Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Sensitive Zn^{2+} Sensor based on Biofunctionalized Nanopores by Combination of DNAzyme and DNA Supersandwich Structures

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1.1 Experimental Section

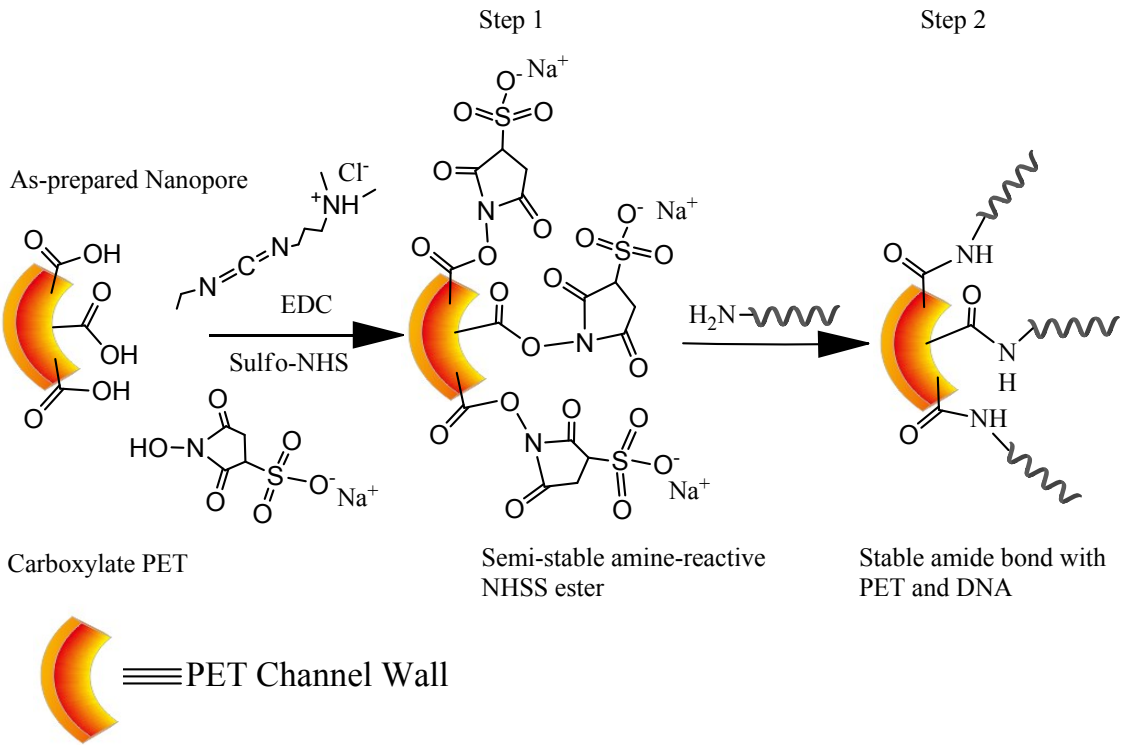
Material. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHSS), Zinc Chloride ($ZnCl_2$) were obtained from Sigma-Aldrich. Tris (hydroxymethyl) aminomethane (Tris) was purchased from Alfa Aesar. Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) and Sodium chloride (NaCl) were purchased from Sinopharm Chemical Regent (Beijing, China). All DNA oligonucleotide sequences were synthesized and purified by TaKaRa Biotech company (Dalian, China). Chemical reagents were all used as received. Deionized water (18.2 MΩ·cm, MilliQ system) was used.

Fabrication of Nanocpores. Cylindrical nanopore array was prepared by using Polyethylene terephthalate membranes (PET, approximately 12 μm thick) through the track etching pretreatment. The PET was irradiated with Au ion beam (11.4 Mev/nucleon in Tsinghua University, Beijing, China). The track density of PET membrane is approximately $1.2\times10^7/cm^2$. Before the chemical etching, the membranes were treated by UV irradiation (4 mW/cm^2) for 30min on each side. Then, the UV radiated membranes were immersed in 2 M NaOH solution at 50 $^oC$for 6.5 min to obtain cylindrical nanopores. After chemical etching, the membranes were taken out from the NaOH solution and completely washed with deionized water for several times. The membranes soaked in deionized water for 8h, in order to remove alkaline residues. The morphology of the nanopores was filmed by field-emission scanning electron microscopy (FESEM, Sirion SEM 200).

Self-assembly of DNA Supersandwich structures in Nanopore. Capture Probe was grafted onto the PET surface through a two-step reaction (detailed in the scheme below). During the track-etching process, the carboxyl (-COOH) groups generated on the surface of nanopore. The etched PET membranes were immersed in 600 μL solution containing 30 mg EDC·HCl and 6 mg NHSS at 4 ^oC for 12 h. During this process, NHSS esters were formed. In the next step, the amine-reactive NHS-esters molecules were covalently coupled to 3'-aminated DNA (the capture probe, 1μM) in 600μL of 20 mM Tris solution (pH=7.5, 100mM NaCl, 10mM $MgCl_2$) overnight. Finally, the modified membrane was washed thoroughly with deionized water.

The purely CP-modified PET filmwas firstly immersed in a centrifuge tube filled with 600 μL solution containing 1 μM AP1 and 1 μM AP2 for 12h. Then, SP and DS were added into a centrifuge tube of a final concentration of 1 μM for 12h.

Electrochemical measurements. The electrochemical characterization of the prepared PET membrane were performed by using two-electrode cells in a Tris buffer solution (20mM Tris pH=7.5, 100mM NaCl, 10mM $MgCl_2$) as electrolytes. The symmetric Ag/AgCl electrodes were used as working and counter electrode.



Scheme 1 chemical modification of the PET nanopores with aminated DNA probes.[1,2,3]

1.2 SEM image of PET membranes

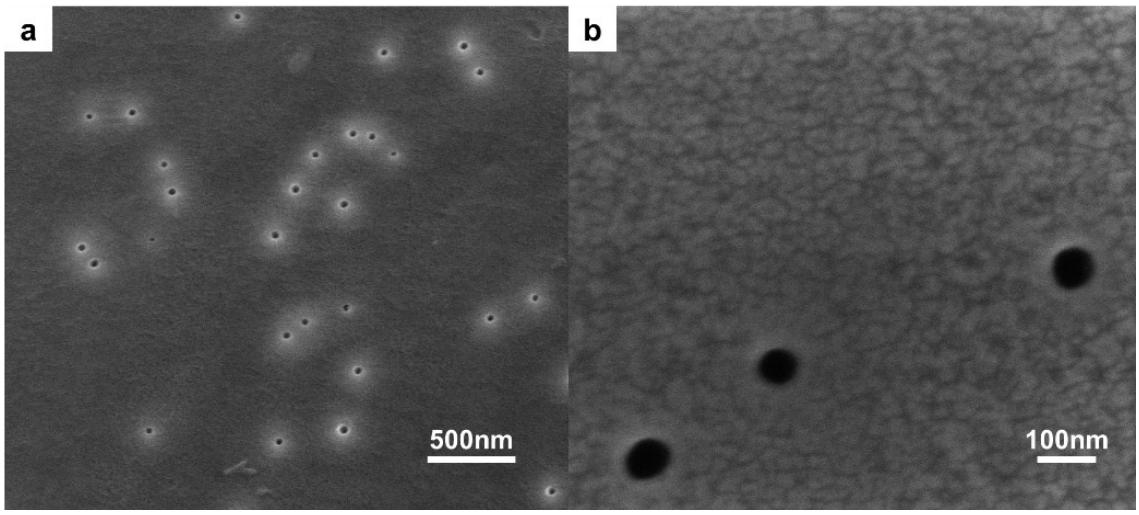


Fig. S1 SEM images of irradiated PET membrane after UV treatment and chemical etching.

1.3 Characterization of the DNA assemblies in nanopores

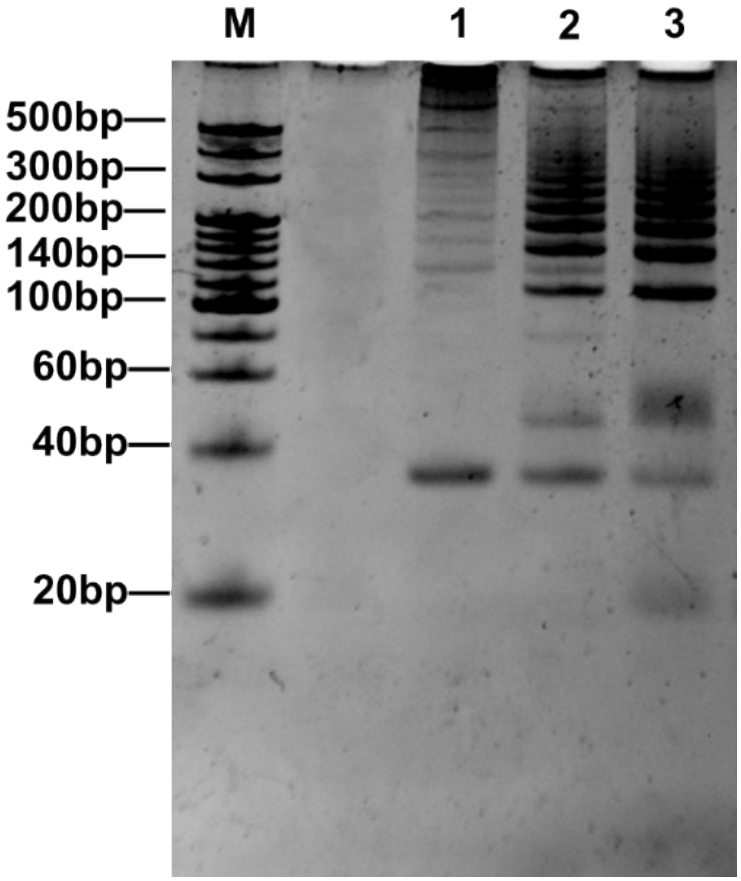


Fig. S2 Agarose gel electrophoresis: 1) 1μM AP1+1μM AP2; 2) 1μM AP1+1μM AP2+0.5μM SP; 3) 1μM AP1+1μM AP2+0.5μM SP+0.5μM DS.

1.4 I-V plot of pure PET membrane and membrane decorated by CPs

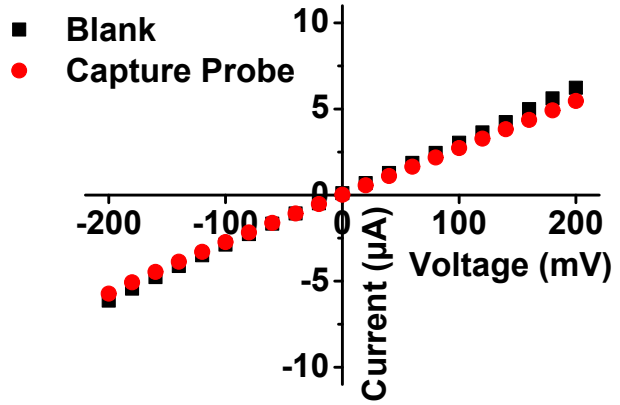


Fig. S3 I-V plot of pure PET membrane (black line) and membrane decorated by CPs (Red line).

1.5 Verification experiments of the current variation in nanopores

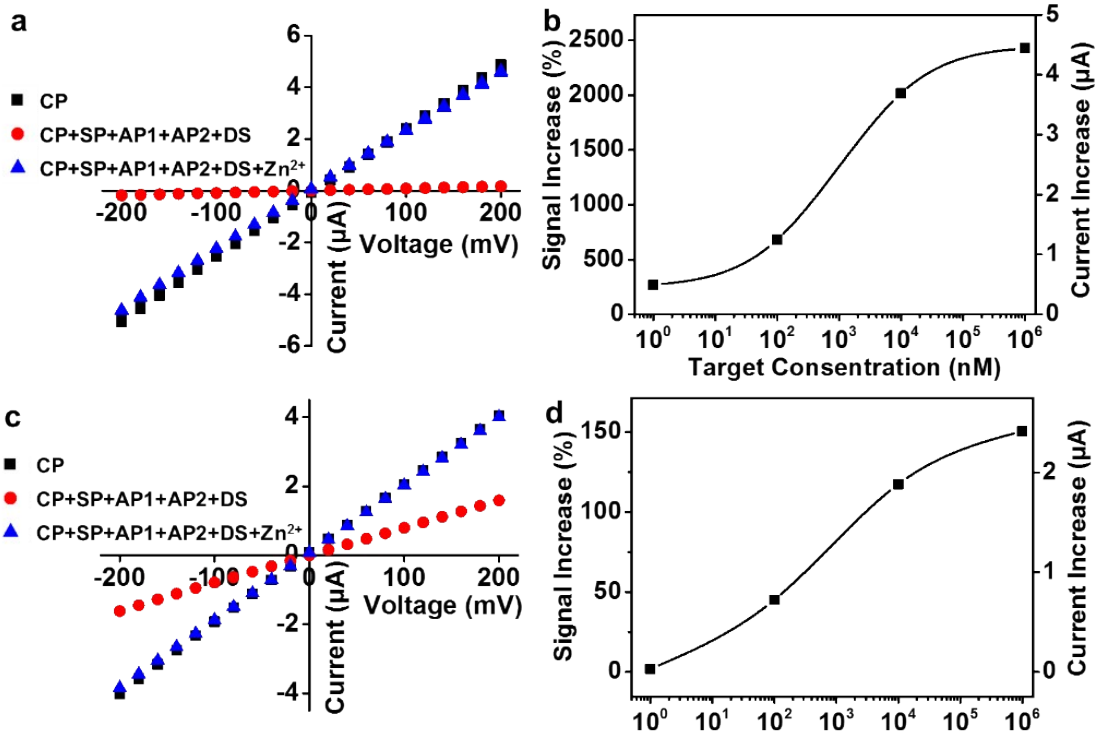


Fig. S4 two verification experiments a) and c) the I-V varation of the nanopore decorated by DNAzyme system after introducing (step 1) or peeling off (step 2) DNA supersandwich structures; b) the signal increasing of conductance (after peeling off DNA supersandwich structures) in a decrement concentration of Zn2+ from 1 mM to 1nM.

1.6 I-V curves for the detection of Pb^{2+}, Cu^{2+} and Hg^{2+}

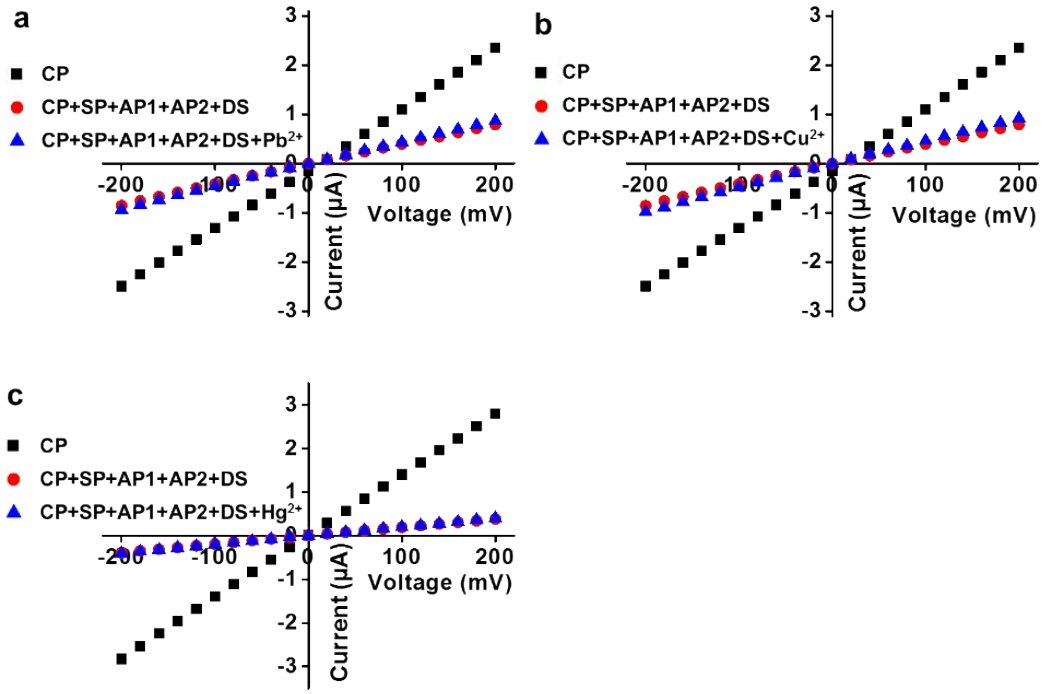


Fig. S5 I-V curves for the detection of Pb^{2+}, Cu^{2+} and Hg^{2+}.

1.7 **Table R1**. The detection limits of various methods for Zn^{2+} sensing

Sensing Method	Detection Limit	Reference
Colorimetric sensor	5.0 μM	4
Fluorescent sensor	1.0 μM	5
	14 nM	6
	3.0 μM	7
Electrochemical sensor	83 nM	8
	2.0 μM	9
	3.6 μM	10
	1.0 nM	Present work

1.8 Reference

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