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Electronic Supplementary Information

Probe-label-free electrochemical aptasensor based on methylene blue-anchored graphene oxide amplification

Jing Rong Chen, Xiao Xia Jiao, Hong Qun Luo* and Nian Bing Li*

Key Laboratory of Eco–environments in Three Gorges Reservoir Region of Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China. P. R. China.

* Corresponding authors: luohq@swu.edu.cn (H.Q. Luo); linb@swu.edu.cn (N.B. Li)

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Experimental details

Materials. Methylene blue (MB), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 6-mercapto-l-hexanol (97%, MCH), and tris-hydroxymethylaminomethane hydrochloride (Tris) were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$ and other chemicals were purchased from Beijing Chemical Reagent Co., Beijing, China. Human α-thrombin (TB), bovine serum albumin (BSA), lysozyme (Ly), hemoglobin (HB), thrombin-binding aptamer (TBA), adenosine triphosphate (ATP), ATP-binding aptamer (ABA), guanosine triphosphate (GTP), cytidine triphosphate (CTP), and uridine triphosphate (UTP) were purchased from Shanghai Sangon Biotechnology Co. Ltd., Shanghai, China. All oligonucleotides were synthesized and purified by Sangon Inc., Shanghai, China. All the chemicals above mentioned were used as received.

All oligonucleotides samples were prepared in 25 mM Tris-HCl buffer (containing 20 mM NaCl, pH 7.4) and stored in the dark at 4°C before use. The TB, Ly, HB, BSA, ATP, CTP, UTP, and GTP solutions at various concentrations were prepared in 10 mM phosphate-buffered saline (PBS buffer, 10 mM, pH 7.4) containing 20 mM KCl and stored in the dark at –20 °C. All the buffer solutions were prepared with Milli-Q water (18.25 MΩ cm) from a Millipore system.

Graphene oxide (GO) was synthesized from natural graphite powder (325 mesh, spectral pure, Sinopharm Chemical Reagent Co., Ltd., China) according to the modified Hummers method [1]. Then, 1 g of as-synthesized GO was dispersed in 1 L water by magnetic stirring and ultrasonication. The concentration of the homogeneous yellow-brown GO dispersion obtained above was about 1 mg mL^{-1}.

Electrochemical measurements. Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV) were all performed on a CHI 660D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd., China). A conventional three-electrode cell was employed for all electrochemical measurements, which involved a modified gold working electrode (2

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mm in diameter), an Ag/AgCl (3 M KCl) reference electrode, and a platinum wire auxiliary electrode.

The electrochemical measurements including CV and EIS were performed in a PBS solution (200 mM, pH 7.4) containing 100 mM KCl and 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. CV curves were scanned from −0.3 to 0.7 V at a scan rate of 100 mV/s. EIS was performed under an amplitude of 5 mV over the frequency range of 10^5 Hz to 0.1 Hz. DPV was performed in 10 mM PBS buffer (containing 20 mM KCl, pH 7.4). DPV parameters applied were: 50 mV pulse amplitude, 200 ms pulse width, 0.5 s pulse period, and voltage range from 0 to −0.5 V. All the electrochemical measurements were carried out at room temperature.

Fabrication of the sensing interface of the MB-anchored GO-based aptasensor. The gold electrode was mechanically polished sequentially with 0.3 and 0.05 μm Al_2O_3 powder followed by ultrasonic cleaning with water, ethanol, and water for 5 min each. Then the electrode was electrochemically cleaned in 0.5 M H_2SO_4 by potential scanning between −0.2 and 1.5 V until a reproducible cyclic voltammogram was obtained. Finally, the electrode was rinsed with copious amount of water and blown dry with high-purity nitrogen. After that, the cleaned gold electrode was immersed in 0.5 μM thiolated-DNA solution and incubated for 15 h at room temperature, followed by thoroughly rinsing with water for several times. Then, ssDNA modified gold electrode was immersed in 2 mM MCH for 1 h to block the uncovered gold electrode surface as well as to make the array of ssDNA on the electrode interface more regularly. For TB-sensor, the TBA modified gold electrode was then immersed in 1 mg/mL GO and 120 μM MB solution at room temperature in turn for some time, followed by thoroughly rinsed with water for several times, and thus the sensing interface of TB-sensor was fabricated successfully. For ATP-sensor, ABA was then immobilized on the surface of gold electrode through DNA hybridization reaction by pipetting 20 μL of 2 μM ABA solution onto the ADNA modified gold electrode, and proceeding for 4 h at room temperature. After that, the ABA-modified electrode was rinsed thoroughly and finally immersed in GO and MB

solution by the same way as mentioned above, and thus the sensing interface of ATP-sensor was also constructed successfully.

Detection of TB or ATP using electrochemical measurements. After accumulation of MB on GO surface, the electrode was rinsed with water thoroughly. Then, the sensing interface of TB-sensor or ATP-sensor was separately immersed into a series of TB or ATP solutions with different concentrations for 1 h. At last, the reduction signal of the MB anchored by GO was measured by using DPV in PBS solution. As for the controlled experiment, the sensing interface was separately treated with 1 μM BSA, Ly, and HB solutions for the TB-sensor or 1 μM GTP, CTP, and UTP solution for the ATP-sensor under similar experimental conditions for 1 h.

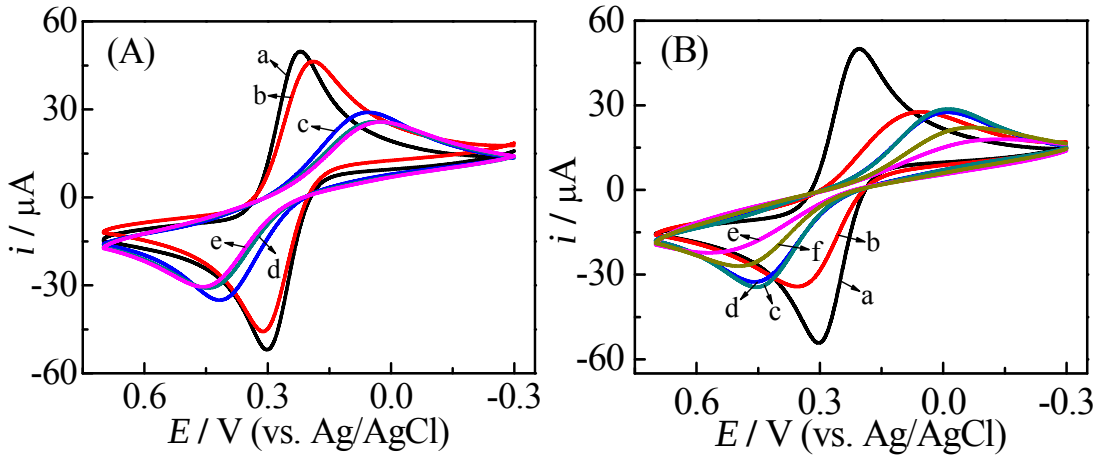

Fig. S1 CVs of two sensors in 5 mM $[Fe(CN)_6]^{4-/3-}$ prepared with 0.20 M PBS buffer (pH 7.4, containing 0.10 M KCl). For TB-sensor (A): bare (a), TBA modified (b), TBA/MCH modified (c), and TBA/MCH/GO modified (d) gold electrodes; (e) is (d) treated with 3.7 nM TB for 1 h. For ATP-sensor (B): bare (a), ADNA modified (b), ADNA/MCH modified (c), ADNA/MCH/ABA modified (d), and ADNA/MCH/ABA/GO modified (e) gold electrodes; (f) is (e) treated with 100 nM ATP for 1 h.

Comparisons of our proposed TB-sensor and ATP-sensor with other aptamer-based assays. In order to testify that our proposed strategy showed a desirable sensitivity, comparisons of the two sensors and other aptamer-based assays with respect to the linear range and the limit of detection (LOD) are listed in Tables S1 and S2, respectively, as follows:

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Table S1 Comparisons of aptamer-based assays for TB detection.

Signal output (Signal amplifier)	*Linear range (M)*	*LOD (M)*	**Ref.**
SPR and QCM-D	$0 - 1.9 \times 10^{-7}$	1.2×10^{-8}	[2]
EIS	$8.0 \times 10^{-11} - 8.0 \times 10^{-10}$	8.0×10^{-11}	[3]
Fluorescence	$2.0 \times 10^{-8} - 5.0 \times 10^{-3}$	1.2×10^{-8}	[4]
Magnetic relaxation time and UV–Vis (Fe_3O_4@Au NPs)	$1.6 \times 10^{-9} - 3.04 \times 10^{-8}$	1.0×10^{-9}	[5]
Fluorescence	$5.0 \times 10^{-10} - 2.0 \times 10^{-8}$	1.8×10^{-10}	[6]
EIS (polyamidoamine dendrimer)	$1.0 \times 10^{-9} - 5.0 \times 10^{-8}$	1.0×10^{-11}	[7]
Colorimetric and UV–vis (ELISA, Fe_3O_4 MNPs)	$1.0 \times 10^{-9} - 1.0 \times 10^{-7}$	1.0×10^{-9}	[8]
QCM-D (Au NPs)	$5.0 \times 10^{-10} - 1.25 \times 10^{-8}$	1.0×10^{-10}	[9]
DPV (Au NPs, hemin/G-quadruplex, horseradish peroxidase)	$1.0 \times 10^{-11} - 5.0 \times 10^{-8}$	2.0×10^{-12}	[10]
DPV (Graphene, quantum dots)	$2.0 \times 10^{-7} - 5.0 \times 10^{-7}$	1.0×10^{-7}	[11]
DPV (nafion@graphene, Au NPs)	$1.0 \times 10^{-11} - 5.0 \times 10^{-8}$	6.0×10^{-12}	[12]
SPR (graphene)	$3.0 \times 10^{-11} - 2.0 \times 10^{-7}$	3.0×10^{-11}	[13]
DPV (PtNPs, graphene, AuNPs@SWCNTs)	$2.0 \times 10^{-11} - 4.5 \times 10^{-8}$	1.1×10^{-11}	[14]
DPV (CdS-CDs)	$7.3 \times 10^{-12} - 7.3 \times 10^{-9}$	4.6×10^{-12}	[15]
CV (nano-Au/thionine)	$1.2 \times 10^{-10} - 4.6 \times 10^{-8}$	4.0×10^{-11}	[16]
DPV (graphene)	$2.8 \times 10^{-11} - 3.7 \times 10^{-9}$	3.05×10^{-12}	our work

Abbreviation: Surface plasmon resonance (SPR); quartz crystal microbalance with dissipation monitoring (QCM-D); electrochemical impedance spectroscopy (EIS); nanoparticles (AuNPs); UV-vis absorbance measurements (UV-vis); Enzyme-linked immunosorbent assay (ELISA); magnetic nanoparticles (MNPs); differential pulse voltammetry (DPV); single-walled carbon nanotubes (AuNPs@S WCNTs); CdS nanoparticle with β-cyclodextrins (CdS-CDs); cyclic voltammetry (CV).

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Table S2 Comparisons of aptamer-based assays for ATP detection.

Signal output (Signal amplifier)	Linear range (M)	LOD (M)	Ref.
Fluorescence	$5.0 \times 10^{-8} - 2.0 \times 10^{-3}$.	2.5×10^{-8}	[4]
Fluorescence (graphene)	$3.0 \times 10^{-6} - 3.2 \times 10^{-4}$	4.5×10^{-7}	[17]
EIS (graphene)	$1.5 \times 10^{-8} - 4.0 \times 10^{-3}$	1.5×10^{-8}	[18]
SWV	$1.0 \times 10^{-8} - 1.0 \times 10^{-3}$	Not given	[19]
SWV (QD)	$1.0 \times 10^{-7} - 1.0 \times 10^{-3}$	3.0×10^{-8}	[20]
ECL (QD, MNPs, DNCs, Au NPs, DNA cycle amplification)	$1.0 \times 10^{-8} - 8.0 \times 10^{-7}$	3.09×10^{-9}	[21]
DPV (DNA ligase)	$1.0 \times 10^{-10} - 1.0 \times 10^{-6}$	5.0×10^{-11}	[22]
EIS (CNTs, Nuclease cleavage, target recycling)	$5.0 \times 10^{-13} - 1.0 \times 10^{-6}$	1.0×10^{-13}	[23]
DPV (graphene, porphyrin)	$2.2 \times 10^{-9} - 1.3 \times 10^{-3}$	7.0×10^{-10}	[24]
DPV	$2.0 \times 10^{-8} - 2.0 \times 10^{-6}$	1.0×10^{-8}	[25]
CC (Au NPs)	$1.0 \times 10^{-9} - 1.0 \times 10^{-5}$	2.0×10^{-10}	[26]
DPV (Au NPs)	$1.0 \times 10^{-10} - 1.0 \times 10^{-7}$	1.0×10^{-10}	[27]
DPV (graphene)	$1.0 \times 10^{-10} - 5.0 \times 10^{-7}$	2.9×10^{-11}	Our work

Abbreviation: Square-wave voltammetry (SWV); quantum dot (QD); electrochemiluminescence (ECL); magnetic nanoparticles (MNPs); dendrimer nanoclusters (DNCs); carbon nanotubes (CNTs); chronocoulometry (CC).

It can be seen from Tables S1 and S2 that the two proposed aptasensors exhibit a much higher sensitivity, which provides a vigorous evidence of our strategy for highly sensitive detection of TB and ATP. However, compared to some other detection methodologies, It can be seen that the two proposed aptasensors have no obviously wide linear range, especially for the TB-sensor (0.028 – 3.7 nM). Nevertheless, by using MB-anchored GO signal enhancement, our methods are simple, easy to operate, low-cost, label-free and still much more sensitive than many others listed in Tables S1 and S2.

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The practical applicability of the two sensors. To further monitor the possibility of the newly developed technique for testing of real samples, the proposed TB-sensor and ATP-sensor have also been applied to detect TB or ATP in human serum samples using the standard addition method. The healthy human serum was firstly obtained from Southwest University Hospital of Chongqing, China, and was then diluted twenty times with 10 mM PBS buffer (containing 20 mM KCl, pH 7.4). At last, a series of samples were obtained by spiking TB or ATP standards into the diluted human serum. Three replicates for each concentration were determined by DPV under the same experimental conditions as mentioned above. The results are shown in Table S3. It was found that the recovery and the relative standard deviation (RSD) values were all acceptable for both the TB-sensor and the ATP-sensor, which clearly indicated the potentiality of these two aptasensors for the analytical application in real biological samples.

Table S3 The recovery of the prepared TB-sensor in diluted human serum.

Sample	Added (nM)	Found[a] (nM)	Recovery (%)	RSD (%)
1	0.120	0.116	96.7	6.22
2	0.280	0.263	93.9	2.36
3	0.600	0.619	103.2	5.64

[a] Mean value of three measurements.

Table S4 The recovery of the prepared ATP-sensor in diluted human serum.

Sample	Added (nM)	Found[a] (nM)	Recovery (%)	RSD (%)
1	10.0	9.23	92.3	4.02
2	100	97.4	97.4	4.68
3	500	542.0	108.4	6.85

[a] Mean value of three measurements.

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