Electronic Supplementary Information

Highly Effective Molecule Converting Strategy Based on Enzyme-Free Dual Recycling Amplification for Ultrasensitive Electrochemical Detection of ATP

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Experimental section

Regents and Materials

Adenosine triphosphate (ATP), cytidine triphosphate (CTP), uridine triphosphate (UTP) and guanosine triphosphate (GTP) were purchased from Worthington Biochemicals (Lakewood, NJ, USA). *N*-hydroxy succinimide (NHS), Chloroauric acid ($HAuCl_4$), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide-hydrochloride (EDC) and 6-mercaptohexanol (MCH) were supplied by Sigma (St. Louis, MO, USA). Carboxylated-magnetic polystyrene microspheres (PSC-COOH) were obtained from Tianjin BaseLine ChromTech Research Centre (Tianjin, China). $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were provided by Beijing Chemical Reagent Co. (Beijing, China). Phosphate buffered solution (PBS) (pH 7.0, 0.1 M) containing 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl was served as working buffer.[1] 5 mM Tris-HCl buffer (pH 8.0) containing 100 mM NaCl, 20 mM KCl and 10 mM $MgCl_2$ was used to prepare various concentrations of oligonucleotide.[2] All oligonucleotide sequences were

synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and listed in Table S1.

Table S1. The oligonucleotide sequences in this work.

Name	Sequence (from 5′ to 3′)
Apt1	ACC TGG GGG AGT ATG TGA GGT AAG ATC G
Apt2	CGA CAT CTA ACC TAG CCC TCA CAG CGG AGG AAG GT
HP1	GAT ATC AGC GAT CTT GCT AGG TTA GAT GTC GAA GCA CCC ATG TTA CTC TCG ACA TCT AAC CTA GC
HP2	AGA TGT CGA GAG TAA CAT GGG TGC TTC GAC ATC TAA CCT AGC AAG CAC CCA TGT TAC TCT
HP3	NH_2-$(CH_2)_6$-AAA ACA CAC AGA GTA TrA GGA TAT CAG TGT GAT ACT G
DNA1	NH_2-$(CH_2)_6$-AAA ACA TTA GCG TCT GAT ATC C
DNA2-MB	CAG TAT CAC AAC GCT AAT-MB

The CHI 660D electrochemical workstation (Shanghai Chen Hua Instrument, China) was used for all electrochemical measurements including electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV) and cyclic voltammetry (CV). The traditional three-electrode consisted of glassy carbon electrode (GCE, Φ = 4 mm) as working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode[3]. The pH meter (MP 230, Mettler Toledo, Switzerland) was utilized to perform pH measurements. Scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan) was applied to characterize the size and surface morphology of AuNPs.

Electrochemical measurements

CV and EIS of electrode modification process were carried out in 2 mL 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl. The CV was taken from -0.2 V to 0. 6 V (*vs*. SCE) at a scan rate of 100 $mV \cdot s^{-1}$. EIS measurements were performed with the frequencies swept from 0.1 to 10^5 Hz. The SWV detection was placed into the

electrolyte of 2 mL PBS (pH 7.0) and the corresponding measurement parameters were as follows: the scanning range: -0.45 V - -0.15 V, step potential: 4 mV, amplitude: 25 mV, frequency: 25 Hz.

Preparation of PSC-HP3

First, 300 μL PSC-COOH was washed and redispersed in 300 μL PBS 7.0. Then, 100 μL coupling reagents (200 mM EDC and 50 mM NHS) were added to activate the carboxyl group of PSC-COOH for 1 h. After that, 100 μL HP3 (5 μM) was mixed with activated PSC and stored at 4 °C for 2 h. During this process, HP3 could be attached on the PSC-COOH surface *via* an acylation reaction. The resulting PSC-HP3 was separated with magnetic separator to remove unbound HP3 and re-suspended in 500 μL Tris-HCl buffer (pH 8.0).

Fabrication of ATP assay

Before use, Apt1, Apt2 and all hairpin oligonucleotides were annealed according to reference[4]. 1 μM Apt1, Apt2, HP1 and HP2 were mixed with different concentrations of ATP and incubated for 3 h at 37 °C. Afterwards, 20 μL prepared PSC-HP3 was mixed with 20 μL of above mixture in a plastic tube for 1 h. During this process, HP3 was autocatalytically cleaved by Mg^{2+} and produced amounts of S1, which was attributed to the formation of active Mg^{2+}-dependent DNAzyme structure. Finally, the mixture containing various concentrations of released S1 was collected with magnetic separator.

Meanwhile, a cleaned bare glassy carbon electrode (GCE)[5] was immersed in 2 mL $HAuCl_4$ (1%) for electrodeposition a thin layer of AuNPs (constant potential: -0.2

V, electrodepositing time: 30 s). Then 15 μL DNA1 (2 μM) was dropped on the AuNPs-modified electrode and incubated overnight at 4 °C. Next, 10 μL MCH (1 mM) was applied to block the nonspecific binding sites for 1 h. Ultimately, 20 μL of mixture containing 10 μL of preceding released S1 and 10 μL of 2 μM methylene blue-labeled DNA2 (DNA2-MB) was casted onto the modified electrode and incubated for 90 min at 37 °C to form "Y" junction structure. MB that approached to the electrode was used as signal probe to indicate the amount of ATP. After thoroughly cleaned with ultrapure water, the modified electrode was stored at 4 °C for further usage.

Results and discussion

Characterization of the electrochemical biosensor

CV measurements were employed to characterize the electrochemical biosensor preparation procedure. As shown in Fig. S1A, a couple of well-defined $[Fe(CN)_6]^{3-/4-}$ redox peak could be acquired at a bare GCE (curve a). After electrodepositing AuNPs on the GCE, an obvious increase in peak current was observed (curve b) due to the prominent electronic conductivity of AuNPs. When DNA1 was assembled on the AuNPs-modified electrode by the interaction of Au-N, the peak current was decreased (curve c) owing to the fact that negatively charged DNA effectively repelled the $[Fe(CN)_6]^{3-/4-}$. As expected, after blocking the nonspecific recognition sites with nonconductive MCH, a further decrease of peak current was obtained (curve d). When the DNA2-MB and the mixture containing S1 were dropped on the electrode, the peak current was decreased (curve e), indicating that the sensing interface was successfully

constructed.

EIS was utilized to further verify the fabrication process of electrodes. Fig. S1B displayed the impedance spectra for the different modified electrodes. A relatively small semicircle can be obtained at the bare GCE (curve a). The modification of AuNPs on the GCE resulted in a smaller semicircle (curve b) in comparison with bare GCE, which was attributed to the excellent conductivity of AuNPs. After DNA1 was self-assembled on the modified electrode, the impedance increased obviously (curve c). Upon blocking with MCH, dramatic increase in the impedance was observed (curve d). Finally, the semicircle diameter increased after assembling DNA2-MB and S1, due to the inhibition of "Y" structure to the electron transfer (curve e). These results were in accordance with CV, which clearly clarified the successful modification of proposed electrochemical biosensor.

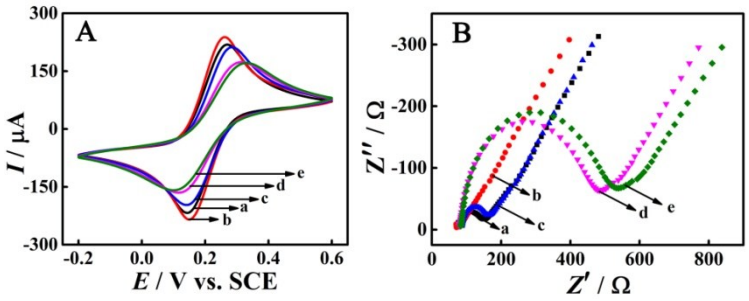

Fig. S1 (A) CV obtained in 2 mL 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl at a scan rate of 100 $mV \cdot s^{-1}$. (B) EIS responses of modified electrodes at different step in 2 mL 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl in the frequency range from 0.1 Hz to 10^5 Hz with 25 mV as the amplitude. (a) bare GCE, (b) AuNPs/GCE, (c) DNA1/AuNPs/GCE, (d) MCH/DNA1/AuNPs/GCE, (e) MCH/DNA1/AuNPs/ GCE treated with S1 and DNA2-MB.

Table S2. Comparison of different methods for ATP assay.

Analytical methods	Detection limit	Linear range	Ref
Chemiluminescent	1.4 nM	2 nM-80 nM	[6]
Electrochemiluminescence	1.5 pM	5 pM-5 nM	[7]
Photoelectrochemistry	0.1 nM	0.3 nM-200 nM	[8]
Electrochemistry	0.1 nM	0.1 nM-2 nM	[9]
Electrochemistry	1 nM	1 nM-20 nM	[10]
Electrochemistry	4.7 nM	20 nM-200 nM	[11]
Electrochemistry	0.45 pM	0.001 nM-50 nM	This work

Table S3. Recovery results for ATP in human serum (n = 3) with electrochemical biosensor.

Sample number	Concentration of ATP Added/nM	Concentration of ATP Found/nM	Recovery/%	RSD/%
1	0.010	0.0104	104	3.2
2	0.10	0.102	102	5.7
3	1.0	0.963	96.3	2.4
4	10	9.26	92.6	6.1

References

1 P. Zhang, Z. F. Lin, Y. Zhuo, R. Yuan and Y. Q. Chai, *Anal. Chem.*, 2017, **89**, 1338-1345.

2 X. Li, Y. Peng, Y. Q. Chai, R. Yuan and Y. Xiang, *Chem. Commun.*, 2016, **52**, 3673-3676.

3 C. Ye, M. Q. Wang, Z. F. Gao, Y. Zhang, J. L. Lei, H. Q. Luo and N. B. Li, *Anal. Chem.*, 2016, **88**, 11444-11449.

4 Y. H. Guo, Wu, J.; Ju, H. X. *Chem. Sci.*, **2015**, *6*, 4318-4323.

5 H. J. Wang, L. J. Bai, Y. Q. Chai and R. Yuan, *Small*, 2014, **10**, 1857-1865.

6 Y. H. Song, X. Yang, Z. Q. Li, Y. J. Zhao and A. P. Fan, *Biosens. Bioelectron.*, 2014, **51**, 232-237.

7 J. J. Lu, M. Yan, L. Ge, S. G. Ge, S. W. Wang, J. X. Yan and J. H. Yu, *Biosens. Bioelectron.*, 2013, **47**, 271-277.

8 Y. X. Liu, H. M. Ma, Y. Zhang, X. H. Pang, D. W. Fan, D. Wu and Q. Wei, *Biosens. Bioelectron.*, 2016, **86**, 439-445.

9 Y. Du, B. L. Li, F. A. Wang and S. J. Dongg, *Biosens. Bioelectron.*, 2009, **24**, 1979-1983.

10 S. F. Liu, Y. Wang, C. X. Zhang, Y. Lin and F. Li, *Chem. Commun.*, 2013, **49**, 2335-2337.

11 H. Zhang, C. C. Fang and S. S. Zhang, *Chem. Eur. J.*, 2010, **16**, 12434-12439.