Probing Solid-State Nanopores with Light for the Detection of Unlabeled Analytes

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SUPPLEMENTARY INFORMATION
1. Rhod-2 - Ca\(^{2+}\) binding constant measurements for different EGTA concentrations

Calcium chloride was titrated into a volume of 1M KCl to the final concentrations as indicated in Fig. S2. The concentration of Rhod-2 concentration was fixed at 5.6 µM, and fluorescence was measured at the confocal spot, positioned 100 µm above a glass coverslip. The experiment was repeated for three EGTA concentrations (10, 50, and 100 mM EGTA). The midpoint of each curve between background fluorescence and saturation was termed the \(k_D\) for a given concentration of EGTA which competes for calcium binding with the calcium indicator Rhod-2. Higher concentrations of EGTA shift the midpoints toward higher concentrations of CaCl\(_2\).

\[
\begin{array}{|c|c|c|}
\hline
\text{[EGTA]} & k_D \sim 5.6 \text{ mM} & k_D \sim 20.5 \text{ mM} & k_D \sim 34.2 \text{ mM} \\
\text{base} & \sim 1.3194 & \sim 1.0376 & \sim 0.87689 \\
\text{max} & \sim 37.256 & \sim 44.302 & \sim 45.549 \\
\hline
\end{array}
\]

**Figure S2** | Measurements of Rhod-2 - Ca\(^{2+}\) binding constants at different EGTA concentrations. Curves show the fluorescence intensity of Rhod-2 under excitation of 532 nm laser vs. calcium chloride and EGTA concentrations, and a fixed Rhod-2 concentration.
2. Fluorescence intensity versus voltage curves at different CaCl$_2$ concentrations (trans) and EGTA concentrations (cis).

A 4 nm nanopore was assembled in the optical setup and illuminated using green laser ($\lambda_{exc} = 532$ nm). Continuous $I$ - $V$ curves were taken as the voltage was ramped from -1V to 1V in 0.01 V increments, for 0, 10, 50, 100, 500, 1000, and 3000 mM calcium chloride (trans) and EGTA concentrations as indicated in the cis chamber.

![Graphs showing fluorescence intensity versus voltage for different CaCl$_2$ concentrations](image)

**Figure S3** | Fluorescence intensity vs. voltage for 0, 10, 50, 100, 500, 1000, and 3000 mM CaCl$_2$ in 10, 20, and 50 mM EGTA, measured using a 4 nm solid-state nanopore.

A summary of the data shown in Figure S3 is given in Figure S4 using the same Y scale for the entire CaCl$_2$ concentration range (0 – 3M) at fixed [EGTA] = 10 mM.
3. Numerical model of Ca\textsuperscript{2+} - bound dye concentration distributions near a solid-state nanopore.

Following previous studies\textsuperscript{1,2} we used the Poisson-Nernst-Planck equations to model ionic concentration distributions near nanopores. We numerically solve Poisson’s equation,

\[ \nabla^2 V = \frac{F}{\varepsilon} (2c_{Ca} + c_K - c_{Cl}) , \]

and the Nernst-Planck equation for each ionic species (potassium, calcium, and chloride),

\[ \nabla \cdot \vec{J}_{Ca} = 0 \]

\[ \vec{J}_{Ca} = -D_{Ca} \nabla c_{Ca} - z_{Ca} \mu_{Ca} F c_{Ca} \nabla V \]

\[ \nabla \cdot \vec{J}_K = 0 \]

\[ \vec{J}_K = -D_K \nabla c_K - z_K \mu_K F c_K \nabla V \]

\[ \nabla \cdot \vec{J}_{Cl} = 0 \]
\[
\overrightarrow{J}_{Cl} = -D_{Cl}\nabla c_{Cl} - z_{Ca}\mu_{Ca}F c_{Cl}\nabla V,
\]
where \(V\) is the electric potential, \(F\) is the Faraday constant (\(\sim 96,485 \text{ s} \cdot \text{A/mol}\)), \(\varepsilon\) is the dielectric constant permittivity of water (\(\sim 7.09 \cdot 10^{-10} \text{m}^3 \cdot \text{kg}^{-1} \cdot \text{s}^4 \cdot \text{A}^2\)), and \(c_i\) is the concentration, \(\overrightarrow{J}\) is the flux, \(D_i\) is the diffusion constant, \(z_{Ca}\) is the charge number, and \(\mu_{Ca}\) is the electrophoretic mobility of each ion species \(i\). The diffusion constant \(D_i\) is calculated from the Einstein-Smoluchowski relation,\(^3\)
\[
D_i = \frac{h k_B T}{q}
\]
where \(k_B\) is the Boltzmann constant, \(T\) is the temperature, and \(q\) is the electric charge of each ion species. The boundaries are set to 1 \(\mu\)m in all directions around the pore forming a sphere of radius 1 \(\mu\)m in a 2D axisymmetric model (Fig. S5). The boundaries in cis and trans are set to constant concentrations and the far and near boundaries are set to 0 V (in cis) and a target voltage in trans. These are reasonable approximations as the cis and trans chambers in the experiment are large enough to be taken as constant concentration sources relative to the duration of an experiment.

We solve for equilibrium spatially distributed steady-state free calcium in the presence of the chelating agent EGTA\(^4\) according to
\[
\left[Ca\right] = \left[C_T\right] - \frac{1}{2}\left([C_T] + [EGTA] + k_D - \sqrt{4\left[C_T\right][EGTA] + ([C_T] + [EGTA] + k_D)^2}\right)
\]
where \(k_D = 150 \text{ nM}\) is the dissociation constant for EGTA-Ca\(^5\), \([C_T]\) is the total calcium concentration (numerically solved for with PNP), and \([EGTA]\) is the concentration of EGTA. We solve for the concentration of calcium-bound Fluo-4 (again, following ref 4) according to
\[
\left[F \cdot Ca\right] = \frac{1}{2}\left([C] + [F] + k_D - \sqrt{4\left[Ca\right][F] + ([Ca] + [F] + k_D)^2}\right)
\]
where \(k_D\) of Fluo-4 for Ca is 345 nM\(^6\) and \([\text{Fluo-4}]\) is an experimentally determined concentration.

To model the total intensity acquired with TIRF illumination mode, we calculated the evanescent \(I(z)\) using:
\[
I(z) = I_0 e^{-z/\delta}
\]
with a skin depth \(\delta\)
\[
\delta = \frac{\lambda}{4\pi \sqrt{n_1^2 \sin^2 \theta - n_2^2}} \sim 100 \text{ nm}
\]
where $n_1$ is the index of refraction of the trans chamber, $n_2$ is the index of refraction of the cis chamber, and $\theta$ is the angle of incidence of the 488 nm excitation laser from cis to trans. We then integrate for total fluorescence ($F$):

$$F = k \int_0^L l(z)[F \cdot Ca](z) \, dz$$

where $L$ is 100 µm, the distance to the cis boundary in our numerical model.

The simulations were run with the potential difference across the pore ramped from -1V to 1V in 50 mV steps for each concentration of CaCl$_2$. We then calculated $F$ for each voltage and CaCl$_2$ concentration and used the parameter $k$ to scale the theoretical curves to the experimentally measured value at 1V. We note that the simulations are only simple approximations to the experimentally observed values, however, they replicate quite clearly the same trends (e.g., both show sigmoidal saturating behavior at similar voltages and fluorescence intensity scaling with CaCl$_2$). Furthermore, while it would be difficult to calculate real fluorescence intensity numbers given that many experimental parameters are lumped into $k$ (photon counts/second for a given experimental conditions a priori), our models clearly show that the gradient of the calcium-bound fluorophore can be finely tuned in understandable ways by varying CaCl$_2$, EGTA, and Rhod-2 or Fluo-4 concentrations, or by varying the voltage, within the range of several nanometers to micrometers with variable decay rates, and are thus valuable guides to experiments.
4. Asymmetric trans/cis calcium concentration increases capture rate and slows translocation

Asymmetric distributions of calcium chloride, with CaCl$_2$ in trans, were shown to both increase capture rate (Fig. S5) and slow down translocations (Fig. S6) without substantially changing the normalized blockade (Fig. S7) levels for 1 kbp DNA at 300 mV in a 4 nm pore relative to similar KCl gradients.

**Figure S5** | Capture rate enhancement of 1 kbp DNA in a 4 nm pore with CaCl$_2$ and KCl gradients.
**Figure S6** | Increasing calcium chloride concentration in trans incrementally slows down DNA translocation (1 kbp DNA, 4 nm pore, V = 300 mV).

**Figure S7** | $I_b$ for translocation shows similar peak blockage levels at three concentration of trans CaCl$_2$ (1 kbp DNA, 4 nm pore, V = 300 mV).
5. Synchronous electrical and optical translocation blockade depth

Synchronous electrical and optical translocations of 1 kbp, 8 kbp, and 10 kbp DNA at 300 mV are shown in Fig. S8.

**Figure S8** | 1 kbp, 8 kbp, and 10 kbp (~1.5 nM, ~192 pM, and 154 pM, respectively) synchronous electrical and optical translocation at 300 mV in 4 ± 0.2 nm pores with 4 µM Fluo-4 in cis and 250 mM CaCl₂ in trans.
6. Comparison of Electrical versus Optical noise during long DNA translocations.

We compared the blocked level noise in the ion currents to those in the optical signal for a set of long DNA translocations ($t_D > 10$ ms, $N = 57$). These events were acquired using a 8 kbp DNA, 2.8 nm pore at $V = 1$ V. Electrical and optical events were acquired simultaneously, and for each event we measured the width at half maximum of the relative blocked ion current distributions during translocations. Our results (shown in Fig. S9) indicate that the optical signal as whole displayed more consistent average blocked levels, and a smaller dispersion overall than the electrical signal (the standard deviations in normalized blockade levels were 0.064 and 0.033, for the mean $I_B$ and $F_B$ of all events, respectively). This is consistent with the absence of flicker low frequencies noise in the spectrum of the optical signal, and may offer a significant improvement over electrical sensing when accurate determination of a current level is necessary.

![Figure S9](image)

**Figure S9** | 8 kbp DNA blockades at 1 V in a 2.8 nm pore with 250 mM CaCl$_2$ in trans and 20 µM Fluo-4 in cis. Left panel: the electrical ($I_B$ and optical $F_B$) fractional blocked levels for all events. Right panel: histograms of all events using equal bins, showing a narrower distribution for the optical signals of the same events.

7. Experimental Setup

A schematic of the optical and electrical components of the experiment is shown in Fig. S10. Both confocal and TIRF modes can be used with two different laser lines, as explained in the
Abbreviations: PD, Photo-diode; Pol, Half-wavelength wave plate; DM, Dichroic Mirror; NF, Notch Filter; PH, Pinhole; APD, Avalanche Photo-Diode; PZT-NP, Piezo-Nanopositioner; CNT, Counter source; AI, Analog Input; AO, Analog output.

Figure S10 | Optical and electrical experimental setup schematic
**Figure S11** | Electrical and optical measurements of Power Spectrum Distributions, measured simultaneously using 75 mM Ca\(^{2+}\), V = 300 mV and 400 µM Rhod-2 (electrical signal filtered at 40 KHz using Butterworth filter).

**SUPPLEMENTARY REFERENCES**

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