Supporting information

Responsive surface bioaffinity binding to construct flexible and sensitive electrochemical aptasensor

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Table S1. The used nucleic acid base sequences in the experiment[a]

Name	Sequence (5' to 3')
IP (for ATP)	Dig-CAATACTCCCCCAGGTTTTT-$(CH_2)_6$-SH
HP-1 (for ATP)	**ACCTGGGGGAGTATTG*CGGAGG*(AAGGT)** *CCTCCG*
HP-2 (for ATP)	**ACCTGGGGGAGTATTG*CGGAGG*(AAGGT** TTTAA)*CCTCCG*
HP-3 (for ATP)	**ACCTGGGGGAGTATTG*CGGAGG*(AAGGT** TTTTTTTTAA)*CCTCCG*
HP-4 (for ATP)	**ACCTGGGGGAGTATTG*CGGAGG*(AAGGT** TTTTTTTTTTTTTAA)*CCTCCG*
HP-5 (for ATP)	**ACCTGGGGGAGTATTG*CGGAGG*(AAGGT** TTTTTTTTTTTTTTTTTAA)*CCTCCG*
IP (for thrombin)	Dig-TGCCCTACCACGGACTTTTT-SH
HP (for thrombin)	**AGTCCGTGGTAGGGCA*GGTTGG*(GGTGA CT**TTTTTTTTTTTTT)*CCAACC*

[a] The HP-1 to HP-5 represent the hairpin-like aptamer probes with different loop sequences shown in the bracket. They are 5, 10, 15, 20 and 25-base loop sequences for HP-1, HP-2, HP-3, HP-4 and HP-5, respectively. The aptamer sequences were shown in bold letters. The underlined letters in IP and HP indicates mutual base complementarities. The italic letters in HP shows the complementary bases in the stem region. The ATP aptamer sequence was based on the reference (D. E. Huizenga and J. W. Szostak, *Biochemistry*, 1995, 34, 656; wherein the ATP aptamer sequence was firstly developed). The 29-mer DNA aptamer for thrombin was based on the reference (D. M. Tasset, M. F. Kubik and W. Steiner, *J. Mol. Biol.*, 1997, 272, 688; wherein this aptamer sequence was originally developed).

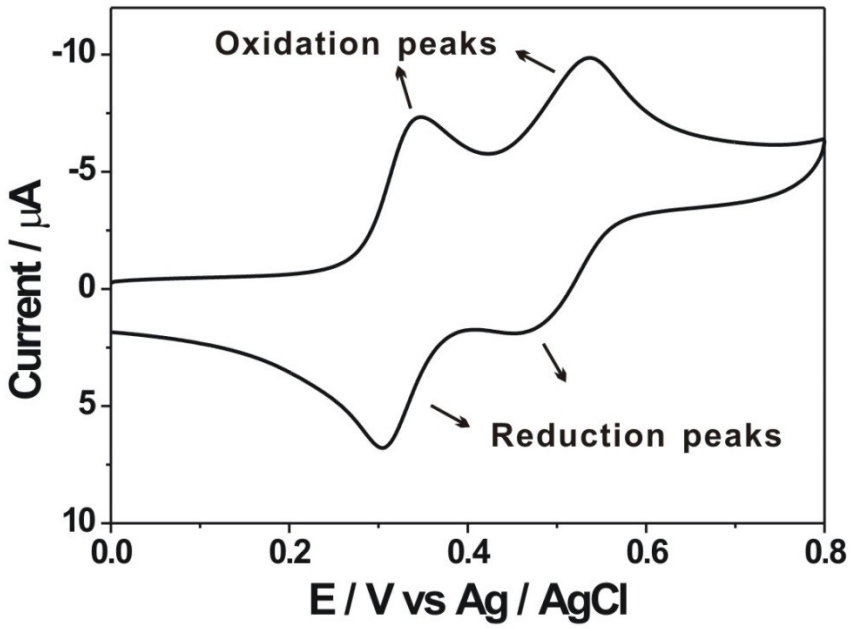

Figure S1. Cyclic voltammogram of bare gold electrode in 0.1 M pH 5.5 HAc-NaAc buffer containing 1 mM TMB and 5 mM H_2O_2. The scan potential and scan rate were 0-0.8 V and 100 mV/s, respectively.

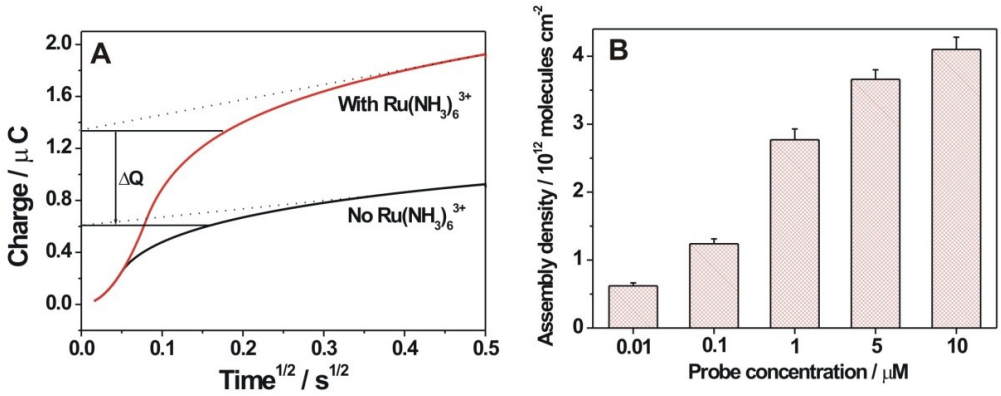

Figure S2. (A) Chronocoulometric curves of duplex DNA probe modified electrodes in the absence and presence of 50 μM $Ru(NH_3)_6^{3+}$. (B) Surface assembly density of duplex DNA probe obtained by using different concentration of duplex DNA probe.

The corresponding calculation method for the surface coverage of duplex DNA probe on the electrode was based on the previous reference (A. B. Steel, T. M. Herne and M. J. Tarlov, *Anal. Chem.,* 1998, **70**, 4670) and shown below:

The charge Q, as a function of time t in a chronocoulometric experiment is given by the integrated Cottrell expression,

$$Q(t) = 2FAC\left(\frac{Dt}{\pi}\right)^{\frac{1}{2}} + Q_c + nFA\Gamma_0$$

where F is the Faraday constant (96487 C/equiv), n is the number of electrons involved in the electrode reaction, A is the electrode area (cm^2), C is the bulk concentration of $Ru(NH_3)_6^{3+}$ ($mol\ cm^{-3}$), D is its diffusion coefficient ($cm^2\ s^{-1}$), t is the time (s), Q_c is the capacitive charge (C), and Γ_0 is the quantity of the adsorbed $Ru(NH_3)_6^{3+}$ ($mol\ cm^{-2}$). The term Γ_0 designates the surface excess and represents the amount of $Ru(NH_3)_6^{3+}$ confined near the electrode surface. The chronocoulometric intercept at t=0 is then the sum of the capacitive charge and the surface excess terms. The surface excess is thus determined from the difference (ΔQ) in chronocoulometric intercepts for the identical potential step experiment in the presence and absence of $Ru(NH_3)_6^{3+}$.

The saturated surface excess of $Ru(NH_3)_6^{3+}$ is then converted to the surface density of the duplex DNA probe with the relationship,

$$\Gamma_{DNA} = \Gamma_0\left(\frac{z}{m}\right)(N_A)$$

Where Γ_{DNA} is the surface density of the duplex DNA probe ($molecules/cm^2$), m is the number of bases in the duplex DNA probe, z is the charge of the $Ru(NH_3)_6^{3+}$, and N_A is Avogadro's number.

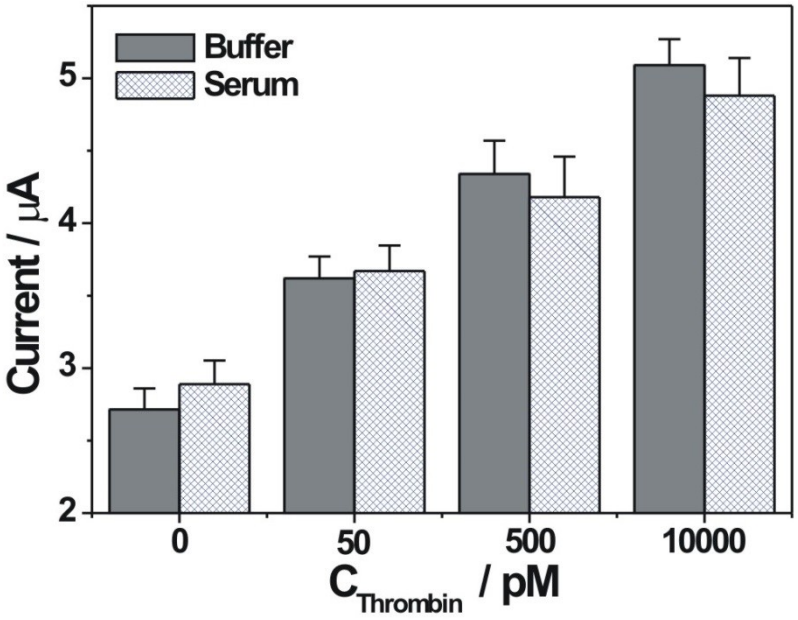

Figure S3. Steady current responses toward the spiked thrombin in buffer and 5% diluted serum.

Table S2. The detection performance comparison toward ATP by electrochemical aptamer-based methods[a]

Method	Detection limit	Dynamic range	Strategy	Ref.
SWV	1.9 nM	10 nM ~ 100 μM	Coulping signal-on and signal-off strategy	1
SWV	1.4 nM	5 nM ~ 1 μM	Target-induced conformational change of dual-hairpin DNA structure	2
SWV	10 nM	10 nM ~ 1 mM	Target-responsive aptamer switch	3
CC	100 nM	100 nM ~ 1 mM	Surface charge change	4
DPV	10 nM	10 ~ 80 nM	Aptamer-complementary DNA oligonucleotides as probe	5
SWV	3.4 nM	10 nM ~ 1 μM	Nicking endonuclease-assisted recycling of target–aptamer complex	6
CC	0.2 nM	1 nM ~ 10 μM	Gold nanoparticle-based signal amplification	7
SWV	5 nM	10 nM ~ 4 mM	nanoprobe-enhanced split aptamer-based electrochemical sandwich assays	8
EIS	15 nM	15 nM ~ 4mM	Graphene enhanced electron transfer	9
CA	0.87 nM	1 nM ~ 10 μM	Responsive surface bioaffinity binding strategy	This work

[a]Abbreviation: square wave voltammogram (SWV), differential pulse voltammetry (DPV), chronocoulometry (CC), electrochemical impedance spectroscopy (EIS), chronoamperometry (CA).

Table S3. Detection performances of current electrochemical aptasensors toward ATP and thrombin

Target	Linear equation	Linear range	Detection limit	Correlation coefficient
ATP	I (μA) = 9.428 + 0.712 $\log C_{ATP}$ (M)	1 nM-10 μM	0.87 nM	0.9948
Thrombin	I (μA) =10.0017 + 0.6103 $\log C_{thrombin}$ (M)	10 pM-100 nM	6.3 pM	0.9931

Table S4. The detection performance comparison toward thrombin by electrochemical aptamer-based methods [a]

Method	Detection limit	Dynamic range	Strategy	Ref.
SWV	6.4 nM	20 ~ 768 nM	Binding-induced conformational change	10
LSV	1 nM	None	Nucleic acid-functionalized Pt nanoparticles	11
SWV	1.7 pM	5 pM ~ 1 nM	Proximity binding-triggered molecular machine	12
DPV	0.1 nM	1 ~ 200 nM	Enzyme amplification	13
EIS	0.267 pM	0.267 ~267 pM	Molybdenum disulphide nanosheets	14
ACV	2.5 pM	10 pM~100 nM	Binding-induced DNA walker	15
SWV	1.2 pM	10 pM~10μM	Gold nanoparticle-decorated MoS_2 nanosheets	16
EIS	10 pM	0.3 ~50 nM	Functionalized graphene nanocomposites	17
CA	6.3 pM	10 pM~100 nM	Responsive surface bioaffinity binding strategy	This work

[a]Abbreviation: square wave voltammogram (SWV), linear sweep voltammogram (LSV), differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS), alternating current voltammetry (ACV), chronoamperometry (CA).

References

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

[2] J. Jia, J. Feng, G. H. Chen, H. Q. Luo and N. B. Li, *Sensors and Actuators B,* 2016, **222**, 1090.

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

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

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

[6] T. Hu, W. Wen, X. Zhang and S. Wang, *Analyst,* 2016, **141**, 1506.

[7] W. Li, Z. Nie, X. Xu, Q. Shen, C. Deng, J. Chen and S. Yao, *Talanta,* 2009, **78**, 954.

[8] T. Zhao, R. Liu, X. Ding, J. Zhao, H. Yu, L. Wang, Q. Xu, X. Wang, X. Lou, M. He and Y. Xiao, *Anal. Chem.,* 2015, **87**, 7712.

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

[10] Y. Xiao, A. A. Lubin, A. J. Heeger and K. W. Plaxco, *Angew. Chem. Int. Ed.,* 2005, **44**, 5456.

[11] R. Polsky, R. Gill, L. Kaganovsky and I. Willner, *Anal. Chem.,* 2006, **78**, 2268.

[12] J. Yang, B. Dou, R. Yuan and Y. Xiang, *Anal. Chem.,* 2017, **89**, 5138.

[13] B. Jiang, M. Wang, C. Li and J. Xie, *Biosens. Bioelectron.,* 2013, **43**, 289.

[14] K. C. Lin, B. Jagannath, S. Muthukumar and S. Prasad, *Analyst,* 2017, **142**, 2770.

[15] Y. Ji, L. Zhang, L. Zhu, J. Lei, J. Wu and H. Ju, *Biosens. Bioelectron.,* 2017, **96**, 201.

[16] S. Su, H. Sun, W. Cao, J. Chao, H. Peng, X. Zuo, L. Yuwen, C. Fan and L. Wang, *ACS Appl. Mater. Interfaces,* 2016, **8**, 6826.

[17] Q. Wang, Z. Zhou, Y. Zhai, L. Zhang, W. Hong, Z. Zhang and S. Dong, *Talanta,* 2015, **141**, 247.