Supplemental Information

Recapturing and Trapping Single Molecules with a Solid State Nanopore

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Materials and Methods

Nanopores were fabricated using a condensed TEM beam[1,2] to put a hole in a ~20 nm thick SiN membrane. For the recapture experiment, pores made in this method were preferred to those fabricated by ion beam sculpting[3,4] because their simpler geometry and symmetric shape made it easier to model the experiment and interpret its results. The unsupported area of the membrane had lateral dimensions greater than 20 microns, so the pore could effectively be represented as a hole in an infinite insulating sheet. To reduce capacitance the SiN membrane was supported on a 2 μm thick silicon dioxide layer, which was on a 3 mm silicon chip with a pyramidal pit fabricated by standard MEMS techniques[3,4]. The total capacitance of the chip, flow cell, and fluid inputs was 13 pF.

The chip containing the nanopore was assembled in a PEEK flow cell with PDMS gaskets. After assembly, the chip, holder, and gaskets were oxygen plasma cleaned for 60s at 100W and 500 mT. Immediately after the plasma cleaning, 1 M KCl solution with 10 mM TE buffer at pH 8 was added to the flow cell, and a baseline current was established. Voltage was sourced and current measured using an Axopatch 200B amplifier in resistive feedback mode with a 4 pole low pass Bessel filter with a 10 kHz

cutoff frequency. The amplifier output was digitized at 200 kHz and continuously recorded to disk using a Digidata 1322A digitizer and pClamp software.

An equimolar mixture of 6 kb and 4 kb DNA fragments was obtained from New England Biolabs. The fragments were provided in TE buffer at a concentration of 0.5 mg/mL. 4 μL was diluted in 50 μL of the 1 M salt buffer, then almost the entire amount was slowly added to, and through, the 1-2 μL volume of the *cis* reservoir of the flow cell, which was contacted by the ground electrode. This ensured a repeatable concentration of DNA in the *cis* chamber.

A forward voltage of 120mV was applied to the flow cell and controlled by a National Instruments DAQ card (PXI-6070E) and custom Labview software. The amplified current signal was passed through a bandpass filter and used to trigger the voltage reversal. After a molecule was detected entering the pore, the forward voltage was maintained for a programmed time before a reverse voltage of -120 mV was applied. The lag introduced by the filters was comparable to the translocation time through the pore (100-200 μs), so even though the reversal was triggered on the leading edge of the event, the delay can be considered as entirely after the molecule's translocation. Once the voltage was reversed, it was maintained at -120 mV for 500 ms, then restored to +120 mV. The return to positive voltage was not triggered and took place 500 ms after the voltage was initially reversed, regardless of whether any molecules translocated during this interval.

For the trapping experiment of figure 4, the feedback control was modified to trigger a voltage reversal 2 ms after the passage of the molecule in either direction. A higher bias voltage (150 mV) was used, and the excess current due to membrane charging was partially compensated using the pipette capacitance compensation on the Axopatch 200B. To be sure that the trapped molecule was not displaced by another molecule entering the pore, a dilute concentration (12 ng/μL) of mixed 10 and 5.4 kbp dsDNA was used. At this concentration, under forward bias, new molecules arrived at the pore at a rate of under 0.4 Hz. Under reverse bias the background arrival rate was an order of magnitude less. We were able to detect, by measuring the event charge deficit (discussed in the analysis section below), substitutions of 5.4 kbp molecules for 10 kbp molecules and vice versa in the trap. All the current blockages displayed in figure 4 of the main text correspond to translocation of a 10 kbp molecule.

Analysis of Molecular Signals

Current blockage signals from individual molecular translocations can be characterized by the time duration of the blockage, the magnitude of the blockage, and by the integral of the current blockage over the length of the event. This last quantity, which we term the event's area[4] or the event charge deficit[5] (ecd), is the amount of additional charge that would have passed through the pore without the molecule's blocking some of the ionic current. This ecd is independent of the conformation of the molecule (folded or unfolded) as it passes the pore[4,5] and depends on the length of the molecule. Figure S2A shows a histogram of ecds for all translocations measured in the forward direction. This histogram is fit to the sum of two Gaussian distributions representing 4kb and 6kb free

translocations. Signals with large ecd (>30 pC) represent molecules that stick to the pore wall at some point in the translocation and hence have longer translocation times. These are not included in the fit. Based on these fit Gaussians and each signal's ecd, we can determine the likelihood that a given blockage corresponds to translocation of a 4kb molecule or a 6kb molecule. We then sort the signals from reverse translocations by whether the forward translocation that immediately preceded each was of a 4kb or 6kb molecule. Figure S2B shows the histogram of ecds for reverse translocations following 4kb (red) and 6 kb (black) forward translocations. Thus the length of a molecule passing the pore in the reverse direction agrees with the length of the molecule that passed immediately before in the forward direction, demonstrating that the triggered voltage reversal recaptures the same molecule whose detected passage triggered the reversal.

Counting rate plots like those in Figure 2a have been assembled and presented according to the following procedure. Calling each molecule translocation signal an "event," each event is labeled "forward" or "reverse" according to whether it occurs during a time when the voltage bias is positive or negative, respectively. In addition, each event is labeled by the time duration of its occurrence since the last transition in bias voltage. The events are binned into quantized time increments 50 ms wide (chosen by a compromise between time resolution and statistical accuracy). The experiment proceeds over many forward and reverse cycles and each bin is incremented by one event if the event occurs within the bin's time boundaries. The counting rate for each bin is determined by dividing the total number of accumulated events in a bin by the total time during an experiment that the bin has been accessible. For reverse events that time is simply the bin time increment

multiplied by the number of forward/reverse cycles. For forward events the situation is more complex because each forward event triggers a bias transition, thereby terminating the sampling of forward events that occur at later times. This causes effective length of a time bin to be foreshortened (depending on the delay time to the reverse transition) and it caused less cycles to be associated with events occurring long times after the positive voltage transition than for shorter times. Both of these effects are accounted for in presenting the forward counting rate data in Figure 2a.

Analysis of the Drift Diffusion Model

The drift-diffusion equation for a spherically symmetric distribution of DNA molecules near a nanopore is

$$(1)\quad \frac{\partial c(r,t)}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r} r^2 \left(\frac{\mp |\mu I|}{2\pi\sigma r^2} c(r,t) + D\frac{\partial c(r,t)}{\partial r}\right)$$

where – implies motion away from the pore and + motion towards it.

With dimensionless units of length, $x = r/L$ and time, $s = t/\tau$, with $L = \frac{\mu I}{2\pi\sigma D}$ and $\tau = L^2/D$, the equation becomes

$$(2)\quad \frac{\partial c(x,s)}{\partial s} = \frac{1}{x^2}\frac{\partial}{\partial x}\left(\mp c(x,s) + x^2\frac{\partial c(x,s)}{\partial x}\right)$$

We have modeled the recapture experiment by solving numerically the drift-diffusion equation for these initial and boundary conditions:

$$c(r,0) = \frac{\delta(r - r_0)}{2\pi r_0^2}$$

(3) $c(r_c, t) = 0$

$$\frac{\partial c}{\partial r}(\infty, t) = 0$$

where r_0 is the initial distance from the pore, and was taken to be the average distance from a wall for a Gaussian chain with one end tethered at the wall, 30 nm for the 4kb DNA and 37 nm for the 6kbp DNA. The capture radius, r_c, at which the molecule was assumed to translocate through the pore with unit efficiency, was chosen to be 5 nm less than r_0. Our results had little dependence on the values chosen for these radii, as long as they were much less than L. The reflecting boundary condition at infinity was chosen to simplify computation.

Figure S2 shows the results of these calculations for 4 kb dsDNA at 3.5 nA in 1 M KCl. Figures S2a-c plot the linear probability density $p(r) = 2\pi r^2 c(r)$, where $c(r)$ is the volume density, and $p(r)dr$ represents the probability a molecule is found between r and r+dr. Figure S2a shows the evolution of the probability density with time as the current is directed away from the nanopore. The initial probability density is a delta function at 30 nm. The peak probability moves away from the pore with time, but due to diffusion, there is still a significant chance the molecule remains within 500 nm of the pore, even after 30 ms. Figures S2b and S2c show the evolution of the probability densities displayed in S2a after the voltage is reversed and the molecule is directed towards the pore. Figure S2b simulates an experiment in which the voltage is reversed after 2 ms, while S2c simulates one in which the voltage was reversed after 16 ms of outbound travel. Note that although the net flux of molecules is inward, the probability distribution

eventually skews away from the pore. This is because molecules close to the pore translocate and are removed from the distribution.

Figure S3a shows the probability a molecule translocates the pore within the dimensionless time t/τ for various dimensionless starting radii, $x_0 = r_0 / L$. Most translocations occur within 1/2τ for starting distances less than L. Figure S3b shows the probability a molecule returns within 1/2 τ vs. starting distance. A molecule that starts at $x_0 = 0.4$ reaches the pore within 1/2τ 85% of the time. For these simulations, the dimensionless capture radius, at which instantaneous translocation was assumed, was 0.01.

With the electric force directed away from the pore, the steady state concentration of DNA in the *cis* reservoir becomes

$$(4)\quad c(r) = c_\infty \exp(-\frac{L}{r})$$

where c_∞ is the concentration of DNA far from the pore. It is unclear *a priori* how closely the concentration approaches this equilibrium value in the 500 ms voltage reversal window, so we solve equation (2) for the initial and boundary conditions

$$(5)\quad \begin{aligned} c(r,0) &= c_\infty \\ c(r_c,t) &= 0 \\ c(\infty,t) &= c_\infty \end{aligned}$$

for 500 ms with the drift directed outward. We use this solution (which approximates (4)) as the initial condition when solving for inward directed drift to produce the predicted forward molecular translocation rates shown in figure 2a.

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2. Kim, M. J., Wanunu, M., Bell, D. C. & Meller, A. Rapid fabrication of uniformly sized nanopores and nanopore arrays for parallel DNA analysis. *Advanced Materials* **18**, 3149-+ (2006).
3. Li, J. et al. Ion-beam sculpting at nanometre length scales. *Nature* **412**, 166-169 (2001).
4. Li, J. L., Gershow, M., Stein, D., Brandin, E. & Golovchenko, J. A. DNA molecules and configurations in a solid-state nanopore microscope. *Nature Materials* **2**, 611-615 (2003).
5. Fologea, D. et al. Detecting single stranded DNA with a solid state nanopore. *Nano Letters* **5**, 1905-1909 (2005).

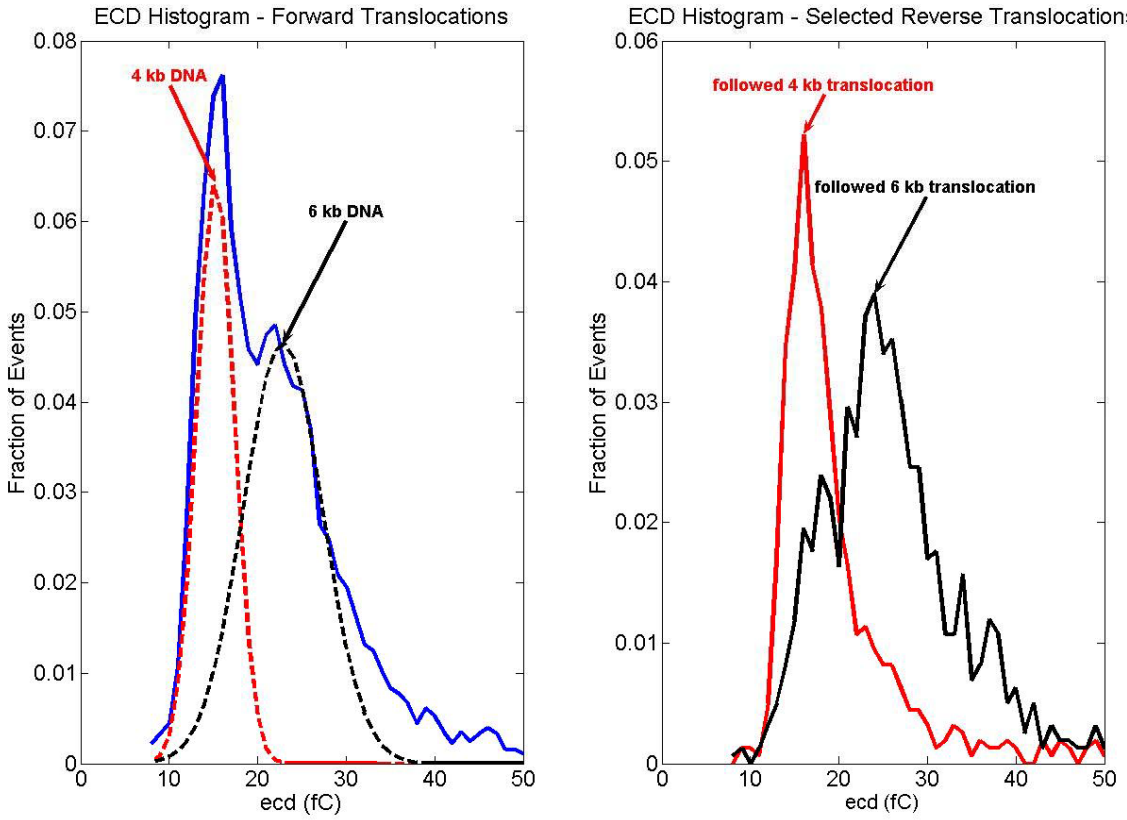

Figure S1. Event charge deficit (time integral of blockage current) histograms for forward and reverse translocations. A) Ecd of forward translocations. The dotted lines represent the two Gaussians whose sum is fitted to the histogram. B) Ecd of reverse translocations sorted according to the ecd of the forward translocations that preceded them. The red trace shows the ecd of reverse events where the forward translocation was determined to have been 4kb with 70% or greater probability, while the black trace represents reverse translocations that follow (with 70% or greater probability) 6kb translocations.

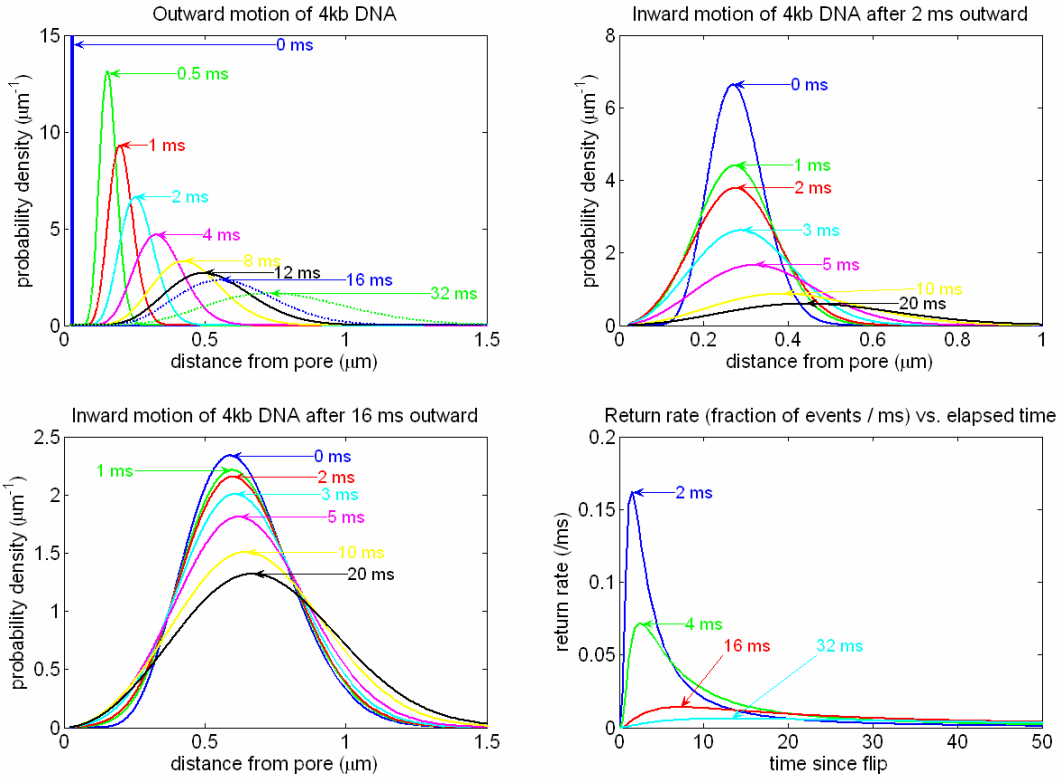

Figure S2. Drift-diffusion solutions for the recapture experiment. a-c Linear probability density $p(r) = 2\pi r^2 c(r)$, where $c(r)$ is the volume density, and $p(r)dr$ represents the probability a molecule is found between r and r+dr. a) After outward movement for the specified times. b) After outward movement for 2 ms, followed by inward movement. c) After outward movement for 16 ms followed by inward movement. d) The return rate vs. time after the voltage reversal for various delays.

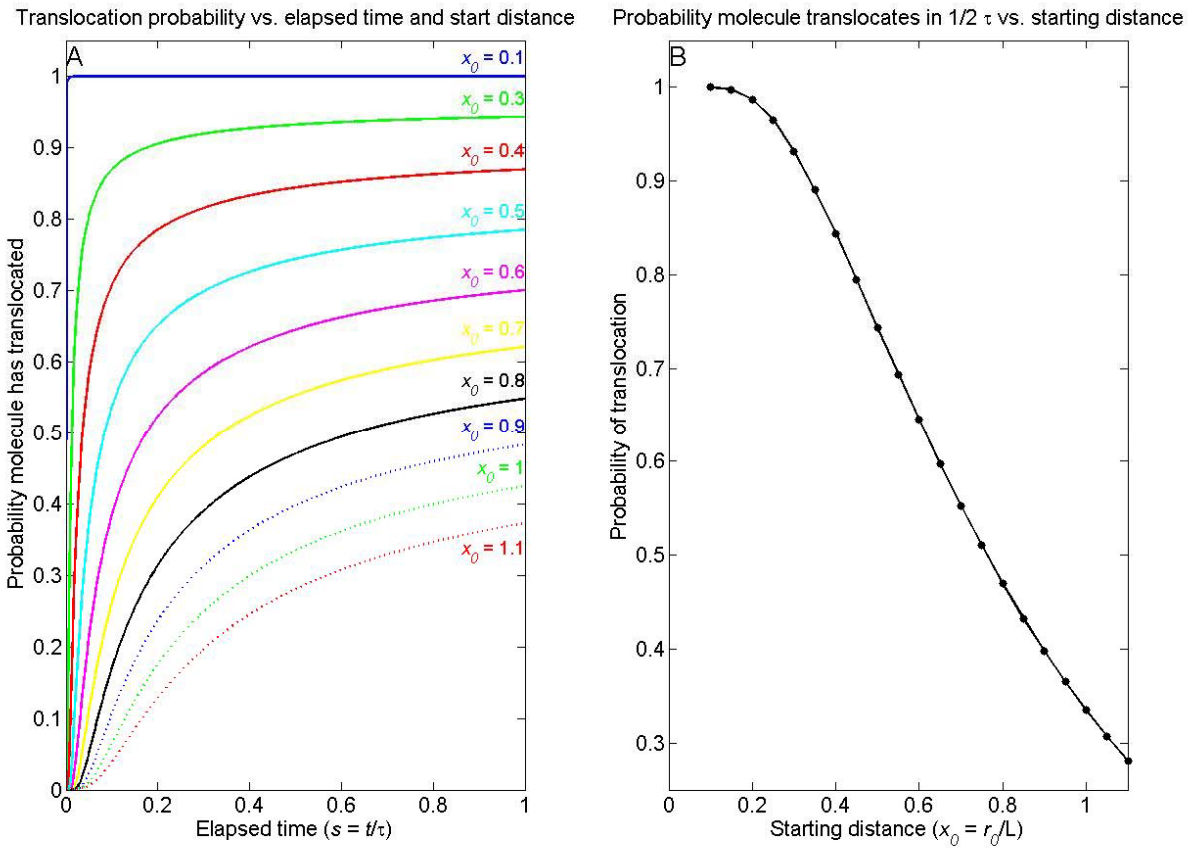

Figure S3. Dimensionless drift-diffusion capture predictions. a) Probability a molecule has returned to the pore within $s = t/\tau$ for various dimensionless starting radii, $x_0 = r_0 / L$. b) Probability a molecule translocates within $s = 0.5\ \tau$ vs. starting distance.