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Conformation and dynamics of single DNA molecules in parallel-plate slit microchannels

Y.-L. Chen, M. D. Graham,* and J. J. de Pablo†
Department of Chemical and Biological Engineering, University of Wisconsin-Madison, Madison, Wisconsin 53703, USA

G. C. Randall, M. Gupta, and P. S. Doyle‡
Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

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The conformation and diffusion of a single DNA molecule confined between two parallel plates are examined using both single molecule experiments and Brownian dynamics simulations accounting for hydrodynamic interactions. The degree of chain stretching and the diffusivity are characterized as a function of the chain confinement and the channel geometry. Good agreement is found between the simulations, experiments, and scaling theory predictions.

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Since the completion of an accurate human genome map, genomic research has focused on identifying the functionality of genetic sequences and the development of tools for fast and reliable analysis of DNA sequences [1,2]. Advanced photolithography techniques have allowed easy fabrication of microfluidic devices that promise to provide accurate, high-throughput methods for studying DNA [3]. Accurate, predictive models of how DNA molecules behave in microfluidic and nanofluidic flows would be of considerable use for device design and optimization. For example, DNA separation devices, such as colloidal postarrays [4] and entropic traps [5], rely on physical mechanisms [6] which are molecular size dependent. Molecular properties (e.g., diffusivity) change upon confinement [7] and require detailed models to correctly predict the resolving power of these devices.

Recent multiscale modeling studies using the Brownian dynamics method with hydrodynamic interactions (BD-HI) have improved our understanding of how long chain molecules behave when confined to channels and subject to pressure-driven flow [8–10]. The BD-HI method explicitly accounts for hydrodynamic interactions between chain segments and perturbations due to confinement by the surrounding walls. Chain stretching under pressure-driven flow and in confinement has been explored systematically, with the finding that chain molecules in square microchannels migrate towards the center of the channel, which is only captured when hydrodynamic perturbations due to the walls are accounted for [10]. Model calculations have also predicted that separation of DNA fragments of different lengths may be achieved in square channel devices [8,10]. In many applications, rectangular slit channels are more commonly adopted because of the ease of fabrication, and many recent theoretical and experimental works have focused on how particles behave in these rectangular slit channels where the cross-sectional aspect ratio is large [11–15]. However, the role played by hydrodynamic interactions in microfluidic flow has not been addressed for the slit channel geometry (see Fig. 1). Furthermore, studies which quantitatively compare simulation and experimental data for single polymers in confined geometries have not been performed. In this work, we describe an application of the BD-HI method to study how DNA chains stretch and diffuse in channels where the chains are bound by two parallel plates. The simulation results are compared to the simulations for DNA in square microchannels and also to experimental measurements.

Following prior work [16], a double stranded DNA molecule (ds-DNA) is modeled as a wormlike chain connected by beads that enforce excluded volume conditions to obtain the correct static properties at equilibrium (i.e., good solvent chain scaling). Stained λ-DNA has a contour length of 22 μm [17], and its radius of gyration in bulk free solution $R_{g,bulk}$ is approximately 0.7 μm. Recent BD-HI model studies of λ-DNA in bulk solution represented the chain molecule as a $N=11$ bead pearl necklace that is connected by $N_s=10$ wormlike chain springs [16]. Each bead is replaced by approximately 20 Kuhn segments in the random-walk conformation, and the beads interact with each other through a Gaussian excluded volume potential. The bead excluded volume ($v=0.0019$ μm^3/Kuhn segment), the Kuhn segment length ($\sigma_k=0.106$ μm), and the hydrodynamic radius of the beads ($a=0.077$ μm) are determined by comparing the model system to experiments of stained λ-DNA in a 43.3 cP solvent at 300 K [18]. This model has been shown to capture the chain dimensions and diffusivity in bulk solution [16], and it is adopted for λ-DNA for the confined system here.

In this study, we focus on the equilibrium conformation and the diffusion of a single DNA molecule confined between two parallel plates separated by a distance H, where

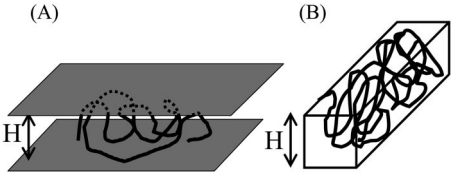

FIG. 1. Illustrations of (A) the slit channel geometry and (B) the square microchannel.

*Electronic address: graham@engr.wisc.edu
†Electronic address: depablo@engr.wisc.edu
‡Electronic address: pdoyle@mit.edu

$\sigma_k \ll H \sim 2R_{g,bulk}$ and the approximations we have adopted are most relevant. The simulation box is periodic in the unconfined directions, and the chain segments are repelled from the confining walls by a short-ranged potential [9]. The positions of the beads on the chain are evolved in time with a semi-implicit Euler scheme according to the Langevin equation rewritten as [16,19]

$$d\vec{R} = \left[\vec{U} + \frac{1}{k_B T}\mathbf{D} \cdot \vec{F} + \vec{\nabla} \cdot \mathbf{D} \right] dt + \sqrt{2}\mathbf{B} \cdot d\vec{W}, \qquad (1)$$

where dt is the time step, $\vec{R}$ represents the positions of the beads on the chain, $\vec{U}$ is the unperturbed velocity field, $\vec{F}$ consists of the internal (chain conformation) and external (wall and bead excluded volume) forces acting on the beads, $d\vec{W}$ is the Brownian perturbation, and $\mathbf{D}=\mathbf{B}\cdot\mathbf{B}^T$ is a $3N \times 3N$ diffusion matrix that accounts for intrachain hydrodynamic interactions between chain segments and perturbations due to the confining parallel walls. **D** is calculated by separating it into particle self-diffusion, Green's function in free solution, and perturbation to the Green's function due to the wall confinement. The Green's function in free solution is given by the Oseen-Burgers tensor [19], but it is replaced by the Rotne-Prager-Yamakawa tensor here for numerical tractability [9,20]. The perturbation due to parallel-plate confinement is more complicated and several approximate theoretical methods have been proposed by considering a particle as a point source [11,21,22]. Recent experimental measurements have verified these predictions for the dynamics of Brownian particles in slit microchannels [12,23]. In this work, the intrachain hydrodynamic interactions due to slit confinement are found by solving the incompressible Stokes flow problem subject to the wall boundary conditions using the finite element method [9,16], and the results are verified against the infinite series solutions of Liron and Mochon [21,22].

Experimentally, Brownian trajectories of single DNA molecules were observed using epifluorescence video microscopy. Double stranded DNA molecules of various sizes (λ-DNA and concatomers referred to as 2λ-DNA and 3λ-DNA) were stained with the dye TOTO-1 (Molecular Probes) at a ratio of 1 dye to 4 base pairs. DNA were subsequently diluted in a buffer of 0.5X Tris-Borate-EDTA (TBE), 4% β-mercaptoethanol, 10-mM NaCl, and 0.1% ascorbic acid (viscosity of 1.08 cP at 294 K). The molecules were observed in rectangular polydimethylsiloxane (PDMS) microchannels fabricated by soft lithography [24] and sealed on the bottom with a glass coverslide. The PDMS portion of the channel was soaked in the buffer overnight to saturate it with solution, thereby preventing the onset of a bulk flow due to solvent permeation into the PDMS [25]. The channels are 2-cm long, 50-μm wide, and have heights (H) ranging from 1.3 to 2.7 μm. Some experiments were performed for λ-DNA in channels having a width of 100 μm and no appreciable difference in the diffusivity was found. The bulk diffusivity was measured in a homemade chamber consisting of two microslides separated by ~100 μm spacers and sealed with vaseline.

The center of mass of a molecule was determined by digitizing images, subtracting the background, and then determining the first moment of the intensity distribution [26]. Only molecules in the central region of the channel (at least 15 μm from the sidewalls) were analyzed so that a direct comparison to the slit geometry simulations can be made. The channels were mounted on an inverted microscope (Zeiss Axiovert 200, 100×NA=1.4 oil immersion objective, Hamamatsu EB-CCD camera) which permits viewing of motion in the x-y plane, where x is taken to lie along the length of the channel, y is along the width, and the thinnest channel dimension H is in the z direction. The gap-averaged, in-plane, self-diffusion coefficient D was determined by computing the mean-squared center-of-mass displacement of several molecules and then using the relation $\langle \Delta x^2(\tau)\rangle + \langle \Delta y^2(\tau)\rangle = 4D\tau$, where τ is the lag time between displacements. For a given channel height and DNA length, traces of $\langle \Delta x^2(\tau)\rangle$ and $\langle \Delta y^2(\tau)\rangle$ were indistinguishable, confirming that interactions with the sidewalls are negligible for molecules near the center of the channel. Following previous studies [27], we compute the unconfined bulk radius of gyration using the Zimm model for good solvents $R_g = 0.203\, k_B T/\sqrt{6}\eta D_{bulk}$ [28], where η is the solvent viscosity and $k_B T$ is the thermal energy. In determining D_{bulk} we only tracked molecules which were at least 20 μm from the chamber's floor and ceiling. For λ-DNA we obtain a value of $D_{bulk}=0.45\ \mu m^2/s$ ($R_{g,bulk}=0.69\ \mu$m), which compares favorably with previous studies [27].

Before presenting the results, we briefly review the scaling predictions derived by de Gennes and co-workers for the stretching and diffusion of a polymer in a thin slit [29–31]. A chain containing N monomers of size a in the bulk has a radius of gyration $R_{g,bulk} \sim aN^{\nu}$, where $\nu \approx 3/5$ for a good solvent. In a thin slit the polymer can be thought of as a chain of blobs (N_{blob}) which span the slit dimension H, each blob containing g monomers; giving $g \sim (H/a)^{1/\nu}$. The number of blobs can then be expressed as $N_{blob}=N/g \sim (R_{g,bulk}/H)^{1/\nu}$. In a slit, the extension X (or size) of the chain is equivalent to a self-avoiding walk of blobs in two dimensions yielding

$$\frac{X}{R_{g,bulk}} \sim \frac{HN_{blob}^{\nu_{2D}}}{R_{g,bulk}} \sim \left(\frac{R_{g,bulk}}{H}\right)^{\nu_{2D}/\nu - 1} \sim \left(\frac{R_{g,bulk}}{H}\right)^{1/4}, \qquad (2)$$

where we have set ν_{2D}, the Flory-Edwards scaling exponent for a chain in two dimensions, equal to 3/4 for a good solvent. In a square capillary, the extension scales simply as the number of blobs times the blob size, giving

$$\frac{X}{R_{g,bulk}} \sim \left(\frac{R_{g,bulk}}{H}\right)^{1/\nu - 1} \sim \left(\frac{R_{g,bulk}}{H}\right)^{2/3}. \qquad (3)$$

In a slit (or capillary) hydrodynamic interactions are assumed to be strongly screened over length scales larger than H and so the net drag on the chain scales as $\zeta_{chain} \sim N_{blob}\zeta_{blob} \sim N_{blob}H \sim (R_{g,bulk}/H)^{1/\nu}H$. Using then the fact that $D_{bulk} \sim R_{g,bulk}^{-1}$ gives

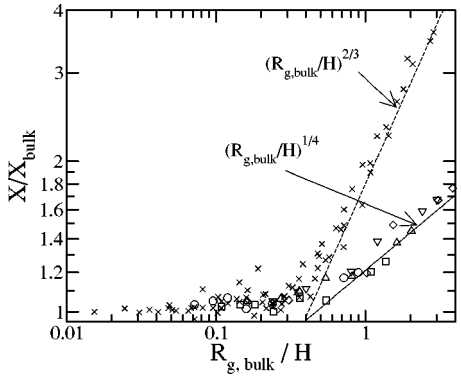

FIG. 2. BD-HI simulation results for the normalized chain stretch in the slit channel as a function of inverse normalized channel width for chain lengths of $N_s=10$ (circles), 20 (squares), 40 (triangles), 80 (inverted triangles), and 120 (diamonds). The crosses are the simulation results for the square channel geometry from Ref. [9]. The solid line is the scaling theory prediction of $(R_{g,bulk}/H)^{1/4}$ and the dashed line is $(R_{g,bulk}/H)^{2/3}$.

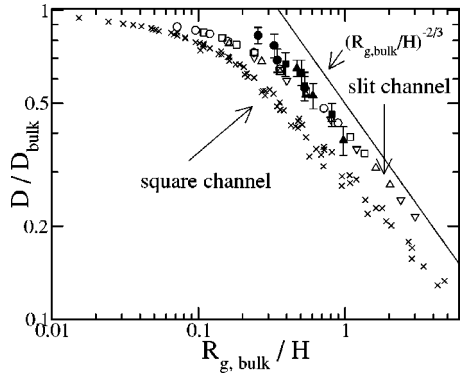

FIG. 3. BD-HI simulation results for the normalized chain diffusivity in the slit channel as a function of inverse normalized channel width for chain lengths of $N_s=10$ (circles), 20 (squares), 40 (triangles), 80 (inverse triangles). Experimental measurements for λ-DNA (filled circles), 2λ-DNA (filled squares), and 3λ-DNA (filled triangles) are shown with error bars. The crosses are the results for the square channel geometry from Ref. 9. The solid line is the scaling theory prediction of $(R_{g,bulk}/H)^{-2/3}$.

$$\frac{D}{D_{bulk}} \sim \left(\frac{R_{g,bulk}}{H}\right)^{1-1/\nu} \sim \left(\frac{R_{g,bulk}}{H}\right)^{-2/3}. \quad (4)$$

This scaling for D is expected to hold for both a thin slit and square capillary.

Under the no-flow condition ($\vec{U}=0$), the equilibrium chain stretch X, precisely defined as the average maximum size of the chain in the x-y plane (the unconfined directions), and the chain diffusivity D in the slit confinement are examined as a function of the slit channel height (H). The chain stretch in bulk free space X_{bulk}, defined as the maximum extension of the chain averaged over an ensemble of configurations scales as $R_{g,bulk}$ and the scaling prediction in Eq. (2) leads to $X \sim X_{bulk}(R_{g,bulk}/H)^{1/4}$. As shown in Fig. 2, the BD-HI simulations confirm the two-dimensional (2D) scaling law in highly confined slit channels, and the crossover from bulk to highly confined behavior occurs near $R_{g,bulk}/H \sim 0.4$, similar to the results found in a square channel geometry [8]. The chain stretch has a much weaker dependence on channel confinement for slit channels than for square channels. We note that equilibrium chain stretching is due entirely to entropic-excluded volume restrictions (bead-wall and bead-bead), and hydrodynamic interactions between chain segments do not play a role.

However, accounting for hydrodynamic interactions is crucial to correctly capture the scaling behavior of transport coefficients. Figure 3 shows the BD-HI results for the chain diffusivity as a function of $R_{g,bulk}/H$. The simulations show that the reduced chain diffusivity D/D_{bulk} scales with $(R_{g,bulk}/H)^{-2/3}$ in the strong confinement limit for both slit and square channels. This finding agrees with scaling theory predictions, Eq. (4), for systems with strong hydrodynamic screening. Quantitative comparisons between the slit and channel geometries show that the chains diffuse faster in slits, which is expected because there is less constraint on the movement of chain segments. The model predictions also compare favorably with the experimental diffusivity measurements on concatenated λ-DNA molecules. This result in and of itself is quite striking given that the parameters for the simulation were chosen to match the bulk solution behavior of λ-DNA measured by Smith and Chu [18] and have not been adjusted. The experimental measurements over a range $R_{g,bulk}/H=0.25-0.9$ appear to follow the scaling law for the highly confined scaling regime within the error bars, while the model predictions show the bulk-confinement crossover to be $R_{g,bulk}/H \approx 0.3-0.4$ and slightly underpredicts the diffusivity near the bulk-confinement crossover.

At the bulk-confinement crossover when the chain first starts to "feel" confined by the walls, the chain diffusivity predicted by the simulations are slightly below the experimental measurements. This may be due to the coarse-grained polymer model and/or the repulsive interactions between the chain segments and the wall. The chain segment-wall repulsive interaction is expected to most strongly affect the chain diffusivity and stretching in the highly confined regime, and additional simulations show that varying the parameters that govern the wall-chain repulsion model only shifts the position of the crossover in terms of R_g/H and does not change the scaling behavior. The quantitative agreement between the simulation and the experiments in the highly confined regime suggests that the coarse-grained chain model is valid when the entire chain is affected by the hydrodynamic perturbations due to the wall. However, near the bulk-confinement crossover when only parts of the chain are near the wall, the wall perturbations to the intersegment hydrodynamic interactions may be exaggerated for the coarse-grained bead and lead to the lower diffusion coefficient of the simulated chain. A more finely coarse-grained model of chain segments near the wall is currently being investigated to better capture the wall hydrodynamic perturbations and the overall chain dynamics.

This comparison demonstrates that the BD-HI method can accurately capture how a single DNA chain behaves under confinement in the slit geometry without flow. The simulation results for the chain stretch and diffusivity are

found to follow scaling predictions in the highly confined limit, and comparisons with the experimental measurements show that the results are in good agreement. Compared to the square channel confinement, DNA chains confined in rectangular slit channels are less stretched and diffuse faster. This comparison allows us to proceed with future investigations into how DNA chains behave under pressure-driven flow and other types of flow conditions in a slit geometry. Other problems, such as the role of hydrodynamic interactions on chain adsorption onto channel walls and the flow of conjugated biological functional groups, may also be addressed.

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