Electronic Supplementary Material (ESI) for Nanoscale.

Probing Driving Forces in Aerolysin and α-Hemolysin Biological Nanopores: Electrophoresis *versus* Electroosmosis

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Electronic Supplementary Information

SI 1 Theoretical Considerations and Orders of Magnitude

SI 1.1 Difference between EOF through solid-state and biological nanopores

Electroosmotic flow (EOF) is the movement of fluid through a capillary under the action of an electric field,[1] but the way in which EOF is generated through solid-state nanopores and biological nanopores is quite different (Figure1).

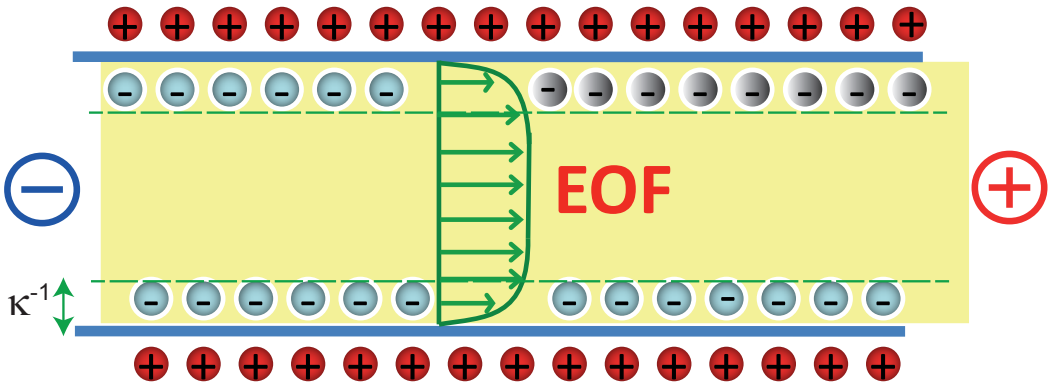
On the one hand, in the case of nanocapillaries or solid-state nanopores, the pore walls are highly charged and the charges are uniformly distributed. When the voltage is applied, the entire double layer (whose thickness is given by the Debye length) is set in motion under the effect of Coulomb forces. As a consequence, when the pore radius is larger than the Debye length $\kappa^{-1} \approx \frac{0.304}{\sqrt{c_{salt}}}$ (at 1M salt concentration $\kappa^{-1} \approx 0,3nm$), the whole fluid is driven under the action of viscous forces, which transfers the momentum from one layer to another, as a "plug flow". The velocity of the driven fluid is given by:

$$v = \frac{\epsilon\epsilon_0 E}{\eta}(\phi - \zeta) \tag{1}$$

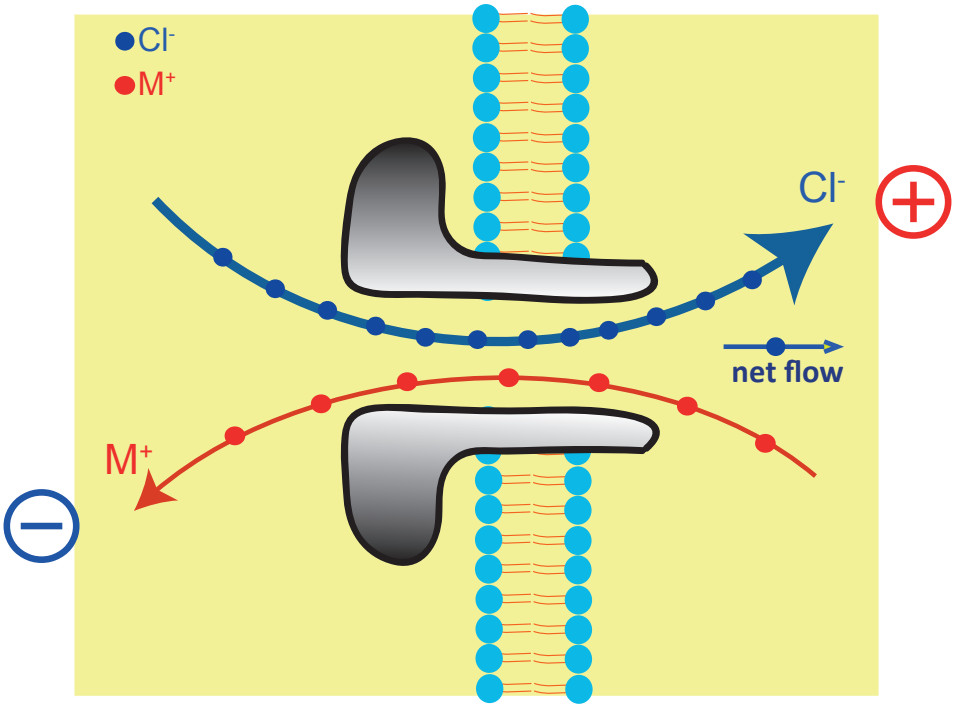
where $\epsilon\epsilon_0$ is the product of the dielectric constant and the permittivity of vacuum, E is the magnitude of the electric field, η is the water viscosity, ϕ is the local electrostatic potential, and ζ is the zeta potential. The zeta potential of the pore is related to the Debye length κ^{-1} and to the charge density of pore walls σ by:

$$\zeta_{pore} = \frac{\kappa^{-1}\sigma}{\epsilon\epsilon_0} \tag{2}$$

Outside of the Debye-Huckel layer, the potential ϕ is equal to zero, hence the electroosmotic



Solid State Nanopores (Plug Flow)



Biological Nanopores (Net Flow)

Figure 1: Difference between EOF through solid-state nanopores (top) and biological nanopores (bottom).

velocity v_{EOF} is expressed as:

$$v_{EOF} = -\frac{\epsilon\epsilon_0 E}{\eta}\zeta_{pore} \tag{3}$$

The electrophoretic velocity v_{ep} of a charged molecule through a pore, under the influence of a zeta potential $\zeta_{molecule}$ is given by:

$$v_{ep} = \frac{\epsilon\epsilon_0 E}{\eta}\zeta_{molecule} \tag{4}$$

The total velocity v_t of the charged molecule through the pore reads:

$$v_t = v_{ep} + v_{EOF} = \frac{\epsilon\epsilon_0 E}{\eta}(\zeta_{molecule} - \zeta_{pore}) \tag{5}$$

The calculated electroosmotic velocity of a neutral molecule through a 5 nm length SiN solid-state nanopore (ζ_{SiN}=10 mV) at 100 mV applied voltage is 0.14 m/s.[2]

On the other hand, in the case of biological nanopores, the pore walls are less charged and the charges are randomly distributed. This gives rise to a new feature known as selectivity, *i.e.* that one kind of ion (depending on the sign of its charge) permeates more easily into the pore. Taking into consideration, the selectivity, the dimension of the channel, the hydration shell and the difference in ion mobility, we obtain a net flow of liquid through the pore and its expression is given by:[3]

$$J_w = N_w \frac{I}{e}\left(\frac{1 - P_+/P_-}{1 + P_+/P_-}\right) \tag{6}$$

where N_w is the number of water molecules transported by an ion, I is the ionic current flowing through the pore, e is the elementary charge and P_+/P is the permeability ratio of cations *vs.* anions given by the Goldman-Hodgkin-Katz (GHK) equation:[4]

$$\frac{P_+}{P_-} = \frac{[Cl^-]_{cis} - \exp(-\frac{eV_r}{k_BT})[Cl^-]_{trans}}{\exp(-\frac{eV_r}{k_BT})[K^+]_{cis} - [K^+]_{trans}} \tag{7}$$

where $[Cl^-]$ and $[K^+]$ are the activities of anions and cations; e,k_B and T are respectively the elementary charge, the Boltzmann constant and the temperature and V_r is the reversal potential that sets the ionic current through the pore to zero under asymmetric conditions of electrolyte concentration.

We measured V_r for the AeL nanopore in KCl and in LiCl as follows: we first introduced 0.2M of the same electrolyte on both the *cis*- and *trans*-sides of the lipid bilayer. After controlling that the setup offset was zero once a single nanopore was inserted (zero current at zero-applied voltage), we concentrated the *cis*-compartment to 1M electrolyte. Under such asymmetric electrolyte conditions, the current through the nanopore is not zero at zero-applied voltage. Then we measured the reversal voltage V_r, *i.e.* the voltage necessary to set the current to zero (Figure 2). We used 3M KCl agarose salt bridges between electrodes and buffer solution in both compartments, in order to ensure electrodes stability. In this case, we have to consider the existence of a liquid junction potential (LJP) on the interface between the salt bridge and the buffer solution, in both compartments.[5] The measured values of V_r were corrected for the LJPs using the Junction Potential Calculator, which used the Henderson equation,[5] supplied by the CLAMPEX 10.5 program. Corrections were -0.8 mV in KCl and -3.2 mV in LiCl. Measurements of V_r were averaged over four independent experiments for each electrolyte. For AeL, at 20°C, we obtained V_r =-20.1 mV ($\frac{P_+}{P_-}$ =0.27) in KCl and V_r =-24.5 mV ($\frac{P_+}{P_-}$ =0.19) in LiCl.

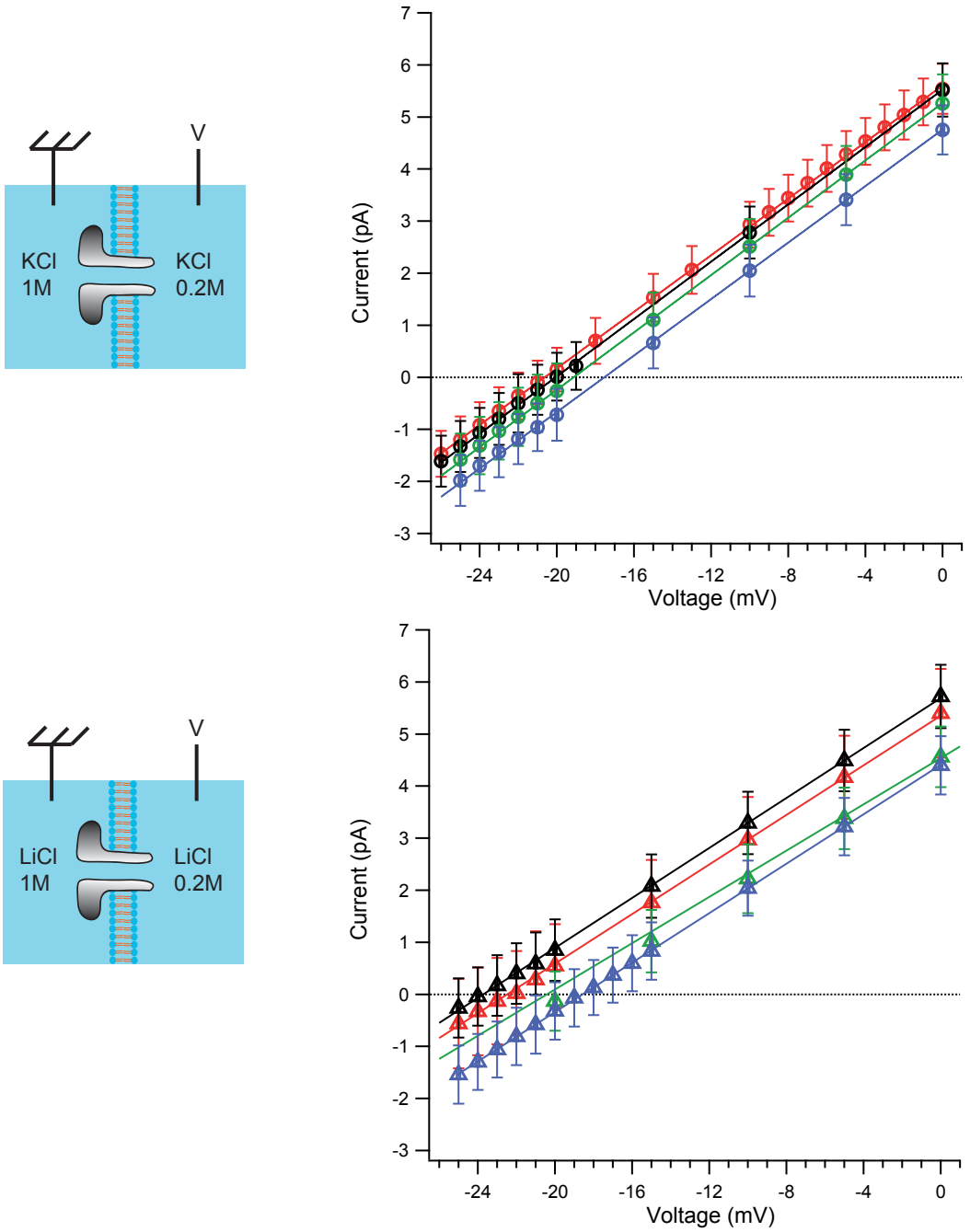


Figure 2: Measurement of the AeL selectivity: current through the AeL nanopore as a function of voltage in presence of 1M-*cis*/0.2M-*trans* electrolyte concentration in KCl (top) and in LiCl (bottom) at 20°C. The reversal voltage V_r is the voltage necessary to set the current to zero. Results of four independent experiments are shown for each electrolyte. Solid lines are linear fits. Cartoons on the left illustrate the recording configuration.

Considering this work and previous work,[6] we measured a value of the ratio $\frac{P_+}{P_-} < 1$, for both AeL and aHL nanopores and in both KCl and LiCl, which means that both pores are selective to anions in these two salts. In parallel, the measured current-voltage (I-V) curve through AeL in KCl and in LiCl shows a higher conductance for positive charges flowing from *cis* to *trans*, *i.e.* AeL shows *trans*-ward rectification, while aHL shows *cis*-ward rectification (figure 3). This reveals that the pore selectivity cannot be predicted from the I-V curve shape in the case of biological nanopores, in contrast with what has been done for solid-state nanopores.[7]

The electroosmotic velocity v_{EOF} through biological nanopores is related to the water flow J_w by:

$$v_{EOF} = \frac{J_w}{\pi r_{pore}^2 c_w} \tag{8}$$

where r_{pore} is the pore radius and c_w is the number of water molecules per unit volume. By taking the measured value of $\frac{P_+}{P_-} = 0.71$ in aHL,[6] the calculated electroosmotic velocity v_{EOF} through this biological nanopore at 100 mV is about 10^{-2} m/s. This value is one order of magnitude smaller than v_{EOF} calculated for a solid-state nanopore (see above).

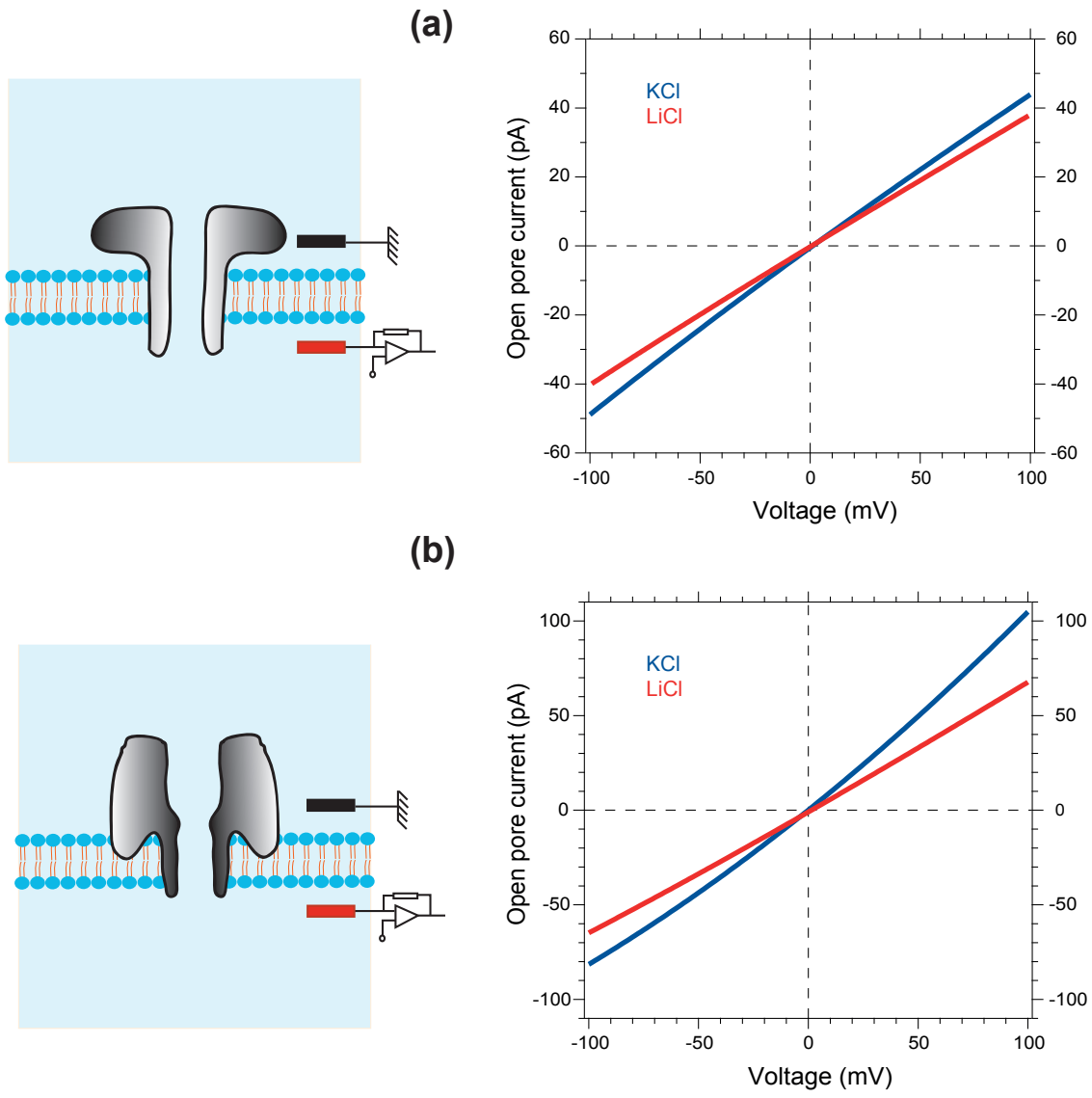


Figure 3: Current-voltage (I-V) curve through AeL (top) and aHL (bottom) in 1M KCl (blue) and in 1M LiCl (red). Cartoons on the left illustrate the recording configuration.

SI 1.2 Injection of neutral flexible chains by electroosmotic Flow

If the flow through nanopores is larger than a critical flow J^* given by:[8–10]

$$J^* = \frac{k_B T}{\eta} \tag{9}$$

The chain enters the nanopore whatever its size and whatever the pores size. This law was elegantly tested by authors of reference.[11] Without a pressure gradient, the critical mean velocity of fluid corresponds to the critical electroosmotic flow velocity and is related to the critical flow v^*_{EOF} by :

$$v^*_{EOF} = \frac{J^*}{d^2_{pore}} \tag{10}$$

Let's evaluate this critical velocity and compare it to the electroosmotic flow estimated above: taking a pore diameter d_{pore}=2nm, the water viscosity η=10^3 Pa.s and $k_B T \approx 4.10^{21}$ J, yield v^*_{EOF}=1 m/s. This value is two magnitudes larger than the v_{EOF} value evaluated through biological nanopores and one magnitude larger than the v_{EOF} value evaluated through solid-state nanopores. This implies that there is a limit in polymer size where EOF cannot overcomes the entropic force $\sim \frac{k_B T}{d_{pore}}$ to confine the chain. In the best case we need to apply a voltage of about 700 mV to reach v^*_{EOF}. This is clearly impossible with biological nanopores as the lipid bilayer would not resist. Nevertheless, the EOF measured through biological nanopores at reasonable applied voltages could be enough to drive neutral macromolecules into the pore when their size is less than or comparable to the pore diameter.

SI 2 Current traces and data analysis

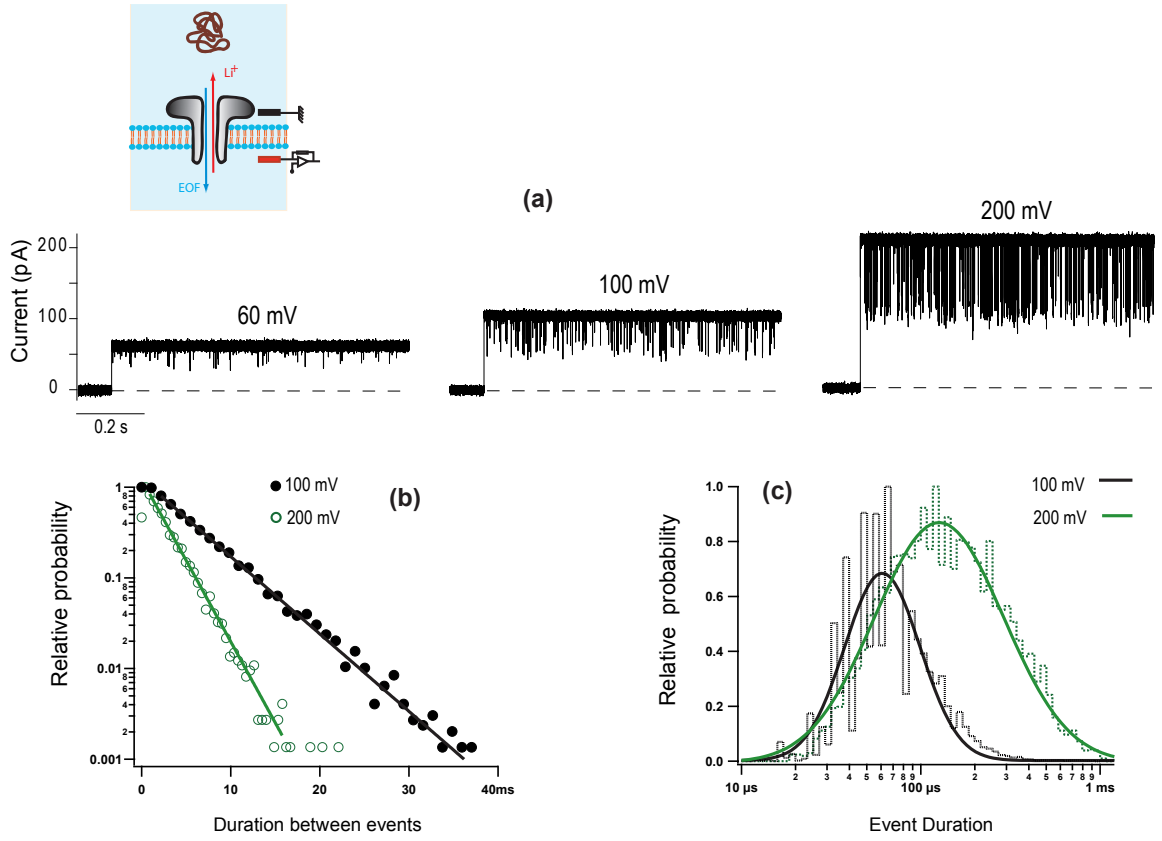


Figure 4: (a) Typical current traces through AeL in 3M LiCl in presence of PEG 1500 added from the *cap*-side of the pore for different positive voltages: +60 mV, +100 mV and +200 mV. Cartoon on the top illustrates the recording configuration and the direction of cation flow and of EOF according to voltage polarity. (b) Inter-blockade duration distributions corresponding to the current traces in (a) at +100 mV and +200 mV. The left axis is in log-scale. The mean inter-blockade duration is estimated from a single exponential fit (solid lines). (c) Blockade duration distributions corresponding to the current traces in (a) at +100 mV and +200 mV. The bottom axis is in log-scale. The mean blockade duration is estimated from a single exponential fit (solid lines).

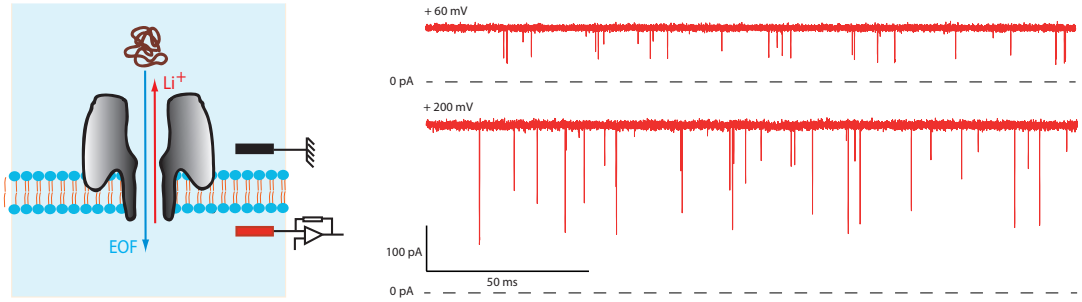


Figure 5: Typical current traces through aHL in 3M LiCl in presence of PEG 1500 added from the *cap*-side of the pore for two different positive voltages: +60 mV and +200 mV. Cartoon on the left illustrates the recording configuration and the direction of cation flow and of EOF according to voltage polarity.

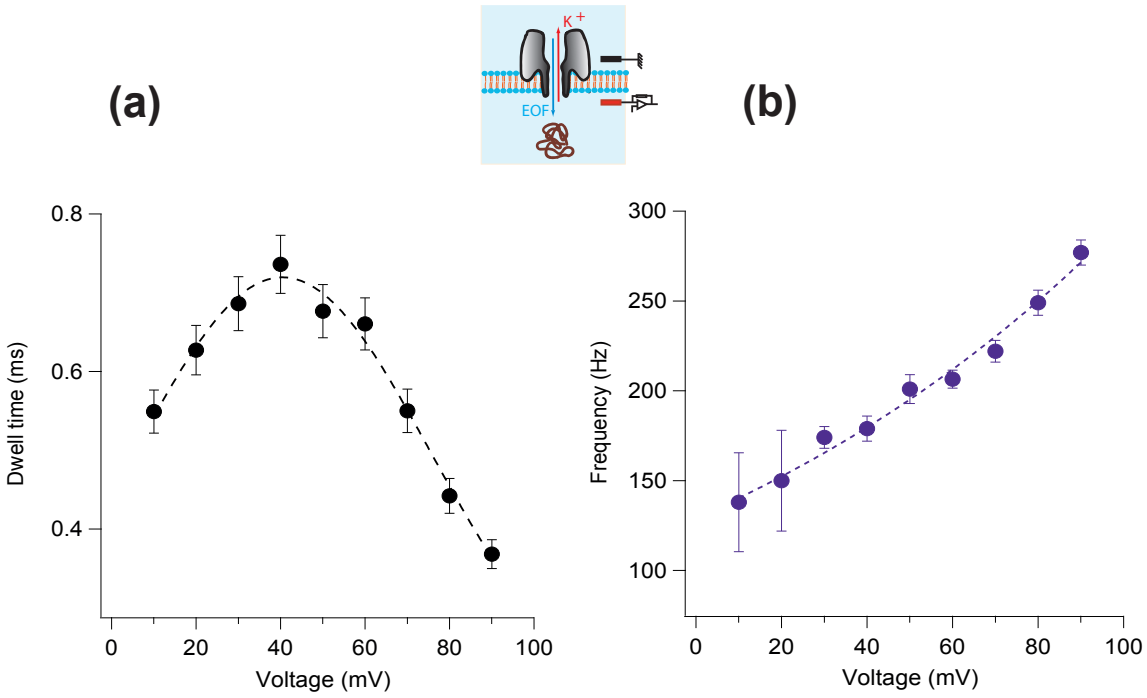


Figure 6: Duration (a) and frequency (b) of current blockades induced by PEG 1500 added from the *stem*-side of aHL in 3M KCl as a function of positive voltage. Dashed lines are guides to the eyes. Cartoon on the top illustrates the recording configuration and the direction of cation flow and of EOF according to voltage polarity.

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