Supplementary Information

Direct sensing of cancer biomarkers in clinical samples with a designed nanopore

Yao Lin, Yi-Lun Ying*, Xin Shi, Shao-Chuang Liu, Yi-Tao Long*

Key Laboratory for Advanced Materials & School of Chemistry and Molecular Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai, 200237, P. R. China.

*Email: ytlong@ecust.edu.cn; yilunying@ecust.edu.cn

Experimental Section

Chemicals and Materials.

Potassium chloride (KCl, purity ≥ 99.5%), Ethylenediaminetetraacetic acid (EDTA, purity ≥ 99.9%) and Tris (hydroxymethyl) aminomethane (Tris, purity ≥ 99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The DNA probe (Sequence: 5'-A_{20}TTAAAGCTCGCCATCAAATAGCTTTCCA_{20}-3') were synthesized and HPLC-purified by Sangon Biotech Co., Ltd (Shanghai, China). Prostate specific antigen (PSA), immunoglobulin (IgG), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Alpha-fetoprotein protein (AFP) was purchased from Fitzgerald (North Acton, MA, USA). The serum samples for PSA detection were provided by Prof. Hong-Yang Wang (Eastern Hepatobiliary Surgery Hospital, The Second Military Medical University, Shanghai, China). Nuclepore track-etched membranes (polycarbonate and hydrophilic) with pore sizes of 0.015 μm were purchased from Whatman Inc. (cat. #110601, New Jersey, USA). The commercial ECLIA kits were purchased from Roche Diagnostic (Shanghai, China). All reagents and chemicals were of analytical grade. All aqueous solutions were prepared with ultrapure water with resistance of 18.2 M Ω cm at 25 °C (EMD Millipore, Billerica, USA) and were filtered with 0.22-μm pore-size filter (Rephile Bioscience Ltd., Shanghai, China).

Sample Preparation.

DNA probes were heated at 95 ℃ for 5 min and then gradually cooled to room temperature in TBS buffer (10 mM Tris-HCl, 150 mM KCl, 5 mM $MgCl_2$, 5 mM KCl, pH 7.4) to form stable structures. DNA probes were incubated with PSA at a 5:1 ratio in TBS for 30 min at room temperature.

Nanopore Fabrication, Detection, and Analysis.

Silicon chips with 10 nm thick, low-stress silicon nitride membranes were purchased commercially from Norcada, Inc. (product # NT005Z and # NT001Z, Alberta, Canada). The SiN_x nanopores were fabricated by controlled dielectric breakdown as previously described[1-3]. Before fabrication of the pore, the nanoproe chips were treated in an oxygen plasma for 30 s on each side. The chip was mounted between two polytetrafluoroethylene (PTFE) flow cells using screws. Nanopore fabrications were performed in 1 M KCl solution (10 mM Tris-HCl, 1 mM EDTA, pH 10). A pair of Ag/AgCl electrodes were immersed into two electrolyte reservoirs to apply a bias voltage and connected to a resistive feedback current amplifier. A custom-designed LabVIEW software was used to acquire data with a DAQ card. The voltage used to fabricate a nanopore was set by the DAQ card. When the measured current exceeded the predetermined threshold current, voltage bias was terminated rapidly by the software. The nanopore chips were immersed with 4 M LiCl (10 mM Tris-HCl, 1 mM EDTA, pH 8) for several hours after fabrication. Translocation experiments were performed in 1 M KCl (10 mM Tris-HCl, 1 mM EDTA, pH 8) and 1 M/0.05 M (*trans*/*cis*) KCl. The DNA probe (pI = 3.5-4.0) and PSA molecule (pI = 6.8-7.5) both are negatively charged in the electrolyte solution (pH=8). Under applied voltages of 100-400 mV, the negatively charged PSA molecule bound with the negatively charged DNA probe as a whole are driven from the cis side to the trans side of the pore. Current traces were collected at a sampling rate of 100 kHz using Axopatch 200B (Axon Instruments, Forest City, USA) with a 5 kHz low-pass Bessel filter. Data analysis was performed using Mosaic software[4] and homemade software[5] and Origin 9.2 (OriginLab Corporation, Northampton, USA).

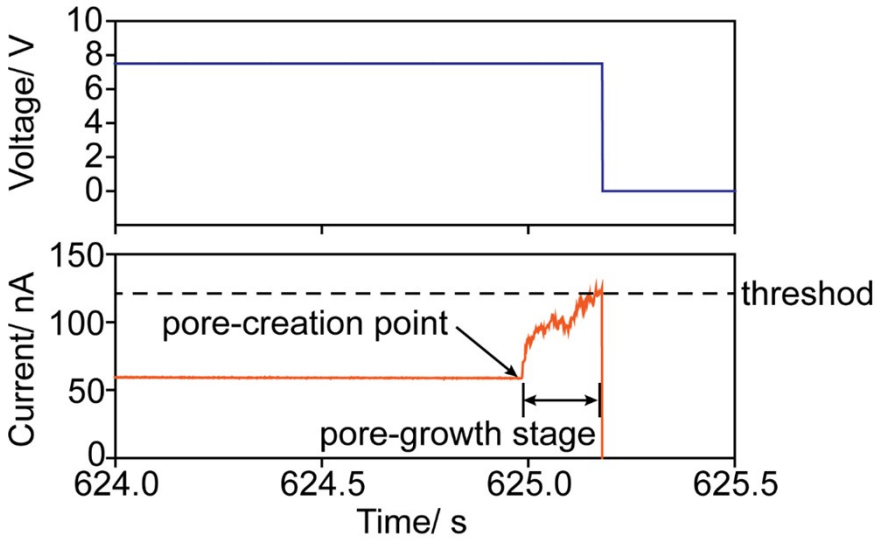

Figure S1. Leakage current of nanopore formation on a 10 nm SiNx membrane in 1 M KCl, 10 mM Tris, and 1mM EDTA (pH 10) solution. The voltage applied for nanopore fabrication is 7.5 V. When the leakage current exceeds a present threshold current of 120 nA, the potential is terminated rapidly.

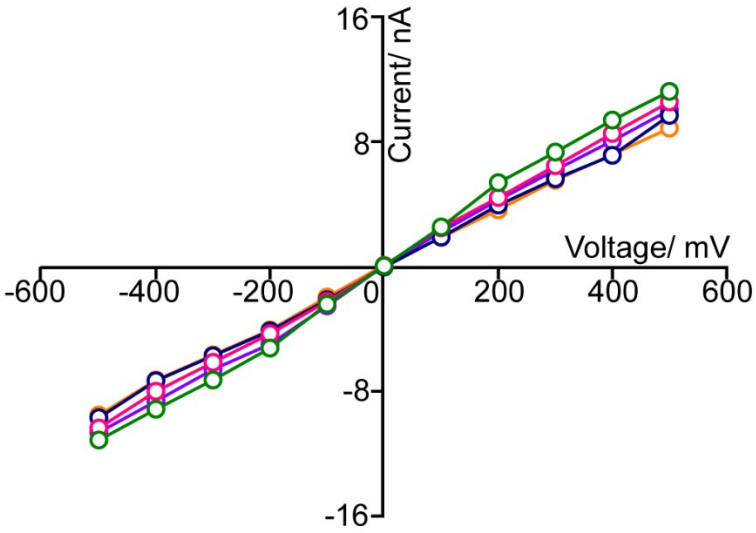

Figure S2. I-V curves of five independently formed nanopores fabricated by controlled dielectric breakdown measured in 1 M KCl (10 mM Tris-HCl, 1 mM EDTA, pH 8). The pore exhibits a linear increase in ion current for applied voltage in the range of -0.5 V to 0.5 V. The pore diameter is approximated to 4-5 nm.

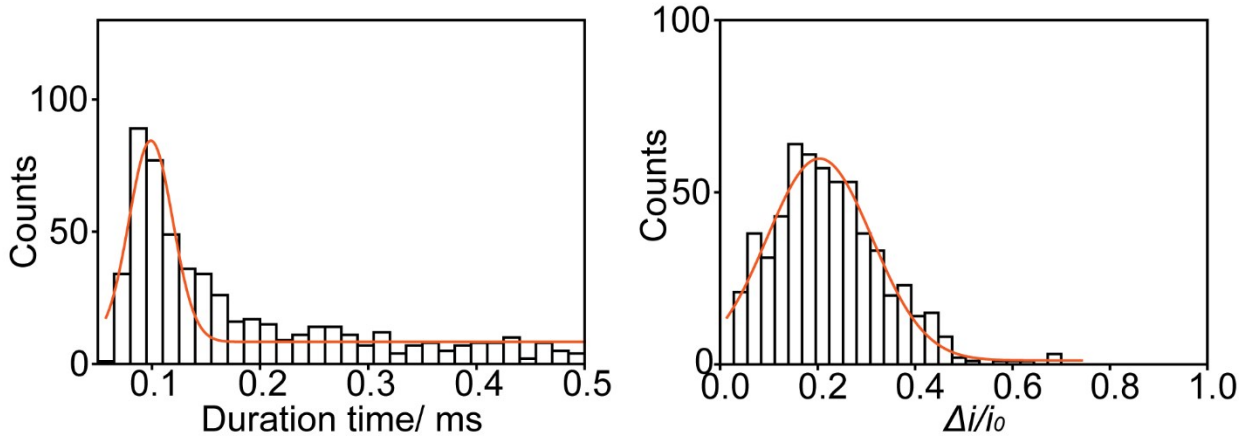

Figure S3. Histograms of duration time and $\Delta i/i_0$ for the DNA probe at 200 mV. The histograms were fit to Gaussian functions.

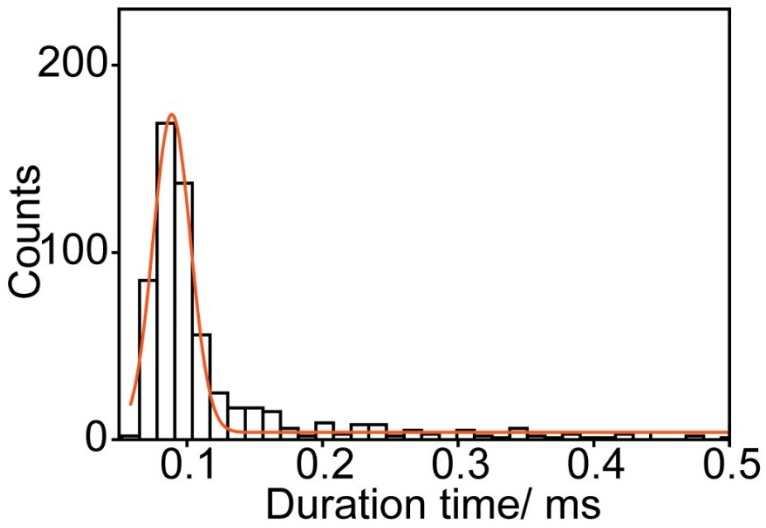

Figure S4. Histogram of duration time for population I of PAS-DNA probe complex at 200 mV. The histogram was fit to a Gaussian function.

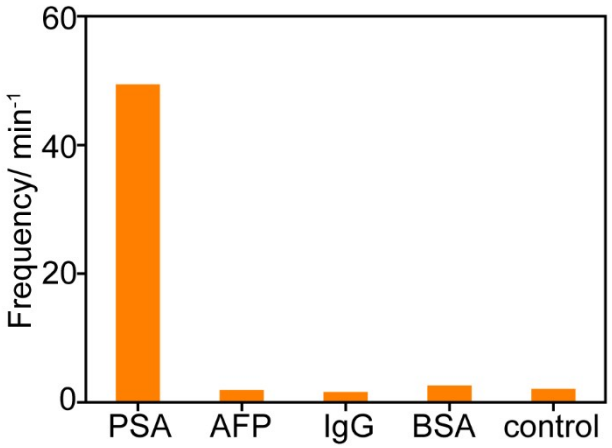

Figure S5. Frequencies of characteristic events in PII ($\Delta i/i_0 > 0.6$) of PSA, interferential protein groups including AFP, IgG, BSA, and control group. The final concentration of PSA is 5 nM, whereas the concentration of AFP, IgG, and BSA is 100 nM. The concentration ratio of protein and DNA probe is 1:5. The control group is in the absence of proteins (only DNA probe). All data were acquired in 1 M KCl solution with applied voltage of 200 mV.

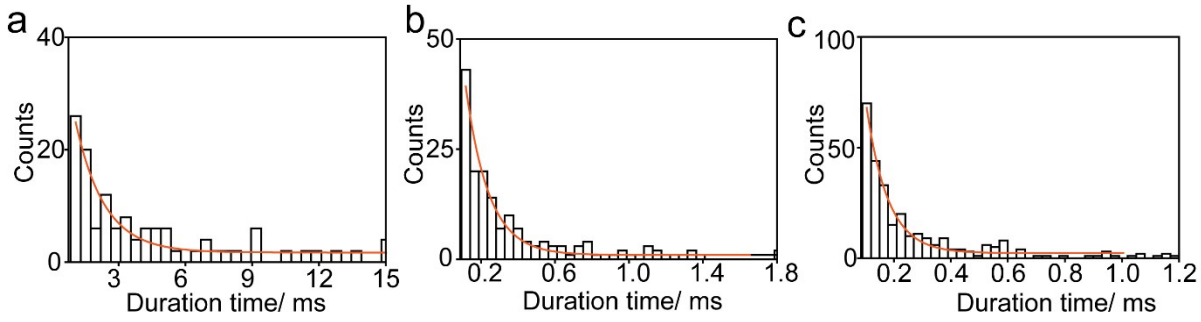

Figure S6. Histograms of duration time for population II of PAS-DNA probe complex at applied voltage of (a) 100 mV, (b) 300 mV and (c) 400 mV, respectively. Histograms were fit to single Exponential functions.

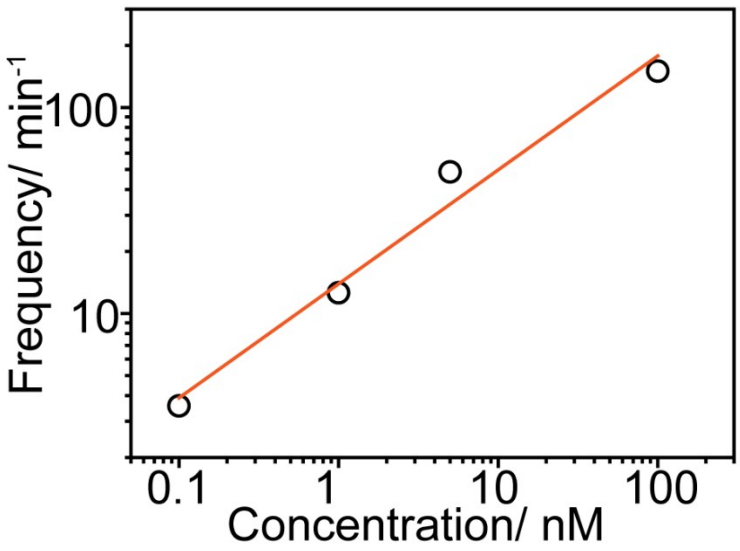

Figure S7. The dependence of the event frequency on the concentration of PSA. The correlation was measured in 1 M symmetrical KCl.

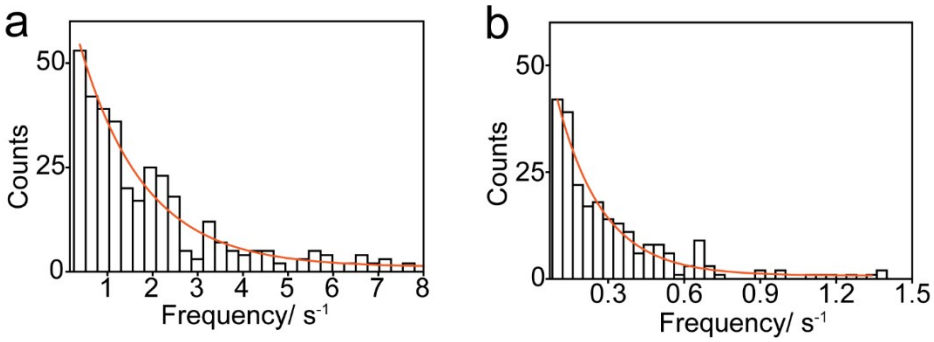

Figure S8. Event frequency of PSA-DNA probe complexes measured in (a) 1 M/0.05 M KCl and (b) 1 M symmetrical KCl. The concentration of PSA is 1 nM. The histograms were fit to single Exponential functions.

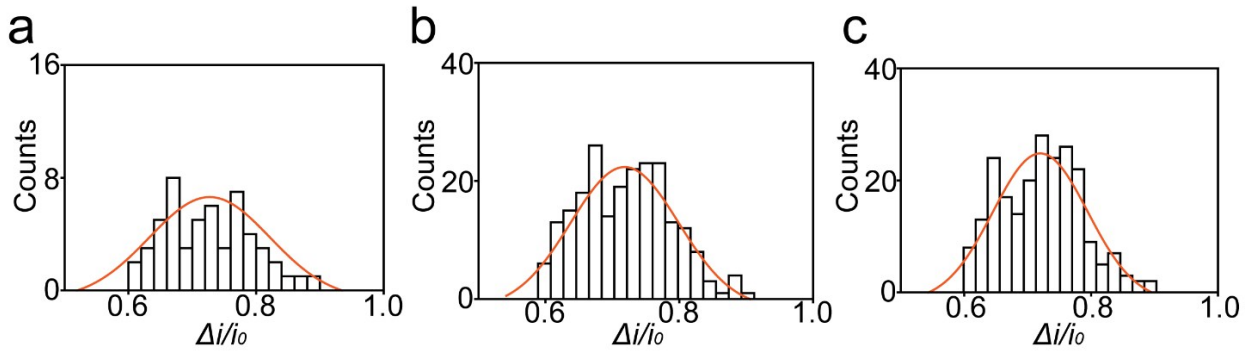

Figure S9. Histograms of $\Delta i/i_0$ for PSA-DNA probe complex in (a) sample 1 (b) sample 2 and (c) sample 3. The histograms were fit to Gaussian functions.

References

(1) Kwok, H.; Briggs, K.; Tabard-Cossa, V. *PLoS One* **2014**, *9,* e92880.

(2) Briggs, K.; Kwok, H.; Tabard-Cossa, V. *Small* **2014**, *10*, 2077–2086.

(3) Yanagi, I.; Akahori, R.; Hatano, T.; Takeda, K. *Sci. Rep.* **2014**, *4*, 5000.

(4) Balijepalli, A.; Ettedgui, J.; Cornio, A. T.; Robertson, J. W. F.; Cheung, K. P.; Kasianowicz, J. J.; Vaz, C. *ACS Nano* **2014**, *8*, 1547–1553.

(5) Gu, Z.; Ying, Y.-L.; Cao, C.; He, P.; Long, Y.-T. *Anal. Chem.* **2015**, *87*, 907–913.