DNA translocation through short nanofluidic channels under asymmetric pulsed electric field

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Investigation of single molecule DNA dynamics in confined environments has led to important applications in DNA analysis, separation, and sequencing. Here, we studied the electrophoretic transport of DNA molecules through nanochannels shorter than the DNA contour length and calculated the associated translocation time curves. We found that the longer T4 DNA molecules required a longer time to traverse a fixed length nanochannel than shorter λ DNA molecules and that the translocation time decreased with increasing electric field which agreed with theoretical predictions. We applied this knowledge to design an asymmetric electric pulse and demonstrate the different responses of λ and T4 DNA to the pulses. We used Brownian dynamics simulations to corroborate our experimental results on DNA translocation behaviour. This work contributes to the fundamental understanding of polymer transport through nanochannels and may help in designing better separation techniques in the future. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4871595>]

I. INTRODUCTION

The transport of polymer molecules through a confined region, such as a nanopore, is ubiquitous in biology and is one of the most fundamental processes of life. Examples of such biological phenomena include passage of mRNA through nuclear pore complexes, injection of DNA from a virus head into a host cell, and protein translocations across biological membranes through channels.¹ The study of DNA as a model polymer in confined environments, such as nanopores,^{2–4} nanochannels,^{5–9} and nanoslits,^{10–15} has led to significant advancements in our understanding of DNA dynamics^{11,16–18} and its application to DNA sorting,^{10,12,19–21} mapping,^{22–25} and sequencing.^{2,26}

DNA translocation through a nanochannel can be viewed as a three stage process—first, one end of the polymer chain reaches the nanochannel entrance, then the chain enters the nanochannel, and finally the chain exits the nanochannel.¹ The second stage, of the polymer chain entering and traversing the nanochannel, has been well studied. However, most of the studies have focused on the movement of DNA either through short nanopores which physically constrain only a small fraction of the polymer^{27–31} or through long nanochannels which completely constrain the DNA leading to equilibrium dynamics studies.^{5–7,16,32,33} Some recent theoretical treatments^{34–37} have given us insight into the situation by characterizing the time scales. Yong *et al.*³⁴ studied the translocation time for various ratio of the channel length L to the polymer length N and found that the translocation time $\tau \sim N^{1+\nu}$ for short channels $L \ll N$, while $\tau \sim L/F$ for long channels $L \gg N$, and is independent of the chain length. Slonkina and Kolomeisky³⁵

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addressed polymer translocation through a long nanopore and reported that the length of the pore is a critical parameter for polymer translocation dynamics and that for polymer chains longer than the pore length, the velocity of translocation is nearly constant. Panwar and Kumar³⁶ simulated the electrophoretic transport of polymers through nano-constrictions and found that the translocation time of the polymer was proportional to the polymer length for all simulated voltages. Sebastian and Paul³⁷ analyzed the escape of a polymer molecule over a one-dimensional energy barrier and concluded that the translocation time depends inversely on the square root of the applied voltage difference. However, experimental investigations of DNA translocation through nanochannels which are shorter than the contour length of the DNA (but longer than the molecule's radius of gyration) are few.^{11,38}

Here, we study DNA translocation dynamics as it is electrophoretically driven through a short nanochannel and calculate the translocation time. We observe that for our system the translocation time is indeed proportional to the DNA contour length and decreases non-linearly with the applied voltage. Brownian Dynamics (BD) simulations are also carried out in order to further validate our experimental observations. Instead of matching the time scale of the experiment, the simulations seek to demonstrate qualitative agreement with the experimental results by solving the dimensionless governing equations.

II. MATERIALS AND METHODS

A four-reservoir device design, as shown in Figures 1(a)–1(c), was used for the experiments. It consisted of two 1.5 μm deep and 1 mm long u-shaped microchannels connected by an array of 200 nm deep, 400 nm wide, and 10 μm long nanochannels. Each microchannel had two reservoirs, one at each end, which facilitated loading of DNA.

The device was fabricated with Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) using a combination of standard photolithography and soft lithography. First, a silicon master was made by patterning 400 nm $\times$ 200 nm wires on a silicon wafer via projection lithography (GCA 6100C Stepper, I-line) using SPR950 photoresist and plasma etching (50 sccm of He, 100 sccm of SF_6 , 300 mTorr, 200 W) (LAM Autoetch 490 Poly/Nitride Plasma Etcher) the exposed silicon surface. Then, contact photolithography was used to pattern 1.5 μm tall micro-ridges over the silicon wires using S1813 photoresist. The final device was made by making a negative replica of the silicon master with PDMS using soft lithography and permanently bonding the resulting PDMS stamp to a glass coverslip. This was done in the following steps: first, the silicon master was treated with vapor-phase hexamethyldisilazane (HMDS) (YES III HMDS Vapor Prime/Vacuum Oven) to facilitate the subsequent demolding of the replica. Then, it was spin coated at 1000 rpm with a 1:1 (w/w) PDMS: curing agent solution and baked at 60 $^\circ\text{C}$ for 0.5 h. Next, a second layer ($\sim$ 0.5 cm thick) of 10:1 (w/w) PDMS: curing agent was added on the master and baked at 60 $^\circ\text{C}$ for 2 h after which the PDMS was demolded from the master. The PDMS stamps were further baked at 170 $^\circ\text{C}$ for >15 h to ensure complete crosslinking of the polymer. Access holes were made by punching holes in the PDMS stamp by using a 3 mm biopsy punch (Harris unicore, Electron Microscopy Sciences). To permanently seal the device, the PDMS stamp and a clean glass coverslip (thickness: 0.16 to 0.19 mm) were exposed to oxygen plasma for 30 s at 50 W and 100 mT and then brought in contact to form an irreversible bond. The sealed device was then filled with 5 $\times$ Tris-Borate-EDTA (TBE, pH 8.3, Sigma-Aldrich, filtered with a 0.22 μm filter) buffer and stored in a humid environment.

Stock solutions of λ -phage DNA (48.5 kbp, Promega) at 5 $\mu\text{g}/\text{ml}$ and T4 GT7 DNA (166 kbp, Wako Chemicals USA, Inc.) at 4 $\mu\text{g}/\text{ml}$ were prepared by staining the DNA with YOYO-1 (Invitrogen) at a dye:base pair ratio of 1:5 in 5 $\times$ TBE. The experimental solutions were prepared by diluting the stock DNA solution in 5 $\times$ TBE buffer containing 0.1% poly(n-vinylpyrrolidone) (PVP, Sigma-Aldrich, MW 40 000) to suppress electroosmosis^{12,39} and 4% 2-Mercaptoethanol (BME, Sigma-Aldrich) to reduce photobleaching. Wide, cut tips were used for pipetting DNA to prevent DNA breakage. The final concentration of DNA for the experiments was 0.5 $\mu\text{g}/\text{ml}$ for λ DNA and 0.4 $\mu\text{g}/\text{ml}$ for T4 DNA. Several hours prior to the experiment, the device was filled with 5 $\times$ TBE containing 0.1% PVP and 4% BME. A Keithley 6517B high resistance

FIG. 1. (a) Schematic of the sealed 4-reservoir PDMS device. (b) Scanning electron micrographs of the device showing two u-shaped microchannels connected to reservoirs at each end; scale bar is $400\ \mu\text{m}$. The dashed box region shows (c) nanochannels interfaced with microchannels on either side; scale bar is $5\ \mu\text{m}$. (d) Snapshot of λ DNA crossing the nanochannels at 5 V. Dotted lines mark the inlet and outlet and the electric field direction is shown by the arrow below. (e) Simulated electric field distributions at the microchannel-nanochannel interface along the nanochannel axis. The applied external voltage is 1 V. (Multimedia view) [URL: <http://dx.doi.org/10.1063/1.4871595.1>]

electrometer, controlled by a computer via custom made LabView program, was used to apply the electric field via platinum wire electrodes dipped into the device reservoirs.

Dynamics of YOYO-1 stained λ DNA and T4 DNA were observed with a Nikon TiE inverted total internal reflection fluorescence (TIRF) microscope with a CFI APO $100\times$ TIRF oil immersion objective (NA = 1.49 and WD = 0.12 mm). A 100 mW 488 nm laser was used to excite the YOYO-1 stained DNA and the emitted light was passed through a bandpass emission filter to eliminate background. Images were captured using an Andor iXon DU897 EMCCD camera with a readout rate of 35 frames/s (for full 512×512 pixels). The field of view was often cropped to increase the frame rate to ~ 52 frames/s. Nikon NIS Elements software was used to record the data and MATLAB was used to analyze the data.

BD simulations were carried out using a DNA model based on the wormlike bead-rod chain.^{40,41} DNA consists of N beads connected by $N-1$ rigid rods of length l . Details of the simulation setup are given in the supplementary material.⁴² The electric field for one set of parallel nanochannels was simulated using Fluent (ANSYS, Inc.) where the electric field was obtained by solving the Laplace equation. The governing equation and boundary conditions are

$$\nabla^2 V = 0 \quad \text{in } \Omega, \quad (1)$$

$$V = V_{\text{given}} \quad \text{or} \quad \frac{\partial V}{\partial n} = 0 \quad \text{on } d\Omega, \quad (2)$$

where Ω denotes the computational domain, n is the unit normal vector on the boundary $d\Omega$. $V = V_{\text{given}}$ is specified at inlet and outlet and $\partial V / \partial n = 0$ is imposed on the insulating wall of the channel. Once V is solved, the electric field E is determined by $E = -\nabla V$. In order to determine the electric field at DNA bead location, a numerical particle-tracking algorithm was conducted in a 3-D nanofluidic computational domain.⁴³ Due to the higher resistance of the nanochannel, field lines converge at the nanochannel entrance resulting in a higher electric field inside the nanochannel (Figure 1(e)). A high field zone (HFZ) was defined just outside the nanochannel inlet, marked by red dashed lines in Figure 1(e), to be the region outside the nanochannel where the electric field value drops to 10% of its value inside the nanochannel.

As mentioned earlier, the DNA translocation process involves multiple time scales. These are typically described as time to drift or diffuse to the channel entrance, time to overcome the entropic barrier, and time to cross the channel. Since the electric field is negligible outside a distance of the nanochannel height from the nanochannel entrance, the diffusion time scale of DNA to the entrance will dominate any time calculation. Thus, to increase throughput, an electric field was used to pre-concentrate the DNA at the nanochannel entrance. For a device of given dimensions, the time to overcome the entropic barrier is inversely proportional to the applied field. If the driving field is well above a threshold value, then the entropic penalty is negligible and the time to overcome this barrier is essentially zero. In this work, we only consider the crossing time of the DNA and define its translocation time as the time taken by a DNA molecule to completely exit the nanochannel after entering from one end. The entropic penalty plays a part only at low applied voltages (<0.5 V) and its effect will be discussed for the case of asymmetric pulses.

III. RESULTS AND DISCUSSIONS

A. Translocation time

The device configuration used for capturing DNA motion is shown in Figure 1(a). Either λ or T4 DNA was loaded into reservoir 1 and then driven into the right microchannel by applying a small potential difference across reservoirs 1 and 2. Once the desired number of DNA molecules had accumulated near the nanochannel entrance, reservoirs 1 and 2 were connected to the negative terminal and reservoirs 3 and 4 were connected to the positive terminal of the power supply and the desired voltage was applied for 1 min to move the DNA across the nanochannels. The motion of DNA in multiple nanochannels was simultaneously captured with a $100\times$ objective (field of view as shown in Figure 1(c)). The translocation time was calculated by analyzing the captured videos for both DNA under a range (1–10 V) of applied DC fields (Figure 1(d)). This was done by creating regions of interest (ROI) around each of the nanochannels (rectangular boxes, $10\ \mu\text{m} \times 0.5\ \mu\text{m}$) and plotting the intensity vs. time graph for each ROI using the NIS software. A typical intensity-time plot for λ DNA at 8 V is shown in Figure 2(a), where the different colors correspond to the different nanochannels. Each peak represents the passage of a single DNA molecule and has a finite width. The peaks are of different intensities due to difference in DNA conformation and length. A higher intensity than average could be due to a DNA passing through the channel in a folded conformation or two DNA strands entering at the same time. A lower intensity could indicate a short piece of DNA caused by cleavage due to shearing or photo-damage. Thus, to exclude events with multiple strands of DNA or cleaved pieces of DNA, intensity threshold values selected from intensity histograms (not shown here) were applied to each data set. At times, a single DNA strand was observed to enter adjacent nanochannels simultaneously and remain there for some time before one of the ends exited into the left microchannel and pulling the other end with it. These events contributed to longer than average translocation times and were also excluded from the calculation. The frequency of translocation events was observed to increase with an increase in the applied voltage. No translocation events were observed below 0.4 V for either DNA. The DNA molecules were observed to come very close to the nanochannel but unable to cross the entropic barrier to get in the nanochannel.^{36,37} Thus, 0.4 V was the threshold electric potential value for this system.

FIG. 2. (a) A typical intensity time curve, shown here for λ DNA at 8 V. (b) Intensity peak corresponding to the translocation of a single λ DNA at 5 V. The numbered data points refer to the snapshots shown in (c). (d) Intensity peak corresponding to the translocation of a single T4 DNA at 5 V where the numbered data points refer to the snapshots shown in (e). Dashed white lines define the ends of nanochannel.

Figure 2(b) shows a single peak for λ DNA translocation at 5 V where the numbered data points identify the snapshots in Figure 2(c). As more of the DNA entered the nanochannel, the intensity rose from the background reaching a peak when the maximum amount of DNA was in the nanochannel (frame 2) and finally decreased as the DNA left the nanochannel. The baseline width of each peak was measured to arrive at the DNA translocation time. In contrast, a single peak for T4 DNA, shown in Figure 2(d), shows two local maxima that correspond to different amounts of DNA being in the nanochannel. Frames 1 and 2 in Figure 2(e) show part of the DNA entering the nanochannel in a stretched configuration. On application of an electric force, the portion of DNA closest to the nanochannel gets sucked in and stretches even as the rest of the chain is oblivious to the force.⁴⁴ This is followed by the propagation of the force to the rest of the chain in the microchannel which due to its smaller radius of gyration is now able to enter the nanochannel in a more coiled configuration as seen in frame 6. Once the nanochannel is fully occupied, the DNA gets gradually pulled towards the exit as shown by frames 7 and 8. Some more examples of T4 DNA translocating through the nanochannel are shown in the supplementary material.⁴² This combination of image and intensity analysis helps to correlate DNA conformations with the observed intensity signal.

Figure 3(a) shows the experimentally calculated translocation time for λ and T4 DNA as a function of the applied voltage. Dimensionless translocation times were also evaluated from BD simulations, shown in Figure 3(b), and were determined as the time difference between when the leading bead of the DNA chain touches the nanochannel and when the last bead of DNA passes the nanochannel outlet. The Peclet number, Pe , defined as the ratio of the DNA electrophoretic advection to its thermal diffusivity, was used as the driving force to calculate the non-dimensional translocation time where time was non-dimensionalized as $\hat{t} = t/(\xi b_k^2/k_B T)$. Here,

FIG. 3. (a) Experimental translocation time for λ DNA (empty square) and T4 DNA (solid square) as a function of the applied voltage across a nanochannel with a cross-section of 200×400 nm and length of $10 \mu\text{m}$. (b) Simulated dimensionless translocation time for λ DNA (empty square) and T4 DNA (solid square) as a function of the Peclet number. Dashed lines represent a power law fit as described in the text.

$Pe = \mu b_k V_p / D l_{\text{nano}}$, where μ is the bead electrophoretic mobility, V_p is the applied voltage, D is the bead diffusivity, and l_{nano} is the nanochannel length. The bead diffusivity was defined as $D = k_B T / \zeta$, the ratio of its thermal energy to its drag coefficient.

Figure 3 shows that the T4 molecules require a longer time to traverse a fixed length nanochannel than the shorter λ DNA molecules and that the translocation time decreases with the electric field. This observed length dependence of crossing time results from the fact that the DNA molecules must uncoil in order to enter the nanochannels smaller than their radius of gyration. Ignoring the entropic barrier time, since the applied voltages are greater than threshold, an order of magnitude estimate of the translocation time may be made from the following relation: translocation time = (channel length + DNA contour length)/DNA velocity. If the mobility of DNA is assumed independent of its length in the nanochannel, then the translocation time should be inversely proportional to the applied potential difference which is what is observed here. Also, for the above mentioned assumptions, the translocation time relation predicts a constant ratio of the translocation time for λ and T4 molecule $\approx (21.5 \mu\text{m} + 10 \mu\text{m}) / (73.4 \mu\text{m} + 10 \mu\text{m}) \approx 0.38$. This estimate is very close to the average ratio calculated from the simulation data (Figure 3(b)) which is $0.39 (\pm 0.05)$. However, the average ratio obtained from the experimental data (Figure 3(a)) is $0.64 (\pm 0.056)$, which is higher than the calculated estimate since the longer T4 DNA molecules do not stretch out completely (as shown in Figure 2(e)), resulting in smaller length and hence a higher ratio.

A power law fit, $t_{\text{trans}} = \alpha V_{\text{app}}^\beta$, of the translocation time data points for both the DNA was calculated and plotted as shown by the dashed lines in Figure 3. The α and β values for the experimental data (Figure 3(a)) were: 395.9 and -0.8 for λ DNA and 655.1 and -0.8 for T4 DNA. Since β values are approximately equal, $t_{\text{trans}} \sim \frac{\alpha_\lambda}{\alpha_{T4}} = 0.6$. Thus, $t_{\text{trans}} \sim \text{DNA contour length}$ as predicted in the previous theoretical analyses.^{36,37} Further, the β obtained from our calculations is very similar to other reported experimental values.⁴⁵ Thus, our experimental system is closer to the nanopore²⁷ system than the DNA than the entropic trap array of Han and Craighead⁴⁶ as the translocation time through the nanochannel is the predominant time scale (by design) and is proportional to the DNA contour length. The α and β values for the simulated data (Figure 3(b)) were: 499.8 and -1.1 for λ DNA and 2128 and -1.5 for T4 DNA. In BD simulation, the DNA-wall interaction was modeled by applying a repulsive force on the wall. The absence of hydrodynamic interactions between the DNA chain and the nanochannel in the simulations may be one of the reasons why the simulation results differ from the experimental observations.

B. Single forward pulse

To demonstrate the utility of the translocation time curves, the region in between the two curves in Figure 3(a) is defined as the selection band. The significance of the selection band is

exploited by observing that if an electric pulse of magnitude and duration equal to the corresponding values for a given point (V , t) within the band was applied to both λ and T4 DNA, it would result in the complete translocation of the λ DNA and but only partial passage of T4 DNA. T4 DNA in this case might end up being either half way across the nanochannel or stretched across the nanochannel with ends protruding from the nanochannel inlet and outlet. Further, if at this stage a reverse pulse of magnitude lower than the threshold potential (0.4 V) was applied for a sufficiently long time, the T4 DNA molecule would be pulled back to the right microchannel, allowing for selective delivery of λ DNA to the other left microchannel.

BD simulations were used to study the effect of such a pulse, that is a pulse of duration between the translocation times of λ ($\hat{t}_\lambda$) and T4 ($\hat{t}_{T4}$) DNA, on a population of DNA molecules. In order to describe the DNA translocation profile, two geometrical parameters x_{leading} and x_{last} are defined as shown in Figure 4(a). x_{leading} is the largest value of x -coordinates and x_{last} is the smallest value of x -coordinates among DNA beads. Thus, the values of x_{leading} and x_{last} determine the location of the DNA strand with respect to the nanochannel. Figures 4(c) and 4(e) show the simulated x_{leading} and x_{last} trajectories of λ and T4 DNA chains under a single pulse: $Pe_1 = 10$, $\hat{t}_1 = 60$, $Pe_2 = 0$, and $\hat{t}_2 = 600$. For clarity, only 20 chains from the simulation were plotted for each DNA. Dark solid lines denote the trajectories of x_{leading} and light dashed lines denote the trajectories of x_{last} . Dark dashed lines mark the nanochannel inlet and outlet. In the case of λ DNA (Figure 4(c)) since $\hat{t}_1 > \hat{t}_\lambda$, both the x_{leading} and x_{last} trajectories are outside the nanochannel outlet at the end of the pulse suggesting that all λ DNA strands completely cross the nanochannel. The trajectories for T4 DNA (Figure 4(e)) show a more complex behaviour. At the end of the pulse, the leading beads for all the 20 strands are able to cross the nanochannel to reach the outlet while the last beads are still in the inlet. Once the pulse is turned off (at $\hat{t} = 60$), both the leading and last beads of most of the strands stay in the outlet and inlet microchannels, respectively, until time $\hat{t} = 240$, suggesting that the DNA is stretched and in a tug-of-war (TOW)¹¹ like situation. After this, the leading beads of 60% of the strands start moving back in the nanochannel and towards the inlet microchannel. This motion is akin to a retraction and recoiling motion⁵ and occurs with a constant velocity (slope of the solid lines between inlet and outlet ≈ 0.063). At the same time (immediately after $\hat{t} = 240$), the x_{last} of 30% of the strands start moving across the nanochannel and recoil to the outlet microchannel with a similar velocity (slope of the dashed lines between inlet and outlet ≈ 0.066).

The foregoing simulation analysis was correlated with single pulse experiments carried out by applying an electric pulse at 4 V to λ and T4 DNA. The duration of the pulse ($t_{\text{exp}} = 200$ ms) was chosen from Figure 3(a) to be between the average translocation times for T4 ($t_{T4} = 233.9$ ms) and λ ($t_\lambda = 133.2$ ms) DNA at 4 V. Figure 4(f) shows the snapshots of a λ DNA molecule as it translocates through the nanochannel under a single pulse of 4 V, 200 ms. Most of the λ DNA molecules observed during the experiments which touched the HFZ at the beginning of the pulse were able to get across the nanochannel. However, some ($\sim 10\%$ of total observed events) λ DNA molecules entered the HFZ in the middle or towards the end of the pulse and were not able to get across the nanochannel completely. Figure 4(g) shows snapshots of a typical T4 DNA responding to a pulse of 200 ms at 4 V. Unlike λ DNA, the T4 DNA was stretched out across the nanochannel with ends hanging out from both inlet and outlet at the end of the pulse. After the pulse was turned off, the T4 DNA was in TOW situation where it was trapped in the nanochannel in quasi-equilibrium for a finite amount of time as both ends of the DNA were pulled by equal and opposite entropic recoiling forces exerted by portions of the DNA outside the nanochannel (Figure 4(g)). This phase ended abruptly (perhaps due to random fluctuations causing one side to win the TOW) with the DNA retracting and recoiling to either of the microchannels.⁵ The total time ($t_{\text{TOW}} + t_{\text{recoil}}$) taken by a T4 DNA to completely return back to a microchannel was about 16–18 s for a DNA that entered the nanochannel in a non-folded conformation. Figures 4(b) and 4(d) show the fluorescence intensity along the nanochannel axis over time for λ and T4 DNA, respectively, as they are subjected to a single pulse of 4 V, 200 ms at $t = 0$ s. This method of visualizing DNA kinematics has been shown to be very useful in investigating DNA conformations as it unfolds, retracts, or recoils^{5,6,23} in

FIG. 4. DNA dynamics under a single forward pulse. (a) Schematic showing the position of the x_{leading} and x_{last} beads with reference to the nanochannel as defined for the simulations. (b) and (d) Time-trace analysis of λ and T4 DNA fluorescence intensity along the nanochannel axis under a single pulse of 4 V and 200 ms. (c) and (e) Simulated trajectories of the leading (x_{leading} shown with solid lines) and last (x_{last} shown with dashed lines) beads of 20 DNA chains under a single applied pulse with, $Pe_1 = 10$, $t_1 = 60$, $Pe_2 = 0$, and $t_2 = 600$; $x = 0 \mu\text{m}$ is the nanochannel inlet and $x = 10 \mu\text{m}$ is the nanochannel outlet. (f) Experimental (left) and simulation (right) snapshots of λ DNA. (g) Experimental (left) and simulation (right) snapshots T4 DNA; dashed lines mark the nanochannel inlet and outlet. Scale bar is $5 \mu\text{m}$. (Multimedia view) [URL: <http://dx.doi.org/10.1063/1.4871595.2>]

nanochannels. A visual comparison of the two DNA profiles shows that once the pulse is applied, the entire length of λ DNA gets across the nanochannel whereas the T4 DNA does not. The top outline of the profiles for both DNAs, Figures 4(b) and 4(d), are similar to the leading bead trajectory in Figures 4(c) and 4(e), respectively, which confirms that the experimental and simulation results are in good agreement. The time-trace analysis shows that for T4 DNA, Figure 4(d), the tug-of-war regime lasts from $t=0.5$ s to $t=12$ s during which the DNA intensity is seen to continuously change both within and outside the nanochannel. The retracting-recoiling phase starts at $t=12$ s and ends at $t=17$ s and the recoiling speed is calculated from the slope of the profile between these times to be $\sim 2 \mu\text{m/s}$.

C. Asymmetric bipolar pulse

A single pulse was able to affect a spatial separation of two DNA molecules of different lengths. However, since the time taken by the longer T4 DNA to completely recoil back in the microchannel was >15 s, the technique would be slow and of low throughput as the interval between two consecutive pulses would have to be greater than the recoil time. To have better control over the DNA recoiling time and direction, an asymmetric bipolar pulse (Figure 5(a)) is

FIG. 5. DNA dynamics under a single asymmetric pulse with a forward pulse of 4 V, 200 ms and a reverse pulse of 0.1 V, 10 s. (a) Pulse schematic (b) Snapshots of λ DNA experimental and BD simulations (right). Scale bar is 5 μm . (c) Simulated trajectories of the leading (xleading shown with solid lines) and last (xlast shown with dashed lines) beads of 20 λ DNA chains under a single asymmetrical pulse. Here, $x = 0 \mu\text{m}$ is the nanochannel inlet and $x = 10 \mu\text{m}$ is the nanochannel outlet. (d) Simulated trajectories of the leading (xleading shown with solid lines) and last (xlast shown with dashed lines) beads of 20 T4 DNA chains under a single asymmetrical pulse. Here, $x = 0 \mu\text{m}$ is the nanochannel inlet and $x = 10 \mu\text{m}$ is the nanochannel outlet. (e) Snapshots of T4 DNA experimental and BD simulations (right). Scale bar is 5 μm . Consecutive frames are 470 ms apart. (f) Time-trace analysis of the T4 DNA. (Multimedia view) [URL: <http://dx.doi.org/10.1063/1.4871595.3>] [URL: <http://dx.doi.org/10.1063/1.4871595.4>]

proposed which would selectively allow the shorter λ DNA to go through the nanochannels during the forward pulse while pulling back the longer T4 DNA during the reverse pulse. The asymmetric pulse has two components- a short forward or positive pulse and a longer reverse pulse. The forward pulse exploits the fact that the longer DNA takes longer to cross and is so chosen to allow only λ DNA to completely cross the nanochannel. The reverse pulse voltage utilizes the fact that there is an entropic penalty for entering the nanochannel and that below a certain threshold voltage DNA cannot get in. Thus, the reverse pulse voltage was chosen to be low enough to prevent λ DNA from re-entering the nanochannel, while the reverse pulse length was chosen based on the single pulse recoil analysis (Figure 4(h)) to allow sufficient time for the T4 DNA to be pulled back. As the electric field inside the nanochannel is stronger than it is in the microchannel, the portion of T4 DNA inside the nanochannel will be pulled towards the inlet microchannel during the reverse pulse, which in turn will pull the entire strand back. On the other hand, the lower electric field in the microchannel together with the free energy barrier at the microchannel-nanochannel interface and the higher molecular diffusivity will prevent the λ DNA from going back into the nanochannel. As a result, these two lengths of DNA can be more rapidly separated.

We used an asymmetrical pulse with the following parameters: forward pulse of 4 V, 200 ms and a reverse pulse of 0.1 V, 10 s. Figure 5 shows λ and T4 DNA under the asymmetrical pulse condition. During the forward pulse the behaviour for both types of DNA was similar to that described earlier for a single pulse situation, namely, that any DNA touching the HFZ was pulled into the nanochannel by the high electric field. The λ DNA (Figures 5(b) and 5(c)) traversed the nanochannel, while the T4 DNA (Figures 5(d) and 5(e)) got stretched in the nanochannel with ends hanging from the nanochannel inlet and outlet. At this point, the low voltage reverse pulse was applied which terminated the TOW regime and aided the retracting-recoiling process of the T4 DNA, thus pulling it out of the nanochannel (Figures 5(e) and 5(f)). It is interesting to note that the reverse voltage was able to pull the T4 DNA back even though most of the DNA was closer to the outlet microchannel, indicating that the force in the nanochannel due to the electric field, even at low voltages is larger than the entropic recoiling force (supplementary material⁴² shows a T4 DNA responding to multiple asymmetric pulses and being pulled back every time). The total time ($t_{\text{TOW}} + t_{\text{recoil}}$) taken by the T4 DNA to completely return back to the inlet microchannel was about 5.6 s and the recoil velocity was 8.33 $\mu\text{m/s}$. Thus, the application of the low reverse voltage sped up the recoil process by about 4.2 times.

Even though the asymmetrical pulse was successful in pulling back the T4 DNA from the nanochannel, a few undesirable events were observed. As predicted by the BD simulations (Figure 5(c)) the translocated λ DNA was also pulled back about $\sim 20\%$ of the time during the reverse pulse. Application of multiple pulses or a longer duration forward pulse (to make sure the λ DNA is past the HFZ region) would reduce this occurrence. T4 DNA was also observed to cross over to the outlet microchannel during the forward pulse if it entered the nanochannel in a folded conformation. These folded conformations can be minimized by pre-stretching the DNA by using pillars in the microchannels and also by using nanochannels of shallower depths.

IV. CONCLUSIONS

The translocation time of fluorescently labelled long DNA molecules passing through a short nanochannel was calculated and was found to decrease with an increase in the applied voltage and decrease in DNA contour length. An electric pulse condition was designed and executed to show the utility of the translocation time curves in designing experiments. Brownian dynamics simulations were carried out using a modified bead-road model which successfully predicted the behaviour of a population of DNA molecules subjected to different pulse conditions. This work contributes to the fundamental understanding of confined polymer dynamics and non-equilibrium dynamics of DNA translocation through nanochannels.

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