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The distribution of DNA translocation times in solid-state nanopores

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Abstract
This paper systematically investigates the effects of solution viscosity, applied voltage and DNA chain length on the distribution of DNA translocation times through 8 ± 2 nm diameter silicon nitride nanopores. Linear dsDNA translocation events were selected based on the magnitude of current blockage and accumulated into scatter plots of current blockage and event duration (translocation time). The translocation time distribution was fitted to the solution of a Smoluchowski-type equation for 1D biased diffusion to a sink. The DNA drifting speed under bias and diffusion constant were extracted from the fits as functions of solution viscosity, applied voltage and DNA chain length. Combined with the Einstein–Smoluchowski relation, this model allowed evaluation of the viscous drag force on DNA molecules. This model also allowed estimation of the uncertainty in determining the DNA chain length due to the influence of friction on the spread of translocation times in a nanopore measurement. The data analysis suggests that the simple 1D biased diffusion model fits the experimental data well for a wide range of conditions. Some deviations from predicted behavior were observed and show where additional phenomena are likely to contribute to the distribution of DNA translocation times.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

The interplay between friction, electrophoretic driving force and thermal motions imposes limits on the resolution of analytical methods based on nanopore translocation of DNA polymers. The distribution of translocation times for DNA passing through voltage-biased nanopores reflects this interplay and can therefore be exploited to evaluate resolution limits for sizing DNA molecules and the uncertainty in more detailed analysis such as detecting the location of a nucleotide on a DNA chain for sequencing analysis. The time duration of a DNA molecule passing through a nanopore determines its temporal resolution. The DNA translocation time in solid-state nanopores has been studied experimentally [1–7] and theoretically [8–14].

This work analyzes the experimental data distribution of double-stranded DNA linear translocation times through biased silicon nitride nanopores [2, 5]. The time distribution was studied as functions of solution viscosity, applied voltage and DNA chain length. To simplify the approach, we first assume that the width of the time distribution is the result of a 1D random walk of DNA molecules in a nanopore; we then compare the parameters obtained from the simple 1D biased diffusion model with the results from other experiments. The data analysis reveals the utility and limits of applicability of a 1D biased diffusion model of DNA translocation.

2. Methods

2.1. Experimental background

This work expands on previous experimental results that measured DNA translocation as a function of solution viscosity, bias voltage and DNA chain length [2, 5]. Briefly, the experiment that detects DNA molecule translocation in a nanopore is illustrated in figure 1(A). The nanopores used
in this work were made in a silicon nitride membrane and are $8 \pm 2$ nm in diameter. As shown in figure 1(A), the silicon nitride membrane contains a nanopore separating two chambers connected electrically only by an ionic solution inside the nanopore. When a voltage is applied across the membrane, a stable open pore current $I_0$ will be observed. After adding negatively charged DNA molecules to the cis chamber, the molecules in the vicinity of the nanopore will be captured by the electric field and forced to pass through the nanopore to the positively biased trans chamber. The translocation of DNA molecules through a nanopore blocks the ionic current flow and produces transient ionic current blockages as shown in the pulses in figure 1(B). An integrated Axopatch 200B system (molecular devices) was used to perform the experiments. The 10 kHz low pass Bessel filter in the Axopatch 200B was selected for most of the measurements in this work. Two sets of data for DNA chain length studies were recorded for a 100 kHz filter as described in the caption to figure 4. The concentration of DNA molecules in the cis chamber was about 10 nM in this work. The salt solution used for the experiments was 1 M KCl or 1.6 M KCl plus glycerol with 10 mM TE buffer at pH 7.5 $\pm$ 0.5. The data used in this work were measured with DNA molecules between 2.2 kbp (chain length $L_c = 738$ nm) and 23 kbp ($L_c = 7820$ nm). The chain length $L_c$ is much longer than the nanopore effective thickness ($H_{eff} \sim 20$ nm [15]). The mean blockade current $\Delta I_b$ was calculated from the current values below the trigger level and the time duration $t_d$ was calculated as the time when the trigger line across the current pulses as shown in figure 1(B) using custom Matlab routines. Figures 1(C) and (D) show scatter plots of $\Delta I_b$ versus $t_d$ to visualize the current blockade event distribution.

The DNA molecules used for the data in this work are from 3 kbp dsDNA (plasmid pSP65) and DNA ladder (Lambda Hind III phage DNA) from New England Biolabs.

2.2. Linear translocation event selection

When the nanopores are $8 \pm 2$ nm in diameter, DNA molecules translocate in either a linear or in a folded configuration [16]. Our earlier studies have shown that the events with one current blockage level are DNA molecule translocation in a linear configuration, and the events with more than one blockage level represent DNA passing through a nanopore in a partially folded form [16]. Several such events are shown in figure 1(B) and a typical event distribution plot is shown in figure 1(C). In this work, we set up a current drop minimum and the current blockade levels below the minimum line (figure 1(B)) or folded events are not selected. The selected current drop level events or linear translocation events are shown in figure 1(D). We focus on the distribution of the time duration $t_d$ histogram (on the bottom axis in figure 1(D)). We fit the time histograms to a first passage time distribution derived from a 1D biased diffusion model that describes a uniformly charged DNA polymer moving in a nanopore.

2.3. First passage time distribution for biased diffusion

We intend to estimate the range over which a 1D biased diffusion model is adequate to describe dsDNA translocation. DNA translocation through a nanopore involves many phenomena. To simplify the problem, we assume free translocation and we ignore complex issues like DNA–pore
interactions and electro-osmotic flow, and DNA (un)coiling in the cis and trans chambers. When the DNA molecule is captured by the strong electric field ($\sim 10^7$ V cm$^{-1}$) near the nanopore, the segment that is going through the pore must first unwind or uncoil. During translocation only a very small segment of the DNA chain is under the electric field. During translocation the uncoiling in the cis chamber will be compensated by the coiling in the trans chamber. The relaxation time of a random coil is of the order of $\sim 100$ ms for a 10 kbp dsDNA [17]; thus this (un)coiling effect is likely to contribute to the distribution of $t_{\text{eff}}$. We therefore expect most of the deviations from 1D biased diffusion behavior to be a result of the insertion process. When the time of entrance becomes significant compared to the translocation times, we also expect deviations from the simple 1D model.

Here we briefly describe the derivation of the first passage time distribution for a charged polymer that has electrophoretic mobility $\mu_x$ and a diffusion constant $D$. In the simple 1D model described below, we assume that DNA molecules are uniformly charged long rodlike polymers with no flexibility. The polymer is located initially at position 0 and moves to a sink (trans chamber) located a distance $H_{\text{eff}}$ away when driven by an electric field $\varepsilon = \Psi / H_{\text{eff}}$. The electric field only exerts an electrical force on the DNA polymer in the longitudinal direction of the nanopore within the range of $H_{\text{eff}}$. The drifting velocity of the polymer is $\nu_d = \mu_x \varepsilon$. Starting with the Fokker–Planck equation for the evolution of the position–time probability distribution function, we solve the 1D Smoluchowski-type diffusion equation below:

$$\frac{\partial P(x, t)}{\partial t} = -\varepsilon \mu_x \frac{\partial P(x, t)}{\partial x} + D \frac{\partial^2 P(x, t)}{\partial x^2}. \tag{1}$$

A general normalized solution of this equation with initial condition $P(x, 0) = \delta(x)$ is

$$P(x, t) = \frac{e^{-\frac{(x-\mu_x t)^2}{4Dt}}}{\sqrt{4\piDt}}.$$ 

To account for the boundary condition at the exit of the nanopore, which we consider to be a sink beyond which the particle cannot return (absorbing boundary), $P(d_{\text{trans}}, t) = 0$, we introduce an image sink of amplitude $A$ at position $x_0$:

$$P(x, t) = \frac{e^{-\frac{(x-x_0)^2}{4Dt}}}{\sqrt{4\piDt}} - \frac{A e^{-\frac{(x-x_0)^2}{4Dt}}}{\sqrt{4\piDt}}.$$ 

The solution that satisfies this boundary condition is (with $x_0 = 2(d - \nu d t)$ and $A = 1$)

$$P(x, t) = \sqrt{\frac{1}{4\pi Dt}}(e^{-\frac{(x-d)^2}{4Dt}} - e^{-\frac{(x+2d-\nu_d t)^2}{4Dt}}).$$ 

Note: this solution is only valid for $x \leq d$. When a DNA molecule has translocated to the trans chamber (outside of $H_{\text{eff}}$) or for $x > d$, equation (1) is no longer valid, so is its solution. The probability that a particle has not translocated (reached the boundary $d$) and survived is

$$\text{CDF}(t) = \int_{-\infty}^d \sqrt{\frac{1}{4\pi Dt}}(e^{-\frac{(x-d)^2}{4Dt}} - e^{-\frac{(x+2d-\nu_d t)^2}{4Dt}}) \, dx.$$ 

The probability that a particle has translocated (reached the boundary and left the system) is

$$1 - \text{CDF}(t) = \text{erfc}\left(\frac{d - \nu_d t}{2\sqrt{4Dt}}\right).$$ 

Substituting the definition of the drift velocity $\nu = \varepsilon \mu_x$, the probability density function of the particle that has reached the boundary is

$$P_{\text{fp}}(t) = \frac{\partial}{\partial t} \text{erfc}\left(\frac{d - \nu t}{2\sqrt{Dt}}\right) = \frac{e^{-\frac{(d - \nu t)^2}{4Dt}}(d + \nu t)}{t \sqrt{4\pi Dt}} \tag{2}.$$

This is the sojourn time (here $t$ is the DNA translocation time $t_d$ extracted from current drop events) distribution and $d$ is the distance to be translocated. For a particle that is small with respect to $H_{\text{eff}}, d$ is just $H_{\text{eff}}$. For a long polymer like DNA measured in this work, $L_c \gg H_{\text{eff}}, d = L_c + H_{\text{eff}}$ is the DNA chain length $L_c$ plus the length of the pore $H_{\text{eff}}$.

We fit $P(t)$ of equation (2) to the current blockade time histograms while holding the $d = L_c + H_{\text{eff}}$ as a constant. We obtain the drifting velocity $\nu$ and the diffusion constant $D$ from the fits.

3. Time distribution analysis

3.1. Solution viscosity

Adding glycerol increases aqueous solution viscosity and decreases solution conductivity. Figure 2(A) shows the event distribution plot of 3 kbp DNA translocation in a nanopore as the glycerol percentage was increased from 0 to 50%. Glycerol decreased the current blockade amplitude $\Delta I_b$ consistent with the decreased solution conductivity. To verify the translocation mechanism for selected events are the same linear translocation, we plot the relative blockade current $I_b$ versus the electric field $\varepsilon$, equation (2) are shown as solid curves. The two fitting parameters—drifting velocity $\nu$ and the diffusion constant $D$—are plotted in figures 2(B) and (C). Both the diffusion constant $D$ and the drifting velocity $\nu$ are inversely proportional to the solution viscosity as expected from the Einstein–Smoluchowski relation for charged particles described below:

$$D = \frac{k_B T}{f} = \frac{k_B T}{C_I \eta}. \tag{3}$$
ψ and Δ1 blockade current H
we have used linear charge density is ∼
of a DNA molecule is
the estimated value of the 'instantaneous electrical charge' (charges
constant that has a unit of 'length' (for a spherical particle,
η of
10−12 kg nm2. Here C_eff is the 'fraction mobility' of a DNA molecule. Q∗ = z∗e0 is the instantaneous average electrical charge 'interacting' with the nanopore electric field while during translocation. The z∗ is the effective number of elementary charges (e0).

3.1.2. Drag force. Using known values of C_l, η, v and Q∗, we can further estimate how the drag force changes as solution viscosity varies, F_drag = f v(L_c/η)(H/η) = C_lηv(L_c/η). The electrical driving force \( F^*E \) assuming that \( F^* \) does not change as solution viscosity changes. Both \( F^*E \) and \( F_{drag} \) are shown in figure 2(D). This analysis suggests that the estimated electrical driving force exerted on a DNA molecule would be ∼23 pN. The drag force calculated decreased as the solution viscosity increased, indicating that either the simple model for calculating the drag force was not accurate or the electrical charge \( Q^* \) could have decreased at higher solution viscosity. It is unlikely that the electrical charge \( Q^* \) could decrease by a factor of two due to viscosity. Thus most likely the drag force calculated from the simple model could not count for all the forces that balance the electrical driving force.

3.1.3. Uncertainty of measuring L_c. In addition, we can also estimate the uncertainty or resolution limit of determining the chain length of DNA, ΔL_c, by the nanopore measurement due to Brownian motion by

\[
\Delta L_c = \sqrt{2D_{1,0}} = \sqrt{2D_{eff}/v}.
\]

(5)
The inset in figure 2(A) shows the calculated values of ΔL_c/L_c from equation (5). Increasing DNA translocation time does

and

\[
v = u^*_cE = \frac{Q^*H_{eff}}{fL_c}E = \frac{Q^*}{C_lηL_c} = \frac{z^*e_0}{C_lL_c} \Psi. \tag{4}
\]

Here \( C_lη = f \) is the friction coefficient. \( C_l \) is a friction constant that has a unit of 'length' (for a spherical particle, \( C_l = 6πr ) \). Here \( C_l \) also includes the parameters we do not include in the equation. Since only a fraction, ∼H_{eff}/L_c of a DNA molecule is in the electric field, here we define \( u^*_c = u_c(H_{eff}/L_c) \) is the 'fraction mobility' of a DNA molecule. \( Q^* = z^*e_0 \) is the instantaneous average electrical charge 'interacting' with the nanopore electric field while during translocation. The \( z^* \) is the effective number of elementary charges (e0).

3.1.1. Values of \( C_l \) and \( Q^* \). Fitting the diffusion constants D versus solution viscosity η (solid curve, figure 2(C)), we obtained a fitting constant \( k_B T/C_l = 130.4 \pm 12(×10^{-18} \text{ kg nm} \ \mu \text{s}^{-2}) \). The estimated value of the friction constant is \( C_l \approx 31.6 \text{ nm} \) with \( k_B T = 4.14 \times 10^{-15} \text{ kg nm}^2 \ \mu \text{s}^{-2} \). Fitting the drifting velocity \( v \) as a function of \( η \), resulted in a fitting constant \( (z^*e_0)ψ/\eta L_cC_l = 14.1 \pm 0.8(×10^{-18} \text{ kg nm} \ \mu \text{s}^{-2}) \). Using \( ψ = 0.12 \text{ V} \) and \( L_c = 1020 \text{ nm} \), the estimated value of the ‘instantaneous electrical charge’ of a DNA molecule is \( Q^* = 23.7e_0-24e_0 \). The estimated linear charge density is ∼24e0/20 nm or ∼1.2e0 nm−1, where we have used \( H_{eff} \sim 20 \text{ nm} \) [15]. This is close to the values measured (∼2e nm−1 or ∼0.3e/phosphate) for the linear charge density of DNA in ∼1 M KCl.
slightly decrease the overall error in measuring DNA's length, but the improvement is very limited. This analysis shows that the error in determining the chain length of DNA, \( \Delta L / L_c \), is about 12–15%.

3.2. Bias voltage

Changing the applied voltage from \( \psi = 130 \) to 20 mV, the time distributions (on the bottom axis) and several event distributions are shown in figure 3(A). We plot the relative blockade current \( \Delta I_b / I_0 \) in the inset of figure 3(A) to verify the translocation mechanism for selected events. The \( \Delta I_b / I_0 \) (right axis) = 1.1 \( \pm \) 0.1%, indicating that DNA molecules in the pore are in the same linear translocation configuration. Fitting the time distributions to equation (2), we obtained the drifting velocities \( \nu \) (figure 3(B)) and the diffusion constants \( D \) (figure 3(C)) as a function of \( \psi \).

3.2.1. Drifting speed. Fitting the drifting speed \( \nu \) as a function of the applied voltage \( \psi \) with a straight line, \( \nu = \text{slope} \times \psi \), the slope = 1.1 \( \pm \) 0.2 (nm V \( \mu \)s\(^{-1}\)). Let the slope = \( u^*_c / H_{\text{eff}} \) = \( u_c / L_c \), using \( H_{\text{eff}} = 20 \) nm and \( L_c = 1020 \) nm. The estimated segmental mobility of DNA translocation in this nanopore is \( u^*_c = 22 \) nm\(^2\) (V \( \mu \)s\(^{-1}\)) = 2.2 \( \times \) 10\(^{-11}\) m\(^2\) V\(^{-1}\) s\(^{-1}\). The estimated electrophoretic mobility of the 3 kbp DNA is \( u_c = 1.12 \times 10^3 \) nm\(^2\) (V \( \mu \)s\(^{-1}\)) or \( \sim 10^{-9} \) m\(^2\) s\(^{-1}\) V\(^{-1}\), which is one order of magnitude smaller than the value (3 \( \times \) 10\(^{-8}\) m\(^2\) s\(^{-1}\)) measured by gel electrophoresis for DNA of similar lengths [18]. The estimated smaller electrophoretic mobility could be due to: (1) the electric force only drags a very small segment of a long DNA chain that could make DNA less mobile; (2) we have ignored the DNA–pore interaction and electro-osmotic flow and (3) the viscous drag on the chain portion in the cis and trans sides is also neglected [19]. All of these three factors could reduce DNA translocation velocity in silicon nitride pores.

3.2.2. Diffusion constant. The average value of the diffusion constant is \( D = 99 \pm 44 \) nm\(^2\) \( \mu \)s\(^{-1}\) (or \( \mu \)m\(^2\) s\(^{-1}\) or \( \sim 10^{-10} \) m\(^2\) s\(^{-1}\) (figure 3(C)). This value is about two orders of magnitude larger than the values (\( \sim 10^{-12} \) m\(^2\) s\(^{-1}\)) measured by gel electrophoresis at lower ionic strength conditions [20]. This shows that the ‘diffusion constants’ obtained by fitting the experimental data to the simple 1D biased diffusion model deviate significantly from the bulk values. This is an important result as it suggests the underlying DNA translocation process is not as simple as the 1D biased diffusion model assumed.

During the translocation process of a DNA molecule in a nanopore, only a very small segment of the long DNA molecule is in the electric field due to \( H_{\text{eff}} \ll L_c \). The segment that is going through the pore must first unwind at the entrance of the nanopore. The motion of the two random coiled ends

Figure 3. (A) Event distribution plot of 3 kbp DNA translocation as a function of applied voltage. Inset, left axis: the uncertainty in determining DNA chain length due to random walk \( \Delta L / L_{\text{DNA}} \); right axis: the relative blockade current \( \Delta I / I_0 \). The error bars are smaller than the symbols. (B) The drifting speed, (C) the diffusion constant and (D) the calculated drag force as a function of voltage. The experiment was performed in 1.6 M KCl with 20% glycerol at pH 7.5 in an 8 ± 2 nm silicon nitride pore.
of the DNA chain in the cis (unwinding) and trans (winding) chambers likely contributes significantly to the distribution of the time duration or the peak width of the 

The peak width of the time histograms is \( \sqrt{4D/\tau} \) in equation (2). If the 1D Brownian motion was the only contribution to the peak width as the model assumed, then the ‘diffusion constant’ values from the fits should be close to the numbers in the bulk. However, if the DNA ‘diffusion constant’ in nanopores is two orders of magnitude larger than the bulk, then it suggests that processes other than ‘1D diffusion’ are likely to contribute significantly to the time duration distribution. As we stated in section 2.3, we therefore expect most of the deviations from 1D biased diffusion behavior to be a result of the insertion process or the unwinding process of the coiled DNA molecules at the nanopore entrance. In conclusion, our analysis shows that the DNA unwinding or the insertion process are likely to contribute to the time duration distribution significantly and could contribute to the deviations observed. However, the unwinding effect is outside the scope of this paper.

If the DNA unwinding and insertion process contribute significantly to the time duration distribution (peak width), since the unwinding of DNA will depend on the electrical force as well as the drag force, thus the diffusion constants obtained from the fits as well as the calculated \( C_t \) are expected to change with the electrical field as shown in figures 3(C) and (D).

3.2.3. Drag force. Using the diffusion constants from figure 3(C), we calculate \( C_t \) using equation (3) and further calculate how the drag force changes as the voltage varies, \( F_{\text{drag}} = f \nu (L_c/H_{\text{eff}}) = C_{\nu} \nu (L_c/H_{\text{eff}}) \). Assuming the instantaneous average electrical charge \( Q^* \) is approximately a constant, the electrical driving force on DNA is \( V_{\text{drag}} = Q^* E \). The estimated \( C_t, F_{\text{drag}} \) and \( V_{\text{drag}} \) are shown in figure 3(D). This analysis suggests: (1) the drag force is about 20 pN on DNA molecules when the bias voltage is above 60 mV, which is in good agreement with the values measured by single-molecule force measurement [21] and (2) the calculated drag force (\( \phi \)) does not agree well the predicted electrical driving force (\( \phi \)).

3.2.4. Entropic barrier. The diffusion constant analysis above also indicates that there is a barrier to DNA entering the pore. This has been studied and reviewed by Muthukumar [22] who proposed that there is an entropic barrier for DNA transport through pores. If we assume there is an entropic barrier when a DNA molecule is entering a pore, when the applied voltage \( \psi \) is less than the barrier height \( \psi_b \), the translocation process is likely partially thermally activated. Three consequences would happen if \( \psi \) is less than \( \psi_b \): (1) the electric field is not strong enough to bend a DNA molecule at the entrance of a nanopore, thus the DNA molecule is less likely to pass through in a folded configuration. (2) More collision events should be observed at lower voltage as a DNA molecule is more likely to be bounced back to the cis chamber if it is in a folded configuration. (3) The 1D biased diffusion model analysis would deviate from the DNA translocation process. All of the three consequences are consistent with the experimental results and data analysis. For example, at \( \psi = 20 \text{ mV} \), most of the DNA current blockage events only had one level of current drop (data not shown). We estimate the entropic barrier height is likely to be of the order of 60 mV as figure 3(D) shows a transition at \( \psi = 60 \text{ mV} \).

3.2.5. Uncertainty of measuring \( L_c \). As shown in the inset of figure 3(A), the accuracy of measuring the chain length of the 3 kbp DNA, \( \Delta L_c/L_c \), improved from \( \sim 30\% \) to \( \sim 12\% \) when the applied voltage \( \psi \) increased from 20 to 50 mV. This indicates that, when the applied voltage \( \psi \) is less than 50 mV, the Brownian motion is likely comparable to the electroophoretic movement. Thus, even a longer translocation time at lower voltage allows more accurate measurement of the current blockage signal; the random motion or ‘diffusion’ effect of the DNA molecule could cancel the benefit of a longer measurement time.

3.3. DNA length

Using the data measured with a DNA ladder that contains a mixture of 2.17 (a mixture of 2027 and 2322 bp), 4.36, 6.56, 9.42 and 23 kbp DNA, we evaluate how time distributions change as a function of DNA length. Figure 4 shows the results of four sets of measurements performed with four different nanopores. In the event distribution plots without glycerol (figure 4(A)) and with 20% glycerol (figure 4(B)) we show that the time durations measured in a more viscous solution are longer as expected. Both sets of distribution plots were measured with a 100 kHz low pass filter. The peaks corresponding to different chain lengths are better separated with 20% glycerol. Longer time durations have wider time distributions as the DNA chain length was increased. The solid curves are best fits of the time histograms to equation (2) while holding the DNA chain lengths as constants. The 2.17 kbp DNA peak is wider and has larger errors, likely because it is an unresolved mixed peak from DNA of 2027 and 2322 bp.

3.3.1. Drifting speed. As shown in figure 4(C), the drifting velocities were faster for the DNA measured without glycerol (square and diamond markers) compared to the data measured with 20% glycerol (circular and triangular markers). For the DNA measured without glycerol, the drifting velocity \( v_d \) decreased approximately with a power law, \( v_d = c L^{-\alpha} \), as the DNA chain length was increased. We fit the drifting velocity \( v_d \) data with the power law and obtained exponents of \( \alpha = 0.37 \pm 0.02 \) and \( \alpha = 0.47 \pm 0.08 \). If we convert the drifting velocity to translocation time using \( t_d = L_{\text{DNA}}/v_d \), then \( t_d \sim L^{1.37} \) or \( t_d \sim L^{1.47} \), respectively. These values are qualitatively in agreement with the value, \( t_d \sim L^{1.26} \), measured by Storm et al [3]. However, for slower translocations (with 20% glycerol in the solution), only when the DNA chain length is between 2.2 and 9.4 kbp, the drifting speed decreased approximately with a power law. For a very long chain length \( L_c \sim 2313 \text{ kbp DNA} \), it seems the drifting speed value reached a constant, \( v_d \sim 5 \text{ nm} \mu \text{s}^{-1} \). Analyzing an even longer DNA, \( \lambda \)-DNA (48.5 kbp or \( L_c = 16490 \text{ nm} \)), the value of \( v_d \) was \( \sim 5.4 \text{ nm} \mu \text{s}^{-1} \) (data not shown), also supporting this observation.
3.3.2. Diffusion constants. The diffusion constants obtained from fitting the time histograms to equation (2) are shown in figure 4(D). The diffusion constant values are $D \sim 10^2 \text{nm}^2 \mu\text{s}^{-1} (\mu\text{m}^2 \text{s}^{-1})$ for $L_c$ between 2.2 and 23 kbp (0.7–9 $\mu$m). Comparing to the values ($\sim 10^0 \mu\text{m}^2 \text{s}^{-1}$) measured by gel electrophoresis [18, 20], the diffusion constants estimated from the nanopore experiment are two orders of magnitude larger and the diffusion constant did not seem to scale with the DNA chain length as a power law, $D \sim L^{-\beta}$.

The two orders of magnitude larger value of $D$ is likely caused by the fact that, in a nanopore measurement, only a very small segment of the DNA chain is in the electric field. That the scale of the diffusion constants ($D$) did not follow the power law may be due to the change of the entropic barrier height with DNA length. As the DNA chain length $L_c$ increases, the entropic barrier height ($H_b$) for DNA entering the pore is expected to be larger, or the translocation process is more thermally activated for longer DNA. Thus a higher voltage is needed for longer DNA translocation to be electrophoretically driven. This hypothesis is consistent with the fact that the transition for $D$ from a decrease to an increase is at longer $L_c$ for faster translocations (no glycerol data) shown in figure 4(D). This hypothesis predicts that at high enough voltages ($\psi$ is higher than the barrier height), the
power law, $D \sim L^{-\beta}$, is expected to be observed. Further investigation is needed to test this hypothesis and to understand the diffusion constant magnitude of scaling with DNA chain length shown in figure 4(D).

4. Summary

In this work we have analyzed the time distribution of DNA linear translocation in $\sim 10$ nm diameter silicon nitride nanopores using a 1D biased diffusion model. This model was used to analyze the experimental data and to quantitatively extract the diffusion constant and drifting speed. The analysis of the changes of these parameters as functions of solution viscosity, bias voltage and DNA chain length allows a better understanding about DNA translocation in solid-state nanopores. Our analysis shows that both the DNA drifting speed and diffusion constant are inversely proportional to the solution viscosity, as expected. The DNA drifting speed is proportional to the bias voltage as expected. The simple 1D biased diffusion model was consistent with many of the features of the data, but the values of the diffusion constants obtained are two orders of magnitude larger than the values in bulk. This suggests the processes of DNA unwinding and insertion at the entrance of the nanopore are likely to contribute significantly to the DNA translocation dynamics. Our analysis shows that more investigation is needed to understand the distribution of DNA translocation times in solid-state nanopores.

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