DNA translocation 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 (2010)] reveal that the DNA capture rate can be significantly influenced by a salt gradient across the pore. We show that osmotic flow combined with electrophoresis can quantitatively explain the experimental data on the capture rate. The osmotic flow is induced by the salt gradient across the nanopore, and can be the dominant mechanism for DNA translocation through nanopores with a salt gradient.

Translocation through solid-state nanopores holds the potential to be a fast and cheap 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 [4], 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 increasing 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 at best 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, 6]. However, the experimental data of Wanunu *et al.* indicate that there is another effect of the same order of magnitude, increasing the capture rate as a function of salt concentration asymmetry.

In this Letter we show that the experimental results of Wanunu *et al.* [5] can be quantitatively reproduced when osmotic flow is included [7]. This key ingredient, which has been missing in the theoretical analysis so far, is driven by a pressure gradient anti-parallel to the salt concentration gradient in a layer close to the pore wall. The reservoirs are kept at constant pressure, and a chemical potential gradient is present accross 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 net flow of the liquid (ions plus water) will only take place if the ions are somewhat restricted from flowing through the pore. In the textbook example of osmosis through a semi-permeable membrane ions are completely restricted from entering the pore due to steric repulsion[8]. However, when the pore diameter is larger than the ion diameter such steric repulsion is absent, and other interactions must be accounted for to properly describe the net liquid flow [7, 9, 10]. In this work we model the ion-pore interactions by a layer depleted from ions due to repulsive interactions between the nonpolar pore walls and the ions, e.g. due to image charges, stripping of the ion hydration shell, or water structure near the wall [11–14]. For large salt concentration gradients, neutral pore walls, and nonpolar membrane material, the osmotic flow that pulls the DNA towards (resp. pushes the DNA away from) the pore turns out to be the dominant factor for enhanced (resp. reduced) capture rate for forward (resp. reverse) salt concentration gradient across the pore. Interestingly, a strikingly similar mechanism has recently been proposed for genome ejection from a (bacterio)phage into a bacterial cell during infection, wherein the osmotic flow from the culture medium into the bacterial cell cytoplasm, up the osmotic gradient, drags the genome along from the phage capsid and releases it into the cell cytoplasm[15].

The geometry we study, similar to an experimental setup, is shown in Fig. 1. Two reservoirs at constant pressure P_0 with salt concentration 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_α, and $\sum_{\alpha=\pm} c_\alpha = C$. The solvent (water) is modeled as a continuum with dielectric constant $\epsilon = 80$, and viscosity η 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. Due to the preference of ions to be solvated in bulk water, they feel a repulsive potential $U(\rho)$ from the pore walls [12, 14], where ρ is the radial coordinate around the cylindrical axis inside the pore. We model such interactions with a region ℓ 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.

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

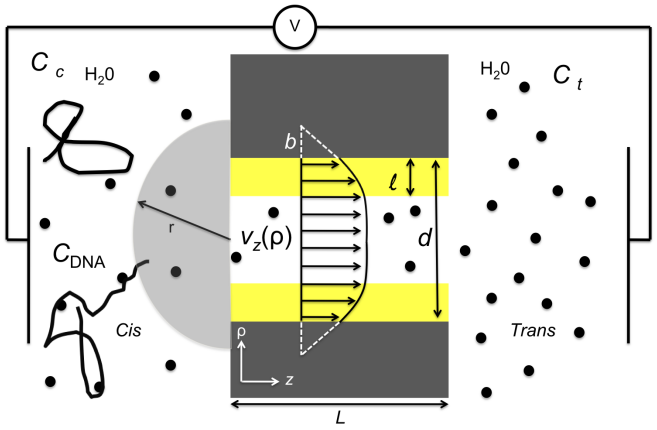

FIG. 1: Schematic of the pore geometry showing a membrane of thickness L connecting two salt reservoirs with salt concentrations C_c(*cis*) and C_t (*trans*) by a pore of diameter d. In the *cis* reservoir there is a bulk DNA concentration of $C_{\rm DNA}$, and a voltage difference V is applied accross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

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:

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

This is equivalent to conservation of particle current, with $\mathbf{J}_\alpha$ the current density, D_α the diffusion coefficient of ion type α 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) [10]

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

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

which from Eq. (1) gives

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

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

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

Since the membrane is hydrophobic [16] we also introduce hydrodynamic slip at the pore walls [17], such that

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

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

$$\bar v_{\rm o} = \frac{k_BT(C_t - C_c)\sigma_{\rm o}}{L}\frac{d^2(1+8b/d)}{32\eta}, \quad (9)$$

where we have introduced the osmotic reflection coefficient

$$\sigma_{\rm o} = 1 - \frac{(a/d)^2}{1+8(b/d)}\left(8(b/d) + 2 - (a/d)^2\right). \quad (10)$$

For $a = 0$ ($\sigma_{\rm o} = 1$), i.e. ions are totally depleted from the pore, we recover the standard slip modified Poiseuille flow due to osmosis through a semipermeable membrane [17]. If we set the slip length to zero, we recover the result of Anderson and Malone for leaky membranes[8], however with an effective solute radius ℓ. From Eqs. (1) and (2) the osmotic flow at a radial distance $r \gg d$ from the pore can be approximated as

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

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 [18]

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

where $\phi_{\rm DNA}$ is the surface potential of DNA, and μ 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 electrophoresis and osmosis. By conservation of DNA particle current, we get the capture rate per bulk DNA concentration

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

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

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

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

Note that the result does not depend on DNA length [5], and that $k = 0$ describes electrophoresis alone.

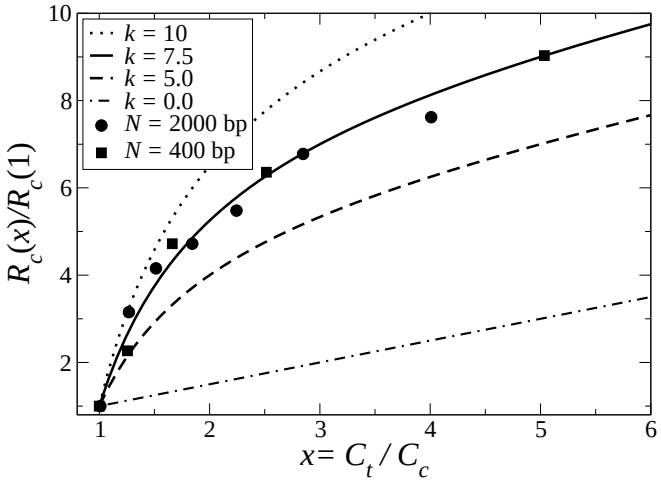

FIG. 2: Capture rate (Eq. (15)) as a function of salt asymmetry, for different values of the dimensionless parameter k (see Eq. (15)). The points are experimental mesurements of Ref. [5]

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 electrophoresis alone (straight line, $k = 0$). The flow due to diffusio-osmosis varies inversly with x, and will therefore saturate fairly quickly, while the flow due to electrophoresis has a linear dependence 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 $k \approx 7.5$.

To compare our predictions with the recent experimental measurements of DNA translocation in salt 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 $\mu = -10^{-8}{\rm m}^2{\rm s}^{-1}{\rm V}^{-1}$ [19] which for water with viscosity $\eta = 1$ mPa $\cdot$ s (at $T = 20°$C) gives from Eq. (13) $\beta e\phi_{\rm DNA} \approx -0.55$. The only free parameters are the depletion length ℓ and the slip length b.

In Fig. 3(a) we plot the different combinations of ℓ and b for different values of k, showing resonable combinations of ℓ and b for the k-regime of interest. The slip length of hydrophobic surfaces are typically in the range $0-30$ nm depending among others on the contact angle and the smoothness of the surface [20]. The contact angle of Silicon Nitride has recently been measured to be $\approx 100°$ [16]. Comparing with other surfaces with similar contact angle, the slip length of Silicon Nitride should be in the range $b \approx 5 - 10$ nm [20], which would correspond to a depletion length $\ell = 0.3 - 0.5$ nm, in agreement with ion depletion caused by image charges [14].

Similarly, in Fig. 3(b) the liquid flow profile inside the pore is plotted for different combinations of the depletion length ℓ and the slip length b, with $k = 7.5$, $x = 5$, and all other parameters fixed to match the experiment of Ref. [5]. When the slip length is larger than the pore diameter, $b > d$, the flow profile becomes almost uniform, in contrast to the no slip case where the flow is zero near the pore walls. When ℓ is small compared to the pore diameter a larger slip length is needed to produce a large enough flow to explain the experimental measurements. This is because a large ℓ also decreases the electrophoretic motion of the DNA molecules, by lowering the ion-acessible area of the pore. For the flow profiles in Fig. 3, the Reynolds number is $Re = vd/\nu < 0.0035$, where $\nu = 10^{-6}{\rm m}^2/{\rm s}$ is the kinematic viscosity of water.

Further, in the measurements by Wanunu *et al.* [5] the capture rate was decreased more than 10 times when the salt ratio was reversed to $x = 0.2$ (at $C_c = 1$M). This substantial decrease in capture rate can be understood by the mechanism that we predict, where the drift of DNA for $x = 0.2$ is actually away from the pore, with $R_c(0.2)/R_c(1) = -5.4$, for $k = 7.5$. Note that a negative capture rate is unphysical, however it tells us that the drift is away from the pore. The observed capture rate is so low since DNA has to diffuse against the drift to enter the pore mouth. To get a better estimate of the capture rate in this situation, a more detailed theory for the capture rate must be developed, which includes the probability to diffuse against the drift. If osmosis is not accounted for ($k = 0$), the capture rate would be $R_c(0.2)/R_c(1) = 0.6$, far less reduced than observed in the experiment of Ref. [5].

To conclude, having approximated the ion-wall interactions due to image charges and water structure by an effective depletion length ℓ, we show that the experimental data of Wanunu *et al.* for DNA translocation in salt gradients can be explained by a combination of electrophoresis and osmosis. Osmosis is the dominent term for salt asymmetries $x < 5$. In fact, we show that the large decrease in capture rate measured in the experiments [5] when the DNA molecules are located in the high salt reservoir, is due mostly to osmosis, and not electrophoresis. Since the pore material (Silicon Nitride) is hydrophobic [16], we also introduced hydrodynamic slip at the pore walls, which enhances the flow due to osmosis. With resonable 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. The measured relative increase in capture rate as a function of salt asymmetry is nearly independent on DNA length [5], indicating that the barrier is nearly constant as a function of salt asymmetry. This is likely due to the weak dependence of the electric field inside the pore on the salt concentration

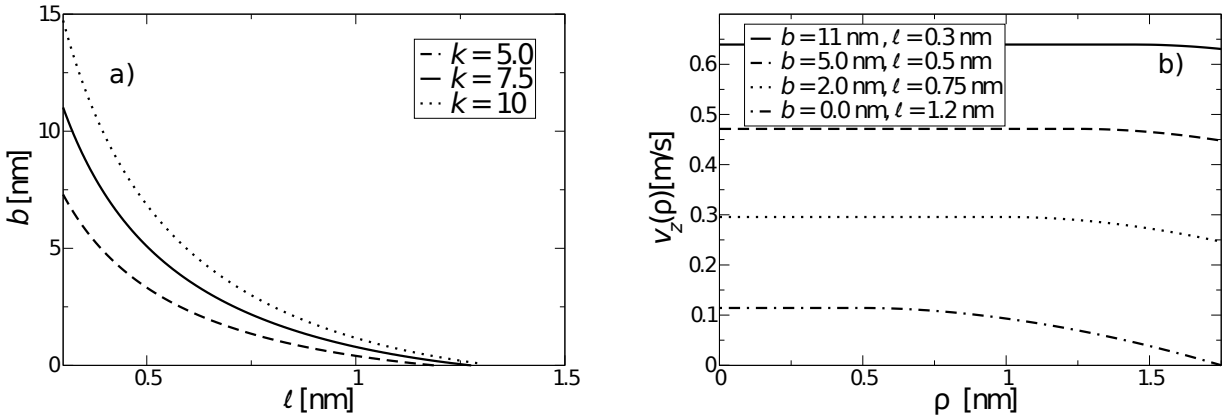

FIG. 3: (a) Combinations of the slip length b and ion wall depletion length ℓ for different values of k (see Eq. (17)), with all other values fixed to match the experimental values of Ref. [5]. (b) The liquid flow profile inside the pore as a function of the radial coordinate ρ for different combinations of the slip length and depletion length with $k = 7.5$ and $x = 5$, all other parameters are fixed to match the experiments of Ref. [5] (see text for details).

asymmetry. We therefore expect our analysis of the relative capture rate to hold also in the barrier limited regime, as indicated by the good fit between the experimental data and our theoretical predictions.

We have also assumed the ion density inside the accessible part of the pore 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 $C_t = 1\mathrm{M}, C_c = 0.2\mathrm{M}$ to $1\mathrm{M}$), see supplementary information of Ref. [5]. Our preliminary calculations of the full Nernst-Planck equations show that for larger salt asymmetries $x > 5$, and/or a larger salt concentration in the trans reservoir, convection will reduce the ion concentration inside the pore towards the value of the concentration of the cis reservoir.

More detailed information on the behavior of the ions can be obtained by solving the Poisson-Nernst-Planck equations, which would extend our results to situations where the induced charge inside the pore is significant (large electric fields), include the influence of convection on the ions, and include induced charge electroosmotic flow. One may also incorporate charges on the pore walls, leading to electroosmotic flow and convection current. A more accurate estimate of the ion wall interactions would require treating the water explicitly, which may be achieved by Molecular Dynamics simulations [11].

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. 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 flow driving the DNA towards the pore. This flow is here shown to be made up of two main contributions; electrophoresis and osmosis. The capture rate due to electrophoresis with salt gradients has been described before [5, 6], however the role of osmosis has not been previously discussed. To understand the osmotic flow it is crucial to account for the repulsive interaction between 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. 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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