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A Conformation and Charge Co-Modulated Ultrasensitive Biomimetic Ion Channel

Sheng-Lin Cai,[a] Yu-Bin Zheng,[a] Shuo-Hui Cao,[ab] Xiu-Hong Cai,[a] and Yao-Qun Li*[a]

[a] Department of Chemistry and Key Laboratory of Analytical Sciences, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, P. R. China.

[b] Department of Electronic Science, Xiamen University, Xiamen, 361005, P. R. China.

Correspondence should be addressed to yaoqunli@xmu.edu.cn

Supporting Information

1. Chemicals and Materials

Prism glass capillary (outer diameter ~1.35 mm, inner diameter ~0.95 mm, Hirschmann, Germany), platinum wire (diameter 25 μm, from Alfa Aesar), Tungsten wire (0.25 mm, 99.95%, Alfa Aesar); Ferrocene (Fc, 99%, Alfa Aesar); Tetra-n-butylammonium hexafluorophosphate ($TBAPF_6$, 98%, Alfa Aesar), 3-aminopropyl-triethoxylsilane (APTES, 99%, Sigma-Aldrich), Glutaraldehyde (25% solution in water, Acros); All DNA sequences and Adenosine 5'-triphosphate (ATP), cytosine 5'-triphosphate (CTP), guanosine 5'-triphosphate (GTP), and uridine 5'-triphosphate (UTP) disodium salt solution were purchased from Shanghai Sangon Co., Ltd (Shanghai, China). The ATP binding aptamer (ABA) used for functionalization is amino-terminated at its 5' end, and its sequence is 5′-NH_2-$(CH_2)_6$-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3′. The non-specific control DNA strands is also amino-terminated at its 5' end and the sequence is 5′-NH_2-$(CH_2)_6$-CCC CCC CCC CCC CCC CCC CCC CCC CCC-3′.

2. Preparation of single glass conical nanopore channel

The single glass conical nanopore channels were prepared from glass capillaries, according to the method reported by White and coworkers with slight modifications.[1-4] Firstly, a platinum wire was electrochemically etched in 15 % $CaCl_2$ to obtain a sharpened tip (Fig. S1(a)). Then, the sharpened tip was sealed into a glass capillary. Finally, the Pt wire sealed in glass was pulled out and etched in the boiled aqua regia solution for ~3h to obtain a single conical glass nanopore channel. The pore radius was evaluated by measuring the steady-state diffusion-limited current of the Pt disk electrode prior to etching according to the equation (1). The radius of single glass conical nanopore used in this study was estimated to be ~10 nm, and its steady-state diffusion-limited current was shown in Fig. S1(b).

$$i_d = 4nFDC_b r \quad [1a] \qquad (1)$$

Where i_d is the steady-state limiting current of the nanodisk electrode measured in 5.0 mM Ferrocene and 0.1 M Tetra-n-butylammonium hexafluorophosphate acetonitrile solution, n is the number of electrons transferred per molecule, F is the Faraday constant, D is the diffusion coefficient (2.4×10^{-5} cm^2/s), C_b is bulk concentration of the redox molecule, and r is the radius of Pt nanodisk, respectively.

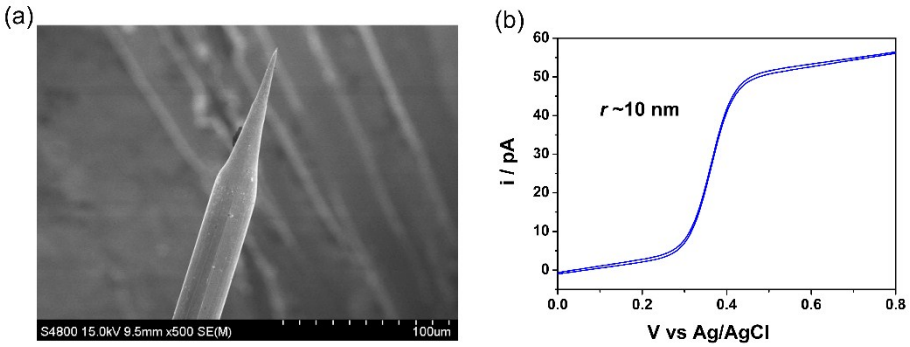

Fig. S1. (a) SEM image of the sharpened Pt wire. (b) The steady-state diffusion-limited current of the Pt disk electrode prior to etching. The radius was estimated to be ~ 10 nm.

3. Immobilization of DNA onto the single glass conical nanopore surface

The immobilization of DNA strands onto the nanopore inner wall has been reported in our previous work.[5] Figure S2 shows the schematic diagram of the modification process of DNA strands to the glass nanopore through siloxane chemistry. Firstly, the nanopore channel was treated with piranha acid (concentrated H_2SO_4/ 30% H_2O_2, V: V = 3:1, 80 ºC, 30 min), followed by washing with ultrapure water and absolute ethanol to obtain clean silica hydroxyl group on the interior surface. Then 5% APTES in absolute ethanol was used to react with the interior pore surface for 30 min, followed by rinsing with absolute ethanol and drying at 120 ºC for 30 min. Afterwards, the resulting nanopore channel was treated with 2.5% glutaraldehyde aqueous solution overnight, followed by rinsing with ultrapure water. Finally, tris(hydroxymethyl)aminomethane hydrochloride solution (Tris-HCl, 20 mM, pH = 7.4, containing 300 mM NaCl, 5 mM $MgCl_2$) with 5'-aminated DNA strands (1μM) was treated with the aldehyde groups terminated surface for ~20 h, followed by rinsing with Tris-HCl buffer solution and ultrapure water, respectively. When testing the response with ATP, the ABA-modified nanopore was incubated with corresponding concentration of ATP solution before measurements for 40 min.

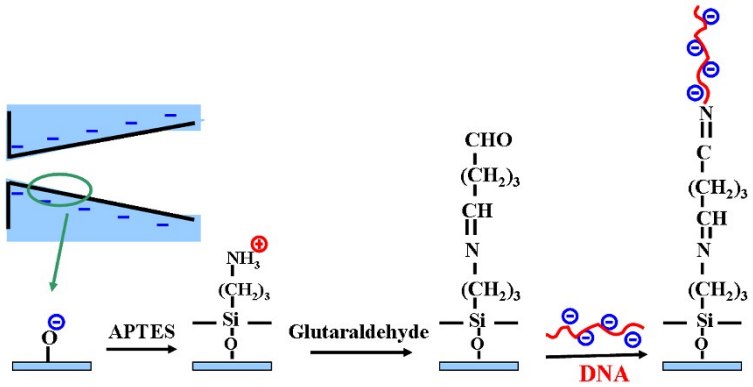

Fig. S2. Schematic description of the covalent modification procedures of glass nanopore surface with DNA strands.

4. Current-voltage measurement

Glass nanopore channels were filled with 1 mM KCl aqueous solution (pH = 7.4) in phosphate buffer (0.1 mM) using a 100 μL microsyringe. An Ag/AgCl electrode (0.5 mm diameter) was inserted into the glass capillary and served as the working electrode, and another Ag/AgCl electrode (0.5 mm diameter) was placed in bulk solution as an auxiliary/reference electrode. In all cases, the nanopore channels were filled with the same electrolyte as the bulk solution. Linear sweep voltammetry experiments were carried out with a CHI 660C electrochemical workstation (Shanghai CHI Instrument Co. Ltd., China). The measurements of the resulting ion current flowing through the nanopore channel were performed by scanning the voltage from –1 V to +1 V with a scanning rate 100 mV/s. All measurements were performed at room temperature.

Various concentrations of ATP and other NTPs are prepared in the same electrolyte solution (1 mM KCl, 0.1 mM phosphate buffer, pH=7.4), used for the measurement of respective I-V curves. When testing the response with ATP, the ABA-modified nanopore was incubated with corresponding concentration of ATP solution for at least 40 min to ensure saturation.

5. CD spectra and electrochemical impedance spectroscopy of ABA binding with ATP

The conformation and charge changes were characterized using CD spectroscopy and electrochemical impedance spectroscopy (EIS), respectively (Fig. S3). Fig. S3A shows the CD spectra of ABA prior to and after the addition of ATP. Significantly increased intensity in positive peaks (~266 nm) as well as a slight shift of this peak to lower wavelengths (~263 nm) were observed upon the addition of ATP, which indicated the transition in secondary structure of aptamers. The CD spectra we obtained here are in good agreement with previous literature.[6] The charge change in the formation of ABA-ATP complexes were characterized using EIS by modifying ABA strands onto a gold electrode. As shown in Fig. S3B, the R_{et} value increased upon the addition of ATP. This is because the binding of negatively charged ATP molecules increase the charge density of the electrode surface and further repelled the redox probe $[Fe(CN)_6]^{3-/4-}$, leading to an enhanced R_{et}. Similar results and mechanism are demonstrated by previously reported works.[7]

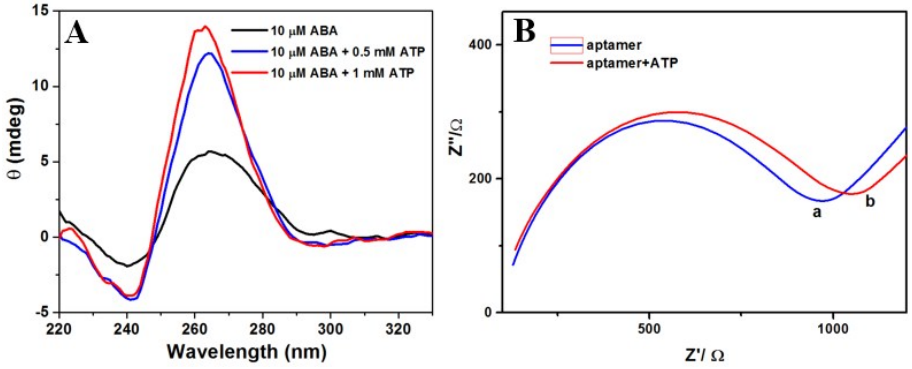

Fig. S3. (A) The CD spectra of 10 μM ABA in Tris-HCl buffer (pH=7.4) prior to and after the addition of 0.5 mM and 1 mM ATP, respectively. (B) Electrochemical impedance spectra of (a) the Au/ mercaptoethylamine/ glutaraldehyde/ aptamer DNA electrode (b) the the Au/ mercaptoethylamine/ glutaraldehyde/ aptamer DNA/ ATP electrode. The impedance spectra were recorded at the formal potential of $[Fe(CN)_6]^{3-/4-}$. The amplitude of the alternate voltage was 5 mV.

6. Monitoring of modification stages

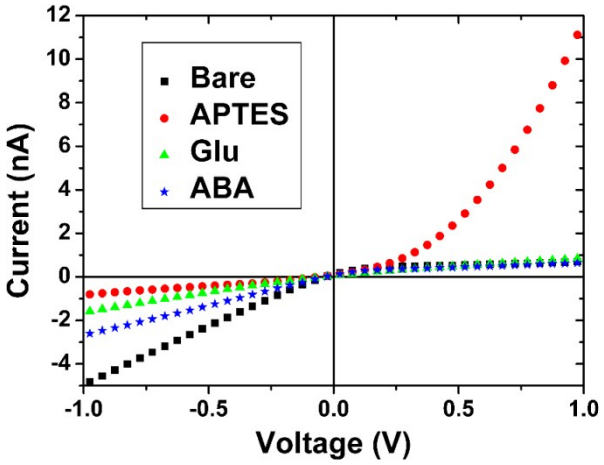

Fig. S4. Monitoring of the modification stages (silica hydroxyl groups ■, amino groups ●,

aldehyde groups ▲, and immobilized aptamer strands ★, respectively) on the inner-surface of single glass conical nanopore channel by recording I-V curves in 1 mM KCl aqueous solution at pH = 7.4.

7. Time-dependent of the current of ABA-modified nanopore in the absence and presence of ATP.

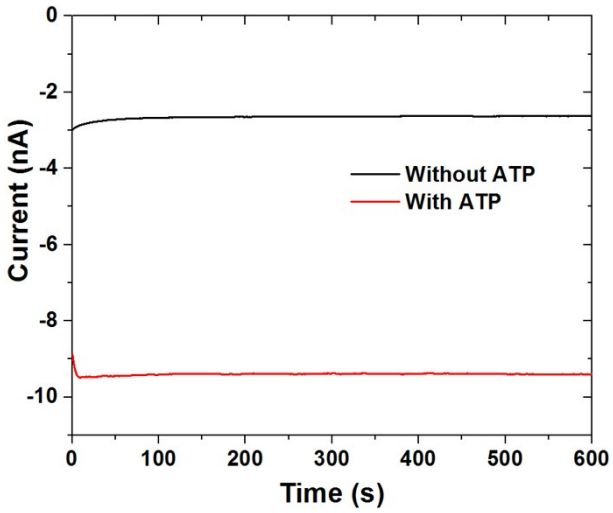

Fig. S5. The current traces at -1V of ABA-modified glass conical nanopore in the absence and presence of ATP. The electrolyte is 1 mM KCl PBS buffer (pH = 7.4).

8. Control experiments with the groups generated during the modification procedure and non-specific DNA strands.

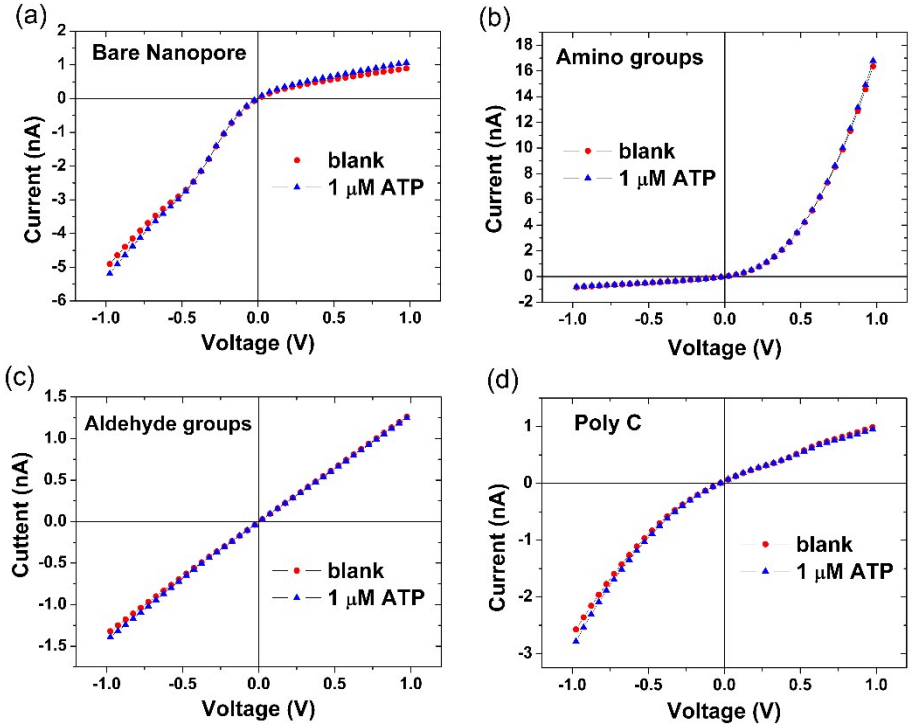

Fig. S6. I-V curves of a single glass conical nanopore in the 1 mM KCl (pH = 7.4) solution prior

to and after the addition of 1 μM ATP bearing (a) silica hydroxyl groups, (b) amino groups, (c) aldehyde groups, (d) non-specific DNA.

9. Response to ATP and ATP analogues

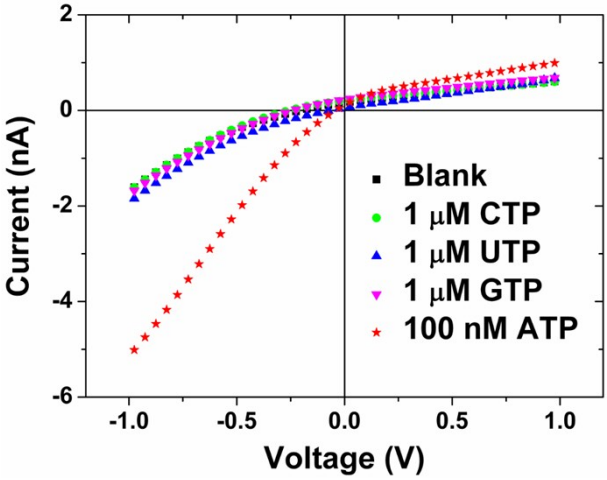

Fig. S7. I-V curves of ABA-modified single glass conical nanopore in 1 mM KCl (pH = 7.4) solution with the addition of 1 μM CTP, 1 μM UTP, 1 μM GTP and 100 nM ATP, respectively. Error bars indicate the standard deviations of three independent measurements.

10. Time-dependent of the current of ABA-modified nanopore prior to and after ultrasonication.

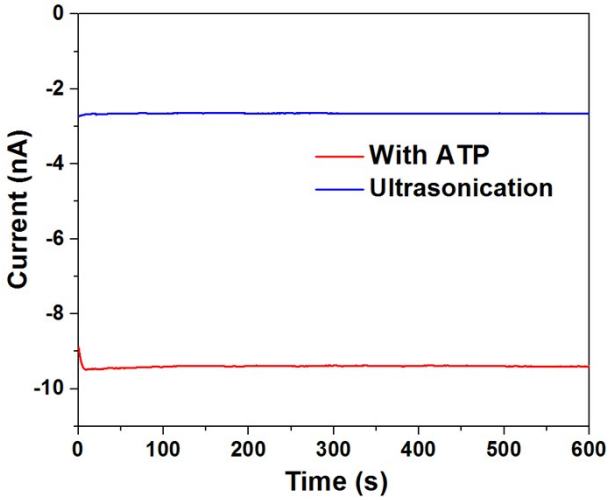

Fig. S8. The current traces at -1V of ABA-modified glass conical nanopore in the presence of ATP and after ultrasonication. The electrolyte is 1 mM KCl PBS buffer (pH = 7.4).

References

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