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Electrostatic Focusing of Unlabeled DNA into Nanoscale Pores using a Salt Gradient

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Abstract

Solid-state nanopores are sensors capable of analyzing individual unlabelled DNA molecules in solution. While the critical information obtained from nanopores (e.g., DNA sequence) is the signal collected during DNA translocation, the throughput of the method is determined by the rate at which molecules arrive and thread into the pores. Here we study the process of DNA capture into nanofabricated silicon nitride pores of molecular dimensions. For fixed analyte concentrations we find an increase in capture rate as the DNA length increases from 800 to 8,000 basepairs, a length-independent capture rate for longer molecules, and increasing capture rates when ionic gradients are established across the pore. In addition, we show that application of a 20-fold salt gradient enables detection of picomolar DNA concentrations at high throughput. The salt gradients enhance the electric field, focusing more molecules into the pore, thereby advancing the possibility of analyzing unamplified DNA samples using nanopores.

Solid-state nanopores are an emerging class of single-molecule sensors for characterizing individual unlabeled biopolymers¹⁻⁷, as well as DNA/protein⁸ and DNA/ligand⁹ complexes. Their unique potential for obtaining sub-molecular information from long, unlabelled double-stranded DNA (dsDNA) molecules is of particular interest in native genome analysis and for proposed nanopore-based DNA sequencing approaches^{10,11}. While nanopore sensing involves reading the properties of long dsDNA molecules during their linear sliding through the pore, it is the initial process of threading into the nanopore that determines the throughput of the method. This capture process consists of two steps:

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Author contributions A.M. and M.W. designed the experiments.

M.W. performed all experiments and analyzed the data.

W.M. performed finite element simulations.

Y.R. and A. G. developed the theoretical model.

M.W., W.M., Y.R., A.G. and A.M. co-wrote the paper.

All authors discussed the results and commented on the manuscript.

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arrival of a molecule from the bulk to the pore mouth, and threading of a polymer end into the pore. Studies of single-stranded DNA capture into lipid-embedded α -hemolysin channels indicate that there is a free energy barrier for DNA capture, associated with threading of the first few bases¹²⁻¹⁵. Such a barrier was not observed for capture of dsDNA of lengths 4-6 kbp¹⁶ and 48 kbp(λ -phage DNA)² into large solid-state pores (diameter >5 nm). Despite the sub-5 nm size regime being the most relevant for many nanopore applications¹⁰, DNA capture into pores in this regime has yet to be characterized.

In this paper, we investigate the capture mechanism of dsDNA capture into sub-5 nm solid-state nanopores, as well as its scaling with various experimental parameters. We show that capture involves two main steps, illustrated in Figure 1: (a) as a DNA coil approaches the pore from bulk (i) to a distance larger than the coil size, its motion transitions from purely diffusive to biased motion, driven by the electric field outside the pore. This field is maintained by an ion current across the pore, which creates a potential profile $V(r)$ outside the pore mouth that attracts the DNA coil from a distance r , orders of magnitude greater than Debye screening length scales (0.1 - 1 nm). Consequentially, a DNA coil experiencing this field is 'funneled' towards the pore mouth (ii). b) Once the DNA coil is within approximately one coil size (r_g) from the pore, a DNA end threads into the pore, a process that involves crossing a free-energy barrier (iii).

We find that the funneling field can be enhanced by applying salt gradients across the pore, thereby increasing capture from the bulk. Applying a 20-fold salt gradient increases the nanopore sensitivity by a factor of >30 , allowing the first nanopore-based analysis of miniscule amounts ($<10^6$ copies) of long dsDNA molecules under physiological conditions. Incidentally, the same salt gradients which augment the capture rate also increase mean translocation times, presenting two simultaneous advantages in nanopore sensing.¹⁰

Figure 1b displays schematically our setup, which consists of a solitary 4 nm pore fabricated in a SiN membrane (high resolution TEM image is shown in the Figure)¹⁷. Both the analyte (*cis*) and the collection (*trans*) chambers are equipped with a miniature Ag/AgCl electrode. Stochastic threading of a dsDNA end followed by complete translocation generates a distinct square-shaped pulse, allowing statistical analyses of dwell-times t_D and mean time-delays between two successive events (δt), used to define the mean capture rates (see Methods).

DNA capture under symmetric salt conditions

For a small cylindrical pore in an insulating membrane separating two electrolyte solutions, application of potential across the membrane produces an electric field profile away from

the pore mouth ($r > 0$), which varies as $V(r) = \frac{d^2}{8lr} \Delta V$, where r is the distance from the pore mouth, d is the pore diameter, l its length, and ΔV is the voltage applied to the electrodes (see Figure 1 and SI-1). In the negatively-biased chamber, this field creates an attractive, funnel-shaped potential landscape¹⁸ for the negatively-charged DNA, resulting in space-dependent drift velocity $v(r) = \mu \nabla V(r)$, where μ is the DNA electrophoretic mobility. As predicted theoretically¹⁹ and confirmed experimentally²⁰, μ in free solution does not

depend on DNA length for DNA longer than a few persistence lengths²¹. This is the result of the free-draining property of an electrophoretically-driven DNA coil in solution – electroosmotic flow (EOF) of water induced by the flow of oppositely charged counterions counteracts the long-range hydrodynamic interactions between DNA segments¹⁹.

Although for $r \gg d$ ($d \sim 5$ nm), $V(r) \ll V$, the effect of $V(r)$ on DNA capture is significant even at large distances from the pore, because it acts on a highly charged macromolecule. Previous studies have shown that a characteristic length scale exists such that DNA motion crosses over from almost purely diffusive at distances $r > r^*$, to drift-dominated motion when $r < r^*$ ^{2,16,22}. We define r^* here by setting $V(r^*) = D/\mu$, where D is the DNA diffusion coefficient,

$$r^* = \frac{d^2 \mu}{8lD} \Delta V. \quad (1)$$

When the capture rate is limited by the time required for the polymer to arrive at the pore, and not by the final threading process, this can be described by Smoluchowski theory for absorption by a hemisphere of radius r^* . The diffusion-limited current is $J_{diff} = R_{diff}c$, where c is the bulk DNA concentration and the diffusion-limited capture rate R_{diff} is given by:

$$R_{diff} = 2\pi D r^* = \frac{\pi d^2 \mu}{4l} \Delta V. \quad (2)$$

Thus, capture should be *DNA length independent* in this diffusion-limited regime. While this result may seem counterintuitive (the free diffusion for larger DNA is slower), the effective capture radius r^* grows with D^{-1} , canceling out the size dependence.

To test the prediction of Eq. 2, we measured the capture rate of dsDNA molecules as a function of their length in the range 400 - 48,502 bp. We display in Figure 2a a semi-log plot of the specific capture rate (events/s/nM) as a function of DNA length. The salient features are: (a) fast growth of capture rate with increasing DNA length from about ~1 kbp to about 8 kbp; and (b) constant capture rate for molecules longer than ~8 kbp. The observed length independence indicates that for long DNA, capture is limited by driven diffusion to the pore, as Eq. 2 suggests. In contrast, for DNA shorter than ~8 kbp, diffusion to the pore is not the rate-limiting step for DNA capture.

We recall that DNA capture requires threading one of the polymer ends into the pore (step (iii) in Figure 1a). This confinement of the DNA end, as well as possible unfavorable interactions of the highly charged DNA with the pore itself, can create a free energy barrier to capture. This barrier was experimentally observed for DNA transport through the 1.5 nm protein pore α -HL by an exponential dependence of the capture rate on voltage^{12,13}, and also explained theoretically.¹⁵ In contrast, recent experiments^{2,16} and theoretical^{22,23} studies of dsDNA transport through large solid-state pores indicate a barrier-free capture process, as indicated by linear dependence of the capture rate on voltage. We measured the capture rate as a function of voltage and DNA length to explore the free energy landscape of capture into small (<5 nm) solid-state nanopores. Figure 2b displays the specific capture rate into a 4 nm pore as a function of applied voltage V for: (I) 400 bp, (II) 3,500 bp, and (III)

48,502 bp DNA. The capture rates of the short (400 bp) and medium (3,500 bp) DNA lengths display exponential increase with the applied voltage, yielding slopes of $9.6 \pm 0.4 \text{ V}^{-1}$ and $7.1 \pm 0.5 \text{ V}^{-1}$ respectively (black lines I and II in the semi-log plot, Figure 2b). In contrast, the capture rates of the longest DNA fragment (III), which is in the length-independent regime, shows a linear dependence on V (see inset).

When DNA capture is governed by an energy barrier, its rate, according to classical Kramers theory, can be written in the form $J = R \cdot c = \omega \exp[(qV - U)/k_B T]$, where U is the height of the threading barrier without any voltage applied and q is the effective charge of a DNA end segment, which is DNA length independent. The pre-factor ω in the expression above is usually interpreted as the threading attempt rate. In order to understand the steep length dependence in the barrier regime, we have to evaluate the influence of the local potential $V(r)$ and DNA length on ω . In this step, the DNA coil is placed at the pore mouth at an average distance approximated by its radius of gyration, r_g . We find that the funnel-shaped potential $V(r)$ near the pore leads to two capture enhancement mechanisms, evaluated in SI-1: (1) An exponential attempt rate enhancement, resulting from the fact that the potential well $V(r)$ traps the DNA a distance r_g from the pore mouth, where threading is repeatedly attempted. Due to the energy barrier, DNA molecules are delivered to the pore mouth multiple times before a successful translocation occurs. Therefore, while the probability of finding a DNA coil within a distance r_g from the pore mouth is $C r_g^3$ in the absence of $V(r)$, the presence of $V(r)$ enhances this probability by the exponential factor $\exp(Neq/k_B T)$. In this case, the effective charge of DNA is proportional to its length (N). This is different from the N -independent electrophoretic mobility of DNA in the bulk (μ above), because when the DNA coil is pressed against the membrane, hydrodynamic (electroosmotic) flow through it is suppressed (the pore diameter is much smaller than the DNA coil). (2) When the DNA coil is placed at the pore mouth, the probability of successful end threading into the pore is determined by the internal dynamics of the coil. While the coil relaxation time is estimated by its Zimm time (τ_{Zimm}), its dynamics is affected by the potential $V(r)$, which attracts the chain ends towards the pore, providing further rate enhancement. Together, these two effects result in an increased capture rate R_{bar} :

$$R_{\text{bar}} = \frac{r_g^3}{\tau} \exp \left[\frac{q\Delta V - U}{k_B T} + \frac{e\Delta V}{K_B T} \frac{\alpha d^2}{al} \sqrt{\frac{N}{4N_p}} \right], \quad (3)$$

where τ is proportional to $\tau_{\text{Zimm}}(kT/eV)(8la/d^2)$, $N_p \approx 150$ is the number of basepairs in a dsDNA persistence length, a is the length per basepair of dsDNA, and α is a constant related to the fact that not all phosphates on the DNA are ionized due to Onsager-Manning condensation. We consider Eq. (3) to be valid only for sufficiently long DNA, i.e. $N > 4N_p$. Since $\tau_{\text{Zimm}} \propto r_g^3$, the only dependence on DNA length in Eq. 3 appears in the second term of the exponent, namely $R_{\text{bar}} \sim e^{C \sqrt{N/4N_p}}$.

When $R_{\text{diff}} > R_{\text{bar}}$, the capture rate is limited by the barrier crossing given by Eq. (3). This can only be realized when the threading barrier is sufficiently large (i.e., for narrow enough nanopores). In this barrier-dominated regime, R_{bar} grows with DNA length proportional to $\sim e^{C \sqrt{N/4N_p}}$, provided that $N > 4N_p$. When R_{bar} becomes sufficiently large, the rate-limiting

step becomes diffusion to the pore. Capture rate in this regime is proportional to R_{diff} and independent of N (Eq. 2). The overall capture rate is thus expected to exhibit a transition from a rapidly growing function with $N(\sim e^C \sqrt{N/4N_p})$ to a length-independent plateau for long enough DNA molecules.

In Figure 2a, this prediction is overlaid with our experimental data. For molecules in the range ~800 – 8,000 bp, the capture rate per nM of DNA molecules can be well-fitted by Eq. 3 (see solid line), which agrees with the hypothesis that in this range the process is dominated by a free energy barrier. In the range 8,000 bp - 48,000 bp, we observe the length independent, diffusion-dominated regime. The fit also provides us the value of the dimensionless constant $C = (e^{-V/k_B T}) \cdot (ad^2/al) = 0.81 \pm 0.15$. The growing capture rate with N in sub-5-nm pores represents a unique example of capture selectivity towards longer DNA molecules, which is surprising, considering the length-independent DNA mobility in free-flow electrophoresis. When R_{bar} is the rate-limiting step, longer DNA molecules are more efficiently threaded than short ones, as their higher charge is advantageous for funneling into the pore mouth. Moreover, the threading selectivity has a very steep dependence on DNA size, scaling as $e^C \sqrt{N/4N_p}$ for molecules in the range 800-8,000 bp.

Increasing capture rate by ion gradients

The strong impact of $V(r)$ on the capture rate suggests that manipulation of the voltage profile outside the pore is of potential utility for increasing throughput. While increasing the applied voltage V also increases $V(r)$, translocation times become exponentially shorter⁷, decreasing the method's resolution. In contrast, we find that an ion gradient across the pore can be used to significantly enhance the magnitude of $V(r)$ in the *cis* chamber, greatly enhancing the capture rate without reducing the translocation times. This finding is illustrated in Figure 3: Using the same pore, we measured R_C at 1M/1M *trans/cis* KCl concentrations, replaced the buffers with 1M/0.2M, and finally exchanged it to 0.2M/1M, while maintaining a constant concentration of 3.8 nM 400 bp DNA in the *cis* chamber (Figure 3a, top to bottom). Continuous data streams were collected for each gradient, and representative two second snapshots are shown. Strikingly, when the *cis* concentration was lowered from 1M to 0.2 M, the capture rate increased 9-fold from ~0.4 to ~3.7 s⁻¹ nM⁻¹. Reversal of the conditions (to 0.2M/1M) effectively suppressed DNA capture. These results were completely reversible, i.e. changing back to 1M/1M yielded the same capture rate as previously obtained.

We measured capture rate over a range of experimental conditions. Figure 3b shows the enhancement in R_C for several DNA lengths, (400 bp, 2,000 bp, 3,500 bp and 8,000 bp), and over a large range of asymmetries (C_{tr}/C_{cis} from 1 to ~32). Clearly, above a threshold value of roughly $C_{tr}/C_{cis} \sim 1.5$ a linear, length-independent increase in the capture rate is observed. In addition to the rate enhancement, our asymmetric salt conditions allow experiments under physiological conditions (down to 125 mM KCl) while preserving the signal-to-noise ratio (see SI-6).

These experimental observations can be explained by the dependence of $V(r)$ on the ionic environment in the pore vicinity. Under symmetric ionic strengths, the electric field across

the pore equally drives K^+ ions from *trans* to *cis* and Cl^- ions from *cis* to *trans*, creating a symmetric steady-state ion flux (neglecting electroosmosis, see SI-6). In contrast, under asymmetric salt conditions, the applied voltage may result in cation selectivity. Consider the case shown schematically in Figure 3c, where the salt concentration in the *cis* chamber is much lower than in the *trans*: under typical fields applied to nanopores ($\sim 10^5$ V/cm, positive voltage to the *trans* chamber), diffusion of Cl^- ions from the *trans* to *cis* chamber down the concentration gradient is hindered by the applied potential (a transported Cl^- ion must overcome electric potential energy of ~ 0.3 eV, corresponding to $\sim 12 k_B T$). In contrast, K^+ ions are pumped from *trans* to *cis*, moving in the direction of both the chemical and electrical potential gradients. As positive ions are continuously pumped into the *cis* chamber, the pore vicinity is effectively polarized and the magnitude of $V(r)$ increases. This effect enhances the capture rate in both the diffusion-limited and barrier-limited regimes.

Under the asymmetric salt conditions our theory (SI-1) predicts that to a first approximation $V(r)$ is proportional to the ratio of bulk ionic concentrations in the *cis* and *trans* chambers: $V(r) = V_{sym}(r)C_{tr}/C_{cis}$, where $V_{sym}(r)$ is the potential under symmetric conditions. Accordingly, the value of r^* is also modified to $r^* = r^*_{sym}C_{tr}/C_{cis}$. The rationale behind this is that the voltage drop in the solution of lower conductance is higher, and therefore, a high/low *trans/cis* salt gradient will yield an asymmetric potential profile, with more of the voltage dropping in the *cis* chamber than the *trans* chamber. Finite-element COMSOL simulations (see Figure 3d and SI-2) confirm that salt-dependent charge imbalance at the pore results in significant increase in the protrusion of the potential into the *cis* chamber for large C_{tr}/C_{cis} . Notably, for $C_{tr}/C_{cis} = 20$ (4M/0.2M KCl), $V(r)$ remains considerable hundreds of nm away from the pore (Figure 3d, red curve).

One may ask whether the funneling effect near the pore also induces a faster DNA sliding, which would be problematic for current nanopore sensing methods¹⁰. We find however, that the enhanced capture rate with increasing salt gradients is accompanied by *longer* DNA translocation times (see SI-5). This can be explained by a combination of two effects: (i) increased EOF of K^+ counterions along the DNA and pore surface provide drag force opposing DNA motion in the pore, as shown by molecular dynamics simulations²⁴ and direct single-molecule force measurements²⁵. The weakly negatively-charged nanopore surface also contributes somewhat to EOF of K^+ ions from *trans* to *cis*, as supported by the pH dependence on conductance of uncoated and amine-modified SiN pores²⁶, as well as by capture measurements of a neutral polymer (see SI-7). (ii) The actual voltage drop inside the pore is a function of the salt gradient conditions, and a higher voltage drop outside of the pore diminishes the electrophoretic field inside the pore.

Tuning up the capture rate using salt gradients facilitates the detection of trace quantities of DNA. We miniaturized the *cis* chamber to 1 μ L (see Figure 1b) and recorded current traces of a 38 pM solution of 8,000 bp DNA ($\sim 10^7$ copies), going from symmetric (1M/1M) to highly asymmetric (4M/0.2M) salt conditions (see Figure 4a and 4b, respectively), resulting in a 30-fold enhancement in capture rate. The large capture rate allowed us to exchange the concentrated DNA solution with a 3.8 pM solution (Figure 4c), while still preserving a steady capture rate of ~ 1 s⁻¹. Collection of >1,000 events over 15 min, followed by characterization of the translocation dynamics, revealed a mean translocation time of about

20 ms— a factor of 3 longer than for the 1M/1M conditions. In total, our 1 μ l chamber contains under 4 attomoles, or under 10^6 copies of DNA.

We have shown here that salt gradients provide a practical method to controllably tune and enhance the capture rate of DNA molecules into sub-5 nm pores. Moreover, we have observed here capture selectivity towards longer DNA molecules, a direct consequence of the interaction of the potential near the pore with the highly charged polymer. DNA detection experiments using nanopores typically employ analyte concentrations in the nM - μ M range for obtaining average capture rates of ~ 1 event/s, corresponding to 10^{10} - 10^{13} copies for a 10 μ l *cis* chamber. Here we presented results that allow us to decrease the DNA concentration to the pM level, enabling characterization of an attomole-sized sample of DNA in a simple configuration. Our model has yielded a number of clear predictions, such as growing $\sim e^c \sqrt{N/4N_p}$ dependence of capture-rate on DNA length in the energy barrier dominated regime, and an N -independent rate for the diffusion-limited regime. This model also explains the universal scaling of the capture enhancement with the salt asymmetry. Finally, the longer DNA translocation times in the presence of salt gradients provides a means to increase the resolution of the method, while simultaneously enhancing its throughput. These findings facilitate future nanopore studies of biomolecular complexes at physiological conditions and studies of native (unamplified) genomes. These findings may also be of use for separation of charged polymers and biopolymers using nanoporous membranes, as the physics should be applicable to nanopore arrays as well.

Methods

Nanopores were fabricated in 25-nm-thick, low-stress SiN windows ($25 \times 25 \mu\text{m}^2$) supported by a Si chip (Protochips, Raleigh, NC) using a focused electron beam, as previously described¹⁷. Nanopore chips were cleaned in piranha solution and assembled on a custom-designed cell under controlled atmosphere (see ref. 27 for details). After the addition of degassed and filtered KCl electrolyte (all electrolytes were adjusted to pH 8.5 using 10 mM Tris-HCl), the nanopore cell was placed in a custom-designed chamber featuring thermoelectric regulation within $\pm 0.1^\circ\text{C}$, rapid thermal equilibration (< 5 min), and an effective electromagnetic shield. Homemade Ag/AgCl electrodes were immersed into each chamber of the cell and connected to an Axon 200B headstage. All measurements were taken inside a dark Faraday cage. When the electrodes were dipped directly in two chambers containing a salt gradient, the voltage offset values were found to purely Nernstian, i.e., $V_{\text{off}} = -0.058 \cdot \log([Cl^-]_{\text{cis}}/[Cl^-]_{\text{trans}})$ V. Therefore, prior to each experiment the voltage across the electrodes was nullified, either by the voltage compensation knob on the Axopatch or by the use of an agarose bridge. DNA solutions were prepared from stock solutions in the respective salt buffers, added to the *cis* chamber, and ~ 3 minutes were allowed in order for equilibration before measurements were made. Linear, blunt-ended dsDNA fragments were purchased from Fermentas (NoLimits[®]) and further verified to be pure by agarose gel electrophoresis. λ -Phage DNA was purchased from New England Biolabs, and was diluted and heated to 70°C for 10 minutes prior to each experiment in order to destabilize concatamers.

DNA translocation data were acquired using custom LabVIEW software, which collects either continuous recording or real-time detection of current pulses. The analog signal from the amplifier was low-pass filtered at 75 kHz and fed to a DAQ card, which sampled the data at 250 kHz/16 bit. Data was exported to Igor Pro software where curve fits were performed (in all cases R^2 values for the fits were greater than 0.97). The DNA sample was then characterized by statistical analysis of the square pulse widths (dwell-times, t_D) of thousands of molecules, as shown in Figure 5a. Capture rates were calculated for each experiment from the mean time-delay between two successive events (δ). Figure 5b displays semi-log time-delay distributions for 400 bp (top) and 2,000 bp (bottom) DNA, where the KCl concentration in the *trans* chamber was kept at 1M, and the *cis* chamber KCl concentrations were decreased to the indicated concentrations. Typically, $>10^3$ events were recorded for each experiment. In all cases, the time-delay distributions fit mono-exponentials $P_C(t) = A \exp(-t/\tau)$ (see Figure 5), as expected from a stochastic capture process with non-interacting molecules (valid in our regime of concentrations). The average capture rates are then reported as τ^{-1} from the fits. For both symmetric and asymmetric salt gradients, we have verified that the capture rate scales linearly with DNA bulk concentration, c , as shown in SI-3. Finite-element simulations were performed using COMSOL multiphysics software. To simulate our experimental situation, we used the full Nernst-Planck equations for the ionic concentrations and Poisson's equation for the electrostatic potential. The system was analyzed in the steady state by placing each chamber in contact with a bath maintained at specified concentrations (this is a reasonable approximation, considering the large size of the *cis* and *trans* chambers in comparison to the integrated ionic current through the pore in a typical experiment). The nanopore was hourglass-shaped in all COMSOL simulations, to match the measured pore geometry elucidated by TEM tomography¹⁷.

Supplementary Material

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Figure 1.

Biomolecular funneling into a solid-state nanopore. **(a)** The electric potential near small solid-state nanopores attracts negatively charged DNA coils into the nanopore, where individual molecules are detected. Away from the pore **(i)**, the electric field is negligible and DNA motion is purely diffusive, but at distances smaller than r^* , the potential $V(r)$ (where r is the radial distance from the pore mouth into the *cis* chamber, see white arrow) is large enough to funnel DNA in towards the pore mouth **(ii)**. At the pore mouth **(iii)**, an energy barrier describes the final process of threading a DNA end into the pore. Inset: Bright field TEM image of a 3.5 nm solid-state pore fabricated in a 25-nm-thick, low-stress silicon nitride membrane. **(b)** Schematic illustration of our solid-state nanopore setup, which features a μ L-volume analyte *cis* chamber.

Figure 2.

Figure 3.

Capture rate enhancement using a salt gradient across the nanopore. **(a)** Continuous traces measured using a 3.5 nm pore with 3.8 nM 400 bp DNA for different indicated ($C_{\text{Tr}}/C_{\text{Cis}}$) KCl concentrations. The average capture rate is specified in each case. **(b)** DNA capture rate enhancement for four different DNA lengths as a function of $C_{\text{Tr}}/C_{\text{Cis}}$, relative to $C_{\text{Tr}}/C_{\text{Cis}} = 1$. **(c)** Cartoon: the creation of an asymmetric salt gradient across the pore, where $C_{\text{Tr}}/C_{\text{Cis}} > 1$, results in selective pumping of cations (K^+) from *trans* to *cis*, creating a local positive concentration of ions near the pore (reddish area), which result in rate enhancement. **(d)** Electric potential maps evaluated numerically in the *cis* side of the pore, for both symmetric (top left) and asymmetric (bottom left) KCl concentrations. The pore mouth is positioned at the bottom center of each image. The potential range outside the pore increases with salt asymmetry. Right: Axial dependence of the potential as a function of distance from the pore mouth r , shown for 4 different salt gradients, as indicated.

Figure 4.

Picomolar detection of unlabeled DNA under asymmetric salt concentrations. **(a)** Continuous current trace under symmetric (1M/1M) salt concentrations (38 pM of 8,000 bp fragment) showing an average rate of 0.4 s^{-1} . **(b)** Under asymmetric salt concentrations (0.2M/4M) a 30-fold increase in capture rate is observed compared with (a). **(c)** Reduction of the DNA concentration 10-fold to $\sim 3.8 \text{ pM}$ (only ~ 4 attomole in the $1 \mu\text{l}$ *cis* chamber) resulted in a high capture rate ($\sim 1 \text{ s}^{-1}$), with markedly longer translocation time as compared with the symmetric salt conditions and good signal/background ratio.

Figure 5.

Measurement of the capture rate from single molecule ion-current blockades. **(a)** A typical ion-current trace flowing through a 3.5 nm pore in the presence of 8,000 bp DNA (1M KCl, 300 mV, 21°C). We define here the time-delay between successive events (δt) and the DNA transport time, or dwell-time (t_D). **(b)** Representative δt distributions measured for 400 bp DNA (top panel) and 2,000 bp DNA (bottom panel), measured at the indicated *cis* chamber KCl concentrations and 1M concentration at the *trans* chamber.