Supporting information

Responsive hairpin DNA aptamer switch to program the strand displacement reaction for the enhanced electrochemical assay of ATP

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Experimental Section

Chemicals and reagents. Tris(2-carboxyethyl)phosphine (TCEP) and 6-Mercapto hexanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). 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. The HPLC-purified oligonucleotide sequences are purchased from Sangon Biotech. Co., Ltd. (Shanghai, China) and listed in Table S1. The urine samples were obtained from healthy volunteers and filtered through a 0.2 mm membrane to remove any particulate matters.

Apparatus. All electrochemical measurements were carried out by using a CHI 660D electrochemical workstation (CH Instruments, Shanghai, China) at room temperature. A conventional three-electrode system was used, which comprised a gold working electrode (2 mm diameter), a platinum wire auxiliary electrode, and a Ag/AgCl reference electrode. Fluorescence measurements were performed using a Hitachi F-4600 spectrofluorimeter with a scan rate at 1200 nm/min (Tokyo, Japan). The excitation wavelength was set to 490 nm and the 24 photomultiplier voltage was 700V.

Electrode pretreatment. The gold electrode was cleaned by immersion in a freshly prepared piranha solution (a 3:1 v/v mixture of concentrated H_2SO_4 and 30% H_2O_2) for 20 min, followed by a thoroughly rinse with ultrapure water. Then the electrode was polished on a microcloth (Shanghai Chenhua Inc., China) with 50 nm alumina slurry to obtain a mirror surface, followed by sonication in acetone and ultrapure water for 5 min each, to remove residual alumina powder. The well-polished electrode was then subjected to electrochemical pretreatment by cycling the potential between -0.2 and 1.5 V in H_2SO_4 (0.5 M) at a scan rate of 100 mV s^{-1} until a stable cyclic voltammogram was obtained, and then the cleaned electrode was allowed to be dried at room temperature.

The immobilization of I-DNA and B-DNA on the electrode surface. Each DNA solution was heated to 95 °C for 5 min and then allowed to cool to room temperature for at least 2 hr before use. The duplex DNA probe was firstly prepared by mixing the same volume of 1 μM I-DNA and 1.5 μM B-DNA in 10 mM PBS (0.2 M NaCl, 10 mM TCEP, pH 7.9). Then the electrode was incubated into above DNA duplex probe solution for 12 hr at room temperature and then thoroughly rinsed with ultrapure water and dried under a stream of nitrogen gas. The electrode was subsequently immersed in 1 mM MCH solution for 1 hour to remove the nonspecific DNA adsorption.

ATP recognition and toehold-mediated strand displacement reaction on the electrode. The ATP recognition and toehold-mediated strand displacement reaction was performed by incubation of the above I-DNA and B-DNA modified electrode into the 50 μL 10 mM PBS buffer (0.2 M NaCl, pH 7.9) containing 1 μM HP1 and different concentrations of ATP for 2 hr at 37 ^{o}C. Then, the electrode was rinsed with PBS buffer and ultrapure water for electrochemical measurement.

Electrochemical measurements. Cyclic voltammetric (CV) and differential pulse voltammetric (DPV) results were recorded in 10 mM PBS buffer (200 mM KNO_3, pH 7.9). The DPV were recorded with the potential window from 0.4 V to -0.1 V, the pulse amplitude of 50 mV and the pulse period of 0.2 S. The electrochemical impedance spectra (EIS) were recorded in 1 mM $[Fe(CN)_6]^{3-/4-}$and 0.1 M KNO_3 with the frequency range from 0.1 Hz to 10 kHz. Before measurements, the electrolyte solution should be thoroughly purged with high purity nitrogen for about 20 min.

Table S1. Sequence of synthesized oligonucleotide probes for ATP detection

Name	Sequence (5' to 3')
I-DNA	HS-$(CH_2)_6$-TACTAGTCATGAGGAGGAAGGTA
B-DNA	TCATGACTAGTA
HP1-a	TTCCTCCTTTACCTGGGGGAGTATTGCGGAGGAAGGTATCATGACTAGTA-Fc
HP1-b	CCTTCCTCCTTTACCTGGGGGAGTATTGCGGAGGAAGGTATCATGACTAGTA-Fc
HP1-c	TACCTTCCTCCTTTACCTGGGGGAGTATTGCGGAGGAAGGTATCATGACTAGTA-Fc
HP2	CCTTCCTCCTTTACCTGGGGGAGTATTGCGGAGGAAGGTATCATGACTAGTA
S1	FAM-TACTAGTCATGAGGAGGAAGGTA
S2	TCATGACTAGTA- Dabcyl

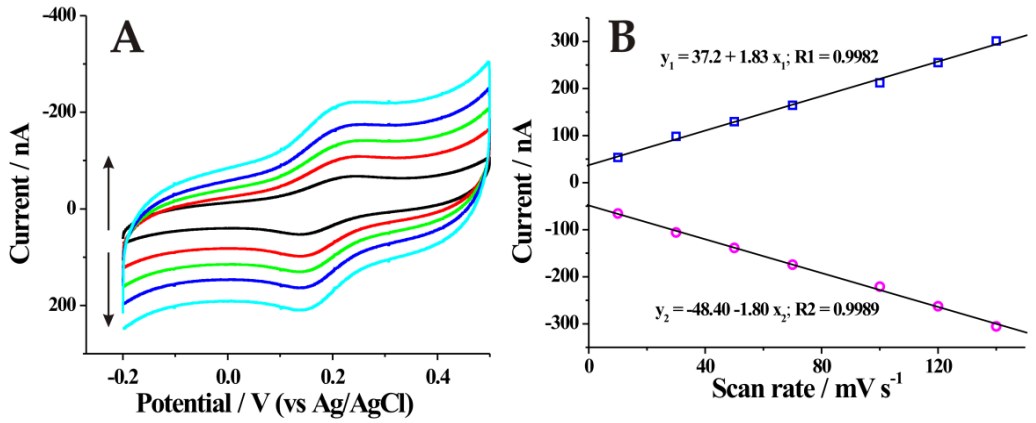

Figure S1. Cyclic voltammograms recorded at various scan rates of 10, 30, 50, 70 and 100 mV/s. (B) The plots of anodic and cathodic peak currents vs scan rates.

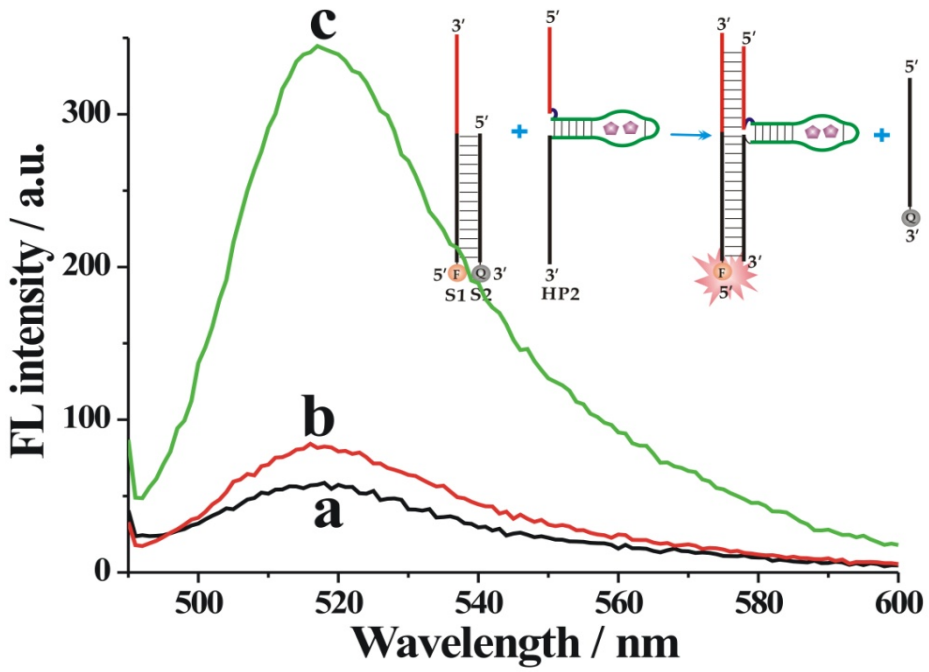

Figure S2. The fluorescence spectra recorded for ATP aptasensor fabrication at different conditions: (a) only the S1-S2 hybrids (S1-S2); (b) HP2 + S1-S2; (c) ATP + HP2+ S1-S2. The final concentration for ATP, HP2, S1 and S2 were 1 μM, 200 nM, 100 nM, and 150 nM, respectively. The S1-S2 were firstly prepared with the hybridization of S1 and S2. The inset schematically demonstrates the responsive toehold-mediated strand displacement reaction for ATP detection. The strands of S1 and S2 were labeled with the fluorophore (FAM) at 5'-end and the quencher (Dabcyl) at 3'-end, respectively. The unlabeled HP2 was used for the substitute of HP1 herein.

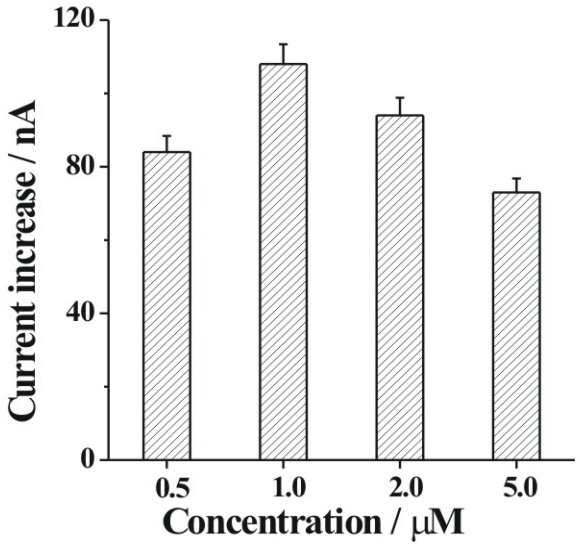

Figure S3. The influence of immobilization concentration of duplex DNA on the electrochemical response toward 10 μM ATP detection. The duplex DNA was prepared with the hybridization of I-DNA and 1.5 time concentration of B-DNA. The used duplex DNA concentration corresponded to the I-DNA concentration. The current increase for y-axis indicates the electrochemical response toward 10 μM ATP after substracting the background value in the absence of ATP.

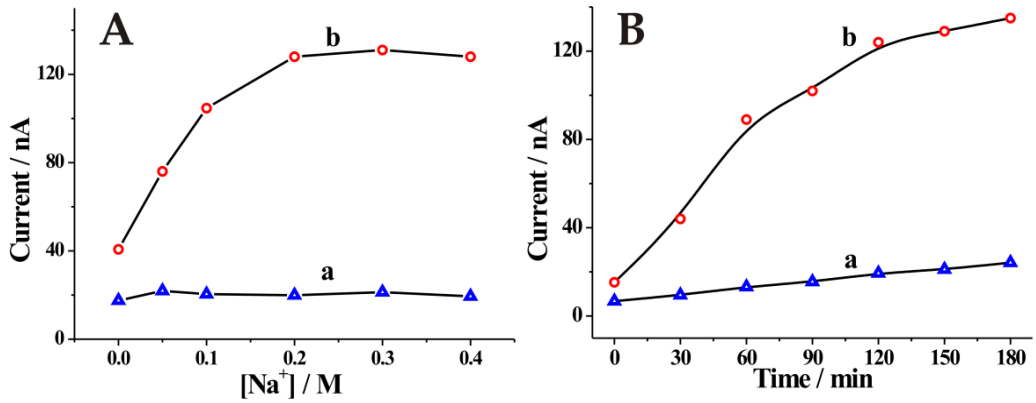

Figure S4. (A) Effects of the Na^+ concentration plotted against DPV peak currents. The reaction mixture contains 1 μM HP1 probe and 10 μM ATP (b) or 0 M ATP (a). (B) The time response of DPV peak current toward 10 μM ATP (b) or 0 M ATP (a).

Table S2. Comparison of detection performance for ATP by currently fabricated aptasensor with the reported electrochemical methods [a]

Method	Detection limit	Signal reporter	Ref.
CC	1.0×10^{-7} M	RuHex	[1]
EIS	1.0×10^{-8} M	$[Fe(CN)_6]^{4-/3-}$	[2]
DPV	1.0×10^{-6} M	Silver nanoparticle	[3]
DPV	1.0×10^{-9} M	Fc	[4]
EIS	1.5×10^{-8} M	$[Fe(CN)_6]^{4-/3-}$	[5]
SWV	3.0×10^{-8} M	PbS	[6]
SWV	1.9×10^{-9} M	Fc and MB	[7]
ACV	1.0×10^{-6} M	MB	[8]
DPV	1.0×10^{-8} M	Fc	[9]
DPV	1.0×10^{-8} M	Fc	[10]
DPV	5.0×10^{-9} M	Fc	This work

[a] Chronocoulometry (CC); Electrochemical impedance spectroscopy (EIS); Differential pulse voltammogram (DPV); Square wave voltammogram (SWV); Alternating-current voltammogram (ACV); Ferrocene (Fc); Methylene blue (MB).

Table S3. Recovery experiments for the added ATP in the diluted urine sample [a]

Sample	Added (nM)	Found (nM)	Recovery (%)
1	20	21±1.7	105
2	100	98±7.2	98
3	500	511±43	102
4	1000	1043±62	104

[a] The standard deviations for the samples were obtained from three independent experiments

References

[1] L. Shen, Z. Chen, Y. Li, P. Jing, S. Xie, S. He, P. He and Y. Shao, *Chem. Commun.,* 2007, 2169.

[2] Y. Du, B. Li, H. Wei, Y. Wang and E. Wang, *Anal. Chem.,*2008, **80,** 5110.

[3] L. Kashefi-Kheyrabadi and M. A. Mehrgardi, *Biosens. Bioelectron.,* 2012, **37**, 94.

[4] S. Liu, Y. Wang, C. Zhang, Y. Lin and F. Li, *Chem. Commun.,* 2013, **49**, 2335.

[5] L. Wang, M. Xu, L. Han, M. Zhou, C. Zhu, S. Dong, *Anal. Chem.,* 2012, **84**, 7301.

[6] H. Zhang, B. Jiang, Y. Xiang, Y. Zhang, Y. Chai, R. Yuan, *Anal. Chim. Acta,* 2011, **688**, 99.

[7] L. Wu, X. Zhang, W. Liu, E. Xiong, J. Chen, *Anal. Chem.,* 2013, **85**, 8397.

[8] X. Zuo, Y. Xiao, K. W. Plaxco, *J. Am. Chem. Soc.,* 2009, **131**, 6944.

[9] Y. Lu, X. Li, L. Zhang, P. Yu, L. Su, L. Mao, *Anal. Chem.,* 2008, **80**, 1883.

[10] X. Zuo, S. Song, J. Zhang, D. Pan, L. Wang, C. Fan, *J. Am. Chem. Soc.,* 2007, **129**, 1042.