Supplemental information for: Pressure Induced Enlargement and Ionic Current Rectification in Symmetric Nanopores

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² Materials and Methods

³ S1: Microfluidics chamber

The microfludic flow-cell was designed to accommodate 5x5 mm Si/SiN membrane devices under a working pressure of up to 10 bar, and a closed electrolyte circulation, providing pressure control and electrical insulation. All flow-cell components in contact with the fluid are made from polyether ether ketone (PEEK). Fluid connections to the flow-cell are made with PTFE tubing and connections made with HPLC grade ferrules and fittings. The fluidic pathways are sealed with mechanical shut-off valves. All liquid exchanges are done by flushing

liquid through these fluidic connections using Luer-lock syringes. Liquids are degassed by 10 pushing the fluid first through a 4 mL internal volume degassing hose (Biotech Fluidics 11 BT-9000-1549) connected to a vacuum pump at 10 mbar absolute vacuum. Nitrile O-rings 12 were used to ensure sealing of the chip between the two halves of the chamber. Chlorinated 13 Ag/AgCl electrodes were sealed using the same fittings and ferrules as the connecting tubing. 14 To ensure there are no air pockets near the electrodes they were partially unscrewed and 15 liquid in the chamber was used to push any air out. When first mounted a nanopore device 16 is always left under compression pressure of 7 bar for ≈ 5 min in order to ensure proper 17 wetting. The setup has recently been described and used to probe wetting behaviour and 18 artifacts due to contaminants or bubbles.¹ 19

²⁰ S2: Measurements

All electrical measurements were done using a Zurich Instruments MFLI lock-in amplifier 21 with the MF-DIG option. Both DC and AC bias was applied using the signal output of the 22 instrument, while the current through the sample was measured using the built in current to 23 voltage converter. All DC (AC demodulator) signals were sampled at 1.83 kHz and acquired 24 using the MFLI lock-in amplifer. The input noise used by the amplifier depended on the 25 current input range used and was generally bellow 200 fA/ $\sqrt{\text{Hz}}$. All measurements were 26 done inside a Faraday cage. All DC IV curves were recorded in a sweep from 0 to +V, down 27 to -V and back to 0 to ensure any hysteresis is visible. Pressure was applied and controlled 28 with 99.99% nitrogen using a 7 bar FlowEZ microfluidics pressure controller (Flugient). All 29 interfacing with the measurement instruments was done using a custom made program in 30 LabVIEW. All measurement data was analysed using a custom made script in Python using 31 SciPy signal analysis tools.² 32



Figure S1: Time trace of an AC pressure sweep measurement. Typical pressure sweep time trace and the matching values of the AC resistance R_{AC} and ICR ratio r. Data matches main text figure 1c data for $a = 12\mu$ m.

All pressure-dependent electrical measurements were done after the pressure level has 33 stabilized to at least 5% of the target value. In the case of DC current measurements an 34 additional wait time of 3 s was performed after the pressure settling. In the case of lock-in 35 measurements the wait time was 15 times the lock-in base time plus 2.5 s. The base time 36 constant used was 500 ms for an AC signal at 1 Hz. Supporting figure S1 shows a time 37 trace of the raw data extracted from a pressure sweep of the system. All pressure sweeps 38 are carried out in the same manner, the pressure is increased in steps dP = 300 mbar from 39 P = 0 to $P = \pm P_{\text{max}}$ while in between each non-zero pressure value a control at P = 0 is 40 carried out. This allows to correct for drifts in the baseline values or to discern whether any 41 hysteresis or wetting artefacts are taking place¹. From this raw time trace the values of the 42 resistance $R_{\rm AC}$, and rectification r are obtained for each of the pressure values. All main text 43 figures have the measurable values corrected for any drift at P = 0 by taking into account 44 the difference between the neighbouring values under pressure and the reference value under 45 no pressure. 46

⁴⁷ Buffer solutions of 1 M KCl with 100 mM Tris buffer was used and the pH was titrated ⁴⁸ to 8, 12, or 3 with HCl or KOH. All buffers were prepared using MiliQ grade water (18.2 MΩ/cm). The conductivity and pH of all solutions was checked before use with a MettlerToledo FiveEasy Plus and pH paper. All solutions were filtered through a 20 nm filter before
use (Whatman Anotop 25 plus). Measurements were done using in-house fabricated 20 nm
thick silicon nitride membranes (described elsewhere³) or commercially bought membranes
from NORCADA.



Figure S2: **DC IV curve noise analysis.** Power spectrum density of noise at 200 mV for an IV curve shown in figure 1 of the main text. Noise levels, especially the 1/f portion of the noise, is low showing good filling of the nanopore. This was the case for all the samples discussed in the main text and was verified at several stages of the measurement protocol.

In order to measure the pressure induced strain and ICR at the precision shown in the main text, samples need to exhibit a high level of stability in time. The measured behaviour is seen in about 15% of the measured samples, for a total of 5 representative samples used in this work. The rest were removed from the statistic due to a high level of noise in the measured values, high level of flicker noise which is known to be connected with improper wetting,⁴ or a large drift of the baseline values with time. Although we used pure and filtered solutions, we note that pores tend to increase in resistance with time, especially when pressure gradients are applied in the system. We attribute this due to aggregation of tiny sub-20 nm particles or dissolved impurities at the nanopore area, which is more pronounced with large pores as the ones we use here due to larger flow rates. Even when changes over the time of the measurement are less than 1%, this is sufficient to mask precise measurements. Figure S2 shows an example of the flicker noise in a well filled pore.

⁶⁶ S3: AC/DC current measurements and analysis

When performing phase sensitive measurements with the lock-in a sinusoidal probe voltage 67 was used, of the form: $V(t) = V_{AC} \sin(2\pi f t)$ with f the frequency of the sine wave. Then a 68 response of the form $I(t) = I_1 \sin(\omega t + \phi_1) + I_2 \sin(2\omega t + \phi_2)$ is measured, where the values I_1 69 and ϕ_1 correspond to the linear (1st harmonic) response and I_2 and ϕ_2 the first correction due 70 to the nonlinear response in the system (2nd harmonic), which are measured independently 71 by the lock-in. The response of the system to the driving voltage V(t) is characterized by 72 the complex valued admittance Y(V, P) such that I = Y(V, P)V. Any non-linearity in the 73 response of the system is in the admittance. We find that at sufficiently low driving voltages 74 and low frequencies, we see no contributions from any non-linearity in capacitance.¹ For 75 small bias voltages ($\ll 1 V_{rms}$) we assume that any non-linearity is found in the conductance 76 G(V), such that $Y(V, P) = G(V, P) + j\omega C$. At sufficiently low frequencies (below ~ 100) 77 Hz) the capacity C will be dominated by the capacitance of the supporting membrane with 78 minimal contributions from leakages through the substrate.^{5,6} We can than proceed with 79 the Taylor expansion from the main text: $G(V, P) \approx G_1(P) + G_2(P)V$, here without any 80 pressure dependent terms, with $G(V = 0, P) = G_1(P)$ and $G_2(P) = \left(\frac{\partial G(V, P)}{\partial V}\right)_{V=0}$. Typically 81 $G_2(P) = 0$ due to time and spatial inversion symmetry, but in the case of nanopores it 82 is known that there can be a break in the symmetry due to ionic current rectification.⁷ 83 Previous studies on nanopores⁸ clearly show that the small biasing voltage response is linear 84 and governed by an equation of the form $G_1 = \kappa \sigma$ with σ the solution conductivity and 85 $\kappa^{-1} = \frac{4L}{\pi d^2} + \frac{1}{d}.$ 86

Analogously to ref.¹, we can obtain a response to the applied sinusoidal voltage as

$$I = \frac{1}{2}V_{\rm AC}^2 + (G_1 V_{\rm AC})\sin(\omega t) + \omega C\cos(\omega t) + \frac{1}{2}G_2 V_{\rm AC}^2\sin(2\omega t - \frac{\pi}{2}),$$
 (S1)

from which we can connect the values measured with the phase sensitive detector to the phenomenological conductance terms:

$$I_{1} \approx G_{1} V_{AC} \sqrt{1 + \frac{\omega^{2} C^{2}}{G_{1}^{2} V_{AC}^{2}}} \qquad \phi_{1} = \tan^{-1} \left(\frac{\omega C}{G_{1} V}\right),$$
$$I_{2} = \frac{1}{2} V_{AC}^{2} G_{1} \qquad \phi_{2} = -\frac{\pi}{2},$$

and calculate the conversion formula for the resistance $R_{ac} = \frac{V_{AC}}{I_1 \cos \phi_1}$.

An approximation to the current rectification r(V, P) = |I(+V, P)|/|I(-V, P)| at a DC voltage V and under a pressure P (see main text for details on the pressure response) can be obtained trough the higher harmonics of the lock-in signal. Taking into account that the linear conductivity is dominant $G_1 \gg G_2 V$ and that we measure at low (DC-like) frequencies $\omega \ll \frac{G_1 V_{AC}}{C}$:

$$r(V,P) = \frac{|I(+V,P) - I(V=0,P)|}{|I(-V,P) - I(V=0,P)|} \approx \frac{G_1 + G_2 V}{G_1 - G_2 V} \approx \frac{I_1 + 2I_2}{I_1 - 2I_2}.$$
 (S2)

Note that a rectification value obtained from an AC signal will be according to this definition always $r \ge 1$ so that information about the directionality of the rectification is lost.

⁹⁶ S4: Additional data



Figure S3: Comparison of compression to pressure gradient. Comparison of the effects of gradient pressure and compression (equal pressure simultaneously applied from both sides) on the resistance and rectification curves. A flat response, even up to much higher pressures of 7 bar, in both resistance and rectification is observed.

⁹⁷ In order to show that the measured effect in the main text is a direct consequence of the ⁹⁸ applied pressure gradient (difference between two sides of the membrane), we show in figure ⁹⁹ S3 a comparison of measurements under a gradient and under compression (pressure applied ¹⁰⁰ simultaneously to both sides). This clearly shows that both the strain enlargement of the ¹⁰¹ nanopore diameter and the ICR effect are caused by a pressure gradient.



Figure S4: **AFM images of membranes.** AFM height images taken of four membranes discussed in the main text after experiments. It is difficult to completely clean membranes after use with salt solution and some residue of salt crystals are always present. While the membranes vary in their zero pressure shape with some being flat but elevated (panel a) and b)) and others wrinkled (panels c) and d)) we saw no correlation that could be extracted between the membrane state and resistance or rectification behaviour. Any intrinsic pre-stress in the membrane is therefore assumed to be small which is verified by the positive pressure behaviour of the resistance as seen on figure 2b of the main text. This figure also shows the difference in size of membrane used with the large sized membrane being represented on panel c) and all other panels being the small membrane type.

Figure S4 shows atomic force microscopy images obtained of used membranes after drying. We saw no correlation between membrane shape and level of residual stress or wrinkling with the measured strain versus pressure response. This indicates that we were working in the regime where the pressure load on the membrane is dominant in respect to any residual membrane deformation. Note that all membranes showed the same strain and ICR effect for



¹⁰⁷ mutltiple subsequent measurements, before partially or completely clogging.

Figure S5: Streaming current and pH dependence. Comparison of streaming current levels for the three pH values discussed in the main text: pH 12, 8, and 3. See text for details.

Figure S5 shows the dependence of the streaming current $I_s = H_s P$ on pressure. As the 108 streaming conductance is proportional to the surface zeta potential $H_s \sim \zeta$, this measurement 109 shows how the zeta potential, and thus surface charge, depends on the pH of the solution. 110 The pH 12 dataset shows an increase in streaming current due to an increase in the surface 111 charge of the silicon nitride while at pH 3 the streaming current has inverted its sign. This 112 demonstrates that at pH 3 the iso-electric point of silicon nitride has been crossed as is 113 expected from literature.⁹ However the positive surface charge is much smaller than at pH 114 8 or 12 as can be seen by the slope of the curve, thus supporting the lack of effect seen in 115 figure 3b of the main text for low pH. 116



Figure S6: **Resistance dependence on pH.** Comparison of the elastic behaviour of the membrane at the two different pH values from the main text. The differences are negligible despite the large variation in pH and thus surface charge (as confirmed by streaming current above). This confirms that the two effects discussed in the main text are decoupled effects.

Figure S6 shows the dependence of the measured strain versus pressure for pH 3 and pH 12 solutions. We note a negligible difference between the two measurements in spite of an exchange of solutions and several hours difference between the two measurements. This proves that the strain effect is independent of the surface charge of the nanopore.

121 S5: Finite element model of a nanopore under pressure

Finite element method simulations were implemented on an axially symmetric system in COMSOL Multiphysics (version 5.5). The system was modelled as in ref.^{10,11} by coupled Poisson-Nernst-Planck-Stokes equations. Electrostatic interactions between bound (surface charge on the silicon nitride-water) and free charges (added salt) were modelled using the Poisson equation for the electrostatic potential $\phi(\mathbf{r})$:

$$\nabla^2 \phi(\mathbf{r}) = -\frac{n_c(\mathbf{r})}{\epsilon_0 \epsilon_r},\tag{S3}$$

with $n_c(\mathbf{r}) = e \sum_i z_i c_i(\mathbf{r})$ the density of free charge carriers, with z_i the valency, c_i the density of the ion species *i*, and ϵ_r the relative permittivity of the material (80 for water, 7.7 for silicon nitride). A bulk concentration of each ion species of $c_0 = 1$ M is fixed on all external boundaries. Here the free charge densities c_i were subject to the Nernst-Planck equations for the ion flux \mathbf{J}_i of species *i* with convection due to fluid flow with a velocity $\mathbf{u}(\mathbf{r})$:

$$\mathbf{J}_{i} = -D_{i}\nabla c_{i} - \frac{D_{i}}{k_{B}T}z_{i}ec_{i}\nabla\phi(\mathbf{r}) + c_{i}\mathbf{u}(\mathbf{r}), \qquad (S4)$$

where D_i are the diffusion constants for the ions $(D_+ = D_- = 2 \cdot 10^{-9} \text{ m}^2/\text{s})$. Fluid flow was obtained using the Stokes equation with an electric body force $\rho(\mathbf{r})\nabla\phi(\mathbf{r})$ and pressure gradient ∇p :

$$\eta \nabla^2 \mathbf{u} = \rho(\mathbf{r}) \nabla \phi(\mathbf{r}) + \nabla p, \tag{S5}$$

with η the dynamic viscosity of water using the built in COMSOL parameters.

The FEM mesh was constructed in COMSOL similar to previous works¹⁰⁻¹² using boundary layer refinement with minimal size at boundaries of 0.1 nm, a growth ratio of 1.1 and 12 boundary layer elements near all corners and at the nanopore opening the mesh was additionally refined until convergence was obtained. First a 1D problem on the boundary of the simulation domain was solved without fluid flow and then used as the boundary condition for the full problem,¹⁰ with additional pressure applied on all the external boundaries on both sides of the chamber. Figure S7 shows the fluid velocity in the whole simulated domain for two different pressures at a voltage bias of 100 mV. The top side of the simulation domain is placed at a potential of 0 V, while the bottom is placed at 100 mV.



Figure S7: Fluid velocity obtained from COMSOL model. Figure shows the fluid velocity obtained from the COMSOL model as described in the text for two characteristic pressures P over the whole simulation domain. In both cases the applied bias is V = 100 mV. White arrows mark the direction of flow (the side under positive pressure), which is the opposite direction of the positive z axis (schematic inset in main text figure 4d).



Figure S8: Space charge density along the pore centerline. The space charge density n_c is shown as a difference between values for positive and negative bias V for three different pressure values, indicating the asymmetry between two pore sides which is then responsible for ICR. Dashed vertical lines mark the pore entrance positions.



Figure S9: Total charge carrier density along the pore centerline The difference in the total charge density in the pore centerline between positive and negative voltage biases V at three different pressure values is shown. Dashed vertical lines mark the pore entrance positions.

In order to explain the origin of the ICR effect from the main text, we plot several quantities which make up the Dukhin number as defined in the main text. Figure S8 and Figure S9 show the concentrations of the local charge density $n_c(z, P, V) = e(c_+ - c_-)$ and total ion concentration $c_{\text{tot}} = c_+ + c_-$ on the axis of symmetry r = 0 through the pore center. Far away from the pore $c_{\text{tot}} = 2c_0$ which is the reservoir total ion concentration. The graphs show the difference between the +V and -V case, to emphasize any asymmetry, which is correlated with the ICR effect. Figure S10 shows the spatial average of $\langle c_{\text{tot}} \rangle$ defined as

$$\langle x \rangle = \frac{1}{\pi R^2} \int_0^R 2\pi r x dr$$

with R equal to the pore radius d/2 inside the pore, and 5d/2 outside (|z| > L/2) so 145 that it picks-up the whole pore entrance region while not approaching the edges of the 146 system where the mesh was less refined. Figure S11 shows the Dukhin number, defined 147 as $\operatorname{Du}(z) = -\frac{\langle c_+(z)-c_-(z)\rangle}{2(c_+(z,r=0)+c_-(z,r=0))}$ through the pore. As described in the main text, it is 148 the variation of Du through the pore which is linked to the existence of ICR. When the 149 difference in Dukhin numbers between the pore entrance and exit at opposite potential biases 150 $\operatorname{Du}(z; P, V) - \operatorname{Du}(-z; P, -V)$ is plotted (figure 3d of the main text) it directly quantifies the 151 difference between the pore entrance and exit. This difference is an even function of z, and 152 qualitatively resembles $\langle n_c \rangle$. 153



Figure S10: Average total charge carrier densities along the pore centerline. The average total charge carrier density $\langle c_{\text{tot}} \rangle$ is shown for three different pressures P and voltage biases V. The jump in value at the exit of the pore is due to the integral picking up the double layer outside of the pore interior, which dominates the signal. Dashed vertical lines mark the pore entrance positions.



Figure S11: Dukhin number variation along the pore centerline. The Dukhin number Du(z, P, V) is plotted for three different pressure P and voltage bias V values, with the Dukhin number at no applied voltage bias subtracted to emphasize the difference under applied bias. Dashed vertical lines mark the pore entrance positions.

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