DNA translocation through nanopores with salt gradients: The role of osmotic flow

Marius M. Hatlo†, Debabrata Panja†, and René van Roij†
†Institute for Theoretical Physics, Utrecht University, Leuvenlaan 4, 3584 CE Utrecht, The Netherlands
‡Institute for Theoretical Physics, Universiteit van Amsterdam, Science Park 904, Postbus 94485, 1090 GL Amsterdam, The Netherlands

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 electrophoretic effects can quantitatively explain the experimental data on the salt-gradient dependence of the capture rate.

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 [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 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 quantitatively 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 electroosmotic and/or electrophoretic phenomena. Electroosmotic 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 Supplementary Online Material]. It is worthwhile to note at this point that electroosmotic 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 electroosmotic 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 [11–13], 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 [12]. 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 Supplementary Online Material.

The osmotic flow of water is driven by a pressure gradient anti-parallel 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 correspondig 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 [14]. In the textbook example of osmosis through a semi-permeable membrane ions are completely restricted from entering the pore due to steric repulsion [14]. However, also when the restriction is only partial, e.g. due to wall-ion interactions in the nm vicinity of the pore walls, an osmotic flow develops [11,12,15,16]. Water and ions confined in a nanopore can behave very differently from the same bulk system [17–23]. 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 [16,22] and theories [21,24] 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 [25]. 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 electroosmosis was found be a weak effect, reflected in a two/three 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 concentration $C_I$ (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 $\sum_{\alpha=\pm} c_\alpha = C_0$. The solvent (water) is modeled as a continuum with dielectric constant $\varepsilon = 80$, and viscosity $\eta$ at temperature $T$. The Debye screening lengths $\kappa_{\varepsilon}$, are defined as $\kappa_{\varepsilon}^{-2} = 4\pi C_0/\rho e^2/\varepsilon$, where $\beta^{-1} = k_B T$, $k_B$ is the Boltzmann constant and $e$ is the elementary charge. Due to the presence of ions to be solvated in bulk water, they feel a repulsive potential $U(\rho)$ from the pore walls [19, 21], 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 trans reservoir.

The pore is assumed to be neutral, i.e. $\sum_{\alpha} q_\alpha c_\alpha = 0$ [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:

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

(1)

$$\nabla \cdot \mathbf{v} = 0.$$  \hspace{1cm} (2)

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

$$\nabla \cdot \mathbf{J}_\alpha = -D_\alpha \left( \nabla^2 c_\alpha (\rho, z) + \nabla \cdot \left( c_\alpha (\rho, z) \nabla U(\rho) - q_\alpha \mathbf{E}(z) \right) \right)$$

$$+ \nabla \cdot \left[ \left( c_\alpha (\rho, z) \mathbf{v}(\rho, z) \right) \mathbf{v}(\rho, z) \right] = 0.$$  \hspace{1cm} (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 (2) [11]

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

$$\eta \nabla^2 v_\rho(\rho) = 0,$$  \hspace{1cm} (4)

which from Eq. (1) gives

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

(5)

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

$$\eta \nabla^2 v_\rho(\rho) = \begin{cases} 0 & \rho < a/2 \\ -k_B T \frac{C_t - C_0}{L} & \rho > a/2. \end{cases}$$

(6)

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 $\delta \approx 3 \text{ Å}$ is the intermolecular spacing for water [26], indicating that we are in the slip-flow regime $0.01 \leq Kn \leq 0.1$, such that

$$v_\rho(d/2) = b \frac{\partial v_\rho(\rho)}{\partial \rho} \big|_{\rho=d/2},$$

(7)

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

$$\bar{v}_\rho = k_B T (C_t - C_0) \sigma_0 d^2 (1 + 8b/d) / L,$$

(8)

where we have introduced the osmotic reflection coefficient

$$\sigma_0 = 1 - \frac{(a/d)^2}{1 + 8(b/d)} \left( 8(b/d) + 2 - (a/d)^2 \right).$$

(9)

For $a = 0$ ($\sigma_0 = 1$), i.e. ions are totally depleted from the pore, we recover the standard slip-modified Poiseuille flow due to osmosis through a semipermeable membrane [27]. If we set the slip length to zero, we recover the result of Anderson and Malone for leaky membranes [14], however with an effective solute radius $\ell$. With $\ell = 0$ (no ionic depletion) Eq. (10) gives $\sigma_0 = 0$ (no flow), showing that ion depletion is crucial for the present analysis. From Eqs. (6) and (7) the osmotic flow at a radial distance $r \gg d$ from the pore can now be approximated as

$$v_{OS}(r) = -\frac{\bar{v}_\rho d^2}{8\pi^2},$$

(10)
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. 2), the electric field on the cis side (for \( |\mathbf{r}| \gg d \)) can be approximated as [28]:

\[
E(r) = \hat{r} \frac{C_p \rho a^2 V}{8C_c L r^2}.
\]

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 [28]:

\[
v_{EP}(r) = \mu E(r) = \frac{\phi_{DNA} \epsilon}{4 \pi \eta} E(r),
\]

where \( \phi_{DNA} \) 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

\[
R_c = -2 \pi r^2 \left[ v_{EP}(r) + v_{OS}(r) \right] \cdot \hat{r},
\]

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:

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

where \( x = C_t/C_c \) and

\[
R_c(1) = \frac{\alpha^2 \phi_{DNA} \epsilon V}{16 \pi \eta},
\]

\[
k = \frac{(\kappa_i d)^2 \sigma_0 (1 + 8 b/d)}{2 \pi \eta (\beta_a \phi_{DNA}) (\beta e V)}.
\]

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 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 \( \mu = -10^{-6} \) m^2 s^{-1} V^{-1} [29] which for water with viscosity \( \eta = 1 \) mPa s (at \( T = 20^\circ C \)) gives from Eq. (15) \( \beta e \phi_{DNA} \approx -0.55 \). 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\- [24] and image charge effects [21], one finds from \( k = 7.5 \) that \( b = 4 \) to 7 nm (see SOM for details), in reasonable agreement with measured values of the slip length at smooth surfaces [25]. In the Supplementary Online Material 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 [21, 23, 24], simulation [22, 30–33], and experimental studies [34, 35], 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 [31], surface charge and affinity for water [22]. Dangling atoms lead to binding of ions to the surface, which can result in current rectification [31]. 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

![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].](image-url)
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 electroosmotic 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 resolvable 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 \( C_1 = 1 \text{M}, C_c = 0.2 \text{M} \) to \( 1 \text{M} \)), see supplementary information of Ref. [5]. The calculations presented in this work are valid to first order in the salt concentration gradient \((C_1 - C_c)/L\), 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: electroosmotic effects and osmotic flow. The capture rate with salt gradients due to electroosmotic effects [3, 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.

We thank Meni Wanunu for providing us with additional information about the experimental data, and Gerard Barkema for useful discussions. This work is part of the research program of the "Stichting voor Fundamenteel Onderzoek der Materie (FOM)", which is financially supported by the "Nederlandse organisatie voor Wetenschappelijk Onderzoek (NWO)".

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* Electronic address: M.M.Hatlo@uu.nl

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SUPPLEMENTARY ONLINE MATERIAL

In the main text we discuss the dominant contributions to enhanced DNA translocation induced by a salt gradient in the recent experiment by Wanunu et al. [1]. In this Supplementary Online Material we also discuss other relevant dynamical processes that can drive DNA towards a nanopore in the presence of both an external electric field and a salt concentration gradient across the pore. We also show plots of the induced osmotic flow inside the pore, and relevant combinations of the ion depletion length ℓ and the slip length b (see main text for details).

I. RELEVANT DYNAMICAL PHENOMENA FOR DNA TRANSLOCATION IN THE PRESENCE OF SALT GRADIENT AND A DRIVING VOLTAGE

For a narrow pore in the presence of an external electric field, there are two dynamical phenomena that can drive DNA towards/away from the pore:

(i) electroosmosis, and

(ii) electrophoresis.

These two mechanisms can be put under the broader group of electrokinetic effects, which describe the motion of fluids and charged solutes under forces of electrostatic origin. Phenomenon (i) is the movement of a fluid medium containing a net mobile electric charge driven by an external electric force; for global neutrality reasons this requires the presence of fixed charged walls, as the screening cloud in the fluid constitutes the net mobile charge that couples the electric field to the fluid motion. Phenomenon (ii) concerns the motion of a (often, confined) charged polymer, such as DNA or RNA, under an applied electric field, e.g., gel electrophoresis, used for segregating DNA by their lengths; it describes the motion of a DNA molecule through the pores of a gel under an external electric field.

In the presence of a salt gradient two further dynamical mechanisms play a role for the DNA capture by the pore:

(iii) osmosis, and

(iv) diffusiophoresis.

Osmosis (iii) is the flow of water from a low osmotic pressure to the high osmotic pressure side, i.e., from the cis to the trans in the experiments of Wanunu et al. Osmosis through membranes that are permeable to ions is often called capillary-osmosis or diffusio-osmosis [2], to distinguish the mechanism from classical osmosis through a semipermeable membrane. Similarly, associated with the concentration gradient of solutes is diffusiophoresis (iv), which describes the movement of macromolecules in a solute concentration gradient [3]. For both diffusiophoresis and (diffusio) osmosis, the ions (solutes) must either be attracted to or repelled by the wall/macromolecule. This repulsion/attraction leads to a lateral pressure gradient in the region where the ions interact with the wall/macromolecule, driving fluid flow or movement of the macromolecule. In the main text we discuss osmosis (iii) and electrophoresis (ii), which we find to be the dominant contributions to the enhanced DNA capture rate for the particular system studied by Wanunu et al. [1] i.e., electroosmosis is subdominant. Below we argue that diffusiophoresis (iv) can also be neglected in relation to electrophoresis.

The flow due to electroosmosis as driven by an electric field $E_p$ in the pore has the exact same form as the flow due to electrophoresis (with a change of sign) [4]

$$v_{EO} = -\frac{\phi_{wall}E_p}{4\pi \eta},$$

so when both the polymer and the pore have surface charge (surface potential) with the same sign, electrophoresis and electroosmosis pull the polymer in opposite directions. In the experiment by Wanunu et al. [1] the electroosmotic flow was found to be negligible compared to electrophoresis, as the pore walls was found to be nearly neutral. The diffusiophoretic flow, which relates to diffusioosmosis as electrophoresis relates to electroosmosis, is induced by the salt gradient in the cis reservoir, and pushes DNA towards the pore (as ions are net attracted to DNA molecules). By conservation of particle current

$$J = -D \nabla C(r),$$

the gradient of the salt concentration in the cis reservoir is

$$\nabla C_c(r) = -\frac{C_1 - C_c}{8L r^2}$$

and the corresponding flow is

$$v_{DP}(r) = D_{DP} \nabla \ln C_c(r) = -D_{DP} \frac{(x - 1)}{8L r^2},$$

where $D_{DP}$ is the diffusio-phoretic mobility. The diffusio-phoretic flow has the same dependence on x as electrophoresis, and will therefore add to the term linear in $x$ in the relative capture rate (see Eq. (15) in the main text). The ratio between the electrophoretic flow and the diffusiophoretic flow is (from Eq. (21) and Eqs. (12) and (13) in the main text)

$$\frac{|v_{DP}(r)|}{|v_{EP}(r)|} = \frac{2D_{DP}(x - 1)d^2}{\mu V a^2 x} \approx 0.1$$

with the electrophoretic mobility of DNA $\mu = -10^{-8} m^2 s^{-1} V^{-1}$, $V = 300 mV$ and $D_{DP} \approx 2 \cdot 10^{-10} m^2/s$ [3]. This is about one order of magnitude lower than electrophoresis and is therefore omitted in the analysis in the main text.

II. OSMOTIC FLOW PROFILE

In Fig. 9a we plot the different combinations of ℓ and b for different values of k, showing resonable combinations
FIG. 3: (a) Combinations of the slip length $b$ and ion wall depletion length $\ell$ for different values of $k$ (see Eq. (17) in the main text), 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 $\rho$ 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 main text for details).

FIG. 4: Capture rate (Eq. (15) in main text) as a function of salt concentration in the cis reservoir, with $C_t = 1$ M, for different values of the dimensionless parameter $k$ (see Eq. (15) in the main text). The points are experimental measurements of Ref. [5].

of $\ell$ and $b$ for the $k$-regime of interest (see main text for details). The slip length of surfaces are typically in the range $0 - 30$ nm depending among others on the contact angle and the smoothness of the surface [5]. Similarly, in Fig. 3(b) the liquid flow profile inside the pore is plotted for different combinations of the depletion length $\ell$ 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 $\ell$ 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 $\ell$ also decreases the electrophoretic motion of the DNA molecules, by lowering the ion-accessible area of the pore. For the flow profiles in Fig. 4 the Reynolds number is $Re = \frac{vd}{\nu} < 0.0035$, where $\nu = 10^{-6}$ m$^2$/s is the kinematic viscosity of bulk water.

In Fig. 4 we plot the relative capture rate as a function $C_c$, showing a linear dependence between the capture rate and $C_c$. This is the exact same plot as Figure 2 in the main text, however plotted in a different manner. This linear dependence stems from the osmotic flow as we predict (see Eq. (15) in the main text), and not from electrophoresis, suggesting that the capture rate has a linear dependence on $C_c$ for weak salt gradients, and an inverse dependence for large salt gradients ($x = C_t/C_c$).

* Electronic address: M.M.Hatlo@uu.nl

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