Fast DNA translocation through a solid-state nanopore

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We report translocation experiments on double-strand DNA through a silicon oxide nanopore. Samples containing DNA fragments with seven different lengths between 2000 to 96000 basepairs have been electrophoretically driven through a 10 nm pore. We find a power-law scaling of the translocation time versus length, with an exponent of $1.26 \pm 0.07$. This behavior is qualitatively different from the linear behavior observed in similar experiments performed with protein pores. We address the observed nonlinear scaling in a theoretical model that describes experiments where hydrodynamic drag on the section of the polymer outside the pore is the dominant force counteracting the driving. We show that this is the case in our experiments and derive a power-law scaling with an exponent of 1.18, in excellent agreement with our data.

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Translocation of biopolymers such as polypeptides, DNA, and RNA is an important process in biology. Transcribed mRNA molecules for example are transported out of the nucleus through a nuclear pore complex. Viral injection of DNA into a host cell is another example. Translocation of DNA and RNA can be studied in vitro, as demonstrated by Kasianowicz et al. using an α-hemolysin pore in a lipid membrane. By measuring the ionic current through a voltage-biased nanopore, one can detect individual single-strand molecules that are pulled through the pore by the electric field. Li et al. showed that solid-state nanopores can also be used for such experiments. We report a set of experiments with silicon oxide nanopores on double-strand DNA with various lengths. Surprisingly, we find a nonlinear scaling between the translocation time $\tau$ and the polymer length $L_0$, in contrast to the linear behavior observed for all experiments on α-hemolysin. In our experiments we find a clear power-law relation $\tau \sim L_0^{1.26}$, for DNA fragments from 2000 to 96000 basepairs (bp). While a complete model for translocation should in principle include hydrodynamic, steric, electrostatic and entropic effects, we argue that in our experiments the dominant contributions to the force balance come from hydrodynamics and driving, and propose a simple model that accurately reproduces the observed scaling.

**Experimental results.** Figure (a) shows the experimental layout for translocation studies. At the heart of the setup is a solid-state nanopore device, fabricated by shrinking a 20-50 nm pore in silicon oxide in a transmission electron microscope to a final size of 10 nm. The nanopore is situated in an insulating membrane which separates two macroscopic reservoirs filled with an aqueous buffer solution. When a voltage bias is applied over the membrane in the presence of DNA molecules, the DNA is electrophoretically drawn through the pore due to its negative charge. The detection technique is simple and elegant: A polyn-
ing solution present inside the pore and thereby reduces the ionic conductivity between the reservoirs. Passing molecules are thus detected as short dips in the ionic current, which is induced by the externally applied voltage (see Fig. 1(b)). Analogous to Li et al. \cite{3}, we find that the molecules with a diameter of about 2 nm can pass the 10 nm pore either in a linear or in a folded fashion. Using an event sorting algorithm discussed elsewhere \cite{2}, we analyze only the linear unfolded translocation events in this work. Fig. 1(b) shows an example of such a linear translocation event, detected with 11.5 kbp linear DNA. The width of the dip is interpreted as the duration of the translocation. We performed three experiments, all at room temperature: One on linear 11.5 kbp DNA, one on linear 48 kbp \(\lambda\)-DNA (here we detected both individual molecules and dimers of two molecules bound together with their complementary sticky ends), and one on a mixture that contains 27491 bp, 9416 bp, 6557 bp, 2322 bp, and 2027 bp fragments. In the last experiment, the length difference between the 2322 and 2027 bp fragments could not be resolved experimentally. The durations of individual linear events were collected in a histogram for each experiment. Figure 1(c) shows the dwell time (determined as the peak position in these histograms) versus polymer length for all 7 DNA fragments. The full width at half maximum of the peaks are taken as error bars. We find a clear power-law scaling in the problem is the characteristic relaxation time scale of the translocating polymer. This Zimm time \(\tilde{\tau}\), given approximately by

\[
t_{Z} \approx 0.4 \frac{n \eta R_{g}^{3}}{k_{B} T},
\]

which can be considered an upper bound on the time it takes the polymer to relax to an entropically and sterically favored configuration. In this expression, \(\eta\) is the solvent viscosity and \(R_{g}\) is the radius of gyration of the polymer. This is the radius of the typical blob-like configuration that the polymer will assume in a good solvent, and it scales with the polymer length as

\[
R_{g} \sim L_{0}^{\nu},
\]

where \(\nu\) is the swelling exponent. It depends on the dimensionality of the system, and theoretically a value of 0.588 is found for self-avoiding polymers in a good solvent \cite{5}. Smith et al. \cite{6} have measured the diffusion constant \(D\) for stained DNA molecules with lengths ranging from 4.3 kbp to 300 kbp. They report a scaling with length \(L_{0}\) as \(D \sim L_{0}^{\nu}\) with \(\nu = 0.611 \pm 0.016\), and conclude that Flory scaling is appropriate for DNA molecules longer than about 4 kbp. At room temperature, the measured velocity is about 0.8 \(\mu\)m per base or slower \cite{7}. A 100-base, single-stranded DNA fragment therefore takes around 80 \(\mu\)s to fully translocate. When we compare this to the Zimm time for the same polymer fragment, about 0.2 \(\mu\)s, we see that relaxation is much quicker than the translocation. We will call such events, for which \(\tau \gg t_{Z}\), slow translocations. Lubensky and Nelson \cite{10} have argued that for single-stranded DNA and RNA through \(\alpha\)-hemolysin, the criterion for slow translocation is indeed satisfied for polymer lengths up to hundreds of nucleotides. They show that the Zimm time for a polynucleotide of roughly 300 bases is comparable to the translocation time per nucleotide.

The criterion for slow translocation is evidently not met in our experiments on solid-state nanopores. A full \(\lambda\)-phage genome (48.5 kbp, or 16.5 \(\mu\)m of double-stranded DNA) is found to take only around 2 ms to traverse a 10 nm silicon nitride nanopore, and find translocation times of 100 \(\mu\)s and 400 \(\mu\)s respectively.

The translocation process consists of two separate stages. First, there is the capture stage. A DNA molecule initially in solution in the negative reservoir has to come close enough to the pore to experience the electrostatic force and get pulled in. We assume that the reservoirs are good ionic conductors, and the driving force is only felt in the direct vicinity of the pore. Capture is thus a stochastic process, since the pore has to be reached by diffusion. In this work, we focus on the second stage, where the DNA passes the pore until it has reached the other side. We assume that one end of the DNA has entered the pore and calculate the time required for complete translocation.

**Slow vs. fast translocations.** We now address the dependence of this duration on the length of the polymer. To this end, we consider a linear polymer consisting of \(N\) monomers, each of which has a Kuhn length \(b\). This polymer is partially threaded through a narrow pore. Time \(t = 0\) sets the moment of initial capture. We will let \(L(t)\) denote the contour length of the untranslocated part of the polymer, so that \(L(0) = Nb = L_{0}\). The dwell time \(\tau\) is therefore determined by \(L(\tau) = 0\). A second time scale in the problem is the characteristic relaxation time scale of the translocating polymer. This Zimm time \(\tilde{\tau}\),
First, consider the driving. As stated, a potential difference across the pore exerts a highly localized force on the negatively charged DNA molecule. We assume the potential drop to occur entirely inside the pore, and therefore only the part of the polymer inside experiences the driving force. This force can then be estimated as $F_{\text{driving}} = 2eV/a$, where $e$ is the elementary charge, $V$ is the potential difference and $a = 0.34$ nm is the spacing between nucleotides. A bias voltage of 120 mV, as is typically used in experiments, thus produces a force of around 110 pN. This value is an upper bound of the effective charge on the DNA, and thereby the driving force. Simulations of Manning condensation on double-stranded DNA yield charge reduction values between 53% and 85% \[12,13\]. Barring complete screening however, we consider our DNA translocations to be strongly driven and this justifies ignoring diffusive contributions.

In the absence of specific DNA-pore interactions, the viscous drag per unit length in the pore can be estimated as $2\pi \eta rv/\left(R - r\right)$, where $R$ is the pore radius, $r$ is the polymer cross-sectional area, $\eta$ the solvent viscosity and $v$ is the linear velocity of the polymer inside the pore. Substituting typical values ($\eta = 1.10^{-3}$ Pa·s, $r = 2$ nm, $v = 10$ mm/s, $R = 10$ nm and a pore depth $\ell_{\text{pore}}$ of 20 nm) we can estimate this drag force to be around 0.3 pN, decidedly smaller than the driving force. We feel this constitutes an essential difference between solid-state pores and protein pores: In sufficiently shallow solid state pores the effect of friction inside the pore is negligible.

Finally we estimate the hydrodynamic drag on the untranslocated part of the polymer, outside the pore. To this end, we approximate the untranslocated part as a sphere of radius $R_g$ (see Fig. 2). As the polymer threads through the pore, the center of mass of this sphere moves towards the pore at a velocity $dR_g/dt$. Assuming Zimm dynamics (and thus that the solvent inside the coil moves with the polymer), the coil experiences a Stokes drag force of $6\pi \eta R_v = 6\pi \eta R_g dR_g/dt$, which for typical parameters yields a drag force of about 24 pN. This assumption is justified by experiments by Smith et al. \[9\], who found clear evidence for Zimm dynamics for DNA longer than 4.3 kbp. Clearly, in this case the hydrodynamic friction on the part of the polymer outside the pore is the dominant force counteracting the driving force. We therefore choose to model fast translocation dynamics as determined only by the cumulative effect of driving at the pore and hydrodynamic friction outside.

Figure 2 depicts the simplified system we consider. The part of the polymer inside the pore experiences a driving force to the right, while the length of polymer before the pore is coiled up. The pore is sufficiently small to allow only linear (i.e. unfolded) passage of a single molecule at a time.

As the polymer is pulled through the pore the blob before the entrance shrinks in size, and thus its center of mass moves towards the pore with a velocity

$$\nu_{\text{blob}} = \dot{R}_g \sim L^{\nu-1} \dot{L},$$  \hspace{1cm} (3)

where the dot denotes a time derivative. Motivated by our consideration of the relative magnitudes of the counteracting forces, we propose the principal effect of hydrodynamics is to resist motion with a Stokes drag on the DNA coil that is proportional to its radius times the velocity,

$$F_{\text{drag}} \sim R \nu_{\text{blob}} \sim R_g \dot{R}_g \sim L^{2\nu-1} \dot{L}.$$  \hspace{1cm} (4)

Force balance must be met at all times, and since there are only two major forces the driving force should balance the hydrodynamic friction: $F_{\text{drag}} = -F_{\text{driving}}$. As the driving force is constant during the whole process, the same holds for $F_{\text{drag}}$. Thus we can extract the linear velocity $\nu_{\text{lin}} = -\dot{L}$ of the DNA inside the pore from Eq. 4:

$$\nu_{\text{lin}} = -\dot{L} \sim -L^{1-2\nu},$$  \hspace{1cm} (5)

which allows us to obtain the dwell time $\tau$ by integration,

$$\tau = \int_0^\tau dt = \int_{L_0}^L \nu_{\text{lin}}^{-1}(L) dL \sim L_0^{2\nu}.$$  

On the basis of this model we thus predict a powerlaw relation between the dwell time $\tau$ and the contour length $L_0$. Taking the theoretical value of 0.588 for the Flory exponent $\nu$ we find an exponent of $\alpha = 2\nu = 1.18$ for this model. If we take the experimentally obtained value for $\nu$ of 0.61 \[9\], we find $\alpha = 1.22$ - in excellent agreement with our experiments, where we find power law scaling with an exponent of 1.26 ± 0.07.
Scaling regimes for translocation. General considerations along the lines of the argument presented in the preceding sections can be used to qualitatively understand the various regimes of polymer translocation. Firstly, it is important to determine the dominant contribution to the friction. In most cases, it suffices to compare the pore friction $F_{\text{pore}} = \xi_{\text{eff}} v_{\text{lin}}$ (with $\xi_{\text{eff}}$ equal to $2m\eta F_{\text{pore}} r^2/(R - r)$ in the absence of specific interactions) to the Stokes drag on the coil $6\pi \eta R g R^2$. If the pore friction dominates, force balance with respect to the constant driving force implies that the translocation time scales linearly with the polymer’s length $\tau \sim L_0$.

A possible reason for a large pore friction could be the presence of specific interactions, but because of the geometric factor in the effective friction constant $\xi_{\text{eff}}$ the shape of the pore could also lead to pore friction dominated translocation. Such linear dependence of $\tau$ on the length for single-stranded DNA ranging from 12 to 400 bases has been reported experimentally by Kiasianowicz [1] and Meller [4]. For the $\alpha$-hemolysin pore they used it is indeed speculated that significant specific interactions with the passing DNA occur.

When the Stokes drag dominates one can derive, without any assumptions on the polymer statistics, that $\tau \sim R^2$. Depending on the length of the polymer different regimes are thus obtained: when the polymer is short compared to its persistence length $R_g \sim L_0$, and we find that $\tau \sim L_0^2$. For polymers of intermediate length the radius of gyration follows the scaling for a Gaussian chain, $R_g \sim L_0^{1/2}$, and consequently the translocation time is predicted once again to scale linearly with length (note, however, that this is a qualitatively different regime than the pore-friction dominated regime identified before). For long polymers (such as those considered in the preceding sections) we have shown that $\tau \sim L_0^{3\nu}$.

So far we have presented scaling arguments assuming Zimm (non-free draining) dynamics. Kantor and Kardar have identified and numerically confirmed yet another regime [14] where $\alpha = \nu + 1$. This behavior can be understood assuming Rouse dynamics (stationary solvent). In this case, the hydrodynamic drag would be given by $F_{\text{drag}} \sim L v_{\text{blob}}$, and one would indeed recover their scaling law $\tau \sim L_0^{1+\nu}$. We speculate that this regime might be observable for semifluid solutions close to $c^*$.

In all cases considered in this section, one can independently determine whether or not the polymer is frozen in its configuration by comparing the Zimm- and translocation timescales. We do not expect this to affect the scaling behavior in the various regimes, but it will affect the prefactors.

Concluding remarks. We have obtained a simple and elegant model description that appears to describe our data well. There are however several effects we neglect but which could have an additional influence on the process that we consider. For instance, we ignore any friction experienced by the DNA that has already passed the pore. We also expect that an electro-osmotic flow is generated inside the pore. This effect is caused by an electrophoretic force on the ions screening the charge on the surface of our pore. As silicon oxide is known to be negatively charged in water, there is a surplus of positive ions near the surface. These positive ions generate a flow of water inside the pore, slowing down the DNA that moves in the other direction. While we have not explored the consequences of these possibilities the observed agreement between theory and experiment suggests that at least for the fast polymer translocations considered here, hydrodynamic drag does indeed dominate the dynamics. Identification and understanding of the dominant effects in polymer translocation through nanopores is relevant not only for biological processes, but also for potential analytical techniques based on nanopores. Rapid oligonucleotide discrimination on the single-molecule level has been demonstrated with $\alpha$ hemolysin [10], and more recently solid-state nanopores were used to study folding effects in double-stranded DNA molecules [3, 2]. Future applications of this technique may include DNA size determination, haplotyping and sequencing.

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