THE EFFECTS OF CONCENTRATION POLARIZATION ON MOLECULE TRANSLOCATION IN A NANOPORE DEVICE
Thomas A. Zangle,1 AmirAli H. Talasaz,1,2 Ronald W. Davis,2 and Juan G. Santiago1
1Stanford University, Stanford, CA, USA and 2Stanford Genome Technology Center, Palo Alto, CA, USA

ABSTRACT
Nanopores offer the potential for label-free detection of individual proteins [1] and have been identified as a key potential technology for low cost DNA sequencing.[2] To date, most studies of nanopore electrokinetic transport [1,3,4] have neglected the effects of concentration polarization (CP) especially in systems with non-overlapped electric double layers (EDLs). We present a combined computational and experimental study of conical nanopores with tip diameters of 40-100 nm. Our modeling and experimental work shows that for typical (mM) buffer concentrations, even non-overlapped EDLs fundamentally affect key transport characteristics such as the rate of molecular translocations.

KEYWORDS: Nanopores, concentration polarization

INTRODUCTION
In nanopores, the pore diameter approaches the EDL thickness and a majority of ionic current is carried by counter ions to the wall charge. This ionic flux imbalance drives CP. CP significantly changes the concentration and electric field in the vicinity of the nanopore. This has important consequences for the stability and biological function of proteins in the vicinity of the pore and for current rectification in conical nanopore devices with non-overlapped EDLs.

We present a computational study of nanopore CP in which ionic species concentrations and electric fields are resolved in a domain consisting of the pore and large, connected reservoirs. We also present experiments which validate the computational study and demonstrate the extent of CP in the vicinity of a nanopore.

COMPUTATIONAL MODEL
Simulations were performed in COMSOL using axisymmetric Nernst-Planck (without electroneutrality) and electrostatics modules. For measurement of dye concentration or translocation (Figures 2 and 3), the low-concentration anionic dye was assumed to have a negligible effect on the background electrolyte. We modeled a pore which has a 100 nm diameter at the top, a 35 nm diameter at the narrow end and which is 100 nm long. The computational domain extends 50 μm away from the pore in all directions.

These studies show the impact of CP on the electric field and ion concentration in and near a nanopore. In particular, CP around a nanopore has a significant effect on the translocation of analyte ions. Figure 2 shows the results of a sample computation performed for the fully-resolved CP case and for the case of a pore without CP computed using boundary conditions similar to Cervera et al.[4] imposed directly at
the pore inlet and outlet. For wall charge densities of -1.6 C/m², the effects of CP can account for 7X decrease in the rate of translocation events due to both increased potential drop in the bulk solution and the effects of CP on the distribution of ions within the pore.

Figure 1. Computed electric field lines and background co-ion concentration in the vicinity of a conical nanopore. 10 V is applied across the pore with a wall charge of -1.6 C/m². The grey-scale shows ion concentration (mM) in the enrichment and depletion zones in the bulk solution surrounding the pore. The same applied potential can cause ion depletion on the near or far side of the pore, depending on the wall charge (c.f. Figure 3).

Figure 2. Simulation results showing the effect of CP on translocation rate (as molar flux) with 1 mM KCl as the background electrolyte. For the CP data, analyte concentration is 1 mM far from the pore. The “no-CP” case uses typical existing model boundary conditions (e.g., from Cervera et al.[4]). These results indicate that CP is a key factor in predicting translocation.

EXPERIMENTAL
We performed experiments with an array of 10 nanopores fabricated by focused ion beam milling of a 100 nm thick, 100 μm wide free-standing silicon nitride membrane. This fabrication process results in conical pores with a tip diameter of 100 nm. The experimental setup is shown schematically in Figure 1. We placed 1 mM Tris-HCl solution (pH=7.5) on the top and bottom of the pore.

The translucent 100 μm silicon nitride membrane supporting the pores allows for fluorescence imaging on either side of the pores. Fluorescence contained within the small square (as in Figure 3 (a) and (b)) indicates analyte only on the far side of the pores. The location and intensity of fluorescence in the four cases explored validate the simulations and confirm the drastic effect of CP on local concentrations (and associated electric fields). We performed experiments both with native, negatively charged nanopores (Figure 3 (a) and (c)) and with poly-L-lysine- (PLL) treated, positively charged nanopores (Figure 3 (b) and (d)). These results show that For +10 V applied, dye translocates through pore. For negative applied potentials, the dye is driven by electromigration towards the ground electrode. Our experiments demonstrate that nanopore concentration polarization has a large effect on the concentration of ionic species which extends over a distance far from the pore, and which depends on the sign of the wall charge.
Figure 3. Four example cases of nanopore CP and validation using fluorescence imaging: (a) negative wall surface charge with -10 V applied to the narrow side of the pore. (b) positive surface charge with -10 V applied. (c) negative surface charge with +10 applied. (d) positive surface charge with +10 V. The top of the pore is grounded in all cases and cross-section views show background co-ion concentration greyscale from simulation results (as in Figure 1). Computed contours (white lines) of Alexa Fluor 488 concentration are also shown. Inset (on the right of each) are experimental images using an anionic dye (Alexa Fluor 488) which is added to the top side of the pore and imaged from below (as indicated by the eye symbol).

DISCUSSION

Our results indicate the importance of resolving the effects of CP for the design and interpretation of nanopore-based assays. Accounting for the effects of CP is crucial for understanding translocation measurements in nanopore devices.

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