# Translocation of DNA Molecules through Nanopores with Salt Gradients: The Role of Osmotic Flow

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Recent experiments of translocation of double-stranded DNA through nanopores [M. Wanunu *et al.*, Nature Nanotech. **5**, 160 (2009)] reveal that the DNA capture rate can be significantly influenced by a salt gradient across the pore. We show that osmotic flow combined with electrophoretic effects can quantitatively explain the experimental data on the salt-gradient dependence of the capture rate.

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Translocation through solid-state nanopores holds the potential to be a fast commercial method for macromolecular characterization and sequencing, such as for long, unlabelled single-stranded and double-stranded DNA molecules [1–3]. Clearly, high throughput and time resolution—effected by enhanced capture rate as well as translocation times respectively—is a necessary precondition for the process to be commercially viable. Although the capture rate or translocation time can be increased by manipulation of the temperature, salt concentration, electric field strength and viscosity, the increase of one is usually accompanied by a decrease of the other [4]. Recently, however, Wanunu *et al.* [5] showed that it is possible to increase the capture rate without decreasing the translocation time by using a forward salt concentration gradient across the pore. The large increase in capture rate as a function of the imposed salt concentration gradient was qualitatively explained by the increase in the electrophoretic motion of DNA towards the pore as a function of salt asymmetry: A constant current of ions is flowing through the pore, creating a long range electric field which acts as a funnel for the ions and the polymers towards the pore [5–7].

These studies [5–7], and some others on similar systems [8–10], have focused on electro-osmotic and/or electrophoretic phenomena. Electro-osmotic phenomena describe flow of liquids with a net mobile charge under applied electric fields, while electrophoretic effects relates to the movement of charged polymers in an electric field. (These terminologies are further discussed in Sec. I of the Supplemental material [11]). It is worthwhile to note at this point that electro-osmotic flow in the experiments of Wanunu *et al.* was found to be in the opposite direction of the observed DNA translocation (see the supplementary material of Ref. [5]), implying that electro-osmotic flow cannot explain the observed enhanced capture rate. In fact, in the presence of an imposed salt concentration gradient across the pore, there is an additional mechanism at play, namely, the capillary osmosis process [12–14], i.e., the flow of water from a lower osmotic pressure (*cis*) side to a higher osmotic pressure (*trans*) side through the pore. The effects of this osmotic flow on the DNA capture rate has been missing in the theoretical analysis so far. In this Letter we show that the osmotic flow is a key ingredient to understand the experiments of Wanunu *et al.* [5]; their results can be *quantitatively* explained with osmotic flow and electrophoretic effects [13]. Note also that the full range of dynamical mechanisms affecting DNA capture in the experimental setup of Wanunu *et al.* are discussed in Sec. I of the SI [11].

The osmotic flow of water is driven by a pressure gradient antiparallel to the salt concentration gradient. The reservoirs are kept at a constant pressure, such that a chemical potential gradient is present across the pore for both the ions and water, causing flow of ions down the salt concentration gradient and water up the salt concentration gradient. However, a pressure gradient inside the pore and a corresponding net flow of the liquid (ions plus water) will only be present if the ions are net depleted from (or net attracted to) the pore [15]. In the textbook example of osmosis through a semipermeable membrane ions are completely restricted from entering the pore due to steric repulsion [15]. However, also when the restriction is only partial, e.g., due to wall-ion interactions in the nanometer vicinity of the pore walls, an osmotic flow develops [12,13,16,17]. Water and ions confined in a nanopore can behave very differently from the same bulk system [18–24]. Both water and ions will be influenced by the pore walls, leading to attraction or depletion of ions and/or water. To capture such a behavior with a simple continuum model we introduce one length scale describing the interaction of the ions with the pore wall (the depletion length), and one length scale quantifying the slip of water flow at the pore wall (the slip length). To quantitatively describe the experimental data of Wanunu *et al.* [5], the ion-wall interactions is found to be repulsive, in agreement with simulations [17,23] and theories [22,25] of kosmotropic (hydrophilic) ions near low dielectric surfaces. Also, the

 

flow of water is found to be in the slip-flow regime, in agreement with experimental studies of flow at smooth surfaces [26]. In the experimental system studied by Wanunu *et al.* [5] we find the osmotic flow to provide the dominant contribution to the enhanced capture rate for weak salt gradients. In the same experiment [5] electro-osmosis was found be a weak effect, reflected in a 2–3 orders of magnitude lower capture rate of neutral PEG compared to charged DNA. Also, the measured purely Nernstian behavior of the diffusion potential suggests nearly neutral pore walls.

The geometry we study, similar to the experimental setup of Ref. [5], is shown in Fig. 1. Two reservoirs at constant pressure $P_0$ with salt concentrations $C_t$ (*trans* side) and $C_c$ (*cis* side) are separated by an impermeable solid membrane of thickness $L$. A cylindrical pore of diameter $d$ connects the two reservoirs. The two electrolytes are composed of monovalent ions of concentrations $c_\alpha$, and $\sum_{\alpha=\pm} c_\alpha = C$. The solvent (water) is modeled as a continuum with dielectric constant $\epsilon = 80$, and viscosity $\eta$ at temperature $T$. The Debye screening lengths $\kappa_{c/t}^{-1}$, are defined as $\kappa_{c/t}^2 = 4\pi C_{c/t}\beta e^2/\epsilon$, where $\beta^{-1} = k_B T$, $k_B$ is the Boltzmann constant and $e$ is the elementary charge. Because of the preference of ions to be solvated in bulk water, they feel a repulsive potential $U(\rho)$ from the pore walls [20,22], where $\rho$ is the radial coordinate around the cylindrical axis inside the pore, measured from the center of the pore. We model such interactions with a region $\ell$ next to the pore walls depleted of ions (see Fig. 1). The part of the pore accessible to ions is described by the diameter $a = (d - 2\ell)$. The polymers (DNA) are located in the *cis* chamber, and the electric field is applied from the *trans* to *cis* side, driving DNA (with negative charge) from the *cis* to the *trans* reservoir.

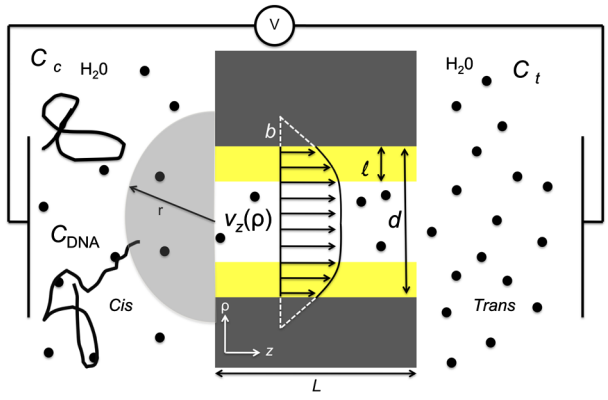


FIG. 1 (color online). Schematic of the pore geometry showing a membrane of thickness $L$ connecting two salt reservoirs with salt concentrations $C_c$ (*cis*) and $C_t$ (*trans*) by a pore of diameter $d$. In the *cis* reservoir there is a bulk DNA concentration of $C_{\mathrm{DNA}}$, and a voltage difference $V$ is applied across the system. The salt ions are depleted within a layer $\ell$ from the pore walls. There is a liquid velocity profile $v_z(\rho)$ in the $z$ direction, which varies with the radial coordinate $\rho$. There is slip of the flow at the pore walls described by the slip length $b$.

The pore is assumed to be neutral, i.e., $\sum_\alpha q_\alpha c_\alpha(\rho, z) = 0$ [5], where $q_\pm = \pm e$, which is a good approximation for nearly neutral pore walls and low induced charge within the pore.

For the system studied here, the Reynolds number is very small, and the flow can be described by the steady Stokes equation combined with incompressibility of the liquid:

$$\eta\nabla^2\mathbf{v}(\rho, z) = \nabla P(\rho, z) + \sum_\alpha c_\alpha(\rho, z)\nabla U(\rho); \quad (1)$$

$$\nabla \cdot \mathbf{v} = 0. \quad (2)$$

In steady state the dynamics of the ions are described by the time-independent Nernst-Planck equations:

$$\begin{aligned}\nabla \cdot \mathbf{J}_\alpha = &-D_\alpha\{\nabla^2 c_\alpha(\rho, z) \\ &+ \nabla \cdot (c_\alpha(\rho, z)\beta[\nabla U(\rho) - q_\alpha \mathbf{E}(z)])\} \\ &+ \nabla \cdot [c_\alpha(\rho, z)\mathbf{v}(\rho, z)] = 0 \end{aligned} \quad (3)$$

This is equivalent to conservation of particle current, with $\mathbf{J}_\alpha$ the current density, $D_\alpha$ the diffusion coefficient of ion type $\alpha$ and $\mathbf{E}(z)$ the local electric field. Assuming fast equilibration of the concentration and the pressure in the radial direction ($\hat{\rho} \cdot \mathbf{J}_\alpha = 0$ and $\hat{\rho} \cdot \mathbf{v} = 0$) we get from Eqs. (1) and (3) [12]

$$c_\alpha(\rho, z) \approx \begin{cases} C_0(z) & \rho < a/2 \\ 0 & \rho > a/2. \end{cases} \quad (4)$$

$$\eta\nabla^2 v_\rho(\rho) = 0, \quad (5)$$

which from Eq. (1) gives

$$\partial_z P(\rho, z) = \begin{cases} 0 & \rho < a/2 \\ -k_B T \partial_z C_0(z) & \rho > a/2. \end{cases} \quad (6)$$

If we further assume that the ion density changes linearly across the pore (which follows from Eq. (3) when diffusion dominates over convection) we get

$$\eta\nabla^2 v_z(\rho) = \begin{cases} 0 & \rho < a/2 \\ -k_B T \frac{C_t - C_c}{L} & \rho > a/2. \end{cases} \quad (7)$$

Since the pore is in the nanometer regime a continuum treatment of the fluid dynamics may not be accurate. The Knudsen number for the system is $Kn = \delta/d \approx 0.1$, where $\delta \approx 3$ Å is the intermolecular spacing for water [27], indicating that we are in the slip-flow regime ($0.01 \leq Kn \leq 0.1$), such that

$$v_z(d/2) = b\frac{\partial v_z(\rho)}{\partial \rho}\bigg|_{\rho=d/2}, \quad (8)$$

where $b$ is the slip length (see Fig. 1). The flow can now be obtained by integrating Eq. (7) twice making use of Eq. (8).

The resulting area-averaged velocity of the flow inside the pore is

$$\bar{v}_o = \frac{k_B T(C_t - C_c)\sigma_o}{L}\frac{d^2(1 + 8b/d)}{32\eta}, \quad (9)$$

where we have introduced the osmotic reflection coefficient

$$\sigma_o = 1 - \frac{(a/d)^2}{1 + 8(b/d)}(8(b/d) + 2 - (a/d)^2). \quad (10)$$

For $a = 0$ ($\sigma_o = 1$), i.e., ions are totally depleted from the pore, we recover the standard slip-modified Poiseuille flow due to osmosis through a semipermeable membrane [28]. If we set the slip length to zero, we recover the result of Anderson and Malone for leaky membranes [15], however with an effective solute radius $\ell$. With $\ell = 0$ (no ionic depletion) Eq. (10) gives $\sigma_o = 0$ (no flow), showing that ion depletion is crucial for the present analysis. From Eqs. (1) and (2) the osmotic flow at a radial distance $r \gg d$ from the pore can now be approximated as

$$\mathbf{v}_{\mathrm{OS}}(r) = -\hat{r}\frac{\bar{v}_o d^2}{8r^2}, \quad (11)$$

where $\hat{r}$ is the radial unit vector, pointing outward from the pore mouth.

In a steady state and using conservation of charge current [Eq. (3)], the electric field on the *cis* side (for $|r| \gg d$) can be approximated as [5]

$$\mathbf{E}(r) = \hat{r}\frac{C_p a^2 V}{8C_c L r^2}, \quad (12)$$

where $C_p = (C_t + C_c)/2$ is the ion concentration inside the accessible part of the pore, and $V/L$ is the strength of the applied $E$-field in the pore. The drift of charged polymers in an electric field is described by electrophoresis [29]

$$\mathbf{v}_{\mathrm{EP}}(r) = \mu\mathbf{E}(r) = \frac{\phi_{\mathrm{DNA}}\epsilon}{4\pi\eta}\mathbf{E}(r), \quad (13)$$

where $\phi_{\mathrm{DNA}}$ is the surface potential of DNA, and $\mu$ is the electrophoretic mobility.

To get an estimate of the number of polymers that translocate through the pore per second, we calculate the flux of DNA generated by the combination of electrophoretic effects and osmosis. By conservation of DNA particle current, we get the capture rate per bulk DNA concentration

$$R_c = -2\pi r^2[\mathbf{v}_{\mathrm{EP}}(r) + \mathbf{v}_{\mathrm{OS}}(r)] \cdot \hat{r}, \quad (14)$$

independent of $r$. Flow towards the pore is antiparallel to $\hat{r}$ (see Fig. 1), and therefore a negative sign in Eq. (14) appears such that $R_c > 0$ for translocation from the *cis* to *trans* reservoir. Combining Eqs. (11), (13), and (14) we find

$$R_c(x) = R_c(1)\left[\frac{1 + x}{2} + \left(1 - \frac{1}{x}\right)k\right], \quad (15)$$

where $x = C_t/C_c$ and

$$R_c(1) = \frac{a^2\phi_{\mathrm{DNA}}V\epsilon}{16L\eta} \quad (16)$$

$$k = \frac{(\kappa_t d)^2\sigma_o(1 + 8b/d)}{32(a/d)^2(\beta e\phi_{\mathrm{DNA}})(\beta eV)}. \quad (17)$$

Note that the result does not depend on DNA length [5], and that $k = 0$ (or $x = 1$) describes electrophoretic effects alone.

In Fig. 2 we plot the predictions of Eq. (15) as a function of salt asymmetry $x$ for different values of the dimensionless parameter $k$ [Eq. (17)]. As the value of $k$ increases the predictions start to deviate from the capture rate due to electrophoretic effects alone (straight line, $k = 0$). The flow due to osmosis varies inversely with $x$ (i.e., linear in $C_c$), and will therefore saturate for large $x$, while the flow due to electrophoretic effects has a linear dependence on $x$ with slope $1/2$. With $k$ in Eq. (15) as a free parameter, we find an excellent fit to the experimental data of Ref. [5] for $k \approx 7.5$ and $x < 5$. Having noted that the calculations presented in this work are valid to first order in the salt concentration gradient $(C_t - C_c)/L$, in Fig. 2 we focus on the regime where $x \leq 5$, as these data points are all for a 1 M salt solution in the *trans* chamber, and represent about 75% of the available data. Note in this context that the experimental data of Ref. [5] also contain four additional data points with larger salt concentrations in the *trans* chamber (2 M and 4 M), and larger values of $x$. These data points lie outside the region where we expect our model to be valid.

To further compare our predictions with the recent experimental measurements of DNA translocation in salt

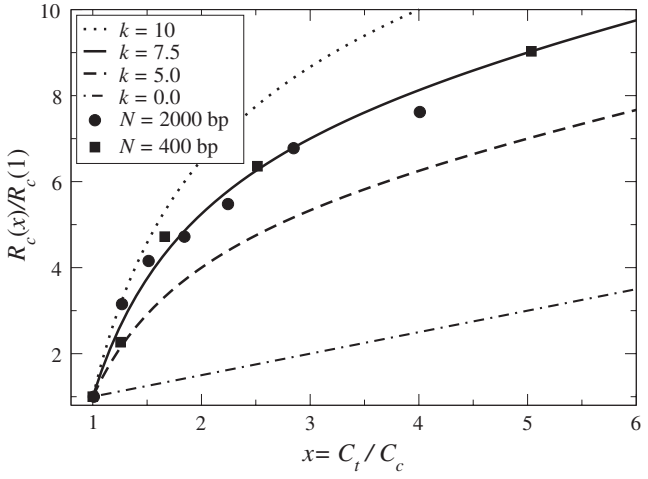


FIG. 2. Capture rate [Eq. (15)] as a function of salt asymmetry, for different values of the dimensionless parameter $k$ [see Eq. (15)]. The points are experimental measurements of Ref. [5].

gradients [5], we fix the system parameters to the experimental values (for DNA length $N = 400$ bp and $N = 2000$ bp); $C_t = 1$ M, $d = 3.5$ nm, $V = 300$ mV and $L = 20$ nm. For the DNA electrophoretic mobility we use $\mu = -10^{-8}\ \mathrm{m^2\,s^{-1}\,V^{-1}}$ [30] which for water with viscosity $\eta = 1\ \mathrm{mPa \cdot s}$ (at $T = 20\,^{\circ}\mathrm{C}$) gives from Eq. (13) $\beta e \phi_{\mathrm{DNA}} \approx -0.55$. The only free parameters are the depletion length $\ell$ and the slip length $b$. For depletion lengths $\ell = 0.3$ to 0.6 nm, which one expects due to finite-size effects of (hydrated) ions like $K^+$ and $Cl^-$ [25] and image charge effects [22], one finds from $k = 7.5$ that $b = 4$ to 7 nm (see [11] for details), in reasonable agreement with measured values of the slip length at smooth surfaces [26]. In [11] we plot combinations of $\ell$ and $b$ corresponding to different values of $k$, as well as the osmotic flow profile $v_z(\rho)$ (see Sec. II).

The ions are assumed to be depleted from the pore walls, an assumption which is based on numerous theoretical [22,24,25], simulation [23,31–34] and experimental studies [35,36], that find nonpolarizable ions to be repelled by an interface between water and a low dielectric material such as silicon nitride ($\epsilon = 7$). This behavior can be modified due to surface chemistry, such as dangling atoms [32], surface charge and affinity for water [23]. Dangling atoms lead to binding of ions to the surface, which can result in current rectification [32]. Unless these effects are very strong, the ions are generally all over depleted from neutral low dielectric interfaces.

To conclude, having approximated the ion-wall interactions due to image charges and water structure by an effective depletion length $\ell$, we show that the experimental data of Wanunu *et al.* for DNA translocation in salt gradients can be explained by a combination of electrophoretic effects and osmosis. To account for the different behavior of water on the nanoscale we have also introduced hydrodynamic slip at the pore walls, which enhances the flow due to osmosis. With reasonable values for both the slip length and the ion depletion length, we find quantitative agreement between theory and experimental measurements.

Throughout the calculation we have focused on the diffusion limited regime, and do not take into account the free-energy barrier felt by the polymers when entering the pore, yet we are able to quantitatively reproduce the relative capture rate enhancement data in the barrier-limited regime. This is most likely a signature of the fact that the barrier height is nearly constant as a function of salt asymmetry. We do expect that the main physics reported here, namely, the role of the osmotic flow, explains the enhanced relative capture rate in the barrier-limited regime; however, including the barrier in our analysis remains a significant challenge.

We have also assumed the ion density inside the accessible part of the pore to be equal to the average of the salt concentration in the two reservoirs. This assumption is supported by the current-voltage relations measured by Wanunu *et al.* for different salt concentrations in the cis chamber (for $C_t = 1$ M, $C_c = 0.2$ M to 1 M), see supplementary information of Ref. [5]. The calculations presented in this work are valid to first order in the salt concentration gradient $(C_t - C_c)/L$, and the results of the measurements by Wanunu *et al.* with higher salt concentration in the trans reservoir ($x > 5$), is outside the region where this model is expected to be valid.

Putting things in perspective, translocation of DNA through nanopores is a complicated problem due to its many aspects, ranging from properties of water in confinement to complicated structures of the translocating molecules and their interactions. However, to understand the recently found increase in capture rate as a function of salt concentration asymmetry, it seems that a detailed description of DNA molecules is not needed, since the main mechanism is the enhanced attraction of DNA molecules towards the pore. This attraction is here shown to be made up of two main contributions: electrophoretic effects and osmotic flow. The capture rate with salt gradients due to electrophoretic effects [5,7] and combined with electro-osmosis [6] has been described before; however, the role of (diffusio)-osmosis has not been previously discussed. To understand the osmotic flow it is crucial to account for the repulsive interaction between kosmotropic ions and a neutral nonpolar wall. We expect that the main physics is captured by introducing a layer near the pore wall depleted of ions. This also means that the osmotic flow is ion specific, and will be reversed when using salt particles that are net attracted to the pore wall. Finally, our analysis shows that osmosis cannot be ignored for nanopores in the presence of salt gradients, even though salt is able to flow through the pore.

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