Supplementary information

An efficient signal-on aptamer-based biosensor for adenosine triphosphate detection using graphene oxide as both electrochemical and Electrochemiluminescence signal indicator

Xiang Huang,‡[a] Yuqin Li,‡[b] Xiaoshan Zhang,[c] Xin Zhang,[a] Yaowen Chen[c] and Wenhua Gao*[ac]

[a] Department of Chemistry, Shantou University, Shantou, Guangdong 515063, P. R. China.

[b] Department of Pharmacy, Taishan Medicine College, Taian, Shandong 271016, P. R. China.

[c] Analysis & Testing Center, Shantou University, Shantou, Guangdong 515063, P. R. China.

** Corresponding author. Tel: +86-22-86502774; Fax: +86-22-82903941*

E-mail address: whgao@stu.edu.cn

‡ Both the authors contributed to the paper equally.

Experimental Details

Synthesis of the ECL probe

Ruthenium bis(2,2'-bipyridine)-(2,2'-bipyridine-4,4'-dicarboxylic acid)-N-hydroxysuccinimide ester [$Ru(bpy)_2(dcbpy)NHS$] as the ECL label of luminescence signal reporter probe ($Ru(bpy)_2(dcbpy)NHS$-ABA, abbreviated as Ru-ABA), was synthesized according to our previous experimental work with some modification.[1] Firstly, $RuCl_3 \cdot 3H_2O$ (0.78 g, 3.05 mM), 2,2'-bipyridine (0.936 g, 6 mM) and LiCl (0.84 g, providing a water-free reaction condition) were put together and under reflux in 60 mL of dimethylformamide (DMF) for 8 h with sustained stirring. Secondly, 50 mL acetone was added to the cooled reaction mixture and followed with an overnight freeze. The green-dark microcrystalline product was filtered through 0.2 μm membranes and washed with water-diethyl ether solution (V: V, 1: 3). The solid obtained as $Ru(bpy)_2Cl_2$ was finally dried under vacuum 40 °C.

The synthesized $Ru(bpy)_2Cl_2$ (0.0934 g, 0.1915 mM), $NaHCO_3$ (0.105 g) and 2,2'-bipyridine-4,4'-dicarboxylic acid (0.04678 g, 0.1917 mM) were added to 10 mL of water-methanol solution (V: V, 1: 4) and refluxed for 4 h. The solution was cooled in an ice bath for 7 h, meanwhile the mixing solution was adjusted to pH 4.4 with hydrochloric acid solution (HCl) to promote the formation of the orange-red crystal. The formed precipitate was filtrated through 0.2 μm membranes and dissolved with methanol and then filtrated once again to obtainorange-red clear filtrate. Then ~ 2 g $NaPF_6$ in 14 mL of water was added in the orange-red filtrate and cooled in an ice bath. And the latter formed precipitate was collected by filtration was $Ru(bpy)_2(dcbpy)(PF_6)_2$ and dried under vacuum 40 °C. Then, 0.153 g (0.74 mM) of N,N'-dicydlohexyl carbodiimide and 0.079 g (0.69 mM) of N-hydrosuccinimide were dissolved in 1.5 mL DMF with stirring and cooled in an ice bath. And then 1 mL DMF containing 0.1322 g $Ru(bpy)_2(dcbpy)(PF_6)_2$ was added in the mixing solution and stirred for 5 h to obtain $Ru(bpy)_2(dcbpy)NHS$. $Ru(bpy)_2(dcbpy)NHS$ was characterized in 0.1 M PBS solution (pH 7.4, 0.1 M NaCl + 0.1 M NaH_2PO_4/Na_2HPO_4) by UV-vis absorption spectrum and showed in Fig. S1.

The $Ru(bpy)_2(dcbpy)NHS$-ABA (Ru-ABA) was synthesized according to our previous experimental work[1] with some modification. Firstly, 1 OD ss-TBA was dissolved in 200 μL water, and then 200 μL of 6.02×10^{-4} M $Ru(bpy)_2(dcbpy)NHS$ inPBS (pH 7.4) was added to the above ss-ABA solution, allowed to shaking at low speed overnight at room temperature. Then, by addition of 100 μL of 3 M sodium acetate trihydrate (NaAc) and 2 mL of ethanol to the mixture, the precipitate reaction was carried out in refrigerator at -20 °C over 12 h. The mixture was immediately centrifuged in a micro-centrifuge at 12000 r/min for 30 min. The supernatant was carefully removed and the precipitate was rinsed with cold 70% ethanol twice and dried in air. The dried precipitate was redissolved in 200 μL of 0.1 M PBS (pH 7.4) and stored under - 20 °C in refrigerator. The resulting solution was used as a stock solution of the ECL probe Ru-ABA.

Results and Discussion

UV-vis absorption spectra of ECL probe

Figure S1 shows that the UV-vis absorption spectra of ATP-binding aptamer (ABA, curve a), $Ru(bpy)_2(dcbpy)NHS$ (curve b) and $Ru(bpy)_2(dcbpy)$-NHS-ABA ((abbreviated as Ru-ABA, curve c). In Figure S1, the characteristic absorption peak at 260 nm is observed for the ABA (curve a). The absorption spectrum of the $Ru(bpy)_2(dcbpy)NHS$ (curve b) exhibits a characteristic peak at 457 nm, assigned to metal-to-ligand charge-transfer band and that at 287 nm, assigned to ligand-to-ligand charge-transfer of $\pi \rightarrow \pi^*$ transitions. After the labeling of ABA with $Ru(bpy)_2(dcbpy)NHS$, the absorption spectrum (curve c) shows both the ABA and the $Ru(bpy)_2(dcbpy)NHS$ characteristic absorption peaks. And the characteristic absorption peak appears at 457 nm, corresponding to the characteristic peak of $Ru(bpy)_2(dcbpy)NHS$. The characteristic absorption peaks at 284 nm and 255 nm corresponding to the characteristic peaks of $Ru(bpy)_2(dcbpy)NHS$ at 287 nm and ABA at 260 nm, respectively. This indicates that $Ru(bpy)_2(dcbpy)NHS$ tag was attached to ABA successfully.

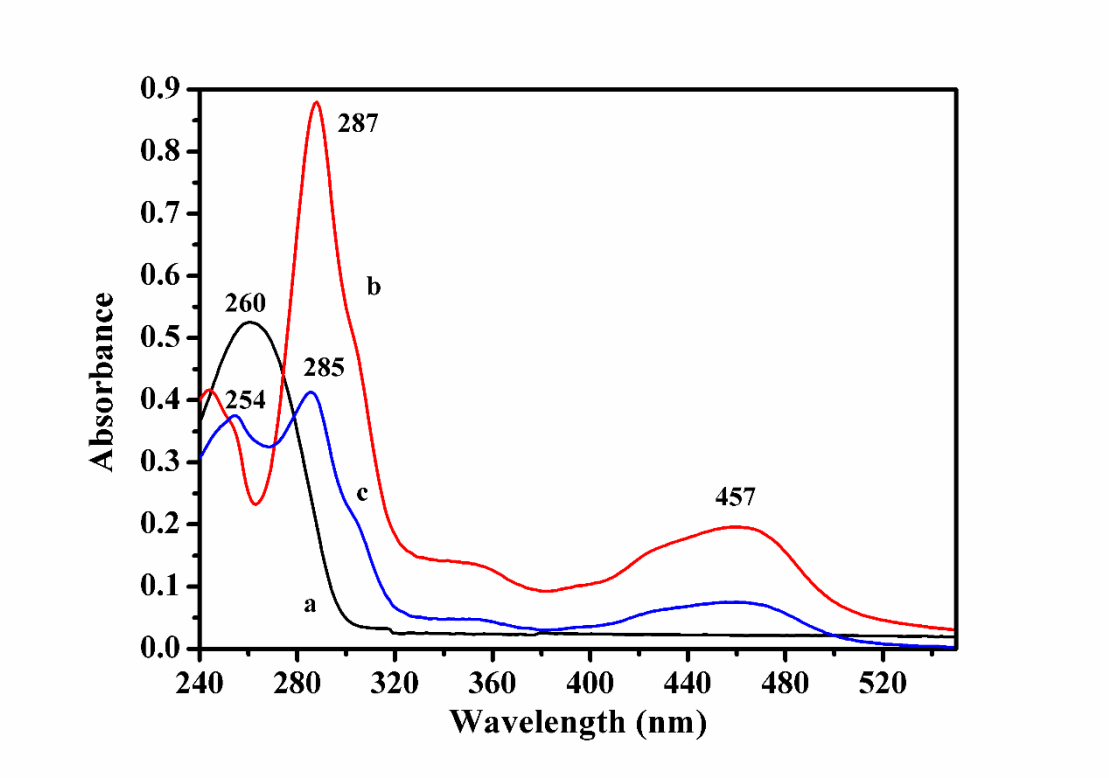

Fig. S1 UV-vis absorption section of (a) ABA; (b) $Ru(bpy)_2(dcbpy)NHS$; (c) $Ru(bpy)_2(dcbpy)NHS$-ABA in 0.1 M PBS (pH, 7.4).

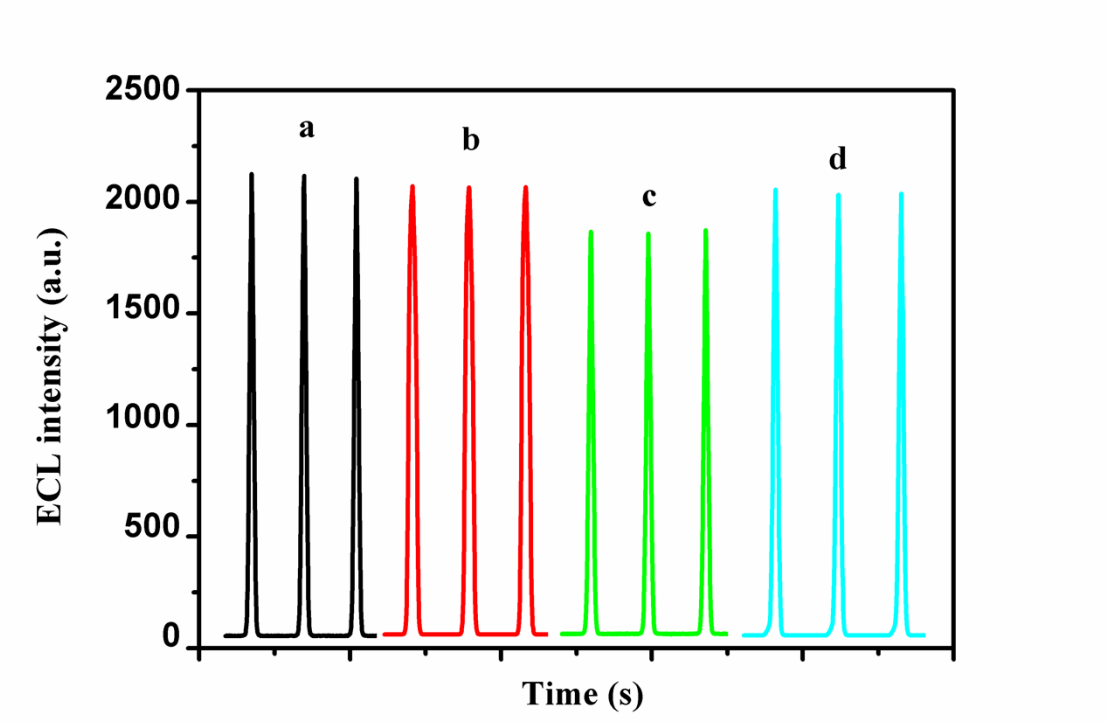

Fig. S2 ECL response of ATP detection in human serum samples directly.

Table S1
Comparison of the sensitivity for different aptasensor assay methods

Analytical method	Detection limit	Linearity range	Reference
Electrochemical method	13.6 nM	0.05 μM – 1.0 μM	2
Electrochemical method	34 pM	0.1 nM -- 20 nM	3
Electrochemical method	6.7 pM	10 pM – 10 nM	This work
Fluorescent	25 nM	50 nM – 20 μM	4
ECL	7.6 nM	8.0 nM -- 2000 nM	5
ECL	31 pM	50 nM -- 100 pM	6
ECL	10 pM	10 pM--100 nM	7
ECL	6 nM	18 nM – 90.72 μM	8
ECL	10 pM	50 pM--10 nM	9
ECL	4.8 pM	10 pM--10 nM	This work

Table S2
Determination results of ATP in human serum samples.

Sample No.[a]	Added (nM)	Found (nM)[b]	Recovery (%)	RSD (%, n=3)
1	1.00	0.96	96.0	4.18
2	2.00	2.15	107.5	5.04
3	5.00	4.97	99.4	6.12
4	10.00	10.42	104.2	5.64

a. All human serum samples were diluted 10-fold with buffer solution (pH 7.4) prior to assay.

b. Each data was given as average value obtained from three successive determinations.

Table S3
Analysis of ATP in human serum samples using the proposed aptasensor directly.[a]

Samples No.	ECL (n=3, $\bar{X}$ ±SD)	Log (ATP) (n=3, $\bar{X}$±SD)	ATP levels (nM) (n=3, $\bar{X}$±SD)
a	2000 ± 70.5	-0.51 ± 0.010	0.31 ± 0.01
b	2100 ± 80.7	-0.42 ± 0.036	0.38 ± 0.03
c	1980 ± 80.4	-0.54 ± 0.053	0.29 ± 0.03
d	2057 ± 100.8	-0.46 ± 0.020	0.35 ± 0.01

a. All values were obtained as average of three repetitive determinations plus standard deviation.

Reference

1. W. H. Gao, A. Zhang, Y. S. Chen, Z. X. Chen, Y. W. Chen, F. S. Lu and Z. G. Chen, *Biosens. Bioelectron*., 2013, 49, 139-145.

2. L. Feng, Z. Zhang, J. Ren, and Qu, X, *Biosens. Bioelectron*., 2014, 62, 52-58.

3. T. Bao, H. Shu, W. Wen, X. Zhang and Wang, S. *Anal Chim Acta*., 2014, **863**, 64-69.

4. F Li, Z. Du, L. Yang, and Tang, B. *Biosens. Bioelectron.*, 2013, ***41***, 907-910.

5. Y. Liu, J. Lei, Y. Huang and H. Ju, *Anal. Chem.*, 2014, ***86***, 8735-8741.

6 L. Chen, Q. Cai, F. Luo, X. Chen, X. Zhu, B. Qiu and G. Chen, *Chem. Commun.*, 2010, **46**, 7751-7753.

7 X. Zhou, R. Duan, and D. Xing, *Analyst.*, 2012, **137**, 1963-1969.

8. H. Huang, Y. Tan, J. Shi, G. Liang and J. J Zhu, *Nanoscale.*, 2010, **2**, 606-612.

9 H. Chen, Q. Chen, Y. Zhao, F. Zhang, F. Yang, J. Tang and P He, *Talanta*., 2014, **121**, 229-233.