Supporting information to:

Lipid-coated nanocapillaries for DNA sensing

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1. Procedure for the preparation of the nanocapillary cells used for ionic current detection

All experiments were done using quartz glass capillaries (outer diameter: 0.5 mm; inner diameter: 0.3 mm; Hilgenberg GmbH, Germany). Capillaries were first cleaned by sonicating in acetone for 5 minutes. After removing the solvent with compressed air, the capillaries are pulled using a laser assisted pipette puller (Sutter P-2000). Pulled nanocapillaries were then placed into a PDMS mould, connecting two reservoirs. The cell is sealed with a cover glass (Menzel-Glaser GmbH, Germany) glued with PDMS by heating for 5 minutes at 150 °C. The assembled cell was then plasma cleaned for 10 minutes (air plasma treatment at 100 W plasma power in a Femto-Diener Electronic plasma cleaner). The solution (150 mM KCl, 10 mM HEPES, pH = 7.5) was filtered (0.22 μm Millipore syringe filters), heated at 70 °C and added to the two reservoirs immediately after the plasma cleaning process. Finally, the assembled cell was placed under vacuum in a desiccator to remove air bubbles in the capillary for approximately 1 minute. Silver electrodes (200 µm diameter) were chlorinated (Ag/AgCl) and inserted in both reservoirs. The electrode in the tip reservoir was connected to ground and the one in the back reservoir was connected with the amplifier headstage. An schematic representation of the nanocapillary cell is shown in figure S1.

![Figure S1](image-url)
2. Methodology used in the current measurements

Ionic current measurements were performed using an Axopatch 200B (Axon Instruments, USA) amplifier in voltage-clamp mode. The signals were digitized with a NI-PCIe-6251 card (National Instruments, USA) and with Axoscope 10.2 software (Molecular Devices). Custom written LabVIEW (LabVIEW 8.6, National Instruments) programs were used to record and process the data. The current vs voltage characteristics were taken by measuring the current between -500 mV to 500 mV. The 5 kHz RMS noise was tested at 500 mV. The power spectrum was recorded at 100 kHz using an internal Bessel filter at 10 kHz or 5 kHz bandwidth. Solution is exchanged in both reservoirs from 150 mM KCl to 500 mM KCl (10 mM HEPES, pH = 7.5) to perform DNA detection measurements. For DNA translocations measurements, λ-DNA (Fermentas) was added to the tip reservoir to a final concentration of 1 nM. The ionic current trace was recorded upon applying 500 mV, using a sampling rate of 300 kHz and an internal Bessel filter at 10 kHz or 5 kHz bandwidth. Current blockades occurring during λ-DNA translocations were recorded whenever a specific threshold was exceeded. Data analysis was performed in Origin8.5.

3. Estimation of the nanopore diameters

The diameter of the nanopore (D) has been estimated by using the simplified equation for conical nanocapillaries shown here (equation S1).¹

\[ D = \frac{4Gl}{\pi gd} \]

"D" is obtained in nm.
"l" is the length of the conical part (~2 mm for these nanocapillaries).
"d" is the diameter of the capillary at the start of the conical taper (0.3 mm)
"g" is the specific measured conductance of the buffer (6.3 S/m for 500 mM KCl and 1.9 S/m for 150 mM KCl)
"G" is the nanocapillary conductance in the respective KCl solution. G (in nS) is obtained by calculating the slope at negative bias.
4. POPC SUVs preparation and size measurement

2-Oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (POPC) (Sigma-Aldrich) was dissolved in chloroform to a lipid concentration of 10 mM. 2 mL of this 10 mM lipid solution were added into a vial and the organic solvent was evaporated under vacuum to form a flat lipid layer. This lipid film is resuspended in an aqueous solution containing 150 mM KCl (10 mM HEPES at pH 7.5) to give a final lipid concentration of 2 mM. SUVs are formed by sonicating (Ultrasonic bath, Grant XB6) this lipid solution for ~30 min at room temperature. SUVs are extruded through 30 nm polycarbonate membranes (Nuclepore Track-Etched Membranes, Whatman) according to the protocols of Avanti Polar Lipids, Inc. Extruded SUVs are stored at 4ºC and they were used within 2 weeks from preparation.

The mean diameter of the extruded SUVs (53 ± 12 nm) was measured via dynamic light scattering (DLS) (Malvern Zetasizer Nano ZS). The actual SUVs diameter was calculated using single Gaussian peak among the diameters of vesicles from a minimum of 9 measurements (figure S3).
5. Noise analysis for a bare and lipid-coated pore

A comparison of the current power spectral density of a nanopore before (black) and after (red) lipid coating measured upon applying +500 mV in a 150 mM KCl (10 mM HEPES (pH =7.5) solution is gathered in figure S4. A significant enhancement in 1/f type low-frequency noise is observed upon coating.

Figure S4. Current power spectra of a nanopore before (black) and after (red) lipid coating.
6. Lipid-coated nanocapillary tip observed under a fluorescence microscope

Nanocapillary coating was performed using 1% mol of a fluorescent lipid in the initial lipid formulation. In all experiments, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2,1,3-benzoxadiazol-4-yl) (ammonium salt) (NBD-DPhPE) (Avanti Polar Lipids) with a $\lambda_{\text{absorption \ max}} = 460$ nm was used as fluorescent lipid. After performing the coating, the nanocapillary was viewed under a Zeiss Axioplan fluorescence microscope ($\lambda_{\text{exc}} = 458$ nm, $\lambda_{\text{em}} = 535$ nm) (figure S5). Scale bar= 20 μm.

![Fluorescence microscopy image of the tip coated with fluorescent lipids. Scale bar= 20 μm](image-url)
7. Current trace histogram for DNA translocations

Figure S6 shows the current trace histogram for DNA translocations (logarithmic scale) when applying 500 mV in a lipid coated nanopore studied at 500 mM KCl in 10mM HEPES (pH=7.5). For each event the mean baseline current is subtracted before generating the histogram. The baseline (1) and translocation peaks (2 and 3) are fitted with a multi-peak Gaussian function (black line).

![Current trace histogram for DNA translocations](image)
8. Success in the DNA detection for lipid-coated and bare nanopores

Figure S7 shows the normalized success in the DNA detection performed with lipid-coated (red bar) and bare (black bar) nanopores. The results are based on experiments carried out in 57 bare and 25 lipid-coated nanopores. Using lipid-coated nanocapillaries a threefold increase of the success rate is achieved.

![Bar diagram of normalized success in DNA detection](image)

Figure S7. Bar diagram of normalized success in DNA detection

9. References