Electronic Supplementary Information

An ion-gating multinanochannels system based on a copper-responsive self-cleaving DNAzyme

Yang Chen,[a] Di Zhou,[a] Zheyi Meng[a] and Jin Zhai[a]*

[a] Beijing Key Laboratory of Bio-Inspired Energy Materials and Devices, School of Chemistry and Environment, Beihang University, Beijing 100191, P. R. China.
Email: zhaijin@buaa.edu.cn

Experimental Section

Fabrication of Nanochannels: The PET films (multiple channels, It4ip, Seneffe, Belgium, 23 μm in thickness, ion track density 10^8 cm^{-2}) were used to fabricate conical multinanochannels by the asymmetric ion-track etching method. Before the chemical etching process, each side of the sample was exposed to the UV light for 1 h. To produce conical nanochannels, etching was performed only from one side; the other side of the cell contained a solution that was able to neutralize the etchant as soon as pores opened, thus slowing down the further etching process. Briefly, the PET membrane was embedded between the two chambers of a conductivity cell at 35 ^oC, one chamber was filled with etching solution (9 M NaOH), while stopping solution (1 M KCl + 1 M HCOOH) was added in the other one. A voltage of 1 V was applied across the membrane. When the ion current reached a certain value (about 10^{-6} A) to make sure the channel conductance reached a certain magnitude, the reaction would be stopped. The membrane was soaked in MilliQ water (18.2 MΩ) to remove residual salts.

DNA modification: Oligonucleotides were synthesized by Sangon Biotech (Shanghai, China). The amino single-stranded DNA(2) (5'-GAATA TAGTG AGCTA CGCTA GAATT C-$(CH_2)_6$-NH_2-3') on 3' end was immobilized onto the PET surface and inner pore wall by a two-step chemical reaction. First, the carboxyls on the PET film were converted into NHSS esters by dipping into an 1mL aqueous solution of 15 mg EDC and 3 mg NHSS for 1 h at room temperature. Then these PET-NHSS esters were reacted with a

solution of 1 μM DNA(2) in water for 2 h at room temperature. To further form double-stranded DNA inside the nanochannels, the precursor in last step were put into a 1 μM DNA(1) (5'-GAATT CTAAT ACGAC TCACT ATAGG AAGAG ATGGC GACTG TTTAG AAGCA GGCTC TTTCT TATGC GTCTG GGCCT CTTTT TAAGA AC) aqueous solution for 2 h at 60 ^{o}C. The functionalized PET membranes were washed with distilled water several times for further experiments.

Ion current measurement: The ionic transportation properties of multinanochannels were studied by measuring I-V curves by the Keithley 6487 picoammeter (Keithley Instruments, Cleveland, OH). The PET multiporous film was mounted between the two chambers of the conductivity cell, and both halves of the cell were filled with symmetric solutions. The base side of the film faced to the anode. Ag/AgCl electrodes were used to apply a transmembrane potential across the film. A scanning voltage varied from -2 V to +2 V at a scanning rate of 40 s. Each test was repeated at least 5 times to obtain the average current value at different voltages.

Characterization: The morphologies of multinanochannels were observed by a FEI Quanta FEG 250 environmental SEM at 10 kV. Circular dichroism (CD) spectra were collected on a JASCO J-810 CD spectrometer from 220 nm to 320 nm at 23 ^{o}C. The DNA was dissolved in a Tris buffer solution (pH = 7.2) containing Tris (5 mM) and HCl (4.5 mM) to give the DNA-Tris buffer solution a final concentration of 1 μM in a 1 mm quartz cell. For gel electrophoresis, the DNA samples were dissolved in a Tris-HCl buffer solution with different amount of Cu^{2+}, and the concentration of DNA was 20 μM. Then the samples were mixed with loading buffer (containing 95% formamide, 5 mM EDTA, 0.025% SDS, 0.025% bromophenol blue, 0.025% xylene cyanol FF) and separated in 12% polyacrylamide gel electrophoresis (acrylamide/bisacrylamide, 29:1) for 1h at 130V.

Supporting Figures

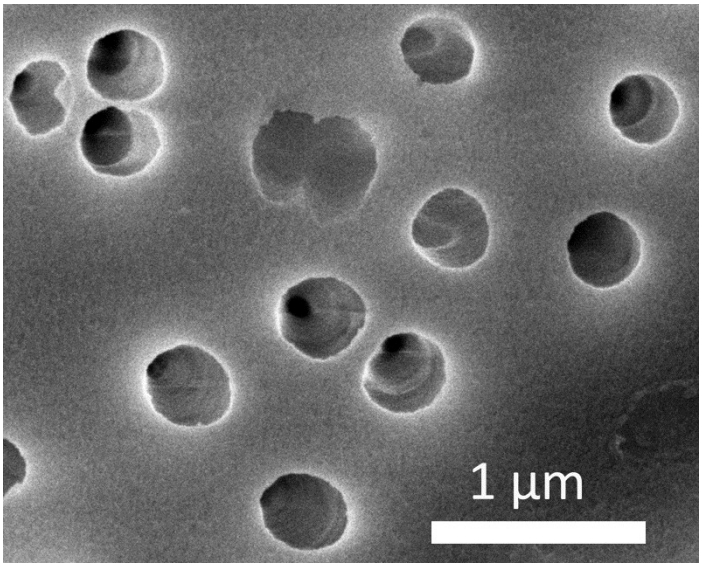

Fig. S1 SEM image of the base side of the multiple conical PET nanochannels. The average diameter is 400 nm.

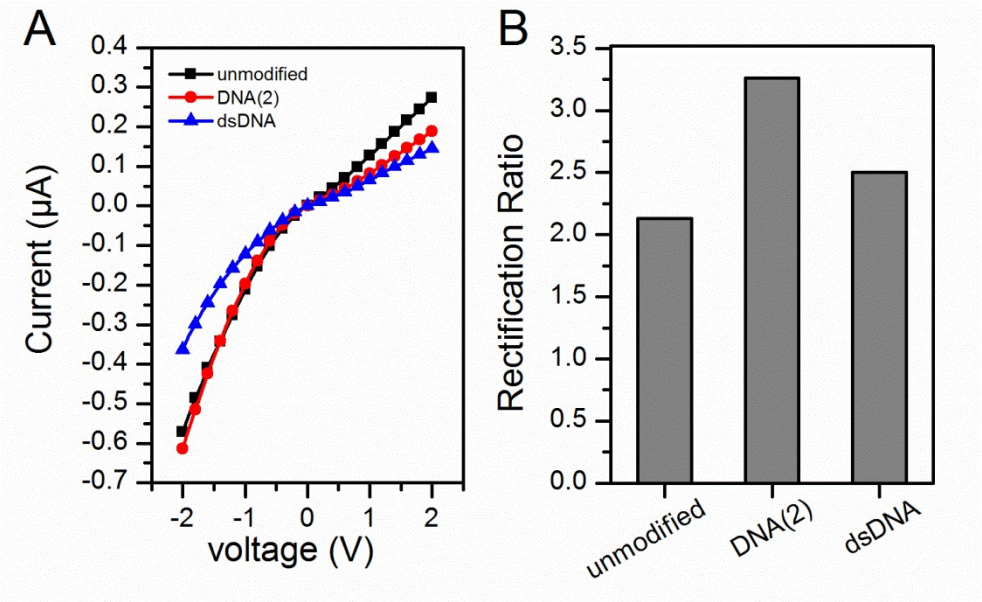

Fig. S2 (A) I-V curves and (B) rectification ratio of multinanochannels before and after modification.

The dsDNA modified in the nanochannel would have synergetic, but antagonistic influences on charge density and steric hindrance. First, the single-stranded DNA(2) is immobilized to the inner wall of the nanochannels. Since DNA(2) is short and single-stranded structure, the steric hindrance can be ignored here. The negative charges brought by it lead to a small increase of the current at -2 V bias. The rectification ratio also increase from 2.1 to 3.3 after DNA(2) modification. After adding DNA(1) to form a dsDNA construction, the current exhibits an obvious decrease due to the minished effective pore diameter. Based on the change of the I-V curve, the steric hindrance of the double-stranded structure is the main influencing factor, though DNA(1) also brings a lot of negative charges to the nanochannel system, which is a secondary factor. The rectification ratio decreases from 3.3 to 2.5 with the declined current. It's noticeable that we mainly consider the current at -2 V because the cations preferentially transport from tip to base in a negative pore at negative bias and the electrostatic condition of tip side influence the whole pore much more.

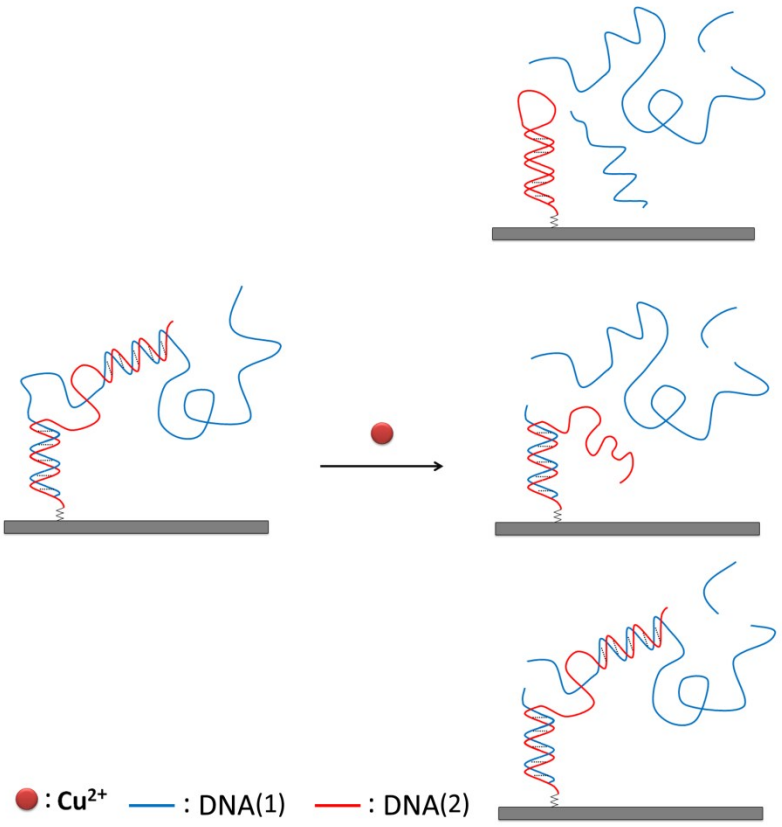

Fig. S3 The possible configuration change of dsDNA after Cu^{2+}-induced cleavage.

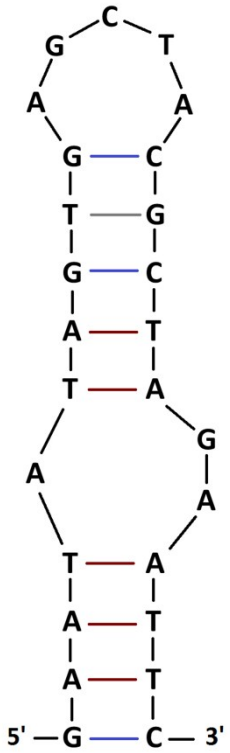

Fig. S4 Secondary structure of DNA(2).

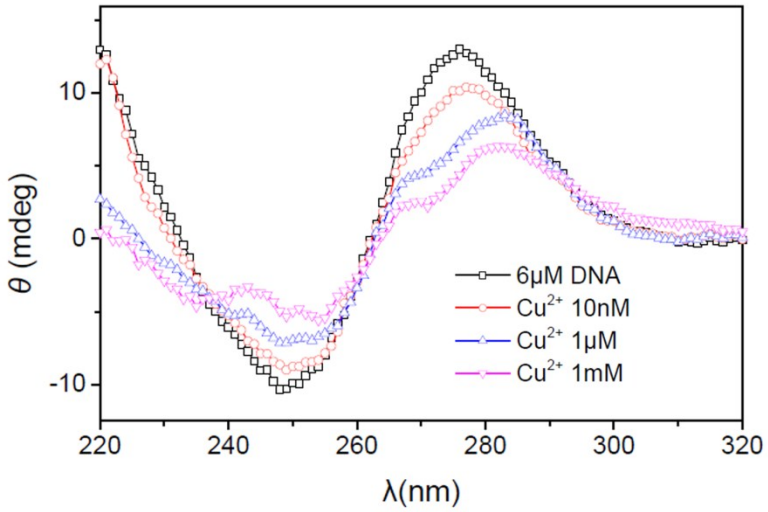

Fig. S5 Circular dichroism (CD) spectroscopy in a 1 μM DNA(1)/DNA(2)-mixing solution with different concentration of Cu^{2+}.

There are two characteristic peaks belonging to the dsDNA at about 250 nm and 275 nm. With the addition of the Cu^{2+}, the intensity of both peaks declines gradually, indicating that the self-cleaving of dsDNA is dependent on copper concentration.

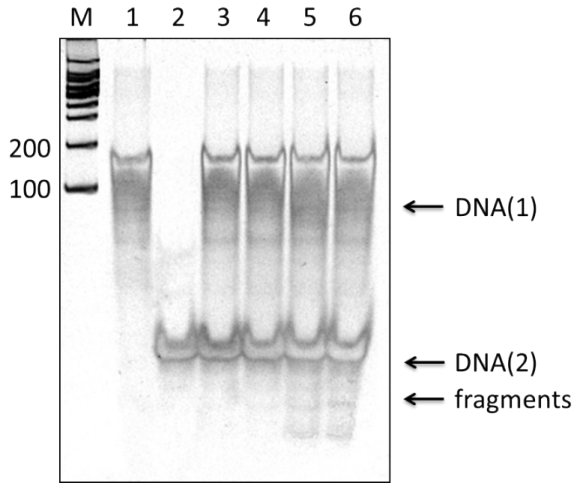

Fig. S6 Polyacrylamide gel electrophoresis with different concentration of Cu^{2+}. Lane M: molecular weight standards; lane 1: DNA(1) without Cu^{2+}; lane 2: DNA(2) without Cu^{2+}; lane 3: DNA(1)/DNA(2)-mixing solution without Cu^{2+}; lane 4: DNA(1)/DNA(2)-mixing solution with 10 nM Cu^{2+}; lane 5: DNA(1)/DNA(2)-mixing solution with 1 μM Cu^{2+}; lane 6: DNA(1)/DNA(2)-mixing solution with 1 mM Cu^{2+}.

The polyacrylamide gel electrophoresis was done at different concentration of Cu^{2+} to demonstrate the digestive ability of DNAzyme. Lane 3-6 are DNA(1)/DNA(2)-mixing solution with no, 10 nM, 1μM and 1 mM Cu^{2+}. Some fragments are shorter than DNA(2) after dissociating from DNAzyme, so the band position of these fragments are more forward than that of DNA(2). As the concentration of copper rises, the band color of the fragments turns darker, which exhibits the increase of the amount of fragments. It illustrates that the digestive ability of DNAzyme is concentration-dependent.