Supporting Information

Homogenous electrochemical aptamer-based ATP assay with signal amplification by exonuclease III assisted target recycling

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Experimetal Details

Reagents

Exonuclease III, NEBuffer 2 were purchased from New England Biolabs and used without further purification. The hairpin-aptamer probes were purchased from Takara Biotechnology Co., Ltd. (Dalian, China) with the following sequences: 5'-CCTCCTACCTGGGGGAGTATTGCGGAGGAAGGTA-ferrocene-3'; 5'-FAM-CCTCCTACCTGGGGGAGTATTGCGGAGGAAGGTA-BHQ-3'. Adenosine 5'-triphosphate (ATP), guanosine 5'-triphosphate (GTP), cytidine 5'-triphosphate (CTP), uridine 5'-triphosphate (UTP) and thymidine 5'-triphosphate (TTP) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All other chemicals used were of analytical reagent grade. All aqueous solutions were prepared with deionized water (specific

resistance >18.2 MΩ/cm) obtained with a Milli-Q reagent grade water system (Millipore Corp., Bedford, MA).

Exo III-assisted ATP recycling

A volume of 50 μL sample containing 2 μM hairpin-aptamer probe, 100 units of exonuclease III, and varying concentrations of the ATP or its analogues, was incubated at 37 °C for a certain period of time.

Electrode Pretreatment and Measurement

Electrochemical measurements were conducted on a CHI 832B electrochemical analyzer (CH Instruments Inc., Shanghai, China) with a conventional three-electrode system comprising an ITO working electrode with surface area of 0.5 cm^2, a platinum wire auxiliary electrode, and a Ag/AgCl reference electrode. Before each electrochemical measurement, the ITO electrode was sequentially sonicated in an Alconox solution (8 g of Alconox/L of water), propan-2-ol, acetone, and water, each for 10 min. Then, the electrode was immersed into 1 mM NaOH solution for 5 h at room temperature and sonicated in water for 10 min. After these procedures, a negatively charged working electrode surface was obtained. The differential pulse voltammetry (DPV) was recorded in the above-mentioned 50 μL incubation buffer (1× NEB buffer 2) containing reaction mixtures with the potential window from 0.2 V to 0.55 V.

Fluorescence measurements

Fluorescence spectra were measured with a F-4600 fluorescence spectrophotometer (Hitachi, Japan) with excited wavelength of 490nm.

Figure S1. The DPV response of 2 μM hairpin-aptamer probe in 1 × NEB buffer 2 (a) and with addition of 150 mM NaCl (b) and 300 mM NaCl (c) in the case of no ATP (red) and 0.1 μM ATP (green). The concentration of Exo III is 2 unit/μL. Error bars are obtained based on three independent measurements.

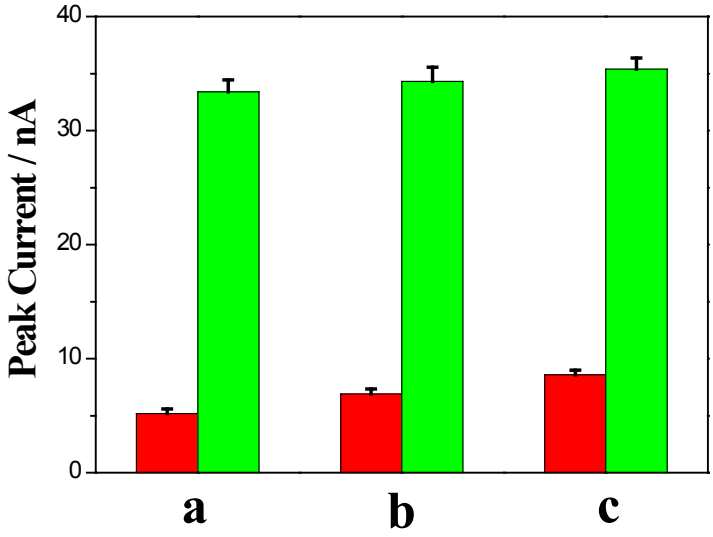

Figure S2. Fluorescence (FL) emission spectra of the FAM dye in the presence of 2 μM hairpin-aptamer probe, with: (a) no ATP, no EXO III. (b) 2 μM ATP, no EXO III. (c) 2 unit/μL Exo III, no ATP. (d) 2 μM ATP, 2 unit/μL Exo III.

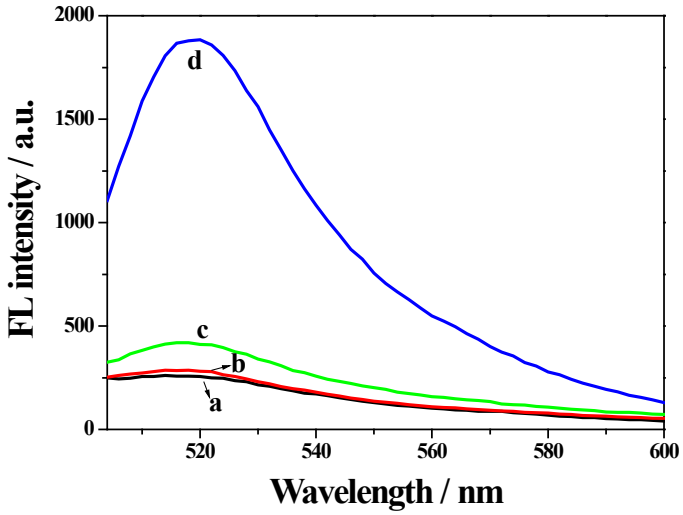

Figure S3. The DPV responses of reaction mixture containing different concentrations of hairpin-aptamer probe and 2 unit/μL Exo III in 1 × NEB buffer 2 in the case of no ATP (red) and 0.1μM ATP (green). Error bars are obtained based on three independent measurements.

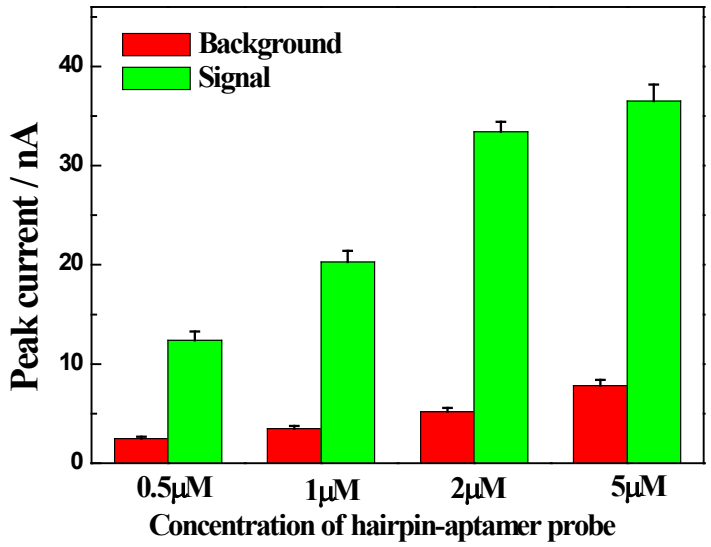

Table S1. Comparison of detection performance for ATP by ours and those reported electrochemical methods

Method	Probe	Detection limit	Strategy	Ref.
DPV	Ferrocence (Fc)	1.0×10^{-5} M	Heterogenous	1
DPV	Fc	1.0×10^{-8} M	Heterogenous	2
DPV	Fc	1.0×10^{-10} M	Heterogenous	3
ACV CC	Methylene blue (MB)	1.0×10^{-6} M	Heterogenous	4
SWV	RuHex	3.0×10^{-10} M	Heterogenous	5
SWV	PbS	3.0×10^{-8} M	Heterogenous	6
DPV	Fc	1.0×10^{-8} M	Heterogenous	7
DPV	Fc	1.0×10^{-9} M	Homogenous	This work

DPV: Differential Pulse voltammetry; ACV: Alternating Current Voltammetry; SWV: Square-wave Voltammetry; CC: Chronocoulometry

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