Supporting Information

Optoelectronic control of surface charge and translocation dynamics in solid-state nanopores

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1. Current versus voltage (I-V) curves of a nanopore with and without laser illumination.

Figure S1 shows the I-V curve for a 9 nm diameter pore drilled in a 30 nm thick SiN membrane, with and without laser illumination (solid and open circles, respectively). The pore conductance increases from 31 nS to 78 nS when illuminated with 5.7 mW of 532 nm light focused at the pore. The graph shows that the nanopore behaves as an ohmic device with a constant conductance throughout the tested voltage range. Immediately after switching the laser off, the conductance returned to 31 nS (data not shown for clarity).

![I-V curve](image)

**Figure S1** I-V curve for a 9 nm pore under no illumination (open circles, 31 nS) and the same pore exhibiting increased conductance under 5.7 mW of a focused 532 nm laser beam (solid circles, 78 nS).

2. Translocation time and capture rate distributions with and without laser illumination.

Figure S2 shows distributions of translocation times (left column) and times between consecutive events (right column) for 10 kbp-long DNA fragments (NoLimits, Thermo Scientific) translocating through a 5.4 nm diameter solid-state nanopore in SiN, collected under three consecutively applied conditions: (i) with no laser illumination (top row), (ii) illuminated by a 2 mW focused beam of 532 nm light (middle row) and (iii) again with no laser illumination (bottom row). A similar number of events (> 1000) were analyzed in all conditions, and all other experimental parameters were kept constant (300 mV voltage clamp, 21 °C, 1M KCl, 20 mM TRIS pH 8.0). All distributions were fitted with a single exponential decay
or, when this was not sufficient, a double exponential decay, and the relevant decay times $\tau$ are indicated for each fit.

![Graphs showing distributions of translocation times and times between consecutive translocations](image)

**Figure S2** Distributions of translocation times (left column) and of times between consecutive translocations (right column) for the passage of 10 kbp DNA fragments through a 5.4 nm diameter pore at 300 mV, 21C and 1M KCl. The top and bottom rows show the distributions before turning the laser on and after turning it off, respectively. The middle row shows the increase in the translocation times and in the times between consecutive DNA captures when the focused 532 nm laser illuminates the pore at 2 mW. Both show a marked increase compared to the conditions without illumination.
Figure S3 Distributions of translocation times (left column) and of times between consecutive translocations (right column) for the passage of 5 kbp DNA fragments through a 5.6 nm diameter pore at 300 mV. The top row shows the distributions before turning the laser on. The bottom row shows the increase in the translocation times and in the times between consecutive DNA captures under 3 mW of laser illumination.

Figure S4 Distributions of translocation times (left column) and of times between consecutive translocations (right column) for the passage of 3.5 kbp DNA fragments through a 7.4 nm diameter pore at 300 mV and 21 C, 1M KCl. The top row shows the distributions before turning the laser on. The bottom row shows the increase in the translocation times and in the times between consecutive DNA captures under 5.7 mW of laser illumination.
In a similar way, Figure S3 shows the distribution times for 5 kbp DNA fragments translocating through a 5.6 nm pore, before (top row) and during illumination (bottom row) with 3 mW. Figures S4 shows the distributions for the passage of 3.5 kbp DNA fragments through a 7.4 nm pore, with no laser and when illuminated with 5.7 mW. All pores used were drilled in 30 nm thick LPCVD SiN. All measurements were performed using a 10 kHz Bessel low-pass filter to reduce higher frequency noise.

3. Measurement of the photoconductive effect response time in solid-state nanopores.

To characterize the temporal response of the photoconductive effect we connected a “laser on/off” TTL pulse from our DAQ card to the Analog modulation control of the iFlex 2000 solid-state laser (QIOptiq). According to the laser specifications (see: http://www.qioptiq.com/diode-lasers.html#Section19866942) the rise time of the system is <200 ns. This was confirmed by measuring the actual rise time with a fast photodiode (PDA36A, Thorlabs) connected to a digital 100 MHz scope (TDS3014C, Tektronix). A LabView program was written to switch the laser on/off while measuring the ion current through a solid-state nanopore positioned at the laser focus, as explained in the main text. The laser intensity rise time measured by the photodiode shows characteristic switching time of < 0.2 μs (Figure S5a). The 

Figure S5 Measurement of the temporal response of the optoelectrical effect in a solid-state nanopore. a) The laser intensity response time upon a TTL trigger measured by a fast photodiode, showing a rise time of <0.2 μs. b) Simultaneous measurement of the pore current and laser intensity upon switching on the laser with the TTL pulse. The bottom panel displays the intensity of the laser pulse as a function of time (going from 0 to 5 mW at t = 25 ms). The simultaneous measurement of the ionic current flowing through a 5 nm pore (V = 300 mV, C = 1 M KCl), shows an abrupt jump of the current from 4.4 nA to 7.1 nA. The delay time from switching on the laser light to the stabilization at the current at the higher level was measured to be 0.45 ms. The ion current response is subject to a transient saturation of the Axon amplifier.
simultaneous measurement of the ionic current flowing through a 5 nm pore ($V = 300 \text{ mV}$, $C = 1 \text{ M KCl}$) and the laser intensity are shown in Figure S5b. We observe an abrupt jump of the current from 4.4 nA to 7.1 nA, concurrent with the laser switching on time. The delay time from switching on the laser light to the stabilization at the current at the higher level was measured to be 0.45 ms, and is attributed to the transient saturation of the Axon amplifier upon an abrupt increase in pore conductance.

4. Modeling the ionic current through a light-induced charged silicone nitride nanopore

For the analytical model described below we consider a cylindrical nanopore of radius $R = 3 \text{ nm}$ with a uniform surface charge density $\sigma$ that generates a surface potential $\varphi_0$. For simplicity and to keep our final solution analytical, we will assume that the surface potential is approximately equal to the potential of the diffuse layer (zeta potential), thus neglecting the Stern capacitance. The nanopore is assumed to be submerged in a symmetrical electrolytic solution of unitary valence as in our experimental conditions. Only the movement of ions in the axial direction will be considered.

As previously described [1-3], the net charge density inside the pore, $\rho(r, \varphi_0)$, can be analytically obtained from the Poisson-Boltzmann equation (relating $\varphi(r)$ with $\rho(r)$) and the Boltzmann distribution (relating $\rho(r)$ with $\varphi(r)$). Using the Debye-Hückel approximation, which is valid for potentials smaller than 50 mV [4], and solving the differential equation in term of the zero-order modified Bessel functions of the first kind ($I_0$, not to be confused with open pore current) one obtains an expression for the radial distribution of the net charge density as a function of the wall potential $\varphi_0$.

$$\rho(r) = -\varepsilon \kappa^2 \varphi_0 \frac{I_0(kr)}{I_0(kR)}$$  \hspace{1cm} (1)

where $\varepsilon$ is the permittivity of the aqueous solution, and $k^{-1}$ is the Debye screening length (3 Å for 1 M KCl and 21C). For potentials greater than 50 mV it is useful to keep in mind that this approximation will underestimate the actual solution.

When an external electric field $E_z$ is applied in the axial direction to the cylindrical tube, the Navier-Stokes equation for the motion of an incompressible Newtonian fluid driven by an electric field can be solved using Eq.1, yielding an expression for the profile of the velocity flow, $v_{EOF}$, due to the net charge density,
\[ v_{\text{EOF}}(r) = -\frac{\varepsilon \varphi_0 E_z}{\eta} \left[ 1 - \frac{I_0(kr)}{I_0(kR)} \right] \]  

(2)

where \( \eta \) is the fluid viscosity coefficient. Eq.2 was obtained by integrating with the boundary conditions that \( v(R) = 0 \) (no-slip condition) and that \( \frac{dv}{dr} \bigg|_{r=0} = 0 \) (no velocity gradient in the center of the pore). Figure S6a shows the profile of \( v_{\text{EOF}} \) across the pore for \( \varphi_0 = -50 \text{ mV} \) and an applied external voltage of 300 mV. The electro-osmotic flow has a characteristically plug-like shape, in contrast to the bullet-shaped profile that results from a pressure differential. For surface potentials approaching the limits of our approximations, the EOF velocity is comparable to the electrophoretic speed of \( K^+ \) and \( Cl^- \) ions (0.76 m/s). The profile of the EOF depends strongly on the Debye length, which in turn depends on the bulk ionic concentration. For small Debye lengths (high salt concentration), the profile is nearly flat over most of the pore cross-section.

The ionic current density due to the EOF can be obtained as the product of the net charge density and the \( v_{\text{EOF}} \)

\[ j_{\text{EOF}}(r) = v_{\text{EOF}}(r) \rho(r) \]  

(3)

\[ j_{\text{EOF}}(r) = \frac{\varepsilon \varphi_0 \kappa^2 E_z I_0(kr) I_0(kR)}{\eta I_0(kR)} \left[ 1 - \frac{I_0(kr)}{I_0(kR)} \right] \]  

(4)

Figure S6b shows the profile of the current density due to the EOF for \( \varphi_0 = -50 \text{ mV} \) and \( V = 300 \text{ mV} \). As the figure shows, this current, as well as \( j_{\text{DL}} \), is only present near the cylinder walls. For comparison, the magnitude of \( j_{\text{bulk}} \) for the above conditions is only 0.15 nC/nm². Keeping in mind that the magnitude of \( j_{\text{EOF}} \) scales with the square of the wall potential, it is not difficult to see how the surface currents can quickly overcome the bulk current for sufficiently high surface potentials.
For the bulk current, \( I_{\text{bulk}} \), we assume a constant value of \( j_{\text{bulk}} \) across the cylinder. As previously mentioned, this approximation undoubtedly fails within a few Debye lengths of the pore walls, but since \( \kappa^{-1} \ll R \) it will be accurate over the bulk of the volume in question and therefore will not introduce significant error to the total calculated current. Thus:

\[
I_{\text{bulk}} \approx en_0KCl(\mu_K + \mu_{Cl})\pi R^2E_z
\]  

(5)

\[
I_{DL} = \mu_K E_z \int_0^R \rho(r)2\pi r dr = 2\pi \varepsilon \kappa \mu_K \varphi_0 RE_z \frac{I_1(kR)}{I_0(kR)} \approx 2\pi \varepsilon \kappa \mu_K \varphi_0 RE_z
\]  

(6)

\[
I_{EOF} = \int_0^R \nu_{EOF}(r) \rho(r)2\pi r dr = \frac{2\pi \varepsilon \kappa \varphi_0^2 RE_z}{\eta} \left( \frac{I_1(kR)}{I_0(kR)} \right) - \frac{1}{2} R \kappa \left[ 1 - \left( \frac{I_1(kR)}{I_0(kR)} \right)^2 \right] \approx \frac{\pi \varepsilon \kappa \varphi_0^2 RE_z}{\eta}
\]  

(7)

For \( I_{DL} \) and \( I_{EOF} \) we use the approximation that \( I_0(kR) \approx 1 \) for \( \kappa^{-1} \ll R \). The resulting total ionic current as a function of surface potential \( \varphi_0 \) is then given by

\[
I_{\text{total}} \approx en_0KCl(\mu_K + \mu_{Cl})\pi R^2E_z + 2\pi \varepsilon \kappa \mu_K \varphi_0 RE_z + \frac{\pi \varepsilon \kappa \varphi_0^2 RE_z}{\eta}
\]  

(8)
It is worth noting that even though $l_{dl}$ and $l_{EOF}$ are nonzero only within the electric double-layer, their combined contribution for $\varphi_0 \approx -50$ mV is equal to a third of the bulk, $\sigma$-independent current $I_{bulk}$, and is therefore quite significant even at low $\varphi_0$.

To relate the nanopore current to the laser power we must first relate the surface charge density $\sigma$ to the laser power $P$. As a first approximation, we assume a linear relationship between them. We will now introduce a proportionality constant, $\gamma$, to relate both quantities

$$\sigma = \gamma P$$  \hspace{1cm} (9)

Finally, the relationship between $\sigma$ and $\varphi_0$ can be obtained from the modified Grahame equation. If the distribution of mobile charges in the solution is described by the Poisson-Boltzmann equation as proposed above, then the charge of an isolated, curved surface satisfies the relationship [5]

$$\sigma(\varphi_0) = \frac{2 \varepsilon \kappa k_B T}{e} \left[ \sinh \left( \frac{e \varphi_0}{2 \kappa k_B T} \right) + \frac{2}{\kappa \kappa R} \tanh \left( \frac{e \varphi_0}{4 \kappa k_B T} \right) \right]$$  \hspace{1cm} (10)

which is known to give the surface charge density to within 5% for $\kappa R > 0.5$ and any surface potential. In our case, $\kappa R = 9.8$ and $2/\kappa R \approx 0.2$, so we can safely discard the term containing the $\tanh$ in favor of the term with $\sinh$. Solving for $\varphi_0$ in terms of $\sigma$ and substituting it into Eq.8 gives the final equation

$$I \approx e n_{kCl}(\mu_k + \mu_{Cl}) n R^2 E_z + \frac{4 \pi e \kappa \mu_k \sinh^{-1} \left( \frac{\beta e y P}{2 \varepsilon k} \right) R E_z}{\beta e} + \frac{4 \pi e^2 \kappa \mathbb{E} (\sinh)^{-1} \left( \frac{\beta e y P}{2 \varepsilon k} \right) R E_z}{\eta \beta^2 e^2}$$  \hspace{1cm} (11)

If one knows the pore radius $R$ from TEM images, Eq.11 has only one free fitting parameter, namely $\gamma$. All other parameters can be either found in the literature or are set by the experimental conditions. Table S1 displays the values used for each parameter when fitting our data.

**Table S1**  Values for the parameters necessary to fit experimental data using Eq.11.

| Parameter | Value in SI units |
|-----------|-------------------|
| $e$       | $1.60218 \times 10^{-19}$ C |
| $n_{kCl}$ | $6.02214 \times 10^{26}$ m$^{-3}$ (1 M KCl) |
| $\mu_k$   | $7.616 \times 10^{-8}$ m$^2$/V.s |
| $\mu_{Cl}$| $7.909 \times 10^{-8}$ m$^2$/V.s |
| $E_z$     | $10^7$ V/m (V = 300 mV, $L = 30$ nm) |
|       |        |
|-------|--------|
| $\epsilon$ | $7.0834 \times 10^{-10} \text{ F/m}$ |
| $\theta$ | $2.4636 \times 10^{20} \text{ s}^2/\text{m}^2\text{kg} \ (T = 21 \ ^\circ\text{C})$ |
| $\kappa$ | $3.2736 \times 10^{9} \text{ m}^{-1}$ |
| $\eta$ | $7.43 \times 10^{-4} \text{ Pa.s}$ |

Eq.11 is expected to fit the data of $I$ vs. $P$ for values of $\sigma < 200 \text{ mC/m}^2$, after which the model should begin to underestimate the data. This is a result of the approximation used at the beginning of the model, which would underestimate the exact solution for $\varphi_0 > |{-50}| \text{ mV}$, or surface charge densities of around 150 mC/m$^2$ (see Eq.10). Figure S6 shows the plots of $I(P)$ corresponding to the five nanopores used to acquire translocations, together with their respective fits to Eq.11 and the extracted photo-reactivity $\gamma$. In all cases we find that the model fits the data remarkably well within its range of validity, after which it underestimates the data. As can be seen in the figure, this is indeed the case for the 5.4 nm and the 5.6 nm pores ($\gamma = 46$ and $29 \text{ C m}^2\text{W}^{-1}$, respectively). The 6.1 nm and 7.4 nm pores ($\gamma = 8$ and $18 \text{ C m}^2\text{W}^{-1}$) are always kept within the validity range of the model due to their low $\gamma$ value. The
exception to the rule is the fit to the highly reactive pore 4.3 nm ($\gamma = 58 \, \text{C m}^{-2} \, \text{W}^{-1}$), which clearly overestimates the data when $\gamma \cdot P > 150 \, \text{mC.m}^{-2}$. We believe that this is due to a different effect, pertaining to the mechanism by which light induces surface charges. For highly reactive pores (i.e. pores with smaller band gaps), we expect the population of chargeable Si dangling bonds to show a saturating effect with increasing laser power [6, 7]. Supporting this interpretation is the PL intensity plot as a function of $P$, which shows a similar saturating curve, only seen in highly reactive pores (data not shown).

5. Salt concentration dependence of the nanopore current at different laser intensities.

Figure S8 displays the dependence of the nanopore open current measured ($V = 300 \, \text{mV}, \, d = 8 \, \text{nm}$) as a function of laser power at three KCl concentrations: 0.01 M, 0.1 M and 1 M. These measurements were obtained from the same nanopore. The nanopore currents were fitted to Eq. 11 using a single $\gamma$ value of 80 C m$^{-2}$ W$^{-1}$, and the parameters in Table 1. The error bars represent standard deviation to the current levels measured at a bandwidth of 100 KHz.
6. Nanopore current noise analysis as a function of laser illumination.

The blue curves in Figure S9 show the dependence of the RMS values of the open pore currents, measured at $V = 300$ mV and 100 KHz bandwidth, as a function of laser intensity, at three different KCl concentrations (0.1 M, 1 M and 2 M, from top to bottom respectively). In all cases we see a nearly linear increase of $I_{\text{RMS}}$ with laser power. Note that the ratio of $I_{\text{RMS}}$ to the open pore current decreases with laser intensity for all salt concentrations measured (red curves) due to the fact that the open pore current grows faster with laser intensity than the noise.

**Figure S9** Measurement of the open pore RMS current noise ($V = 300$ mV) as a function of laser power and KCl concentration (0.1 M, 1 M and 2 M, from top to bottom). In all cases we observe an increase of the RMS current noise with laser power (blue markers and lines on left axes), and a decrease of the ratio of the RMS noise with the open pore current (red markers and line on right axes).

7. Ubiquitin expression and purification protocol.

The wild type ubiquitin gene was cloned into pET3a vector and transfected by electrophoresis into BL21 E. Coli cells. Cell culture was grown in LB medium with AMP antibiotic for selective expression. Induction of expression of the protein was done using IPTG. Later, cells were lysed and the cell's
proteins were precipitated using HClO₄ excluding Ubiquitin and other supernatant dissolved proteins. Then the solution was filtered, dialyzed and ran through a cation exchange column and eluted at ~150 mM NaCl. Purity of the sample was confirmed using gel electrophoresis against a protein ladder.

8. Nanopore photo-reactivity and e-beam exposure

Four nanopores having similar diameters were drilled using TEM in a freshly deposited SiN membrane. Two nanopores were drilled by exposing the membrane for 60 s, and two were drilled with 500 s of exposure to produce different local N/Si stoichiometry. These pores were assembled in our confocal microscope and their surface charge densities were measured by fitting our model to their open pore current versus laser intensity curves. Figure S10 shows the measured current versus laser power and the corresponding fits. The data suggests that the pores drilled with a low e-beam dose (Figure S10a) had relatively low photo-reactivity (γ = 27 C m⁻² W⁻¹), whereas the pores exposed to a high e-beam dose (Figure S10b) had much higher photo-reactivity (γ = 70 C m⁻² W⁻¹). This establishes a direct correlation between e-beam dosage and the optical reactivity of a nanopore.

![Figure S10](image)

**Figure S10** Measurement of the photo-reactivity of four similar nanopores drilled by exposing the SiN membrane to the e-beam for either 60 s (left, empty symbols) or 500 s (right, solid symbols). The model presented in section 4, was used to determine the pores photo-reactivity by fitting Eq. 11 to the date (the open pore current as a function of laser intensity).

9. Nanopore Drilling Method

The solid-state nanopores used in this study were fabricated in a 30 nm thick low-stress silicon nitride (SiN) membranes deposited by LPCVD (CNS Harvard). Nanopores were drilled through the SiN using a highly focused transmission electron microscope beam (JEOL 2010F TEM, 10⁸ – 10⁹ e⁻/nm² s, 200 keV) to
sputter away material from the thin membrane [8]. Some pores were exposed for either 60 s or 500 s to alter the local N/Si stoichiometry. To adjust these nanopores to their final diameters, ion beam sculpting was performed by broadening the e-beam slightly ($\sim 10^6$ e/\(\text{nm}^2\) s) to induce pore shrinking as previously described [8].

**10. Confocal Optical Nanopore Setup**

Figure S11 shows a schematic diagram of the optical setup used for all experiments described in this work. An expanded beam from a 532 nm laser (New Focus) was focused onto the SiN$_x$ membrane using a water immersion, 1.2NA, 60X objective (Olympus UPlanApo) and a closed-loop XYZ nano-positioner (Physik Instrumente) to ensure the continuous alignment. The final power was continuously measured using an amplified photo-diode (Thorlabs), and adjusted using motorized half-wave plates after the beam had been linearly polarized. The emission side consisted of a long pass filter (Semrock) and a CCD camera (Thorlabs) for alignment. Nanopore electrical voltage and current were amplified using an Axoptach 200B (Molecular devices), and sampled using a 16bit Daq board (National Instrument). The piezo stage positions were directly read from the PI controller during scanning using a fast digital board interface (National Instrument) synchronized with the Analog to Digital acquisition. Data acquisition was performed with a custom-written LabView program (National Instruments), and analyses were done using LabView and Matlab (MathWorks).

![Figure S11](image-url) – Diagram of the setup with all the optical components. See Methods section in main text for full description.
11. Nanopore unclogging using laser pulses

Solid-state nanopores that became clogged during a translocation experiment could be cleared by applying a brief, ~5 mW laser pulse (532 nm) centered at the pore location. As shown in Figure 1c (main paper) the pore returns immediately to its open current level and remains stable. The RMS noise of the unclogged pores was nearly identical before and after clearing. This method was routinely applied between 10-50 times during a translocation experiment (when voltage reversal was not sufficient) to keep the pore open for the whole day. Pores were stored overnight in a high humidity environment, and the process would be repeated the following day. In this way, pores were routinely kept functional for weeks at a time. Eventually, the pore would degrade or become permanently blocked overnight and re-cleaning with piranha solution would become necessary.

On the other hand, if a nanopore was not immediately open after being cleaned with piranha, the optoelectronic unclogging method was only 10% efficient (n > 50). We believe that this is due, in part, to the difficulty in aligning the pore with the focused laser beam without counting with an ionic signal to locate it. A fine raster scan, as shown in Figure 1c (main paper), was sometimes effective in unclogging the pore in these situations.

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