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

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Abstract

Nanopores can be used to detect and analyse biomolecules. However, controlling and tuning the translocation speed of molecules through a pore is difficult, limiting the wider application of these sensors. Here we show that low-power visible light can be used to control surface charge in solid-state nanopores and can influence the translocation dynamics of DNA and proteins. We find that laser light precisely focused at a nanopore can induce reversible negative surface charge densities as high as 1 C/m^2 , and that the effect is tuneable on sub-millisecond timescales by adjusting the photon density. By modulating surface charge, we can control the amount of electro-osmotic flow through the nanopore, which affects the speed of translocating biomolecules. In particular, a few mW of green light can reduce the translocation speed of double-stranded DNA by more than an order of magnitude and the translocation speed of small globular proteins such as ubiquitin by more than two orders of magnitude. The laser light can also be used to unclog blocked pores. Finally, we discuss a mechanism to account for the observed optoelectronic phenomenon.

Introduction

The manipulation of electrical surface charge in a rapid and reversible manner is of great importance for a wide variety of biosensing applications, since electrostatic interactions influence the affinity and residence time of biological molecules in the sensing zone.¹

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Author Contributions

N.D.F. and A.M. conceived and designed the experiments; N.D.F., D.B. and T.G. performed the experiments; A.S. drilled all pores; N.D.F., A.S., D.B., T.G. and A.M. analyzed the data; N.D.F., T.M. and A.M. developed the model; N.D.F., A.S., D.B., T.G., T.M. and A.M. co-wrote the paper.

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Charge manipulation in *nanoscale* sensors, such as nanopores, nanochannels and nanotubes,²⁻⁵ is particularly useful because of the scale of these devices. Solid-state nanopores are a prototypical class of nanoscale sensors, employing electrophoretic forces to thread and translocate charged single nucleic acids or proteins through a nanoscale aperture in an ultrathin membrane.⁶⁻⁸ Nanopore surface charge can affect its direct, Coulombic interactions with charged biomolecules, and can indirectly influence sensing by generating electro-osmotic flow. This net flow can modulate molecular capture rate⁹ and molecular residence time in the sensing vicinity via hydrodynamic drag.^{10,11} This effect has previously been characterized using optical tweezers¹² and by varying the nanopore's zeta potential by changing the solution's pH.¹³

The ability to modulate the surface charge of a nanopore and, consequently, the associated electro-osmotic flow could allow control of biomolecule translocation speed. This could in turn lead to the development of advanced single molecule, low-cost characterization techniques for a broad spectrum of clinical samples, including genomic DNA, RNAs and proteins.¹⁴ This challenge must be met without compromising the ability of the sensor to probe extremely dilute analytes in a reasonable time frame.¹⁵ Recent efforts towards this goal have primarily involved the fabrication of metallic electrodes at the nanopore surface to induce an electrical field.^{16,17} Yet to date, there has been no experimental report of *in situ*, opto-electrical manipulation of high-density surface charge in nanopores or other nanoscale sensors.

We show here that an off-the-shelf, low-power visible laser beam can be used to directly manipulate the surface charge of a solid-state nanopore. The ability to dynamically increase surface charge has three immediate useful consequences. First, it allows the translocation speed of DNA to be tuned without chemically modifying the nanopore surfaces or altering the buffer properties. Second, it can be used to reliably unblock clogged nanopores, keeping them functional over prolonged periods of time. Third, it permits the detection of extremely low molecular weight proteins, which otherwise are nearly invisible to the resistive pulse sensing technique.

The photo-conductive phenomenon in solid-state nanopores

A silicon nitride membrane containing a single nanopore was scanned in a custom-made confocal microscope with a tightly-focused green laser (532 nm), as illustrated in Figure 1a. The continuously recorded ionic current (I) flowing through the pore at a fixed voltage reveals an increase when the laser spot overlaps with the nanopore location. Figure 1b illustrates this effect with an intensity surface plot of the ionic current I flowing through this pore as a function of laser spot position. A line scan through the image reveals a clear symmetric peak in the ionic current, more than double the baseline current level (FWHM ~500 nm, right panel).

This effect is evident in all tested pores (4-20 nm diameter) even for laser powers of just a few mW. Although a tightly focused IR laser at high power (~1 W) can produce a small increase in current by locally heating the solution,¹⁸ laser-induced heating can be ruled out here for two reasons: the electromagnetic absorption of water at 532 nm is extremely

small,¹⁹ and the laser powers used in our experiments are only a few mW. A temperature increase of $\sim 35^\circ\text{K}$ would be required to produce the observed increase in I , yet heating is estimated to account for a mere 0.005°K per mW of focused light based on measurements performed at 800 nm.²⁰ We thus conclude that direct interaction of light with silicon nitride must cause this increase in the ion current via a *photo-conductive effect*.

Permanent nanopore blocking by small molecules, tiny air bubbles, or other nanoscale particulates is a common limitation for nanopore sensors, severely reducing their functional lifetime. In some cases, a series of electrical pulses clears the pore,²¹ but often the system must be disassembled and re-cleaned. We find that in many of these cases a short exposure to a ~ 5 mW focused laser beam will immediately clear the nanopore. Figure 1c (left) shows a raster scan (bottom left to top right) of a fully blocked nanopore ($I \approx 0$ at $V = +300$ or -300 mV). As soon as the laser beam reaches the nanopore location, the pore is cleared. Thereafter, I stabilizes at the open pore level of 12 nA. This is shown chronologically (Figure 1c, right) as a time trace of I during the laser scan. We have found that light-induced nanopore unblocking is a highly robust and efficient tool to extend the functional lifetime of solid-state nanopores from a few hours to several days, as detailed in the Supporting Information (SI).

Light-induced retardation of DNA and proteins

Over 1,000 translocation events of 10 kbp DNA were acquired in a nanopore (5.4 nm) in the dark (laser power $P=0$). At $t=150$ s the laser radiation was switched on ($P=2$ mW, pre-aligned with nanopore) as the software continued to record translocation events. Representative events before, during, and after illumination are shown in Figure 2a. Three features are immediately apparent: (i) the open pore current (I_O) and the current during translocation (I_B) both increased when the laser was turned on, (ii) the average event amplitude $I = I_O - I_B$ remained at the same level of 0.78 ± 0.10 nA under both conditions, and (iii) the mean event dwell-time t_D increased by roughly a factor of 10 under illumination, as compared to dark conditions. These features are illustrated in Figure 2b, which shows I_O and I as a function of time throughout the experiment (top panel). A 150-event running window average over all translocations (bottom panel) indeed shows a tenfold increase in the translocation time from ~ 1 ms to ~ 10 ms under laser illumination. We define the Retardation Factor (RF; here $\text{RF}=10$) as the increase in mean translocation time under laser light relative to darkness. Upon switching off the laser illumination at $t=300$ s, I_O returned to base level (3.6 nA) and the mean translocation dwell time was restored to ~ 1 ms, suggesting that this effect is completely *reversible*. This experiment was repeated for nanopores of various sizes, and in all cases we observed a marked retardation of translocation time under laser illumination (see SI for analysis).

We next tested the photo-conductive effect for analytes other than DNA. Previous studies have sensed proteins such as bovine β -lactoglobulin or avidin^{13,22} in solid-state nanopores, and antibodies such as anti-biotin Fab fragments or anti-biotin in lipid coated nanopores.²³ However, the detection of small proteins (MW < 10 kDa) in their native state remains a major challenge, since these proteins translocate through the pore too quickly to produce a resolvable signal given current bandwidth limitations.²⁴

We selected ubiquitin (MW ~ 8.5 kDa, 4 nm diameter) as a representative small protein, relevant to a broad range of biological processes,²⁵ to demonstrate that the photo-conductive effect can slow small protein translocations and enable their detection and characterization. Under dark conditions, purified wild-type ubiquitin (see SI) translocating through a ~5 nm pore yielded sporadic, brief downward spikes (red trace, Figure 2c). The dwell time of these spikes was estimated to be <12 μ s, but neither this nor the current amplitude of ubiquitin translocations could be accurately determined due to bandwidth constraints even at 100 kHz,²⁶ suggesting that the true translocation dwell time of these proteins is shorter than estimated.

Laser illumination of the same nanopore at $P=4$ mW resulted in a marked increase in both the frequency and average dwell time of detected ubiquitin events (blue trace, Figure 2c). The average translocation time increased by at least two orders of magnitude, allowing full characterization of both amplitude and dwell times for ubiquitin translocations. The increase in event rate under illumination may be attributed to events that were previously undetectable under dark conditions due to bandwidth limitations. As with DNA translocations, this effect was completely reversible.

A typical dwell time distribution and fractional blockade current (I_B) for illuminated ubiquitin translocations are shown in Figure 2c ($N > 500$). Two prominent timescales are observed for ubiquitin translocation (340 ± 5 and 890 ± 70 μ s), as well as two peaks in the blockade currents (0.88 and 0.78), approximated by a sum of two Gaussians. We hypothesize that the two peaks observed in these distributions may be associated with different orientations of the asymmetric “mushroom-like” ubiquitin (inset) during translocation. This hypothesis is consistent with previous reports for other protein/DNA complexes.^{27,28}

Light-induced modulation of electro-osmotic flow

To elucidate the physical origin of light-induced retardation of translocation, we first characterized the pore current I at varying laser intensity P for a range of nanopore sizes (4-20 nm diameter, d). Figure 3a displays ionic current enhancement ($I(P)/I(0)$), for four representative pores ($d = 5, 10, 15, 20$ nm). In all cases I is linear for $P < 5$ mW, above which I deviates slightly from linearity. Linear fits for $P < 5$ mW characterize the response of each pore to light as the initial slope $\delta I / \delta P$. A plot of $\delta I / \delta P$ as a function of diameter for 14 pores (4-20 nm) exhibits a clear linear dependence on d (Figure 3b). This suggests that when the SiN_x thickness L and nanopore drilling conditions are maintained, the pores response to light per their surface area (πdL) is a constant. From Figure 3b we obtain a value of 2.2×10^{-3} nA mW⁻¹ nm⁻².

The linear dependence of $\delta I / \delta P$ on nanopore diameter suggests that photo-conductance current enhancement is a surface phenomenon rather than a volumetric one (which would exhibit $\propto d^2$ dependence). Solid-state nanopores possess weak surface charge, which can dominate pore conductivity at salt concentrations lower than ~100 mM.²⁹⁻³¹ However, it has been theorized that high charge densities on nanopore walls can affect electrical conductivity even under high salt conditions.^{32,33} Surface charges induce a diffuse double layer containing an excess of oppositely charged ions, of thickness comparable to the Debye

screening length κ^{-1} . Ionic current is enhanced by the electrical double layer in two ways: (i) by creating a local imbalance of counter ions within κ^{-1} , which (ii) drags water molecules with its motion, creating an electro-osmotic flow (EOF) which in turn pulls along additional current (see Figure 4a). The total ionic current for a nanopore with a charged surface is:

$$I = I_{bulk} + I_{DL} + I_{EOF} \quad (1)$$

where I_{bulk} is the current due to ions outside the electrical double layer, I_{DL} is the current due to ions forming the double layer, and I_{EOF} is the current due to net water flow induced by net movement of ions within the double layer. I_{EOF} is proportional to the product of the flow velocity field and the net charge density $\rho(r)$, which is only nonzero within κ^{-1} . For small κ^{-1} compared to the pore diameter, (i.e. $\kappa^{-1} \approx 0.3$ nm at 1M KCl at 21°C), only I_{bulk} scales with cross sectional area ($I_{bulk} \propto \pi d^2$) whereas I_{DL} and I_{EOF} scale with circumference ($I_{DL}, I_{EOF} \propto \pi d \kappa^{-1}$). Thus, surface charges can, in principle, explain increased I as well as its linear scaling with d . Moreover, if the analyte remains mostly within the bulk pore volume where there is no charge imbalance, κ^{-1} distance away from the pore walls, we expect I to be independent of surface charge – and thus independent of P . This is consistent with our observations (Figure 2).

Previous theoretical work has predicted that surface charge on a nanopore will affect (i) translocation times^{10,11} and (ii) polymer capture rate.^{9,34} A negatively charged nanopore produces an EOF towards the cathode (*cis* chamber, Figure 4a), increasing drag on a negatively charged translocating polymer, resulting in longer translocation times and reduced polymer capture rates. Conversely, positive surface charges would enhance capture rate and reduce translocation times. Our results (Figure 2) indicate that the RF for DNA translocation grows with laser intensity. Moreover, DNA capture rate was reduced by 30%, from 11 s⁻¹ to 8 s⁻¹, for 2 mW of laser light (see SI). These observations lead us to hypothesize that visible light can induce negative surface charges on silicon nitride, which in turn slows DNA translocation through the drag created by EOF moving in the opposite direction of DNA.

To validate this hypothesis we approximate that for low laser powers the surface charge density (σ) grows linearly with laser intensity, such that $\sigma = \gamma P$ where γ is the photo-reactivity of the pore (C m⁻² W⁻¹). The total ionic current I can be obtained as a function of surface charge density using the following three individual contributions (see SI for full derivation): (i) I_{bulk} is the electrophoretic movement of each ion species through the nanopore; (ii) I_{DL} may be determined from the net charge distribution ρ for a given wall potential, derived analytically from the Poisson-Boltzmann equation and from the Boltzmann distribution, assuming a cylindrical nanopore and using the Debye-Hückel approximation;⁹⁻¹¹ (iii) I_{EOF} is a product of ρ and the EOF velocity profile described by the Navier-Stokes equation. The Grahame equation may be substituted to give σ as a function of wall potential.³⁵ The contributions to the ion current are:

$$I_{bulk} \approx en_{KCl} (\mu_K + \mu_{Cl}) \frac{\pi d^2}{4L} \Delta V \quad (2.1)$$

$$I_{DL} \approx \frac{2\pi d \varepsilon \kappa \mu_K}{L} \sinh\left(\frac{\beta e \gamma P}{2\kappa \varepsilon}\right) \Delta V \quad (2.2)$$

$$I_{EOF} \approx \frac{2\pi d \kappa \varepsilon^2}{L \eta \beta^2 e^2} \left[\sinh^{-1}\left(\frac{\beta e \gamma P}{2\kappa \varepsilon}\right) \right]^2 \Delta V \quad (2.3)$$

for elementary charge e , permittivity of aqueous solution ε , parameter $\beta = 1/k_B T$ (Boltzmann constant k_B , absolute temperature T), pore length L , applied voltage clamp ΔV , and solution viscosity η . The number density of potassium or chloride ions is n_{KCl} , with electrophoretic mobilities μ_K and μ_{Cl} (see SI for numerical values). Nanopore diameter d can be determined from TEM images, enabling a quantitative estimation of the nanopore photo-reactivity γ via Eq.2 (see SI Figure S7). We additionally measured the dependence of I on KCl concentration (0.01 M to 1 M) at different P and found it consistent with the model (see SI Figure S8).

Additional DNA translocations were performed using a range of DNA lengths, nanopores, and laser powers to determine the role of surface charge in slowing translocation speed. The results are summarized in Figure 4b (left), where RF is plotted against P for five nanopores (4.3, 5.4, 5.6, 6.1, and 7.4 nm diameters) and four DNA lengths (0.4, 3.5, 5, and 10 kbp). t_D was measured for >1,000 events per laser intensity, and the t_D histograms were fitted with exponential functions as previously described.²⁶ These measurements suggest that RF per mW of light widely varies with pore size and DNA length.

Fitting Eq. 2 as described above to obtain γ for each of the pores, we then transform the DNA RF into surface charge density σ using the relationship $\sigma = \gamma P$. Remarkably, all data points collapse onto a single curve (Figure 4b, right). Thus, light-induced slowing is dependent only on the induced surface charge density σ . Controlling the RF value can be achieved either by selecting a highly optically reactive pore (high γ) under low laser intensity, or using a less optically reactive pore under higher laser intensity.

Nanopore photo-reactivity depends on e-beam exposure during the formation of the nanopore

Drilling nanopores in SiN_x with an electron beam reduces the N/Si ratio around the pore due to preferential ablation of N atoms.³⁶ Moreover, the principal defects in silicon-rich CVD amorphous SiN_x materials are Si dangling bonds, which can trap electrons or holes to become charged.³⁷⁻³⁹ When the N/Si ratio approaches 0.8, the band gap is within visible light energies.^{39,40} When the N/Si ratio of the material surrounding the pore is low enough, we propose that visible light can excite electrons from the ground state across the band gap, trapping them in Si dangling bonds.⁴¹ Eventually, trapped electrons would recombine with holes, but a high density of arriving photons could maintain a steady state of negatively charged Si dangling bonds, creating a net surface charge density.

We TEM-drilled four additional nanopores, all with similar diameters, in a freshly deposited SiN_x membrane. Two were exposed to the e -beam for 60 s, and two were exposed for 500 s, producing different local N/Si stoichiometry (see SI).⁴² As expected, the pores drilled with a

low e -beam dose had relatively low photo-reactivity ($\gamma=27 \text{ C m}^{-2} \text{ W}^{-1}$), whereas the pores exposed to a high e -beam dose had much higher photo-reactivity ($\gamma=70 \text{ C m}^{-2} \text{ W}^{-1}$), establishing a direct correlation between e -beam dosage and optical reactivity.

Conclusions

We have shown that just a few mW of visible laser light focused on a nanopore can induce surface charge densities up to $\sim 1 \text{ C m}^{-2}$, much larger than the weak native charge of SiN_x . Increased σ produces net charge near the pore walls, which, under external voltage, creates an electro-osmotic flow moving in opposition to an anionic translocating molecule. This flow slows translocating DNA and proteins, allowing detection and characterization of small analytes, such as ubiquitin. Previous approaches to slowing analyte translocation have introduced permanent modifications to the nanopore walls, inducing stronger interactions of analytes with the pore surface during translocation.^{13,23,43,44} By contrast, the optoelectronic effect allows completely reversible, *in-situ* control of σ and translocation speed without permanently altering the surface properties of the nanopore or buffer.^{45,46} This is particularly useful for detection and characterization of molecules in their native form. Moreover, ultra-fast tuning of surface charge by light completely decouples the capture process from subsequent translocation. Solid-state lasers, as well as other light sources, can be readily switched on/off in sub- μs timescales, and hence induce current jumps in the nanopores within milliseconds (see SI). The dynamic range of the photoconductive effect is determined by local N/Si stoichiometry, which in turn depends on e -beam exposure during nanopore drilling. We predict that this method will facilitate a broad range of emerging nanopore sensing applications, including genotyping using Peptide Nucleic Acid (PNA) probes⁴⁷ and quantification of epigenetic markers.^{48,49}

Ultra-fast and reversible modulation of surface charge is fundamentally important for the development of novel nanosensors. By exploiting the intrinsically high sensitivity of the nanopore ionic current to small modulations of surface charge, we were able to explore and characterize a new optoelectronic effect, model the increase in electric conductance under visible laser illumination, and propose an underlying mechanism. We have shown here three useful applications of the technique: (i) clearing blocked nanopores and thus significantly extending their useful lifetime; (ii) slowing and tuning the translocation speed of DNA; and (iii) detecting and characterizing translocation events of very small proteins, such as ubiquitin. Together, these milestones broaden the range of molecules that can be probed by solid-state nanopores, enhance nanopore sensitivity, and extend the lifetime and throughput of individual pores.

Methods

Nanopores were drilled on low-stress (silicon-rich), amorphous LPCV-deposited SiN_x membranes 30 nm thick. Chips were then cleaned using Piranha solution and kept in water until mounted on the Teflon holder, rendering two independent chambers connected by the nanopore and filled with an electrolytic solution (1M KCl, 20 mM TRIS, pH 8.0, 21° C). A pair of Ag/AgCl electrodes was used to apply an electrostatic potential difference across the chambers separated by the insulating SiN_x membrane. The resulting ionic current through

the nanopore was recorded using a patch-clamp amplifier (Axopatch 200B, Molecular Devices Cooperation). The whole apparatus was shielded from external electromagnetic noise by a Faraday cage. The electrical readout from the amplifier was filtered with a 10 kHz low-pass Bessel filter and digitized using a 16 bit A/D card (National Instruments) controlled by a custom-written LabView (National Instruments) program (see SI for a detailed diagram of the apparatus).

The nano-chip was secured to a closed loop nano-positioner (Physik Instrumente) with sub-nm accuracy, also controlled by the LabView program. The nano-positioner was mounted in a custom-built confocal setup, in order to illuminate the nanopore with a focused laser beam. A 532 nm line from a laser diode (New Focus) was cleaned by a Glan-Thompson polarizer (Thorlabs), with the final power adjusted on demand by a half-wave plate mounted on a motorized rotating holder. This resulted in a final dynamic range of 2 μ W to 17 mW with 500 intermediate values. The laser beam was expanded to completely fill the back aperture of a 60 \times , 1.2 NA water immersion objective (Olympus UPlanApo). The emission side contained a long-pass filter (Chroma HQ560LP) to remove any elastically scattered light, followed by a motorized mirror that acted as a shutter for the APDs and allowed direct visualization of the membrane using a CCD camera (Thorlabs DC111).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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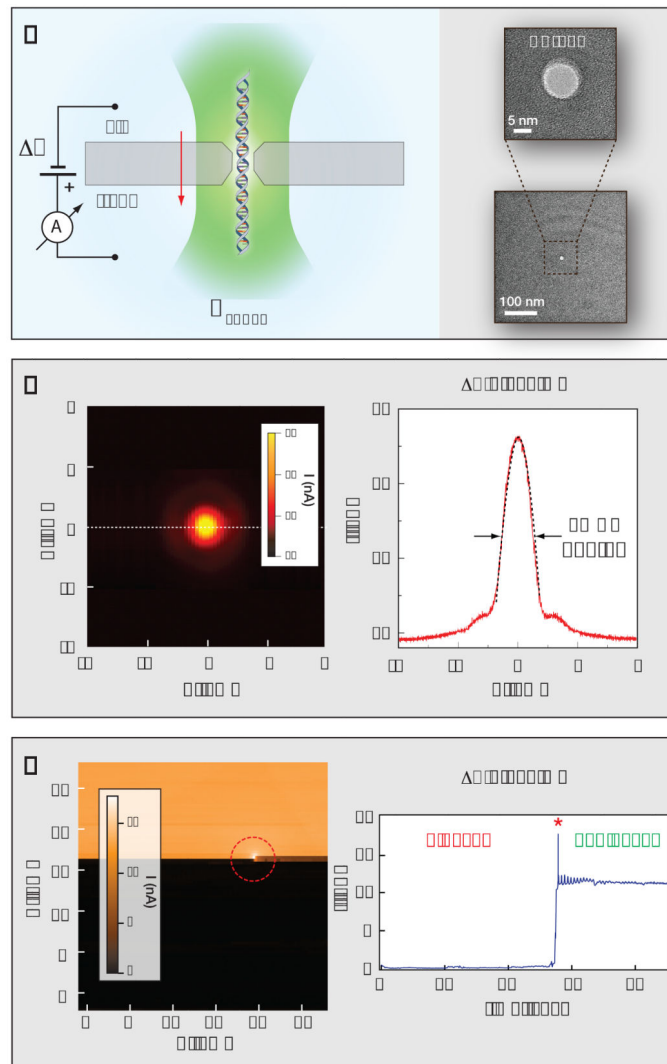


Figure 1. The photo-conductive effect in solid-state nanopores

a) Cartoon illustrating the optical nanopore setup. A solid state nanopore is positioned at a laser beam focus by a nano-positioner, and the ion-current flowing through the pore is measured before and during translocation of DNA molecules. The inset shows a high-resolution Transmission Electron Micrographs of a typical pore (10 nm). b) Left: surface plot showing nanopore ionic current enhancement as a 10 mW focused laser beam ($\lambda = 532$ nm) scans the $4 \times 4 \mu\text{m}^2$ SiN membrane at $1 \mu\text{m/s}$. When the focused beam reaches the nanopore it produces a significant increase in measured current (2.4-fold increase for this pore). Right: line profile through $Y=0$, fitted using a Gaussian function with FWHM of ~ 500 nm, consistent with the diffraction limit. c) Clearing a blocked solid-state nanopore with light. Left: a 5 mW laser intensity raster scan of the entire SiN window ($30 \times 30 \mu\text{m}^2$, lower left to top right). The color map represents the current flowing through the pore at $V = 300$ mV. As soon as the laser beam overlaps with the nanopore location, it clears the pore and thereafter the ionic current stabilizes at the open pore level of 12 nA. Right: time trace of I during the laser scan.

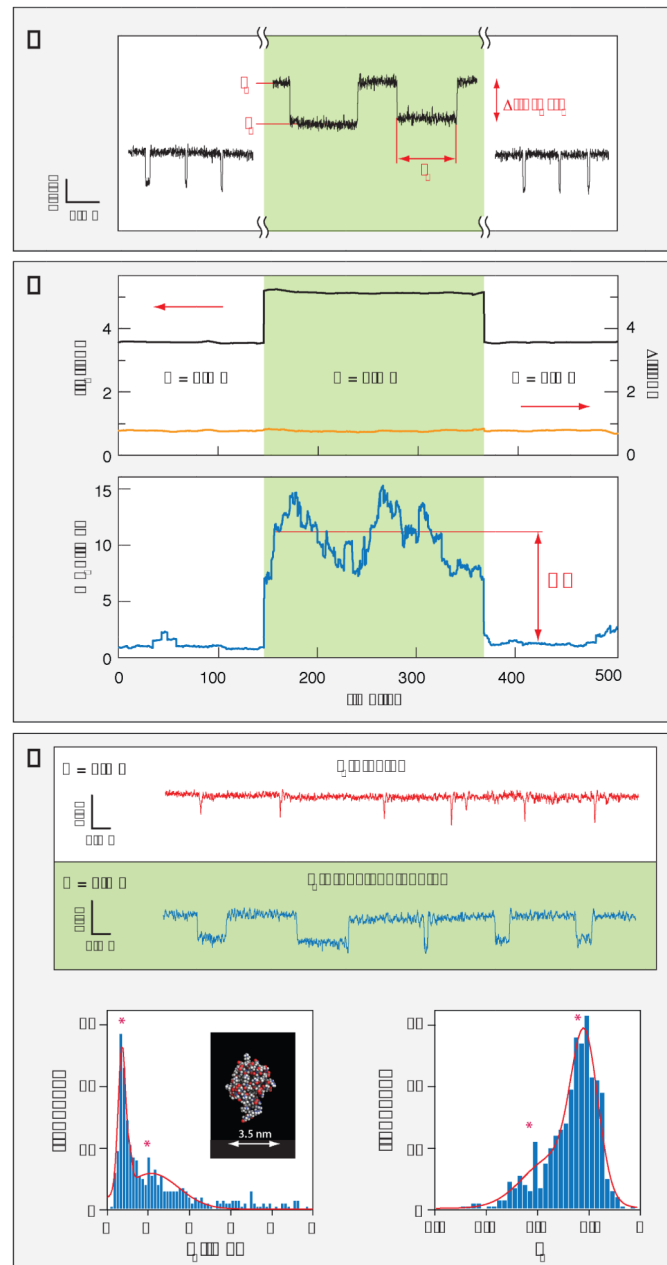


Figure 2. Slowing down DNA and protein translocation speed with light

a) Representative translocation events with and without illumination, showing the increase in I_O , I_B and t_D . b) Time traces showing the open pore current I_O , the blocked current amplitude I_B (top panel), and the mean translocation time $\langle t_D \rangle$ (lower panel). The net effect of the laser illumination is to increase I_O and t_D while keeping I_B constant. All data points in $\langle t_D \rangle$ represent a running average over 150 translocation events, initialized at the moment the laser is switched on/off. The Retardation Factor, RF, defined as the mean t_D with light divided by the mean t_D in darkness. In this example a factor of ~ 10 is obtained with $P = 2$ mW. c) Detection of the small molecular weight protein ubiquitin in its native state using a 5 nm nanopore, enabled by illumination of the chip with laser light. Typical translocation time

traces are shown at $P = 0$ (red) and $P = 4$ mW (blue). With $P = 4$ mW we observe at least two orders of magnitude increase in the events' dwell times. A typical translocation time distribution and fractional blockade current (I_B) of the ubiquitin under 4 mW of focused light are shown at the bottom ($N > 500$). Under these conditions, we typically observe two prominent timescales for ubiquitin translocation (340 ± 5 μ s and 890 ± 70 μ s), as well as two peaks in the blockade currents (0.88 and 0.78), approximated by a sum of two Gaussians (black lines). The inset is a cartoon of the crystallographic structure of wild type human ubiquitin (PDB 1d3z).

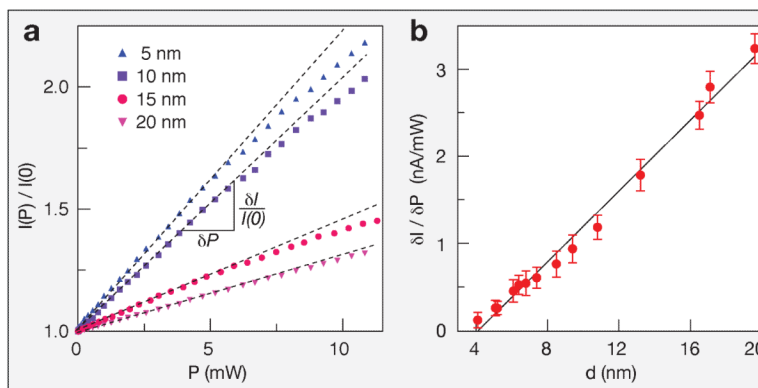


Figure 3. Ionic current enhancement as a function of laser power and pore diameter

a) Plots of the ionic current enhancement $I(P)/I(0)$ as a function of laser power for a 5, 10, 15, and 20 nm-diameter pores, as indicated. b) Pore response to light, $\delta I/\delta P$, as obtained from linear fits to plots of $I(P)$, as a function of pore diameter. The dependences on the current enhancement shown in a) and of the pore response to light shown in b) on pore size suggest that the origin of the photo-conductance effect resides on the pore's surface (i.e. pore walls) and not on its volumetric content.

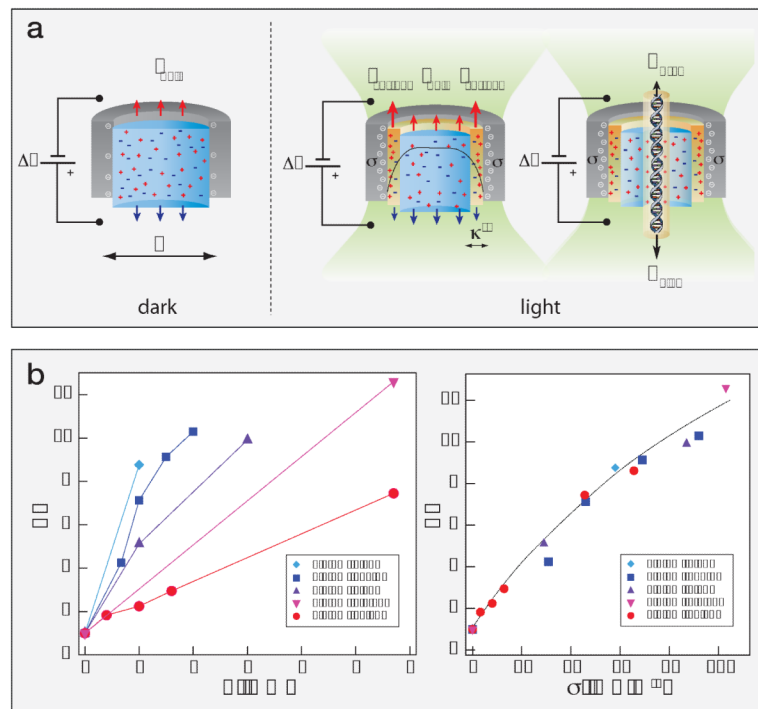


Figure 4. Light-induced surface charge density modulates the EOF and DNA translocation speed

a) Cartoons showing the bulk and surface ionic current terms discussed in the text, and the origin of the electro osmotic force acting to retard the DNA translocation for the same nanopore in darkness (left) and under laser illumination (right). Here $I_{surface} = I_{DL} + I_{EOF}$ (Eq.1) b) Left, the Retardation Factor (RF) as a function of laser intensity measured for different nanopore sizes: 4.3, 5.4, 5.6, 6.1, and 7.4 nm (diamonds, squares, upward triangles, circles and downward triangles, respectively) and DNA lengths: 400 bp (squares), 3.5 kbp (downward triangles), 5 kbp (upright triangles and diamonds), and 10 kbp (circles). Each data point was calculated from at least 1,000 events per laser intensity. Right, the RF as a function of the nanopore surface charge density calculated using the open pore current versus laser intensity for each pore (Eq. 2). Remarkably, all data points collapse onto a single curve, regardless of DNA length or nanopore diameter.