Supplementary Information
Measuring temperature effects on nanobubble nucleation via a solid-state nanopore

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Experimental Section

1. Reagent and materials.

All reagents were analytical grade, phosphate buffer solution (PBS) was purchased from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). $NaBH_4$ and ethanol (≥ 99%) was obtained from Shanghai Guoyao Co., Ltd. (Shanghai, China). The tetrabutylammonium hexafluorophosphate ($TBAPF_6$) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All solutions for analytical studies were prepared with ultrapure water (reaching a resistivity of 18.2 MΩ·cm at 25℃) obtained using a Milli-Q System (EMD Millipore, Billerica, MA, USA). A pair of platinum wire (0.5 mm, Alfa Aesar Co., Ward Hill, MA, USA.) was used as the electrode.

2. SiN_X membrane leakage current recording during nanopore fabrication and ionic current recording during nanobubble nucleation

The leakage current was monitored and recorded by home-designed circuit[1,2]. Firstly, the 10 nm thick SiN_X membrane chip was fixed between two polytetrafluoroethylene

(PTFE) flow cells by crews. Two cells were filled with 200 μL 1 M potassium chloride (KCl), pH 8. Two Pt electrodes were immersed into two cells respectively to apply a constant direct current voltage. And one electrode was connected to a current amplifier. Another one electrode was connected to a DAQ card or a voltmeter to apply potential. A custom-designed LabVIEW software and home-designed program were used to control the voltage potential and collect the current. Once the current exceeded the threshold current value, the voltage potential was switched to 0 V. The ionic current during nanobubble nucleation was measured by Axopatch 200B (Axon Instrument, Forest City, USA) with 100 kHz sampling and 5 kHz low-pass filter.

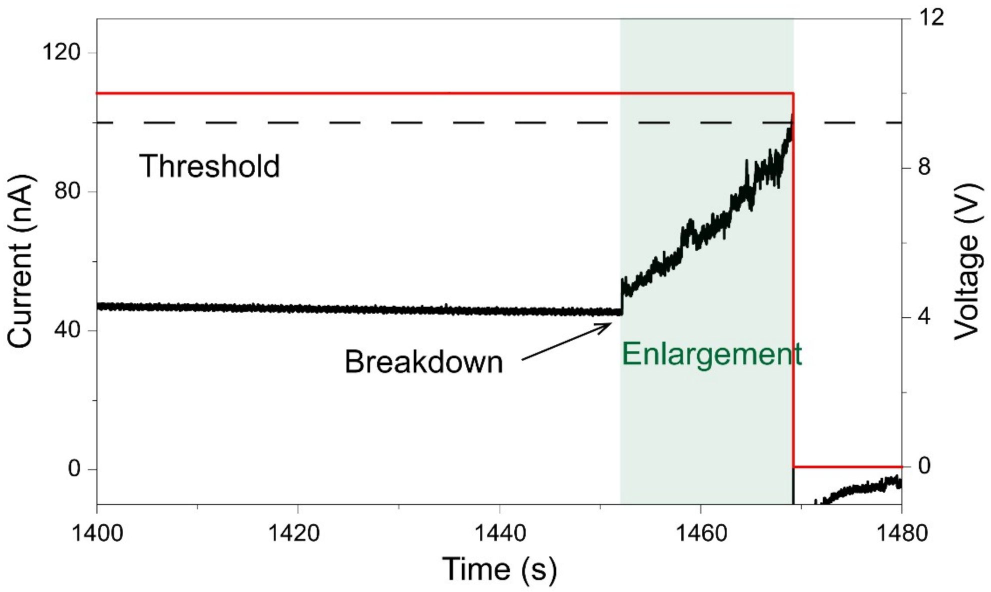

Fig. S1 Current-voltage-time trace for dielectric breakdown fabrication of a SiN_X nanopore. Applying a constant direct voltage of 10 V (red solid line), the leakage current (black solid line) was monitored. An abrupt increased current was observed, as the black arrow signed, suggesting a newly pore formed. Then the keeping increased current was related to the enlargement of the nanopore (green background). Until the current reached the current threshold of 100 nA, the voltage was turned off. And the nanopore stopped to further enlarge.

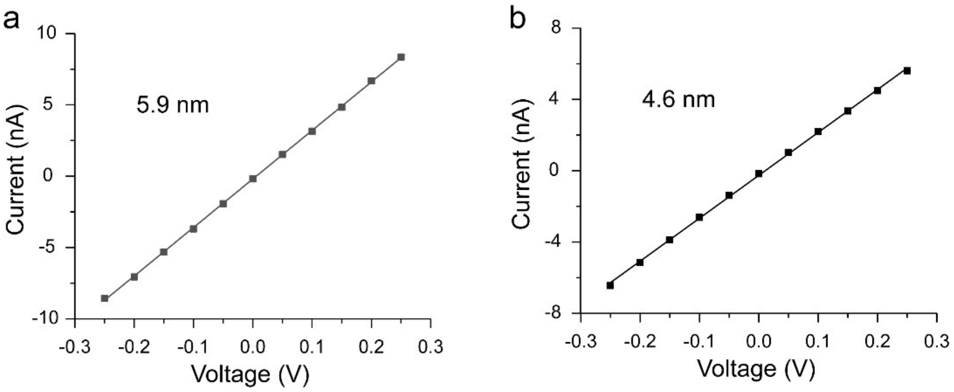

Fig. S2 I-V scatter plot and fitting curve for nanopore with diameter of 5.9 nm (a) and 4.6 nm (b).

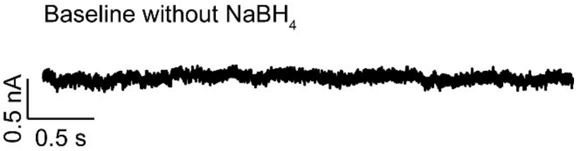

Fig. S3 Raw ionic current trace without $NaBH_4$ in EtOH solution.

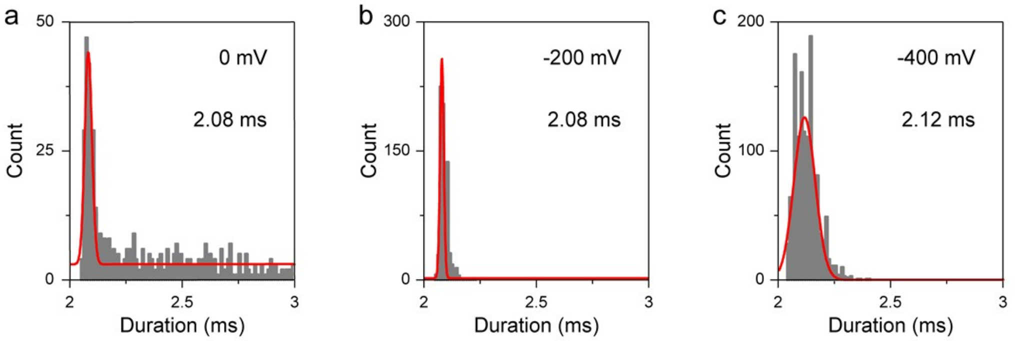

Fig. S4 The voltage-dependent signal duration for nanobubble nucleation with a $d \sim 2.9$ nm SiN_X nanopore at 0 mV (a), -200 mV (b) and -400 mV (c). The duration histograms were fitted by Gaussian function. The fitted durations are 2.08 ms, 2.08 ms and 2.12 ms at 0 mV, -200 mV and -400 mV, respectively.

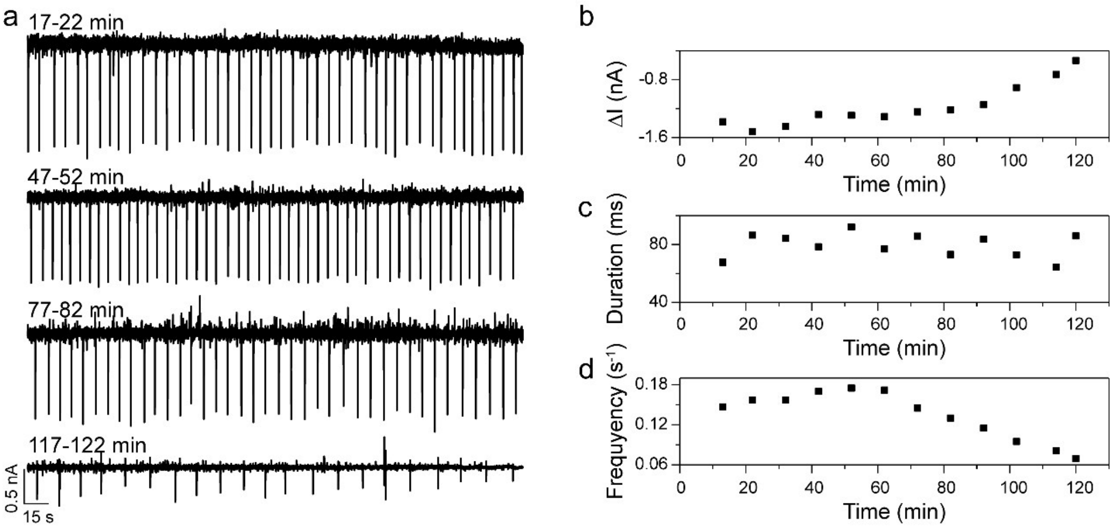

Fig. S5 Time-dependent collection of the ionic current signals from nanobubble nucleation with a $d \sim 5.9$ nm SiN_X nanopore. (a) Raw ionic current traces at different time. Scatter plot of ΔI (b), duration (c) and frequency (d) with time. The ΔI, duration and signal frequency keep stable within 60-min measurement. However, the signal frequency drifted after 60-min measurement duo to the highly miscible of two liquids with the reaction time inside the nanopore. Therefore, we collected the signals from fresh prepared solution within 60 min at each temperature.

References:

1 Q. Li, L. Yao, Y.-L. Ying, S.-C. Liu and Y.-T. Long, *Sci. Sin. Chim.*, 2017, **47**, 1445–1449.

2 Q. Li, Y.-L. Ying, S.-C. Liu, Y. Lin and Y.-T. Long, *ACS Sensors*, 2019, **4**, 1185–1189.