$ for λ -DNA ($L = 19 \mu\text{m}$) in water ($\eta = 0.89$ cP) at room temperature ($T = 25^\circ\text{C}$). The line calculated from Eq. 7 gives $c_1 = 0.86 \pm 0.79$ and $c_2 = 1.2 \pm 0.19$ at a 95% confidence level. The error bars for the standard error of the mean experiments are calculated from uncertainty in linear fit to uncorrelated MSD vs δt and are smaller than symbol size.

Table 1

Summary of channel dimensions and statistics. Movies that produced anomalous diffusion for the ensemble of DNA molecules in that movie (see Figure 1b for an example) were excluded from further analysis. The number of molecules denotes the number of trajectories within the set of accepted movies that were used to compute the time and ensemble-averaged mean-squared displacement via Eq. 9.

Width (nm)	Height (nm)	D_{eff} (nm)	Number of Movies		Number of molecules
			Acquired	Accepted	
137	118	117	10	8	57
137	171	143	12	11	65
176	171	163	8	8	38
205	215	200	11	10	63
280	260	260	15	14	74