Supplementary Material

Ensuring Fair Assessment of Solid State Nanopore Sensors with Baseline Current Reporting

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Materials and Chemicals

0.2 mm Ag wires (Warner Instruments, Hamden, USA) were used to fabricate the Ag/ AgCl electrodes in-house. Microinjectors of 34 gauge were purchased from World Precision Instruments. Lithium chloride and Tris-EDTA-buffer solution (10 mM Tris-HCl and 1 mM EDTA) were purchased from Sigma-Aldrich. Piranha solution was made by mixing concentrated sulfuric acid (H_2SO_4) with hydrogen peroxide (H_2O_2). The 10 nm thick SiN_x membranes were used in our experiments (Norcada, Canada). The square membrane with a 10 × 10 μm^2 window is at the center of a 200 μm thick silicon frame. Quartz capillaries with inner and outer diameters of 0.5 mm and 1 mm were purchased from Sutter Instrument. We filtered the testing solutions with a 0.2 μm syringe filter (Whatman). DNA templates (20 kbp and 2 kbp dsDNA with a concentration of 0.5 μg/μL) were purchased from ThermoFisher.

SiN_x nanopore fabrication

The SiN_x membrane was mounted into the flow cell with a transparent quartz coverslip bottom. The cis and trans chambers were filled with 2 M LiCl in Tris-EDTA buffer. The flow cell was mounted on a nano-positioner (Physik Instrumente, P-611.3S NanoCube). The 488 nm laser (Coherent OBIS 488 LS) was used for laser-enhanced ionic current mapping. The Keithley 2636 was used to apply voltage bias and collect current signals through Ag/AgCl electrodes. The moving Z-score method was adopted for the nanopore fabrication; details about this method can be found in our previous work [1]. The nanopore formation was checked by IV characterization between ± 0.3 V and laser-enhanced ionic current mapping.

Glass nanopore fabrication

The quartz capillaries were cleaned in Piranha solution for 30 minutes to remove organic residues, then rinsed with DI water, and dried in an oven at 120 °C for 15 minutes. A two-line recipe, (1) heat 750, filament 5, velocity 50, delay 140, and pull 50; (2) heat 710, filament 4, velocity 30, delay 155, and pull 215, were used to pull the capillaries with a laser pipet puller (P-2000, Sutter Instruments, USA). This recipe typically produces nanopore size around 10 nm.

Nanopore sensing and data analysis

2 M LiCl in Tris-EDTA buffer was used for all DNA experiments. A 0.3 V was applied across the nanopore by a 6363 DAQ card (National Instruments, USA). A trans-impedance amplifier (Axopatch 200B, Molecular Device, USA) was used to amplify the resulting current and then digitized by the 6363 DAQ card at a 100 kHz sampling rate. Finally, a customized MATLAB (MathWorks) software was used to analyze the current time trace and extract the DNA translocation information. The threshold of the event peak was set at 6 times of standard deviation of the current traces.[2]

References

1. Roshan, K. A.; Tang, Z.; Guan, W., High fidelity moving Z-score based controlled breakdown fabrication of solid-state nanopore. *Nanotechnology* **2019,** *30* (9), 095502.
2. Carter, B., Op amp noise theory and applications. In *Op amps for everyone*, Elsevier: 2009; pp 163-188.

Electrical characteristics of SiN_x and glass nanopores.

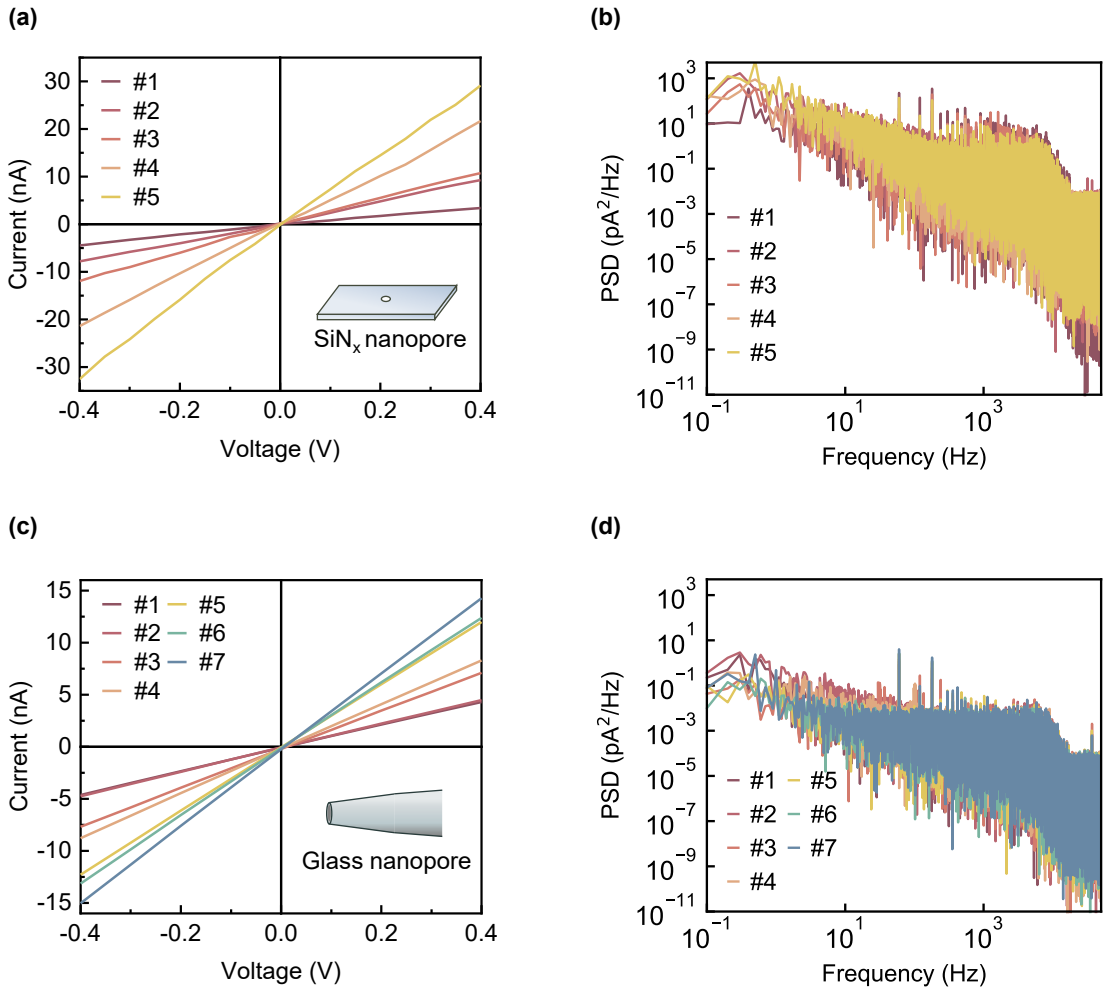


Figure S1. Electrical characteristics of SiN_x and glass nanopores. (a) and (b) illustrate the current-voltage (IV) and power spectrum density (PSD) profiles for SiN_x nanopores, respectively. (c) and (d) present profiles for glass nanopores.