Supporting Information

Ultratrace Detection of Glucose with enzyme-Functionalized Single Nanochannels

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Materails

Poly(ethylene terephthalate) (PET, 12 μm thick) membranes were irradiated with single heavy ion of energy 11.4 MeV/nucleon at UNILAC linear accelerator (GSI, Darmatadt, Germany). The Glucose Oxidase enzyme (from *Aspergillus niger*, ≥ 100,000 units/g lyophilized powder), D-glucose (≥ 99.5 %) and L-glucose (≥ 99%) were purchased from Sigma-Aldrich. 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide (EDC, ≥ 98 %), N-hydroxysulfosuccinimide (NHSS, ≥ 97 %) were purchased from Alfa-Aesar. Hydrochloride acid (HCl, 37%), sodium hydroxide (NaOH, ≥ 96 %), potassium chloride (KCl, ≥ 99.8 %), potassium dihydrogen phosphate (KH_2PO_4, ≥ 99.5 %), potassium hydrogen phosphate (K_2HPO_4, ≥ 99 %) and formic acid (HCOOH, ≥ 88 %) were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (SCRC, China). All solutions were prepared in MilliQ water (18.2 MΩ).

Characterization of nanochannel aperture

One accelerated heavy ion from UNILAC linear accelerator penetrated a PET membrane to create a damaged zone (latent track) in the centre. Then, the PET

membrane was treated with UV light (wavelength 320 nm) both sides for 1h. Then the membrane was mounted between two identical conductivity cells which were filled with 9 M NaOH and 1 M KCl + 1 M HCOOH respectively. The optimal etching temperature is 30 ℃ and 1 V voltage is needed too. The etching rate of latent track (v_t) is faster than the bulk materials (v_b) because of UV light sensitizing procedure. The half-cone angle of as-prepare conical channel (α) is determined by the difference of v_t and v_b. Various nanochannels with different diameters can be prepared by controlling the desirable ionic current after the breakthrough. Subsequently, the 9 M NaOH was replaced by stopping solution (1 M KCl + 1 M HCOOH) and maintaied the voltage for 30 min. Finally, the membrane was soaked in Milli-Q water (18.2 MΩ) in order to remove residual salts.

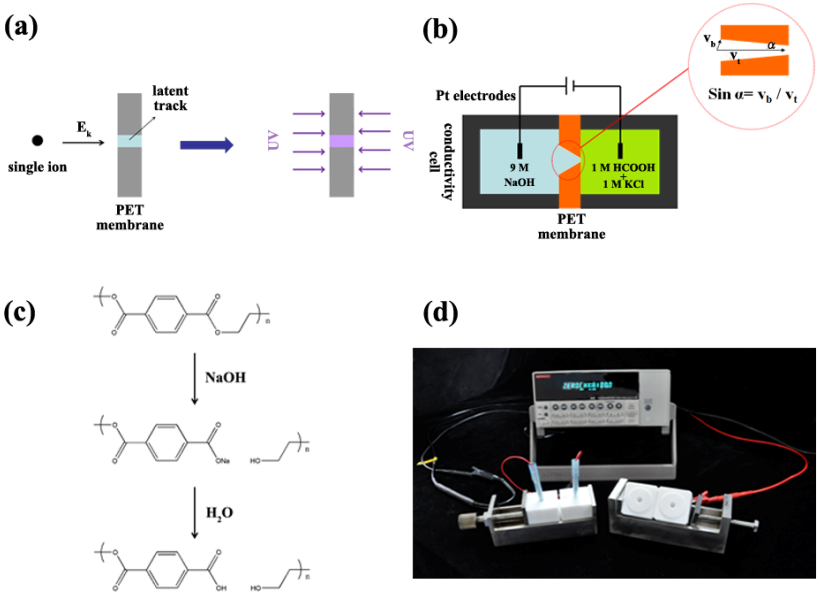

Figure S1. The preparation of conical single PET nanochannel. (a) The formation and sensitizing procedure of PET single latent track. (b) Scheme of asymmetric etching a conical single PET channel in a conductivity cell. (The inset shows the comparision of etching rates

between latent track and bulk materials, α denotes the half-cone angle). (c) Chemical etching reations of a latent track PET membrane in NaOH. (d) The photo of etching devices that include picoammeter, conductivity cells and Pt electrodes.

The diameter of the large opening (base side) D of the conical channel was determined by scanning electron microscopy (SEM) in the 10^7 multiple PET membrane which etched under the same procedure with the conical single nanochannel. The diameter of the small opening (tip side) d has to estimated by the following equation :

$$d = \frac{4LI}{\pi D \kappa U}$$

where L represents the length of the channel which equals to the thickness of the membrane, U and I are the applied voltage and measured transmembrane ionic current respectively. D is the large opening diameter of the nanochannel, and κ is the special conductivity of the electrolyte (0.11173 $\Omega^{-1}cm^{-1}$ for 1 M KCl solution at 25 ℃). The measured I = 3.8 nA while U = 0.2 V was applied in 1 M KCl solutions. As shown in Figure S2, the base diameters was 0.86 μm and the tip diameter was ~ 30 nm.

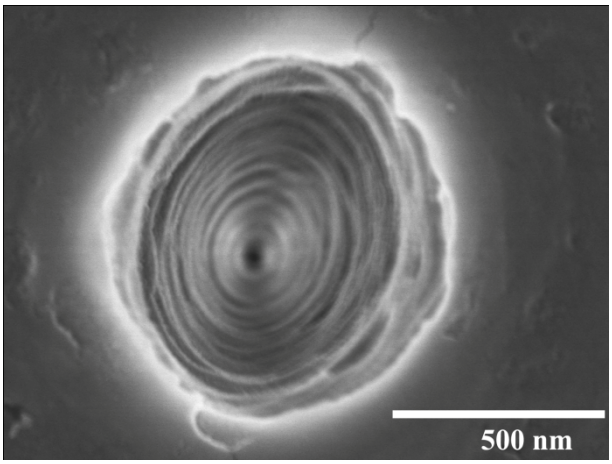

Figure S2. SEM image of typical conical single PET nanochannel from base side (top view).

GOx enzyme modification

For the sake of effective amidation, we choose carbodiimides as the zero-length biochemical crosslinker. The carboxyl groups generated on the channel inner surfaces were first activated into NHSS ester intermediates in a 15 mg ml^{-1} EDC (1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide) and 3 mg ml^{-1} NHSS (N-hydroxysulfosuccinimide) aqueous solution for 1 h at room temperature. Before modification the membrane was washed with 0.1 M PB buffer (pH 5.5) several times. Glucose oxidase lyophilized powder (Sigma-Alderich, E.C. 1.1.3.4) was dissolved in 0.1 M PB buffer to obtain a 10 mg ml^{-1} solution. Then the satisfying PET membrane was immersed in the GOx solution overnight at room temperature in order that the activated NHSS ester intermediates could covalently coupled with the primary amine groups exist in GOx enzymes. The GSNC should be bathed in the 0.1 M PB buffer for 1h at room temperature to clean up the unreacted enzymes and other reagents before measuring its ioninc current.

D-glucose sensing procedure

The as-prepared single conical membrane was mounted between two electrical etching cells and both of them were filled with 2 ml 0.1 M KCl in 0.05 M oxygen saturated PB buffer (pH 5.5) as the test solution. Ag/AgCl electrodes were employed to apply a transmembrane potential across the membrane, and a picoammeter/voltage source (Keithley 6487, Keithley Instruments, Cleveland, OH) was used as a voltage supplier and a ionic current detector. A scanning triangle signal from +1 V to -1 V was applied at the base side while the tip side was connected to the ground electrode.

Current-Voltage (I-V) measurement

The *I-V* characteristic of PET conical nanochannel before and after GOx modification was measured for the sake of determining whether the GOx molecules had been attached on the channel walls properly and figuring out what had happened to the nanochannel's ionic transport properties. By comparison, we can observe in Figure S3 that a significant decrease of nanochannel's currents emerged after the GOx immobilized on the nanochannel surface. This can be attributed to the size of native GOx molecule was estimated to about 8×7×5.5 nm^3 in solutions,[1] so that significant steric hindrance occurred at the tip side, decreased the effective size of nanochannel's small opening. On the other hand, we were informed that both of the etched PET nanochannel and GSNC were negatively charged in the test solution (0.1 M KCl + 0.05 M PB, pH5.5) due to the isoelectric points of carboxyl group and GOx enzyme were ~ 3.8 [2] and ~ 4.2 [3] respectively. 14 side amino groups of lysine residues on the enzyme surface could form amide bonds simultaneously with inherent carboxyl groups on inner channel surface. [4] Except certain unreacted amino groups for improper conjugate conformations, the surface coverage of GOx molecules was estimated to be 1 molecule per dozens of squre nanometers.[5] Meanwhile, the carboxyl group coverage after heavy ion irradiation and chemical etching process was approximately 1 group per square nanometer.[6] Thus, the surface charge density of modified nanochannel decreased compared with naked channel, leading to the declining ability of nanochannel to transport contra-ions flux. At a word, the distinction between these two *I-V* curves confirmed the achievement of the enzyme

modification. Steric hindrance and decrease of nanochannel surface charge density exhibit a synergistic effect in decreasing ionic current after the GOx enzymes were immobilized on PET nanochannel surface.

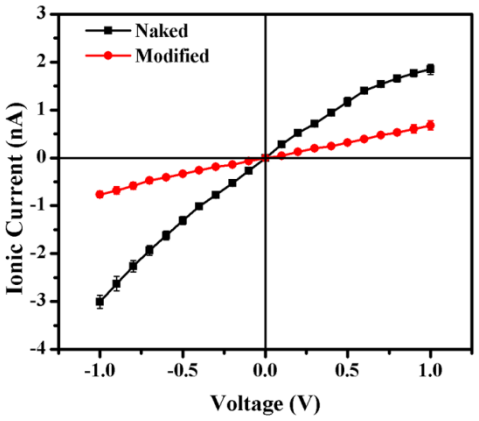

Figure S3. I-V characteristics of the single conical PET nanochannel in 0.1 M KCl + 0.05 M PB solution (pH 5.5). The black curve (rectangle) represents the I-V characteristic of the naked nanochannel that bears negative charge. During the etching process, the ester bonds in PET polymer chains were easily hydrolyzed by alkali generating terminated carboxyl (-COOH) and hydroxyl (-OH) groups. The deprotonation of carboxyl groups in pH 5.5 solution results in negative groups ($-COO^-$). The red curve (circle) shows a significant ionic current decrease after the GOx enzymes were covalently attached to channel surface. The phenomenon can be ascribed to the GOx enzymes blockage and decline of surface charge density.

In order to verify the role of GOx enzyme throughout the experiment, naked conical nanochannel without GOx-modification was also tested by adding D-glucose solutions.

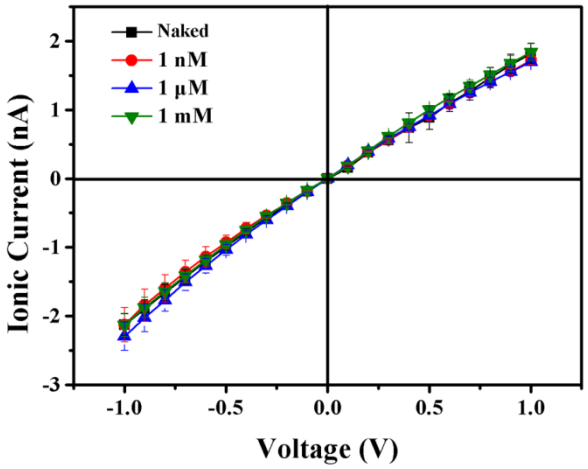

Figure S4. I-V characteristics of naked conical nanochannel filled with test solution (0.1 M KCl + 0.05 M PB, pH 5.5) containing various concentrations of D-glucose: 1 nM (●, red), 1 μM (▲, blue), 1 mM (▼, green). The constant I-V curves suggested no glucose sensing happened in the nanochannel without GOx immobilization.

According to Figure S4, a conical nanochannel does not incorporating GOx enzyme turns out to have no responsive signal to D-glucose. This implies that the presence of GOx enzymes and enzymatic reactions are the key for such a biosensor to produce electrical signals.

X-ray photoelectron spectra characterization

X-ray photoelectron spectra (XPS) data were obtained with an ESCALab220i-XL electron spectrometer from VG Scientific using 300W Mg Kα radiation. All peaks were referenced to C 1s (CHx) at 284.8 eV in the deconvoluted high resolution C 1s spectra, and the analysis software used was provided by the manufacturer.

Table S1 The analysis of element content of the single conical PET nanochannels that without (Sample 1) or with (Sample 2) GOx enzymes modification.

Elements	C	O	N
1 (%)	74.54	25.46	-
2 (%)	72.17	22.15	5.68

XPS characterizations of the GSNC

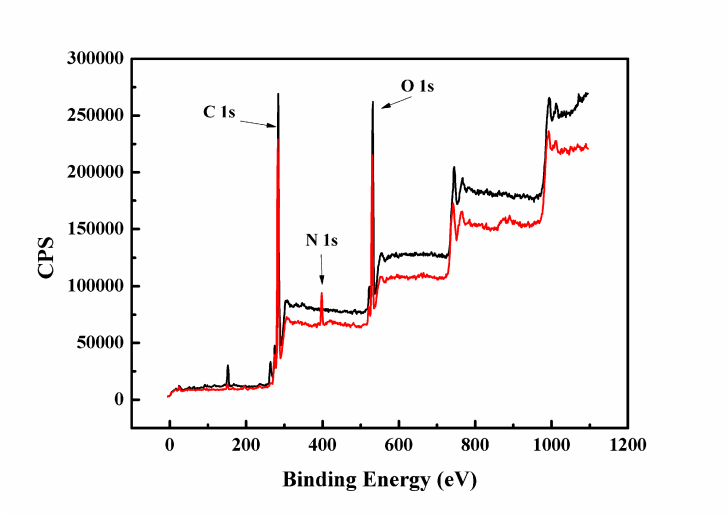

Figure S5. XPS spectra of the nanochannels.

The red line indicates GOx modified sample while black line represents no modification. From the red line, nitrogen element confirms the successful modification of GOx enzymes in the single PET nanochannel.

pH monitoring of the enzymatic reaction

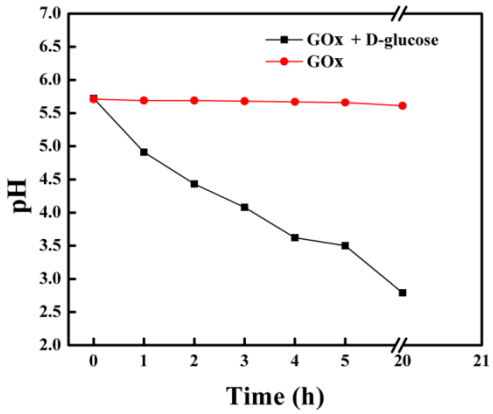

Figure S6. pH alteration of GOx enzymatic reation.

0.5 ml 0.1 M D-glucose aqueous solution was added in 2 ml 10 mg·ml^{-1} GOx phosphate buffer solutions at room temperature (25 ℃). On the other hand, no D-glucose was added in the GOx phosphate buffer solutions as comparative test. The pH values of GOx enzymatic reactions were monitored by pH meter (METTLER TOLEDO PE20k) all the time. Constantly slope down of pH values versus time indicating that acidic productions could be produced after GOx enzymatic reactions.

Zeta potential measurement of flat PET surfaces

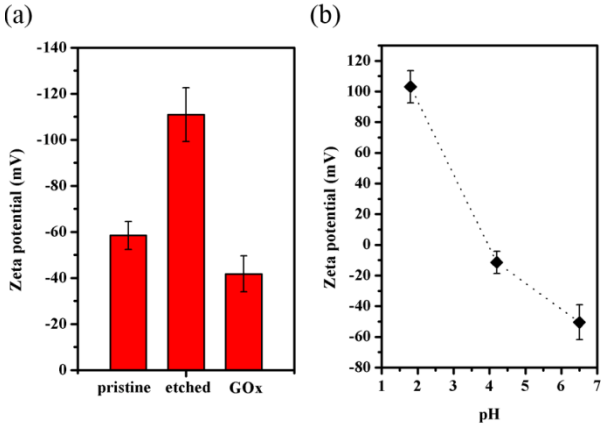

Figure S7. Zeta potential of the flat PET surfaces (a) at each consequential modification steps (pH 6.5). (b) at different pH after GOx modification.

Zeta potential was beleived to reflect the surface charge conditions at solid-liquid interfaces. In this work, zeta potential of flat PET surfaces was measured by DelsaNano C (Beckman Coulter Inc.). As shown in Figure S7a, the absolute value of zeta potential of etched surface is higher than pristine surface due to more carboxyl groups and hydroxy groups were exposured after chemical etching in NaOH. Subsequently, zeta potential fell down after the etched PET were modified with GOx enzymes. This is coincide with the I-V curves in Figure 1a that the ionic conductance decresed after GOx modification. Zeta potential of flat surfaces with pH variation was also carried out to simulate the pH changes in channel confinement. As shown in Figure S7b, zeta potentials increased with declining pH from 6.5 to 2.0. The polarity of surface zeta potential reversed from negative to positive when the pH is below 4 (pKa of GOx equals to ~ 4.2) due to the protonation of GOx.

Specificity verification measurement

Before L-glucose was added in the nanochannel system as substrate, the single conical shaped PET nanochannel with the similar conformation was modified with GOx enzymes by the same path mentioned above.

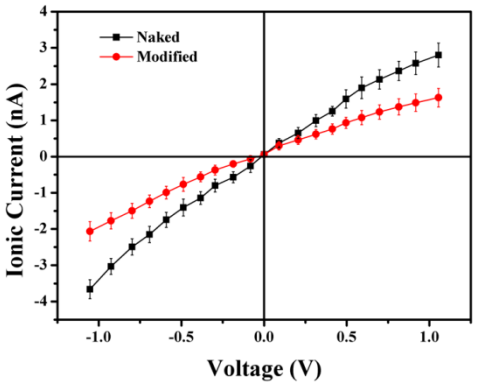

Figure S8. I-V curves of the single conical PET nanochannel in 0.1 M KCl + 0.05 M PB solution (pH 5.5).

As shown in Figure S8, a significant decrease of nanochannel current was observed after GOx modified in the channel surface.

References:

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