Electronic Supplementary Information

A Highly Sensitive Electrochemical Cytosensor Based on Triple Signal Amplification Strategy by Using both Nanozyme and DNAzyme

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Contents

S1. Results and Discussion

S1.1 Characterizations of electrodeposited Au NPs

As mentioned in the experimental section, after 15 cycles of continuous scanning in $HAuCl_4$ solution, the well-dispersed Au film was deposit on the surface of electrode. Figure S3A showed the cyclic voltammetry (CV) results of bare GCE and GCE/Au NPs in $[Fe(CN)_6]^{3-/4-}$ solution. Obviously, the redox peak of GCE/Au NPs electrode was higher than bare GCE, which ascribed to fascinating electronic transmission capacity of Au NPs and their larger specific surface area. Clearly, the EIS results were in accordance with the CV measurements. Figure S3B depicted that Au NPs/GCE had an obvious decrease of faradaic impedance compared to bare GCE. Then the stability of Au NPs/GCE was further explored by continuous CV scan in ferricyanide solution for 30 cycles. As shown in Figure S3C, no obvious variation of current value was observed for 30 cycles scan, indicating that Au NPs/GCE had an acceptable stability. Furthermore, SEM was performed to characterize the morphology of Au NPs/GCE. In Figure S3D, the Au NPs with semi-spherical structure were uniform distribution on the surface of GCE. Meanwhile, energy dispersive X-Ray analysis (EDX) indicated the successful electrodeposition of Au film on GCE electrode (Figure S3E).

S1.2 Optimization of experimental parameters

To improve the electrochemical performance of cytosensor, a series of experimental parameters should be optimized. The optimized paraments were conducted in the basis on the electrochemical cytosensor towards 1×10^5 cell mL^{-1}.

Above all, the preparation of Au NPs layer on the electrode surface was a key factor for the construction of the cytosensing platform, which depended on the electrodeposition scan cycles in chloroauric acid solution. As shown in Figure S5A, the optimized electrodeposition condition was 15 cycles to achieve the maximum sensing signal. Excess electrodeposition would generate a dense gold film, which had negative effect on cytosensing. For one reason, it was hard to control the distance between modified capture aptamers as the compact DNA layer would prevent the recognition between capture aptamer and its target due to the steric hindrance effect. For another reason, too dense gold film was not beneficial for electronics transfer.

Moreover, the concentration of the capture aptamer applied for electrode modification was another important parameter. It mainly affected the efficiency of cells capture and further influenced the sensitivity. As shown in Figure S5B, the electrochemical signal gradually improved with increasing concentration of capture aptamer and reached to the plateau at the concentration of 2 μM. Therefore, the concentration of capture aptamer was fixed at 2 μM to fabricate the cytosensor. To assure the highest loading efficiency of target cells on modified electrode, targeted cancer cells incubated with capture aptamer for different periods and the relevant current signal were recorded. From Figure S5C, with extension of incubation time, the electrochemical signal increased rapidly and maintained stable after about 60 min, achieving the saturation binding. Meanwhile, the influence of the nano-probes' amount on cytosensing performance was also investigated (Figure S5D). The optimized volume of nanoprobe (1 mg mL^{-1}) was 10 μL. In addition, as illustrated in Figure S5E, the optimal pH was 7.4, which may be ascribed to the break of cell and aptamer linkage and weak bioactivity in acid or alkaline solution. Hence, PBS (pH 7.4) was selected as the buffer solution for further studies. Finally, the incubation temperature of target cell and modified electrode was also investigated. From the figure S5F, the optimal incubation temperature was 37℃ because too high or low temperature may influence the binding efficiency between target cell and modified electrode.

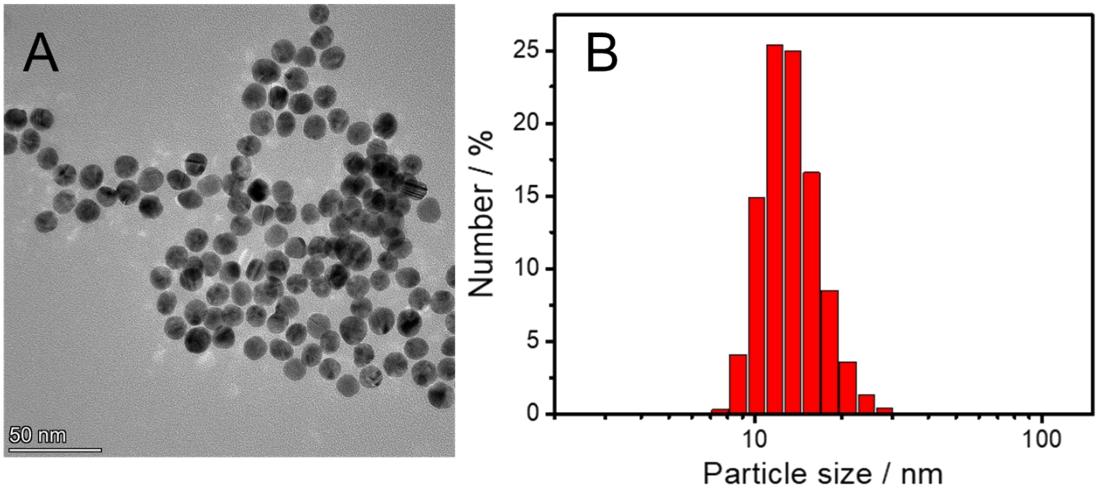

Figure S1. (A) Typical TEM images of the prepared Au NPs. (B) The DLS size distribution of the prepared Au NPs.

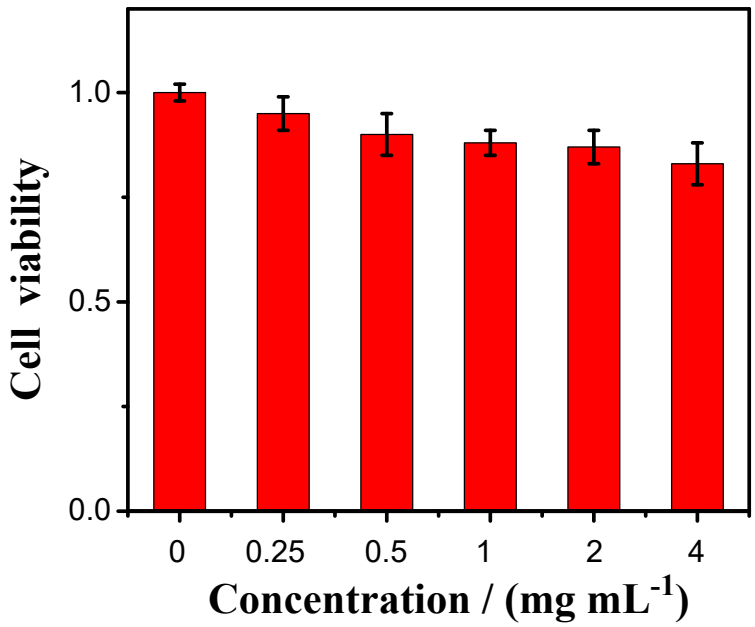

Figure S2. Viabilities measurement of HepG2 cells after incubated with different concentrations of Fe_3O_4@Au nanocomposites for 12 h (n=5). The viability of untreated cells was considered as 100%.

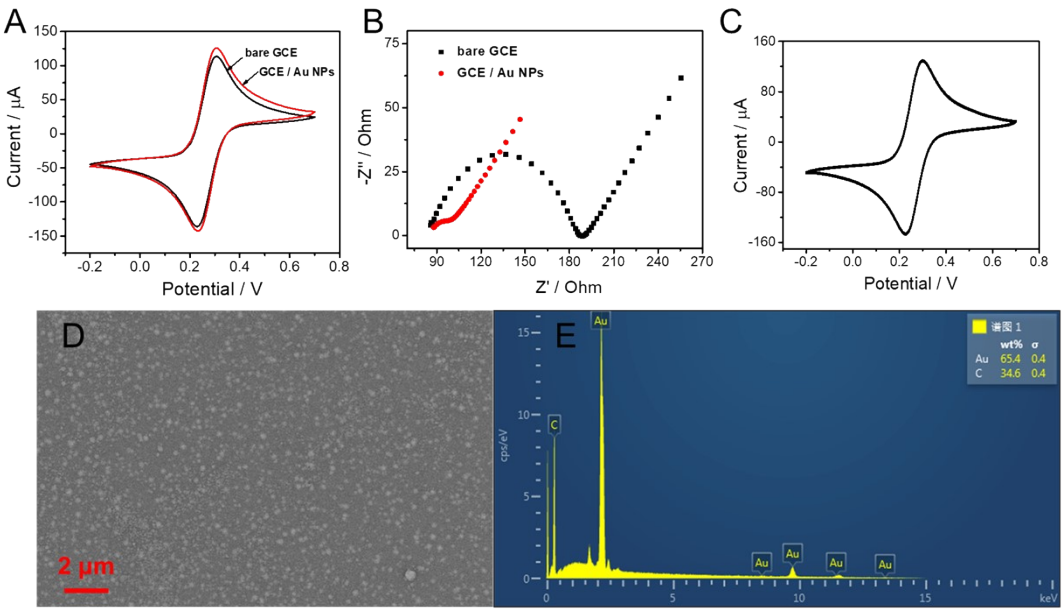

Figure S3. (A) Cyclic voltammograms and (B) Nyquist plots of EIS signal of bare GCE and GCE/Au NPs electrodes. (C) Stability of the GCE/Au NPs electrode under the successive CV scans for 20 cycles in the solution of 5 mM $[Fe(CN)_6]^{4-}$ containing 0.5 M KCl. Scan rate: 100 $mV \cdot s^{-1}$. SEM image (D) and EDX (E) of GCE/Au NPs electrode.

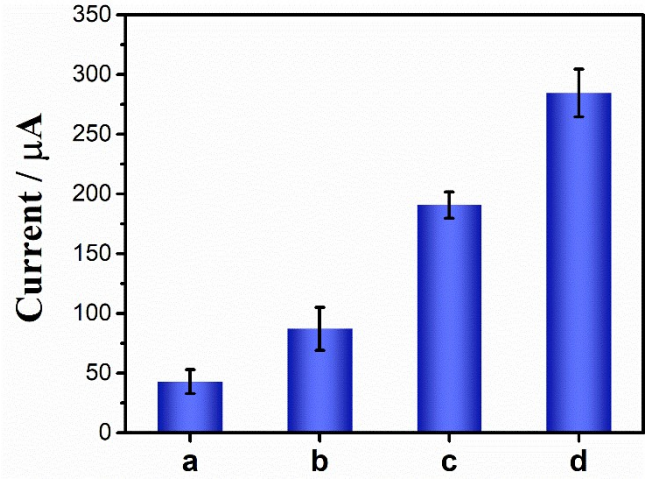

Figure S4. The current measurement of the cytosensor incubated with different signal probes: (a) Au NPs-SA-BSA; (b) Fe_3O_4@Au-SA-BSA; (c) Fe_3O_4@Au-hemin/G-quadruplex-BSA; (d) Fe_3O_4@Au-Tb-hemin/G-quadruplex-BSA. Error bars indicate the s.d. (n = 3).

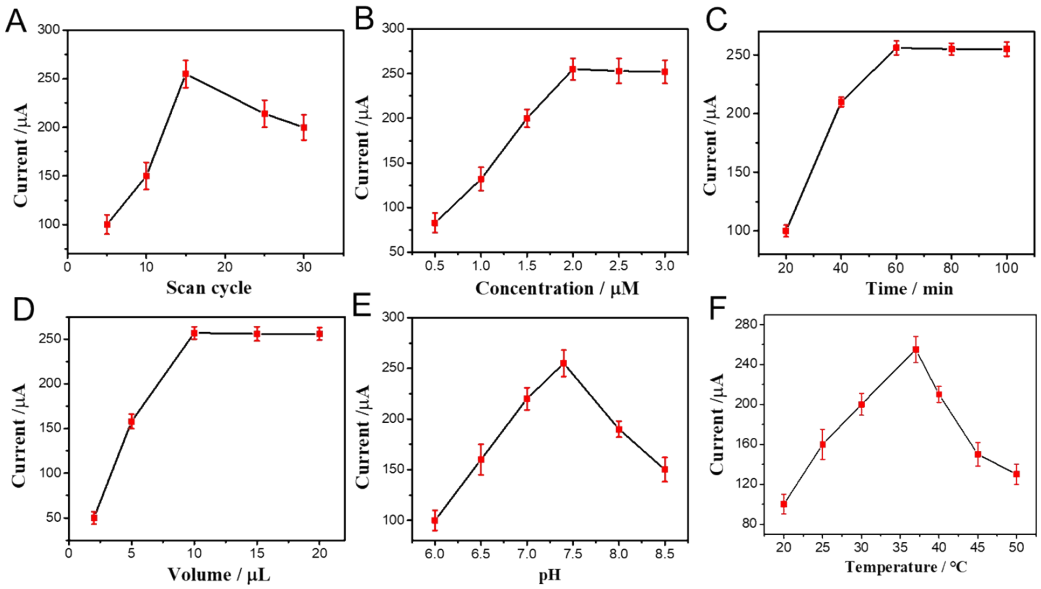

Figure S5. Optimization of electrochemical cytosensor. (A) scan cycles for the deposition of Au NPs; (B) the concentration of capture aptamer; (C) the incubation time of HepG2 cell; (D) the amount of nanoprobe(1 mg mL^{-1}); (E) effects of pH and (F) incubation temperature.

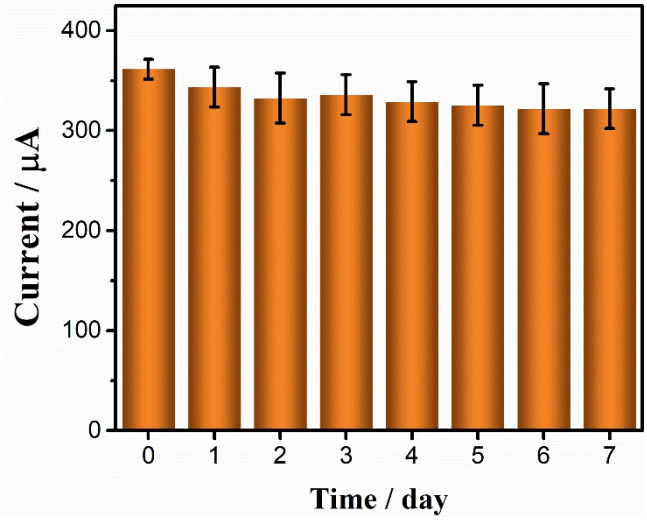

Figure S6. Stability of the cytosensor stored at in the dark.

Table S1. Comparison of electrochemical cytosensors in detecting HepG2 cells

Cytosensor type	Analytical method	Linear range (cells mL^{-1})	Detection limit (cells mL^{-1})	Reference
Electrochemiluminescent immunosensor	Electrochemiluminescence	3×10^2-1×10^4	256	[1]
Microcantilever aptasensor	Microcantilever assay	1×10^3-1×10^5	300	[2]
Magnetic immunosensor	Inductively coupled plasma mass spectrometry	40-8×10^3	15	[3]
Electrochemical cytosensor	Impedance spectroscopy	1×10^2-1×10^5	30	[4]
Electrochemical aptasensor	Amperometry	**1×10^2 - 1×10^7**	**20**	**This work**

Table S2. Detection of HepG2 cells in blood sample

Sample	Spiked cells (cells mL^{-1})	Detected cells (cells mL^{-1})	RSD (n=3, %)	Recovery (%)
1	1×10^3	0.85×10^3	6.31	85.3
2	1×10^4	0.95×10^4	5.13	95.2
3	1×10^5	0.91×10^5	6.56	91.1

S2. Experimental Section

S2.1 Chemicals and reagents

Hemin and toluidine blue (Tb) were purchased from Sigma-Aldrich. Sodium citrate ($Na_3Cit{\cdot}2H_2O$, ≥99.0%), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, ≥99.0%), ethylene glycol (EG, ≥99.0%), Tetrachloroauric (III) acid hydrate ($HAuCl_4{\cdot}4H_2O$), anhydrous sodium acetate (NaAc, ≥99.0%), hydrogen peroxide (H_2O_2), bovine serum albumin (BSA) and ammonia ($NH_3{\cdot}H_2O$, 25 wt%) were ordered from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). (3-Aminopropyl) triethoxysilane (APTES, 97.0%) was obtained from Aladdin Chemical Reagent Company. All oligonucleotides stock solutions was composed of 20 mM Tris-HCl buffer (pH 7.4) containg 1 mM EDTA. The phosphate buffered saline (PBS) solution was composed of 0.1 M KCl, 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4. The PBS (pH 7.4, 0.1 M) was used as electrolyte for all electrochemical measurement. Dulbecco's phosphate buffered saline (D-PBS) was purchased from Thermo Fisher scientific and used as cell washing buffer.

All oligonucleotides with amino labeling were synthesized and purified by Sangon Biotech Co., Ltd (Shanghai, China). The sequences of the aptamers were listed in Table S3. All the chemicals and reagents were of analytical pure. Ultrapure and distilled water was used in the whole experiment.

Table S3. The sequence of the aptamer used in this work.

aptamer	sequence (5'-3')
Capture aptamer (TLS11a)	5'-NH_2-$(CH_2)_6$-ACA GCA TCC CCA TGT GAA CAA TCG CAT TGT GAT TGT TAC GGT TTC CGC CTC ATG GAC GTG CTG -3'
Signal aptamer	5'-NH_2-$(CH_2)_6$-ACA GCA TCC CCA TGT GAA CAA TCG CAT TGT GAT TGT TAC GGT TTC CGC CTC ATG GAC GTG CTG-Spacer18-<u>TTT GGG TAG GGC GGG TTG GG</u>-3'

The underlined part indicated the sequence that can form the G-quadruplex, and the spacer-18 connection were composed of PEG, which was designed to reduce interference.

S2.2 Apparatus

The morphological characterizations were carried out by transmission electron microscope (TEM, JEOL JEM-2100, Japan) and field emission scanning electron microscope (FESEM, Ultra Plus, Germany). X-ray power diffractometer (XRD) pattern was obtained by Bruker D8 diffractometer (Bruker, Germany). Ultraviolet-visible (UV-vis) absorption spectra was operated by Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). Magnetic measurements were carried out using a Lakeshore-7404 vibrating sample magnetometer (VSM).

The results of chronoamperometric (i-t) and cyclic voltammetry (CV) in the electrochemical experiment was recorded by CHI 660B electrochemical workstation (Shanghai Chenhua instrument Co., Ltd.). Electrochemical impedance spectroscopy (EIS) experiments were performed on AUTOLAB PGSTAT 302 N electrochemical station (Metrohm Technology Co. Ltd., Switzerland).

S2.3 Preparation of Fe_3O_4@Au nanocomposites

Before use, freshly-prepared aqua regia (HNO_3 /HCl=3:1, v/v) was used to wash all glassware in the following experiments, then rinsed and dried thoroughly with deionized water. According to the previously reported literature[5], Au NPs with an average particle size of 13 nm was prepared. Briefly, $HAuCl_4$ solution (1 mM, 100 mL) was stirred violently and boiled at 100℃ for 10 min, then the sodium citrate aqueous solution (38.8 mM, 10 mL) was quickly added to the above solution and stirred at 100℃. After the color of the solution changed to wine red, keeping boiling for another 15 min and cooling down to room temperature. Finally, the obtained Au NPs were stored in a refrigerator at 4℃ and the concentration was about 1mM.

Amino-functionalized Fe_3O_4 NPs was prepared according to the previous literature with slight modifications[6]. In short, under the condition of stirring, $FeCl_3$ (0.65 g,·4 mM) and Sodium citrate (0.2 g,·0.28 mM) were first dissolved in ethylene glycol (28 mL) to form a uniform solution. Next, sodium acetate (1.2 g) was added to the above mixture and stirred intensely for 30 min. Subsequently, above-mentioned solution was transferred into a high temperature reactor and reacted at 200℃ for 10 h. After cooling to room temperature, the product was collected and washed several times with ethanol and ultra-pure water with the assistance of external magnet and dried at 50℃. The dried black Fe_3O_4 powder (0.02 g) was added to the mixture of absolute ethanol (30 mL) and ultrapure water (2 mL) under sonication for 15 min. Subsequently, 2 mL of ammonia (25%) and 200 μL of APTES were sequentially added to the above solution and mechanically stirred at room temperature for 8 h. After the reaction, the prepared amino functionalized Fe_3O_4 NPs were washed for several times using deionized water and absolute ethanol with the aid of an external magnet. Finally, the product was dispersed in 1 mL of distilled water.

Fe_3O_4@Au nanocomposites were synthesized according to the previous report with slight modifications[7]. Briefly, 1 mL of amino-modified Fe_3O_4 NPs (1 mg mL^{-1}), 10 mL of Au NPs solution and 1 mL of water were mixed and mechanically stirred at room temperature for 3 h. The sample was washed with PBS (pH 7.4) for three times with the aid of external magnet and dispersed in 2 mL water to obtain Fe_3O_4@Au nanocomposites (1 mg mL^{-1}).

S2.4 Cell culture and toxicity test

Human hepatoma HepG2 cells, human normal liver L02 cells and human breast cancer MCF-7 cells were cultured in Dulbecco's modifed Eagle's medium (DMEM), containing 10% fetal bovine serum (FBS), penicillin (80 U mL^{-1}) and streptomycin (80 μg mL^{-1}) at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were collected at the logarithmic growth phase by digesting with trypsin, centrifuging at 1000 rpm for 5 min, separating and washing trice with D-PBS solution. Finally, the precipitation was dissolved in PBS to obtain the uniform cell suspension.

Toxicity of Fe_3O_4@Au nanocomposites in vitro was determined by methyl thiazolyl tetrazolium (MTT) method. Briefly, HepG2 cells were cultured in 96-well plates for 24 h with the cell density of about 1×10^5 cells per well. Next, the cell culture medium in the orifice plate was discarded and replaced with fresh D-PBS to wash the cells. Then, 100 μL fresh medium containing various concentrations of Fe_3O_4@Au nanocomposites (0, 0.25, 0.5, 1, 2 and 4 mg mL^{-1}) was subsequently added to each well and incubated for 12 h. After that, 10 μL MTT solution (5 mg mL^{-1} in PBS) were injected into each wells and further incubated for 4 h. Then carefully absorbed and abandoned the culture supernatant in the hole, followed by addition of 100 μL of DMSO into each hole and shook for 10 min to make the crystal fully dissolved. Finally, the absorbance of each hole at 490 nm was recorded by microplate reader. The cell viability rate (VR) was described as a percent of the control culture value and calculated according to the following formula:

Cells viability (%) = (As - Ab)/ (Ac – Ab) × 100%

As, Ab and Ac represented the absorbance of the experimental group, blank group and control group, respectively.

S2.5 Electrochemical measurement

All electrochemical measurements were carried out with a conventional three-electrode system: GCE as the working electrode, saturated calomel electrode (SCE) as the reference electrode and platinum wire as the counter electrode. Bare glassy carbon electrode (GCE) was polished to the mirror surface with 1.0, 0.3, and 0.05 μm Al_2O_3 slurry on the suede and rinsed with distilled-water. Then, the electrode was washed ultrasonically in the anhydrous ethanol for 2 min. Afterwards, the cleaned electrode was rinsed with distilled-water thoroughly and dried at room temperature (RT).

Cyclic voltammetry (CV) test for conductivity characterization were conducted in 0.1 M PBS containing 5 mM $K_3[Fe(CN)_6]$ with the voltage range of -0.2- 0.6 V at scanning rate of 100 mV s^{-1}. Electrochemical impedance spectroscopy (EIS) measurement was performed in 0.1 M PBS containing 5 mM $K_3[Fe\ (CN)_6]/K_4[Fe\ (CN)_6]$ at the amplitude of 10 mV in the scanning frequency of 0.1 to 10^5 Hz.

The amperometric i-t measurement was carried out in 10 mL of working solution (PBS, 0.1 M, pH 7.4) at -0.4 V. Before measurement, the working solution was bubbled with N_2 for 5 min to eliminate the interference of O_2. Under mild stirring, when the background current was stable, H_2O_2 solution (10 μL, 5 M) was added to the solution, and the change of the current signal was recorded.

References:

1 D. Liu, L. Wang, S. Ma, Z. Jiang, B. Yang, X. Han and S. Liu, *Nanoscale*, 2015, **7**, 3627-3633.

2 X. Zhang, B. Chen, M. He, H. Wang and B. Hu, *Biosen. Bioelectron*., 2016, **86**, 736-740.

3 X. Chen, Y. Pan, H. Liu, X. Bai, N. Wang and B. Zhang, *Biosen. Bioelectron*., 2016, **79**, 353-358.

4 J. Liu, J. Cai, H. Chen, S. Zhang, and J. Kong, *J. Electroanal. Chem*., 2016, **781**, 103-108.

5 M. S. Onses, C. J. Thode, C. C. Liu, S. Ji, P. L. Cook, F.J. Himpsel and P. F. Nealey, *Adv. Funct. Mate*r., 2011, **21,** 3074-3082.

6 J. Liu, Z. Sun, Y. Deng, Y. Zou, C. Li, X. Guo, L. Xiong, Y. Gao, F. Li, D. Zhao, *Angew. Chem. Inter. Ed*., 2009, **48**, 5875-5879.

7 X. Zhang and S. N. Ding, *Sen. Actuators B Chem*., 2017, **240**, 1123-1133.