Supporting Information

Simultaneous Observing the Spatial and Temporal Dynamics of Single Enzymatic Catalysis by a Solid-State Nanopore

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Experimental Section

Chemicals and Materials

Potassium nitrate and methyl alcohol were purchased from m Sinopharm Chemical Reagent Company Co., Ltd. (Shanghai, China). Potassium hydroxide, hydrocholoride acid and hydrogen peroxide were obtained from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Cysteamine hydrocholoride and 3-aminopropyl-trimethoxysilane (APS) were purchased from J&K Scientific Ltd. (Beijing, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), tris(hydroxymethyl)aminomethane (Tris), 2-morpholinoethanesulfonic acid (MES) and glucose were purchased from Aladdin Chemical Co. Ltd. (Shanghai, China). Glucose oxidase was purchased from Sigma–Aldrich (St. Louis, U.S.A.). All chemicals were analytical grade as received. Ultrapure water (18.2 MΩ cm from Millipore system, Billerica, MA, U.S.A.) was used for solution preparation.

Preparation of the bipolar SiNx nanopore (BSN) and further modification with glucose oxidase

Solid-state nanopore-based BSN was prepared via metal deposition on a SiNx film, followed by the pore forming process with the controlled dielectric breakdown method. In detail, an ultrathin 10 nm free-standing SiNx membrane supported on 200 μm Si frames was obtained commercially (Norcada, Canada). Titanium (5 nm) and 30 nm gold were successively deposited on the SiNx film by electron beam evaporation techniques (Denton), and titanium was typically used as an additional adhesion layer to ensure the adhesion of the gold layer on the SiNx film. A metal-coated SiNx film was thus obtained and used for nanopore fabrication.

The experimental setup for pore formation was described in detail previously.[1] Briefly, the Si frame with metal coating was mounted between two reservoirs containing 1 M KNO3 electrolyte solution buffered at pH 8 (10 mM Tris-HCl). A pair of Pt wires was immersed in these two separated solutions and further used to apply a biased voltage. A constant voltage of 10 V was employed here to form a pore, with an instantaneous increase in the leakage current indicating that breakdown occurred on the metal-coated SiNx film. The voltage was terminated immediately while the current reached the preset threshold of 160 nA, which was calculated with the desired pore size. This prepared gold-coated solid-state nanopore was known as a bipolar SiNx nanopore (BSN) and further used for electrochemical analysis.

A step-by-step chemical modification was employed to prepare glucose oxidase-modified BSN. First, an amino group functionalized interface was first established by immersing the prepared nanopore in 20 mM cysteamine hydrochloride for 2 hours, forming a stable Au-S bond at the gold surface. I-V curves was immediately collected in this asymmetric solution, exhibiting the obvious increase of current rectification. Glucose oxidase was dissolved in 0.1 M MES buffer solution at pH 6 with 0.1 M EDC and 0.05 M NHS added to activate the terminal carboxyl group. After the BSN was thoroughly washed with alkaline and water solutions, it was immersed in a mixed solution of glucose oxidase. With a 24-hour

incubation time, glucose oxidase could be chemically linked to the BSN surface via its activated carboxyl group to the surface amino group. To clarify, the length of the SiNx nanopore is only approximately 40 nm, which is relatively short. As a result, the enzyme molecule would predominantly traverse the nanopore through diffusion. Additionally, due to the low concentration of enzymes in the solution (1.25 μM), it would be challenging for more than one molecule to be trapped inside a nanoconfined pore.

Electrochemical measurement for enzyme electrocatalysis

The glucose oxidase-modified gold nanopore was mounted in a liquid cell, separating two reservoirs that were filled with 200 μL 1 M KNO_3 electrolyte. A pair of Ag/AgCl electrodes was inserted into each solution referenced as the working and counter electrodes. Biased voltages were applied on the electrodes, with the *cis* side grounded. Glucose with a final concentration of 1 mM was added to each side solution for the measurement of enzyme electrocatalysis. A current amplifier (Axon 200B, Axon Instrument, Forest City, U.S.A.) was employed for the collection of both I-V curves and current traces with a 100-kHz sampling rate and 5-kHz low-pass filter. The raw data were processed and analyzed with our self-developed software and OriginLab 9.1 (OriginLab Corporation, U.S.A.).

References

1. Li, Q., Ying, Y. L., Liu, S. C., Lin, Y. & Long, Y. T. Detection of Single Proteins with a General Nanopore Sensor. *ACS Sensors* **4**, 1185–1189 (2019).

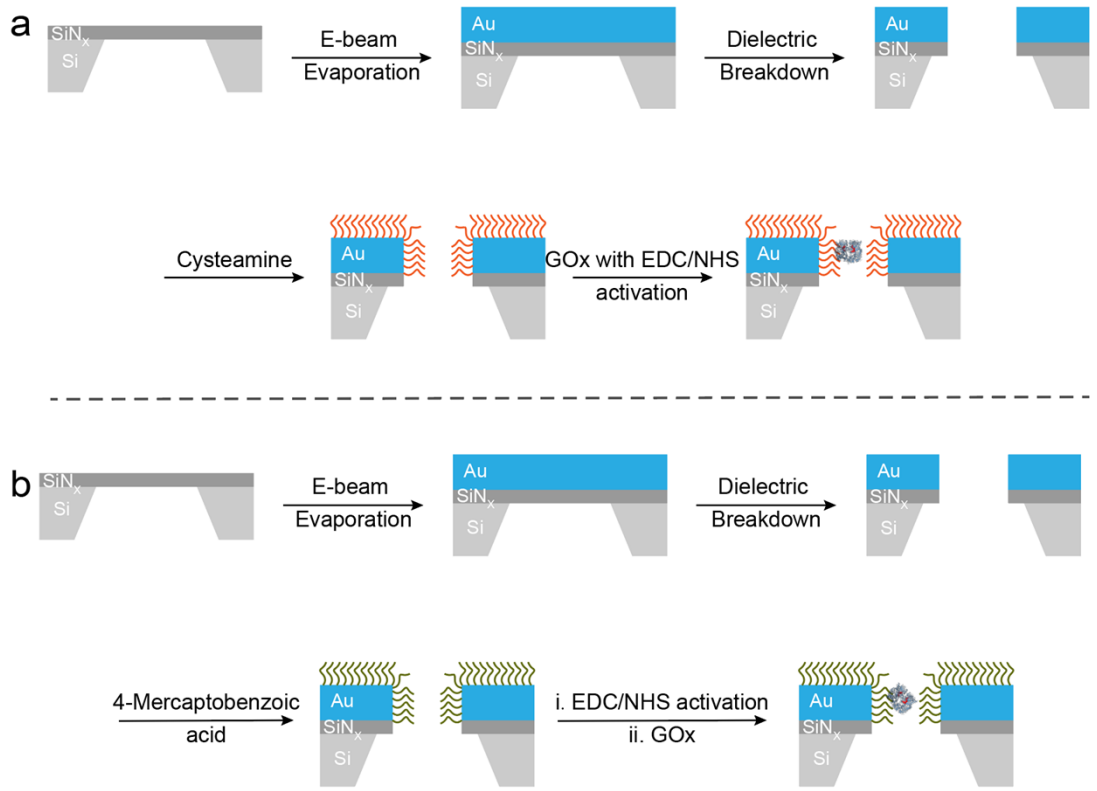

Fig. S1 The preparation of bipolar SiNx nanopores and the modification of single GOx. Procedures for C-terminal (a)and N-terminal (b) binding of single GOx inside the nanopore.

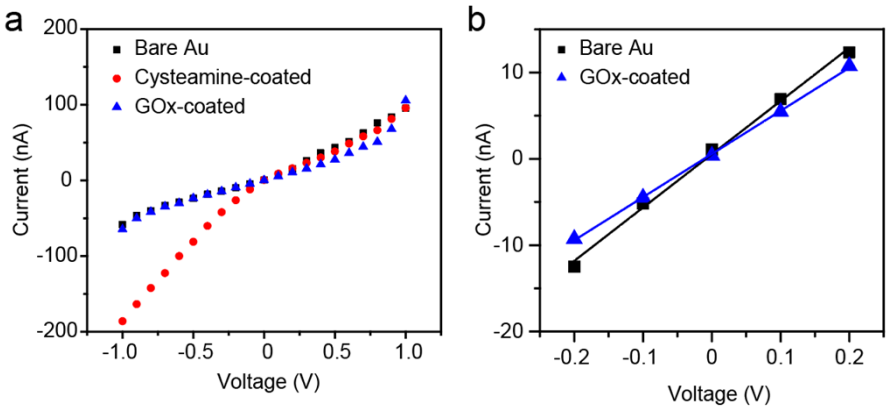

Fig. S2 (a) Representative I-V responses of the bare gold-coated (black) and GOx-modified solid-state nanopore (blue) in 1 M KNO_3, and the I-V curve of the cysteamine-modified (red) pore was measured in asymmetric solutions. (b) The enlarged I-V curve of the GOx modification inside the gold nanopore at -0.2 V to 0.2 V.

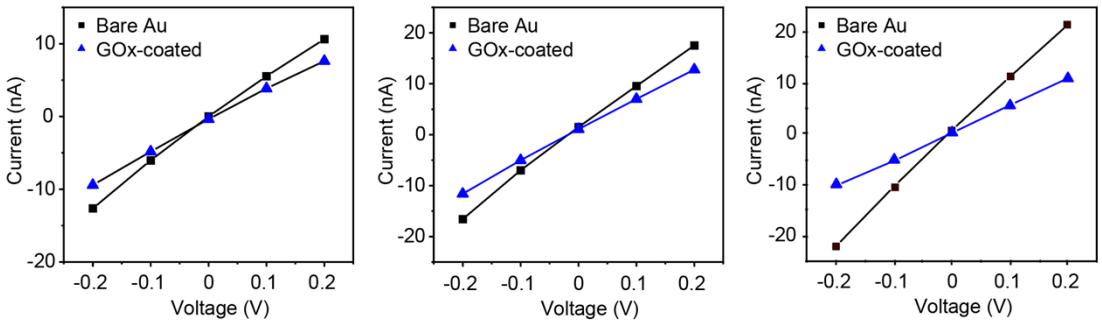

Fig. S3 Three examples of I-V curves showing the GOx modification inside the gold coated nanopore which were measured through sweeping the applied bias voltage from -0.2V to 0.2 V in 1 M KNO_3.

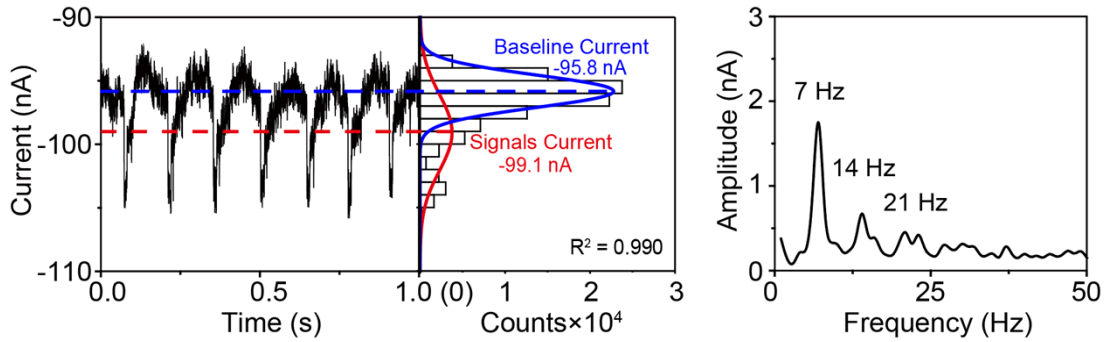

Fig. S4 Illustration of the signal analysis. Raw ionic current trace at -0.8 V falls into all-point histogram (left) with two current distributions for baseline current (blue) and signal (red). Signal frequency analysis by fast Fourier transform (Right), giving the fundamental frequency of 7 Hz and high order harmonics of 14 Hz and 21 Hz

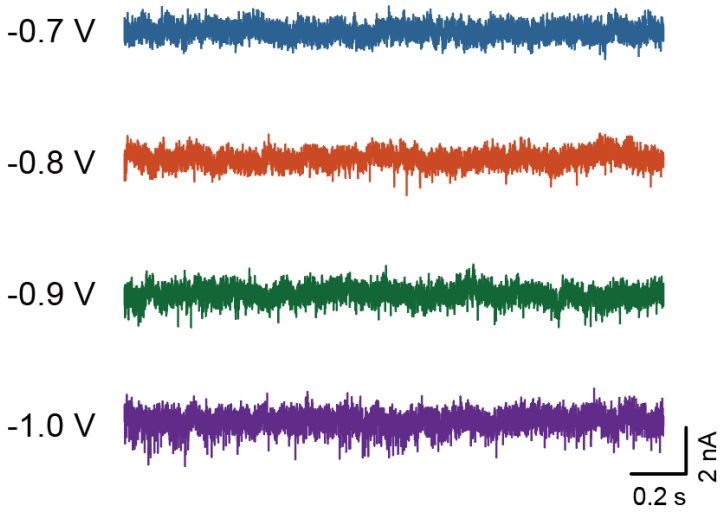

Fig. S5 Raw ionic current traces based on the GOx modified BSN in 1 M KNO_3 at applied voltages ranging from -0.7 V to -1.0 V.

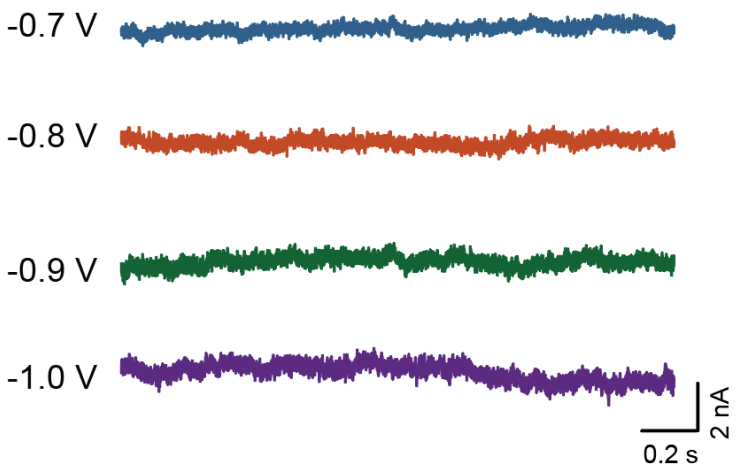

Fig. S6 Raw ionic current traces based on the GOx-coated SiNx nanopores in 1 M KNO_3 with 1 mM glucose at applied voltages ranging from -0.7 to -1.0 V.

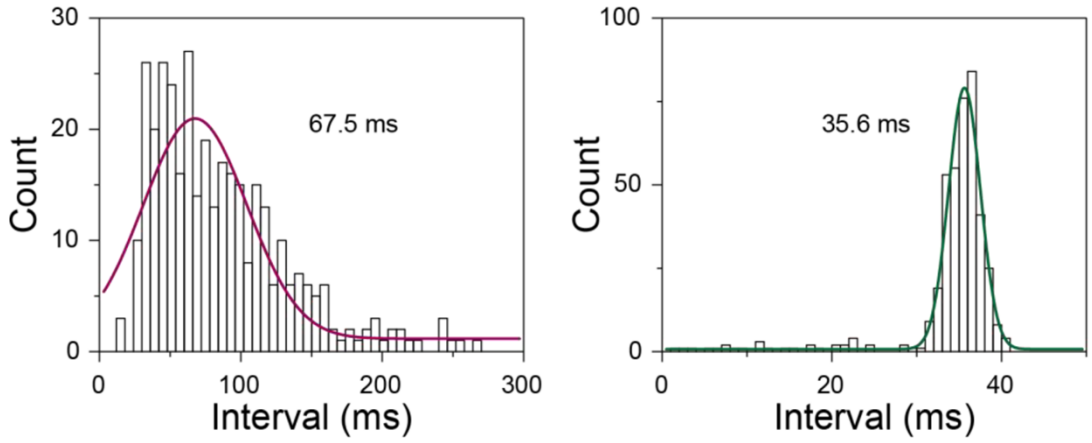

Fig. S7 Histograms and the fitting curves for the interval time in the spike signals in state 1 (left) and state 2 (right) for amino binding GOx, showing frequencies of 14.9 and 28.1 respectively.