

# Recapturing and trapping single molecules with a solid-state nanopore

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The discovery and accompanying theory showing how a molecule which has translocated all the way through a nanopore can be recaptured and interrogated multiple times through the same nanopore is particularly relevant to implementing accurate sequencing. If the initial passage of a molecule provides an incomplete or poor quality read-out, real time software could drive that single molecule back to be re-sequenced multiple times without having to re-sample the entire genome.

The development of solid-state nanopores<sup>1–7</sup>, inspired by their biological counterparts<sup>8–15</sup>, shows great potential for the study of single macromolecules<sup>16–21</sup>. Applications such as DNA sequencing<sup>6,22,23</sup> and the exploration of protein folding<sup>6</sup> require control of the dynamics of the molecule's interaction with the pore, but DNA capture by a solid-state nanopore is not well understood<sup>24–26</sup>. By recapturing individual molecules soon after they pass through a nanopore, we reveal the mechanism by which double-stranded DNA enters the pore. The observed recapture rates and times agree with solutions of a drift-diffusion model. Electric forces draw DNA to the pore over micrometer-scale distances, and upon arrival at the pore, molecules begin translocation almost immediately. Repeated translocation of the same molecule improves measurement accuracy, offers a way to probe the chemical transformations and internal dynamics of macromolecules on sub-millisecond time and sub-micrometre length scales, and demonstrates the ability to trap, study and manipulate individual macromolecules in solution.

In this letter, we present a detailed view of the dynamics of single molecule capture by a solid-state nanopore on millisecond timescales and sub-micrometre length scales. We monitor the current through a nanopore and detect blockages in the current when DNA passes through the pore, partially obstructing the current path. After translocating a solid-state nanopore, a single DNA molecule is allowed to continue to move under the influence of the pore's proximal electric field and diffusive forces for a pre-set time period. The electric force is then reversed to bring the same molecule back to, and then through, the nanopore. Both passages are detected by a blockage of the ionic current through the pore. In previous work with solid-state nanopores<sup>16,17,19–21,25,27</sup>, the capture dynamics could not be studied directly because the location of a molecule was unknown until it entered the nanopore. Here, the molecule is known to be inside the pore at both ends of a measured time interval, the length of which directly reveals the essential characteristics of the molecular motions involved.

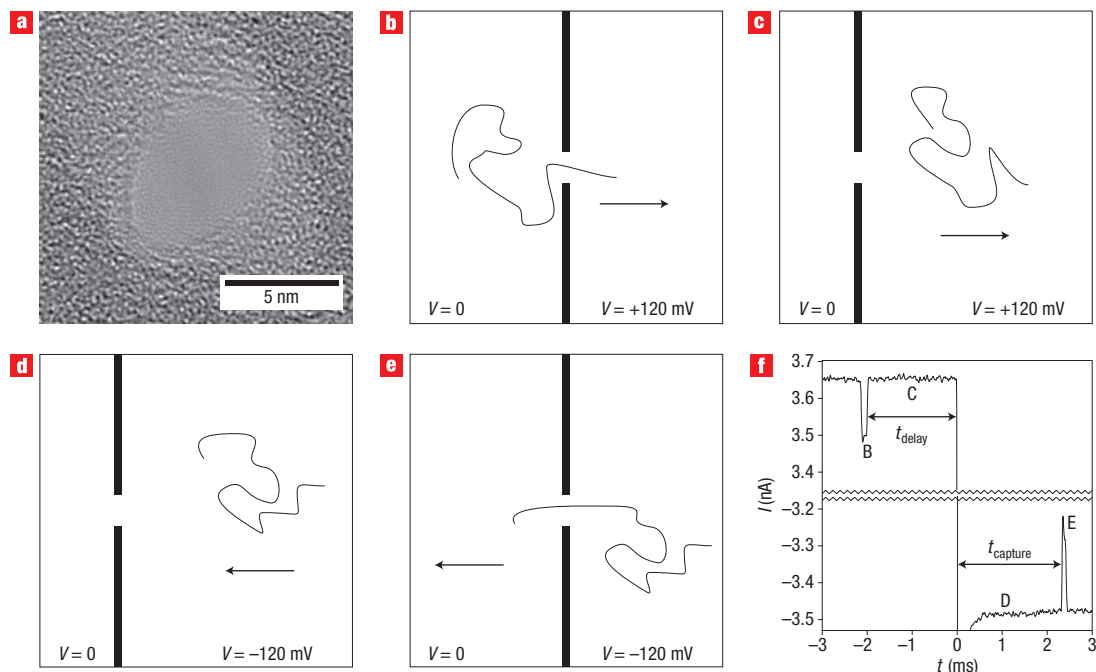
We studied a 5 nm × 7 nm nanopore in a ~20-nm-thick SiN membrane (Fig. 1a) that joined two reservoirs of aqueous 1 M KCl maintained at pH 8 by 10 mM Tris, 1 mM EDTA buffer. Electrical contact to the reservoirs was made with Ag/AgCl electrodes. An equimolar mixture of 6 and 4 kilobase-pair (kbp) double-stranded DNA (dsDNA) fragments was added to

the reservoir contacted by the ground electrode. The other reservoir was biased at +120 mV. Ionic current blockages were monitored to detect the passage of DNA through the pore<sup>16</sup>. After a molecule was detected passing through the pore (Fig. 1b), the bias voltage was maintained at 120 mV for a programmed time,  $t_{\text{delay}}$ , between 2 and 32 ms (Fig. 1c), then reversed to -120 mV for 500 ms (Fig. 1d,e). The voltage was then returned to +120 mV, regardless of when or if a molecule translocated in the reverse direction (see Supplementary Information for details on methods and materials). Fast voltage switching has previously been used to probe the escape of single-stranded DNA (ssDNA) from a protein pore<sup>13</sup>.

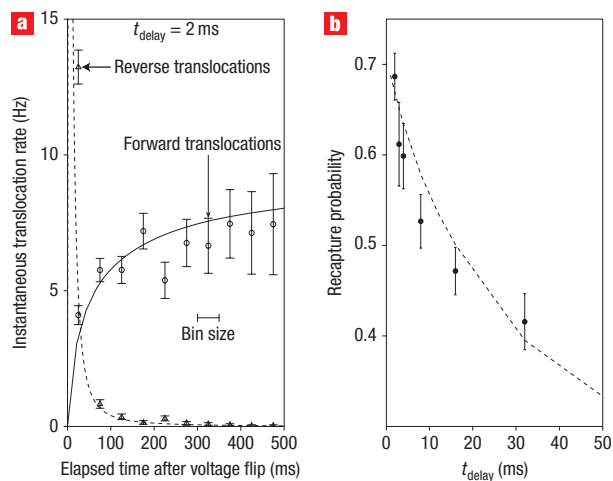
Figure 1f shows a representative current trace. A molecule is detected translocating the pore in the forward direction by an ionic current blockage (B), 2 ms are allowed to elapse (C), then the voltage is reversed (D), and the molecule is seen to translocate the pore in the reverse direction, made evident by a second current blockage (E). Immediately after translocating the pore and before the voltage reversal, the molecule is driven away from the pore by the near-pore electric field and random thermal forces. We varied  $t_{\text{delay}}$ , the time between the first translocation and the voltage reversal, and measured  $t_{\text{capture}}$ , the time until the molecule re-enters the pore after voltage reversal, to probe the behaviour of the molecules at different distances from the nanopore.

All electronic signals due to forward and reverse passages of the molecule through the pore are analysed individually. They show a characteristic blockage current and unfolded translocation time that scales appropriately with the length of the molecules<sup>16</sup>. Based on the structure of these signals (see Supplementary Information for details), we discriminate between 4 kbp and 6 kbp molecules<sup>16,21</sup>. Within the limits imposed on length discrimination by the statistical spread in translocation times and the sticking of molecules to the pore during translocation, we verify that if a 4 kbp molecule passes the pore in the forward direction, the recaptured molecule is also 4 kbp, and likewise for 6 kbp molecules.

Figure 2a shows the rate at which molecules are captured by the pore versus time after voltage bias reversal for  $t_{\text{delay}} = 2$  ms. In the forward direction, the capture rate of molecules is suppressed just after the voltage bias is switched from negative to positive because the molecules near the pore have been repelled by the reversed voltage for the previous 500 ms. In contrast, in the reverse direction, 87% of returning molecules arrive within 50 ms



**Figure 1** Overview of the recapture experiment. **a**, Transmission electron micrograph of the SiN nanopore used. **b–e**, Schematic representation of the experiment. The arrow represents the direction of the electric force on the DNA molecule. A single DNA molecule passes through the nanopore in the forward direction (**b**). After passing through the pore, the molecule moves away from the pore under the influence of the electric field for a fixed delay time (**c**). The field is reversed, and the molecule moves towards the pore (**d**). The molecule passes through the pore in the reverse direction (**e**). **f**, A representative current trace for an experiment with a 2 ms delay before voltage reversal. A gap of 6.6 nA is omitted from the middle of the trace. The letters mark the correspondence between the current trace and the schematic illustrations of molecular motion (**b–e**). Molecules cannot be detected passing the pore during the first 300  $\mu$ s after voltage reversal while the capacitance of the nanopore/flow cell system charges.



**Figure 2** Capture rates and recapture probabilities. **a**, Instantaneous capture rates when a reverse voltage is applied at  $t_{\text{delay}} = 2$  ms after the molecule is first detected entering the pore. Each point represents the average rate at which molecules entered the pore within a 50 ms time interval after voltage reversal (for example, the point at 25 ms represents the rate within the interval 0 and 50 ms after the voltage flip). The solid (forward-biased capture) and dashed (recapture) lines represent the predictions of the drift-diffusion model discussed in the text. **b**, Fraction of molecules recaptured within 500 ms of voltage reversal, as a function of time delay between forward translocation detection and voltage reversal. The dashed line represents the prediction of the drift-diffusion model discussed in the text. On both plots, the error bars represent the uncertainty due to counting statistics.

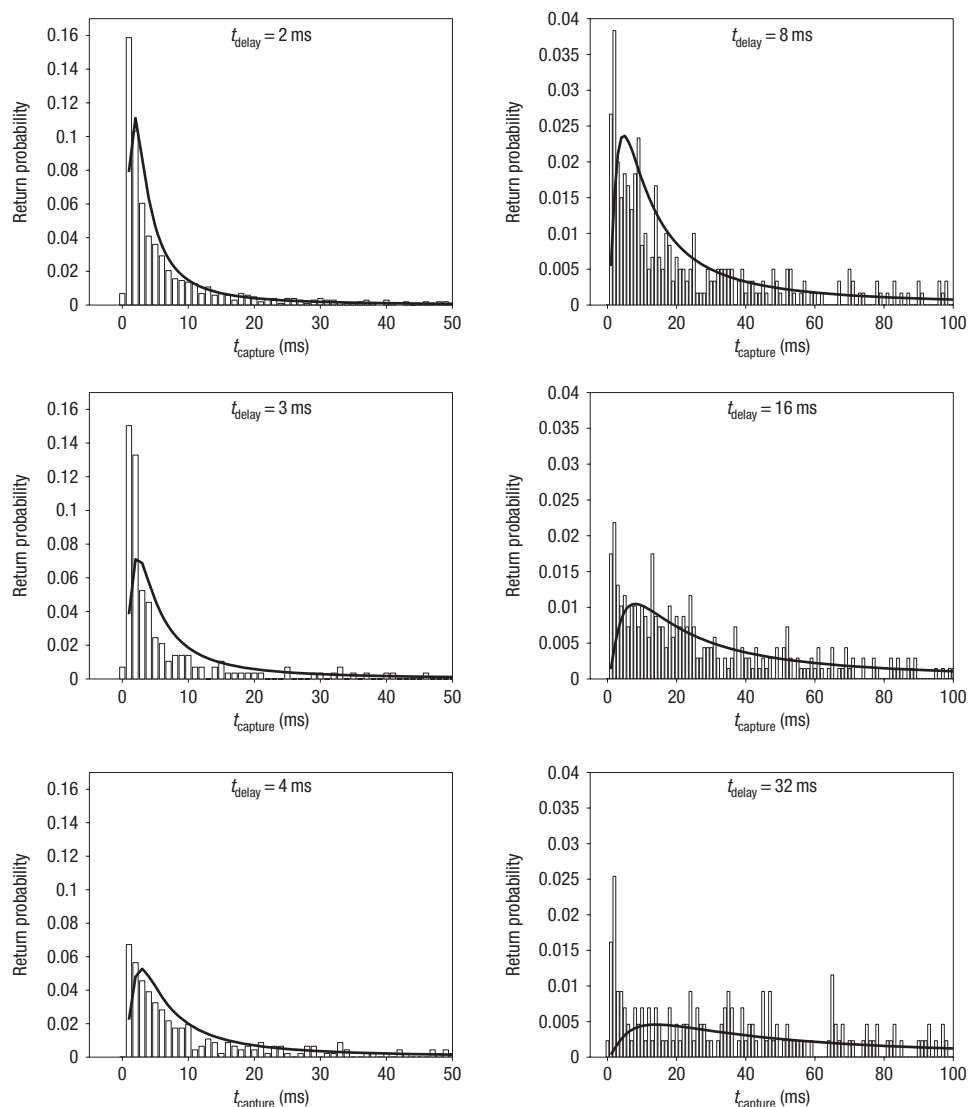
after the bias is turned negative. The high recapture rate just after the voltage is reversed is due to the return of the molecule that previously passed the pore in the forward direction and triggered the voltage reversal. Molecules that pass through the pore and are not rapidly recaptured form a background recapture rate two orders of magnitude lower than the rates discussed above.

Figure 2b shows the recapture success rate, the fraction of forward translocations followed by a reverse translocation at any  $t_{\text{capture}}$  within the 500 ms voltage reversal window, as a function of  $t_{\text{delay}}$ . Figure 3 shows histograms of  $t_{\text{capture}}$  for each  $t_{\text{delay}}$  collected for many events. For  $t_{\text{delay}} < 4$  ms, most molecules arrive at the pore and are translocated through in less than 10 ms. Both the distribution of return times and the overall recapture success rate depend strongly on the delay before reversal.

We compare our observations with a theoretical model in which the DNA's motion is determined by an electric force on the charged phosphate backbone and random thermal forces due to collisions with water molecules. Competition between thermal and electrical forces leads to a characteristic length beyond which the latter dominates the former.

On average, diffusion drives a molecule away from the pore, located at  $r = 0$ . We can define the radial diffusion velocity  $v_d(R, t)$ , as  $E[dr/dt|r(t) = R]$ , the expectation value of the rate of change of a diffusing molecule's distance from the pore. This is equivalent to

$$v_d(R, t) = \lim_{\Delta t \rightarrow 0} \frac{E[r(t + \Delta t)|r(t) = R] - R}{\Delta t} \quad (1)$$



**Figure 3** Capture time histograms for returning molecules for different delays before voltage reversal. Each bar represents the fraction of forward translocated molecules recaptured in the 1 ms interval centred about the corresponding time. Note the axes have different scales for the left and right histograms. The bold lines represent the predictions of the drift-diffusion model discussed in the text.

where

$$\begin{aligned}
 E[r(t + \Delta t)|r(t) = R] &= \int \rho^2 d\rho d\Omega \sqrt{R^2 + \rho^2 + 2R\rho \cos(\theta)} \times \frac{\exp(-\rho^2/4D\Delta t)}{(4\pi D\Delta t)^{3/2}} \\
 &= R + \frac{D\Delta t}{R} \quad (D\Delta t \ll R^2)
 \end{aligned} \quad (2)$$

so

$$v_d(r) = D/r \quad (3)$$

where  $D$  is the DNA's diffusion constant.

An electrical current density  $\mathbf{J}$  results from an electric field  $\mathbf{E}$ , given by Ohm's law,  $\mathbf{J} = \sigma\mathbf{E}$ , where  $\sigma$  is the electrical conductivity of the ionic solution. At distances much greater than the

diameter of the pore, the current density and electric field will be (hemi)spherically symmetric, and related to the experimentally observed current  $I$  through the biased nanopore by  $\mathbf{E}(r) = (\mathbf{J}(r)/\sigma) = (I\mathbf{r}/2\pi r^2\sigma)$ .

DNA in free solution is known to move with a constant electrophoretic mobility  $\mu^{28,29}$ . If we ignore the conformational degrees of freedom of the DNA molecule and assume its charge is distributed symmetrically about its centre of mass, located at  $r$ , the radial electrophoretic velocity  $v_e(r, t)$  is given by  $v_e = \mu I/2\pi r^2\sigma$ .

Comparing  $v_e$  to  $v_d$ , we see there is a characteristic distance  $L = (|\mu I|/2\pi\sigma D)$  beyond which the average velocity away from the pore due to diffusion is greater than the electrophoretic velocity<sup>30</sup>. For our experimental conditions, this length is 940 nm for 4 kbp dsDNA and 1.2  $\mu\text{m}$  for 6 kbp dsDNA. (In contrast, for 100 bp ssDNA and a, for example, protein pore with 100 pA of current, this length is less than 1 nm, and we do not expect recapture of recently translocated molecules by voltage reversal would be possible.)



**Figure 4** Current versus time traces from a single-molecule trapping experiment. **a**, A single 10 kbp dsDNA molecule passes the pore 12 times over 250 ms. The main panel shows the current through the pore versus time. For clarity, 2.4 nA are excised from the centre of the current axis, and the time axis has also been compressed. The short pulses (marked with arrows) show current being blocked as the molecule passes through the pore. At 2 ms after each passage, the voltage bias (plotted below the current) is reversed. As in Fig. 1, the molecule is initially captured at positive voltage bias. The exponential settling at the beginning of each transition results from charging of the membrane capacitance. **b**, Expanded current traces resulting from separate passages of the molecule through the pore. Each is labelled from i to vi to identify the portion of the current trace in **a** from which it was taken.

Assuming a (hemi)spherically symmetric distribution of non-interacting dsDNA molecules, the volume concentration  $c(r, t)$  of DNA obeys the drift-diffusion transport equation

$$\frac{\partial c(r, t)}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} r^2 \left( \mp |\mu I| c(r, t) + D \frac{\partial c(r, t)}{\partial r} \right) \quad (4)$$

where the minus sign is used when the electrical force is directed away from the pore and the plus sign when this force is towards it. This equation can be solved numerically, with appropriate boundary and initial conditions, to model the voltage reversal experiment (see Supplementary Information). With no free parameters, this drift-diffusion model predicts the correct ratios between recapture success rates at different  $t_{\text{delay}}$  (Fig. 2b) and the relative distributions of  $t_{\text{capture}}$  for all  $t_{\text{delay}}$  (Fig. 3), but overstates the actual number of molecules recaptured at all  $t_{\text{capture}}$  and  $t_{\text{delay}}$ . A single parameter fit, which scales the number of recaptures predicted by an overall factor of 70% for all  $t_{\text{capture}}$  and  $t_{\text{delay}}$ , makes a good match to the observed recapture success rates and capture time distributions. The dashed lines in Fig. 2 and solid lines in Fig. 3 represent this fit, with no other free parameters. In the forward direction, the same equation models the capture of molecules initially driven away from the pore by an electric force generated by the reversed voltage. The solid line in Fig. 2a is a single-parameter (the steady-state flux of molecules through the pore) fit of the drift-diffusion model to the observed forward capture rates.

Approximations made in the model, including assuming a spherically symmetric electrical field on all length scales<sup>31</sup> and ignoring the possibility of nonspecific binding of the DNA to the membrane surface, could account for the missing 30% of returning molecules. At long values of  $t_{\text{delay}}$ , we see a higher

return rate at short times than predicted by the model. This could be due either to molecules that stick briefly to the membrane surface and are not driven as far away, or to extended configurations of molecules that leave parts of them far closer to the pore than their centres of mass. We have disregarded effects of electro-osmotic flow, which, due to the negatively charged surface of the pore, would oppose the DNA's electrophoretic motion.

Numerical analysis of the drift-diffusion equation shows that of the molecules that return to the pore from distances less than the characteristic distance  $L$ , discussed above, most do so within a time  $L^2/2D$  (here, 220 ms for the 4 kb DNA and 450 ms for the 6 kb DNA). A molecule that starts at  $0.4L$  (400–500 nm) has an 85% chance (neglecting the overall 70% pre-factor) of translocation in this time (see Supplementary Information for further details of the calculations).

We also probed the time required for DNA to enter the nanopore. The factor  $t_{\text{capture}}$  consists of  $t_{\text{return}}$ , the time it takes a molecule to arrive at the pore, and  $t_{\text{ent}}$ , the time it takes a molecule to enter after arriving.  $t_{\text{return}}$  is predicted by the drift-diffusion model discussed above, and its distribution depends strongly on  $t_{\text{delay}}$ .  $t_{\text{ent}}$  is not included in the drift-diffusion model and does not depend on  $t_{\text{delay}}$ , as it involves the behaviour of the molecule after it has already reached the pore. The histograms of  $t_{\text{capture}}$  presented in Fig. 3 depend strongly on  $t_{\text{delay}}$ , in the same manner as the drift-diffusion calculations of  $t_{\text{return}}$ , which indicates the recapture time is determined mainly by  $t_{\text{return}}$ . The recapture rate is also highest immediately after the voltage is reversed, which is inconsistent with the notion<sup>24,32</sup> that the molecule requires a significant amount of time post-arrival to enter the pore. Hence, upon arriving at the pore, the typical molecule in this experiment translocates in less than a millisecond.

Besides exploring molecular dynamics there are other advantages and applications to recapturing molecules that have passed the pore in the forward direction, including convincing evidence that an electronic signal corresponds to a molecule translocating the pore. This provides a way to distinguish molecular signals from background noise on a single-molecule basis and is valid even for polydisperse samples and analytes<sup>17,18</sup> for which no sensitive assay like the polymerase chain reaction exists. Recapturing the molecule would also allow one to measure changes in molecules (such as hybridization changes, changes in protein conformation, stripping of binding proteins) induced by passage through the nanopore. Immediate voltage reversal can also be used to study the conformational dynamics of a polymer. The Zimm relaxation time<sup>33</sup> for 4 kb dsDNA is 300  $\mu$ s and 610  $\mu$ s for 6 kb dsDNA. In this experiment the configuration of the molecule during the reverse translocation was not influenced by the previous translocation. However, with an increase in viscosity<sup>20</sup> and/or molecule length, the relaxation time can be extended sufficiently to enable us to probe and possibly manipulate the non-equilibrium conformation induced in the molecule by passage through the nanopore and to explore the influence of a molecule's initial conformation on translocation through the pore.

Extending the single recapture experiments presented so far to repeatedly recapture the same molecule realizes a new kind of single-molecule trap based on nanopore technology. Figure 4 presents the electronic signals from such a trap (see Supplementary Information for details of the setup). In this particular experiment (in a new nanopore), a single 10 kbp dsDNA molecule from a mixture of 5.4 and 10 kbp molecules was passed back and forth 12 times over a period of 250 ms. (Single molecules have so far been trapped for as many as 22 passes over 500 ms.) Current blockage induced by the passage of the molecule through the nanopore revealed information about the molecule (length, conformation and interaction with the pore<sup>16</sup>) and, with triggered voltage reversals, provided the feedback mechanism to maintain the trap. Thus, biologically interesting molecules can be trapped, detected and analysed in free solution without any labels or chemical modifications. Repeated electronic interrogation of a single molecule potentially provides a means for greatly enhancing the accuracy with which each molecule can be characterized by a nanopore and allows measurement over time of dynamical properties such as the molecule's conformation and chemical state.

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## NANOPORES

## Molecular ping-pong

Experiments designed to pass the same DNA molecule through a solid-state nanopore many times will greatly improve the quality of single-molecule measurements.

## Derek Stein

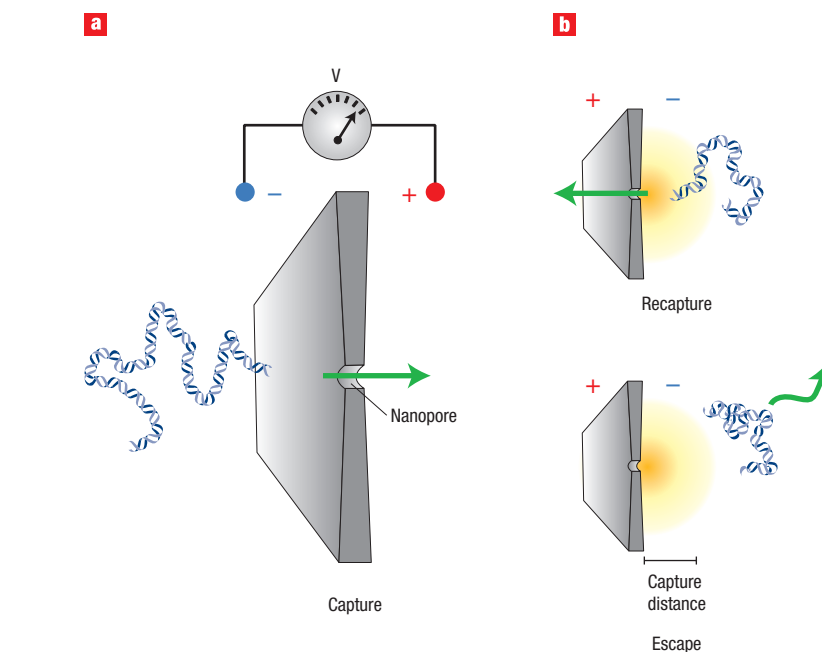
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**D**NA lives in a tumultuous world, constantly being jostled in unpredictable ways by other molecules because of their thermal energy. Amazingly, the machinery of the cell thrives off this agitation as it faithfully reads and copies the genetic information stored along a single DNA molecule. Synthetic devices aspire to such exquisite control and sensitivity, but first they must cope with the random forces that are inherent to the molecular scale. On page 775 of this issue, Marc Gershow and Jene Golovchenko from Harvard University report how they keep a DNA molecule within reach of a solid-state nanopore detector by bouncing it back and forth with electric fields<sup>1</sup>. The technique offers a way to perform multiple measurements on the same molecule and to better understand its dynamic behaviour as it approaches and leaves the pore.

A nanopore is nothing more than a tiny hole in a thin insulating membrane. When the membrane separates two reservoirs filled with a high salinity ionic solution and DNA, a voltage difference applied between the reservoirs drives a current of ions through the pore (Fig. 1a). DNA, which is negatively charged in solution, is driven through the nanopore with the ionic current. This event, known as 'translocation', occurs at speeds of about  $10^7$  bases per second — which means the DNA strands that are typically studied in the laboratory pass through the nanopore in milliseconds or less. If the diameter of the nanopore is comparable to that of DNA, the insertion of a single molecule induces a measurable dip in the current called a 'current blockade'. Building on this simple principle, individual DNA molecules can be electrically detected and manipulated in their native environment.

The inspiration for nanopore devices came from biology. The protein channels found in membranes are nature's nanopores, and they play a vital role in trafficking molecules in and out of the cell and



**Figure 1** DNA capture and recapture in a solid-state nanopore **a**, The nanopore membrane is submerged in an ionic solution. When a voltage is applied across the membrane, the DNA molecule, which is negatively charged in solution, will be attracted to the pore and pulled through it from the negatively-biased to the positively-biased side. **b**, If the polarity of the voltage is reversed while the DNA is still within the capture distance of the nanopore, the probability for recapture is highest; otherwise, the molecule is more likely to escape.

sub-cellular compartments. They regulate the flow of energy, information and matter through openings approximately one nanometre in diameter. Sakmann and Neher<sup>2</sup> pioneered the study of these fascinating machines in living cells by measuring the tiny electrical currents that a single ion channel can carry.

The first translocation experiments in the mid-1990s turned ion-channel research on its head. John Kasianowicz of NIST and colleagues at Harvard University and the University of California, Santa Cruz used the channel  $\alpha$ -haemolysin, which is particularly stable and wide enough (1.4 nm) to pass a nucleic acid, as a tool to study other molecules<sup>3</sup>. The ability of the nanopore technique to simultaneously detect a molecule while constraining it to translocate

along its length sparked a dream that the sequence of bases along a single strand of DNA might be read off at high speed.

That particular dream did not materialize, however, owing to the limitations of the proteins and, more importantly, the ionic signal. This should not come as a great surprise because membrane channels did not evolve in order to electrically detect the DNA sequence. Synthetic nanopores, on the other hand, were developed to circumvent shortcomings of their biological counterparts<sup>4,5</sup>. They preserve the ability to shuttle molecules along their length while providing a robust and versatile platform onto which electronic, optical and chemical probes can be integrated. Several groups are currently pursuing such detection strategies in order to increase the sensitivity

of nanopore devices to the chemistry of different DNA bases.

At present, a number of single-molecule properties can be determined with nanopore technology. For example, the duration of the current blockade correlates with the length of the translocating DNA strand. A molecule's folding conformation can also be detected. The strong electric fields at the nanopore can grab a long DNA molecule somewhere in the middle, bend it into a hairpin shape, and pull it through. The hairpin places two segments of DNA together in the nanopore, which is detectable as a dip in the ionic current that is twice that of a single strand passing through the pore<sup>6</sup>.

Despite these successes, a number of open questions remain about how a molecule is transported to the nanopore, and in what conformation it presents itself. In previous measurements, once DNA was outside the pore, no information was available about where it was or what it was doing. The clever experiments by Gershow and Golovchenko<sup>1</sup>, illustrated schematically in Fig. 1, have changed that.

The Harvard team detected the translocation of DNA through a nanopore, and then reversed the polarity of the driving voltage a short time after the DNA had passed through it. If the delay was short enough, they could catch the same molecule and force it to re-enter the pore (Fig. 1b). The process could be repeated multiple times, trapping the DNA in a game of molecular ping-pong.

By increasing the delay time between when the DNA first exits the pore and when the voltage is reversed, Gershow and

Golovchenko were able to determine just how far DNA can stray before it escapes the grip of the applied electric field. Their results are well explained by a drift–diffusion transport model, lending support to the notion that electrophoresis and Brownian motion are the dominant mechanisms for this common set of experimental conditions. The model also yields a characteristic escape length of one micrometre, beyond which the molecule is most likely to escape instead of being recaptured. Interestingly, this length is around 200 times the diameter of the nanopore, which illustrates how a tiny device can have a strong influence over a surprisingly long length scale.

Gershow and Golovchenko also found that when a molecule was recaptured, it inserted itself into the pore without significant hesitation. This is a noteworthy result because it had been previously suspected that DNA — a long polymer that tends to form a complicated coil in solution — could spend considerable time searching for a conformation that would allow it to translocate.

The experiments reported here represent a significant step for an emerging nanotechnology. The ability to repeatedly interrogate a single molecule, above all, has important implications. From a purely practical standpoint, increasing the number of times a single molecule can be sampled will greatly improve the accuracy of any one measurement, such as a measurement of the length of the DNA strand. It should even be possible to select a particular molecular conformation in the nanopore

with sufficient attempts. To illustrate this capability, the Harvard team shows data corresponding to the same DNA molecule translocating the nanopore in both linear and folded conformations.

The ability to carefully repeat experiments is also key to progress in the field of single-molecule biophysics. Our understanding of DNA translocations is built on measurements of ensembles of identical molecules, but exciting advances are increasingly focused on tests of the same molecule. In 2006, researchers at Delft University of Technology demonstrated that a single DNA molecule can be lowered into, and removed from, a nanopore with nanometre control via an optically trapped bead<sup>7</sup>. This method greatly slows down the translocation process to provide more time to study a single molecule in detail. In contrast, the voltage-reversal technique of Gershow and Golovchenko entails fast translocations that are performed multiple times, but it is relatively straightforward to implement. As almost any new measurement of the properties of DNA can be checked more accurately with this technique, everyone in the nanopore field may soon be playing molecular ping-pong.

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## **Supplemental Information for NNANO-07080641-A**

### **Recapturing and Trapping Single Molecules With a Solid State Nanopore**

**Marc Gershow and J.A. Golovchenko**

#### **Materials and Methods**

Nanopores were fabricated using a condensed TEM beam<sup>1,2</sup> 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<sup>3,4</sup> 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  $\mu\text{m}$  thick silicon dioxide layer, which was on a 3mm silicon chip with a pyramidal pit fabricated by standard MEMS techniques<sup>3,4</sup>. 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 were obtained from New England Biolabs. The fragments were provided in TE buffer at a concentration of 0.5 mg/mL. 4  $\mu$ L was diluted in 50  $\mu$ L of the 1 M salt buffer, then almost the entire amount was slowly added to, and through, the 1-2  $\mu$ L 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  $\mu$ s), 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/ $\mu$ L) of mixed 10 and 5.4 kbp dsDNA was used. At this concentration, under forward bias, new molecules arrived 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<sup>4</sup> or the event charge deficit<sup>5</sup> (ecd) is the amount of additional charge that would have passed through the pore without the molecule blocking some of the ionic current. This ecd is independent of the conformation of the molecule (folded or unfolded) as it passes the pore<sup>4,5</sup> 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

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

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

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

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  $t/\tau$  for various dimensionless starting radii,  $x_0 = r_0/L$ . Most translocations occur within  $1/2\tau$  for starting distances less than  $L$ . Figure S3b shows the probability a molecule returns within  $1/2\tau$  vs. starting distance. A molecule that starts at  $x_0 = 0.4$  reaches the pore within  $1/2\tau$  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

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

where  $c_\infty$  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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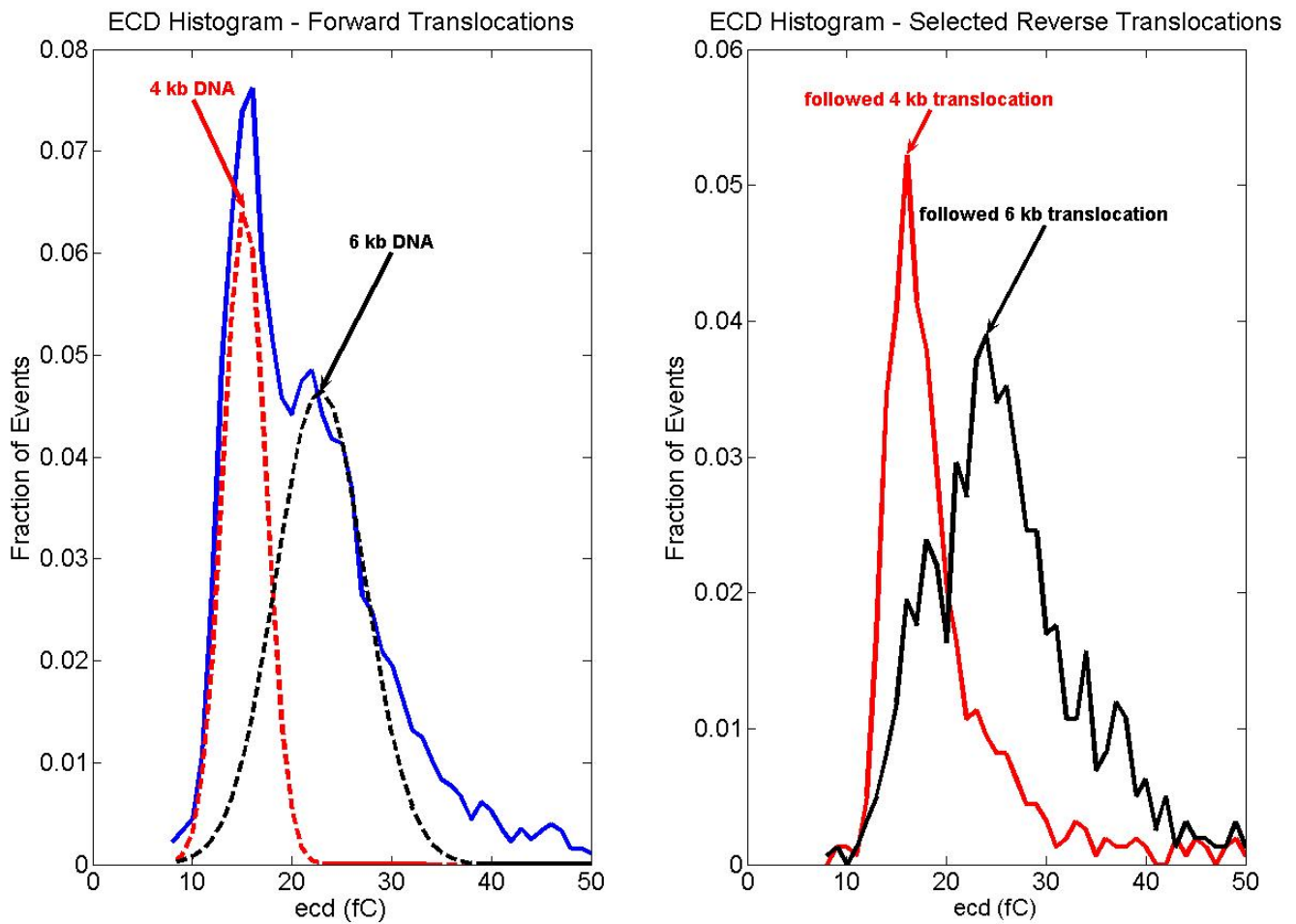


Figure S1 Event charge deficit (time integral of blockage current) histograms for forward and reverse translocations. A) Ecd of forward translocations. The dotted lines represent the two gaussians whose sum is fitted to the histogram. B) Ecd of reverse translocations sorted according to the ecd of the 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.

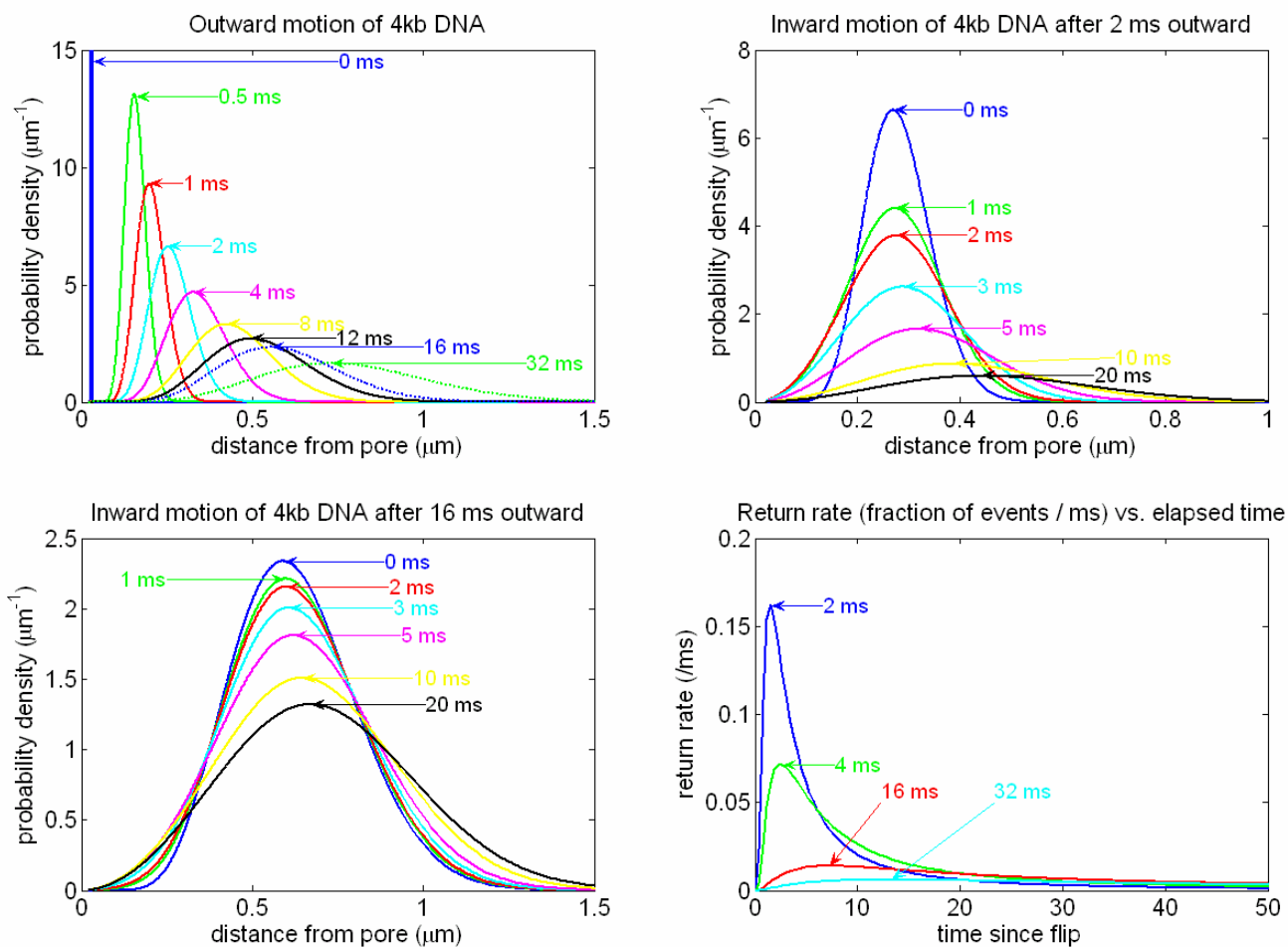
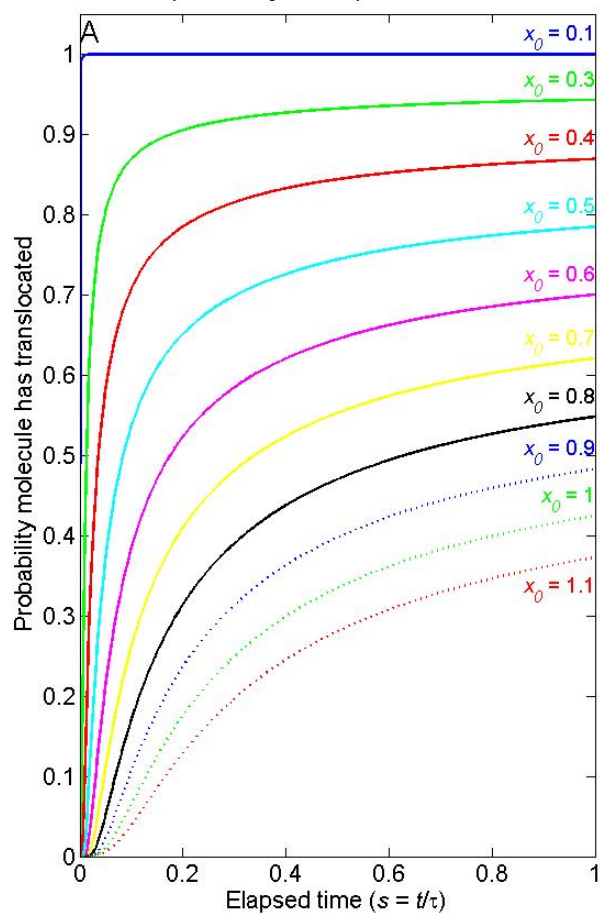


Figure S2- Drift-diffusion solutions for the recapture experiment. a-c Linear probability density  $p(r) = 2\pi r^2 c(r)$ , where  $c(r)$  is the volume density, and  $p(r)dr$  represents the probability a molecule is found between  $r$  and  $r+dr$ . a) After outward movement for the specified times. 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.

Translocation probability vs. elapsed time and start distance



Probability molecule translocates in  $1/2 \tau$  vs. starting distance

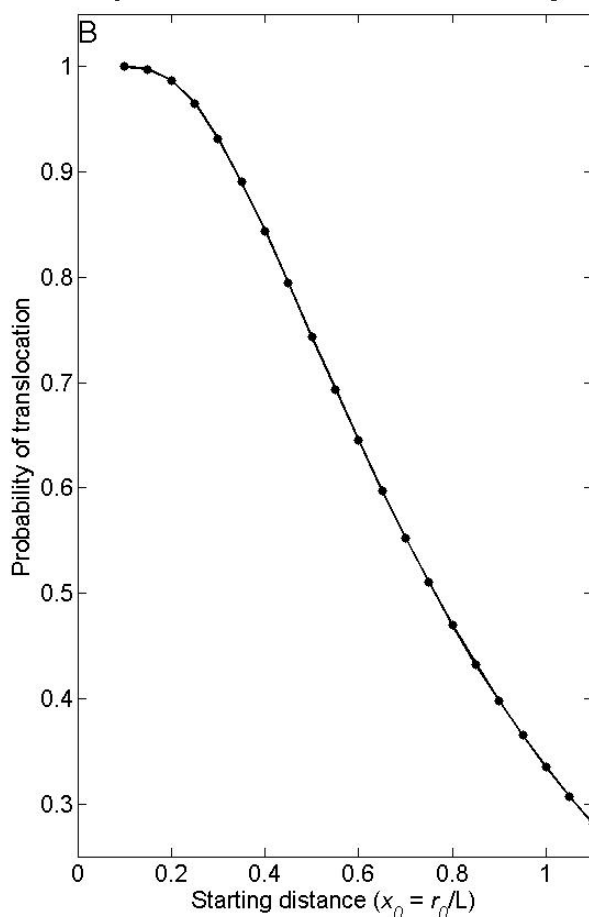


Figure S3: Dimensionless drift-diffusion capture predictions. a) Probability a molecule has returned to the pore within  $s = t/\tau$  for various dimensionless starting radii,  $x_0 = r_0/L$ . b) Probability a molecule translocates within  $s = 0.5 \tau$  vs. starting distance.