Supporting Information

A new signal amplification strategy using semicarbazide as co-reaction accelerator for highly sensitive electrochemiluminescent aptasensor construction

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1. Preparation of different TBA 2 signal probes for comparison

Probe *A*: TBA 2-AuNCs signal probe. Briefly, 200 μL of 2.5 μM TBA 2 solution was added into 300 μL of the prepared AuNCs solution (1 mg/mL) for 12 h under stirring. After washed twice by deionized water, the mixture was collected by centrifugation. Finally, 50 μL of 0.25% BSA was added to the above solution for 1 h to block nonspecific binding sites. The prepared TBA 2 signal probe was stored at 4°C for further use.

Probe *B*: TBA 2-Sem-AuNCs signal probe. Initially, 200 μL of 10 mM Sem solution and 500 μL AuNCs solution (1 mg/mL) were mixed for 12 h under stirring, followed by washing twice by deionized water. Afterwards, 200 μL of 2.5 μM TBA 2 solution and 100 μL 0.5% (*w/w*) GA cross-linking solution were added into the above dispersion for 12 h with stirring. Then the resultant product was washed for several times by deionized water. At last, we added 50 μL of 0.25% BSA into the above mixture for 1 h to block nonspecific binding sites. The prepared TBA 2 signal probe was stored at 4°C when not used.

Probe *C*: TBA 2-AuNPs-Sem-AuNCs signal probe. Primarily, 200 μL of 10 mM Sem solution was added into 500 μL AuNCs solution (1 mg/mL) with stirring for 12 h. Next, the above materials was centrifuged and washed for several times by deionized water. Subsequently, 200 μL of prepared AuNPs (1 mg/mL) solution was added under stirring for 12 h and the resultant solution was washed twice with deionized water to remove the excess AuNPs. Then 200 μL of 2.5 μM TBA 2 solution was added into the above mixture for 12 h with stirring. After washed twice by deionized water, the

solution was mixed with 50 μL of 0.25% BSA to block nonspecific binding sites. The final TBA 2 signal probe was stored at 4°C until use.

2. CV and EIS characterization of stepwise fabrication of the aptasensor

The stepwise fabrication process of the ECL aptasensor was also characterized by CV and EIS in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution (Figure S1). As shown in Figure S1 A, a pair of apparent redox peaks could be observed on the GCE (curve *a*). When the GCE was modified with CdTe QDs@C_{60}NPs composite, an obvious decrease in the peak current was observed (curve *b*). It could be attributed to the insulation effect of the CdTe QDs@C_{60}NPs film. After Dp AuNPs was electrodeposited onto the electrode surface, the peak current was enhanced (curve *c*), suggesting that Dp AuNPs could promote the electron transfer. However, the peak currents decreased successively when TBA 1 (curve *d*) and blocking reagent of BSA (curve *e*) were respectively incubated onto the electrode. As expected, when the electrode was incubated with TB, the peak current declined again (curve *f*). Such results showed that TBA 1, BSA and TB were all non-electroactive micromolecule, which could dramatically increase the steric hindrance and obstruct the electron transfer tunnel of the electrode surface. Meanwhile, in the EIS (Figure S1 B), the semicircle diameter equaled the electron-transfer resistance and the EIS results were consistent with the CV characterization.

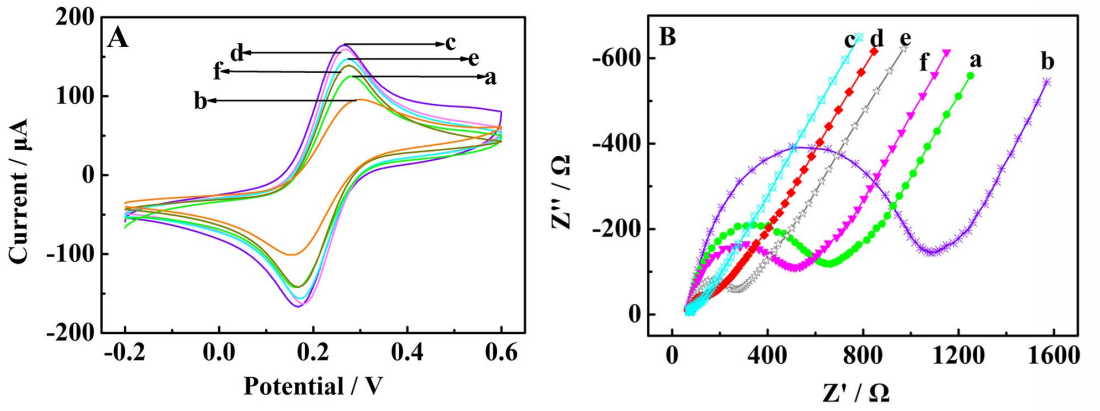

Figure S1. CV (A) and EIS (B) responses of (a) bare GCE; (b) CdTe QDs@C_{60}NPs/GCE; (c) Dp AuNPs/CdTe QDs@C_{60}NPs/GCE; (d) TBA 1/Dp AuNPs/CdTe QDs@C_{60}NPs/GCE; (e) BSA/TBA 1/Dp AuNPs/CdTe QDs@C_{60}NPs/GCE and (f) TB/BSA/TBA 1/Dp AuNPs /CdTe QDs@C_{60}NPs /GCE in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution.

3. Optimization of the working potential of CdTe QDs and the adsorption layers of AuNPs and Sem

Among the factors influencing the performance of the aptasensor, the working potential of CdTe QDs and the adsorption layers of AuNPs and Sem were both important in this experiment. Firstly, the GCE was scanned in QDs+$S_2O_8^{2-}$ solution with the working potential setting at -1.1~0 V, -1.2~0 V, -1.3~0 V, -1.4~0 V, -1.5~0 V and -1.6~0 V respectively. It could be seen in Figure S2 A that only at -1.5~0 V, the QDs produced the highest ECL intensity. Thus, -1.5~0 V was adopted as the optimal working potential of QDs for subsequent study. Afterwards, we prepared different TBA 2 signal probes with the different adsorption layers of AuNPs and Sem and the adsorption layers were set as 0, 1, 2, 3 and 4 respectively. Then the same batch of aptasensors were reacted with the same concentration of TB (0.1 nM), followed by incubating with the same concentration of the above TBA 2 signal probes. At last,

their ECL responses were recorded in 0.1 M $S_2O_8^{2-}$ solution. As illustrated in Figure S2 B, the ECL intensity of aptasensor increased with the increasing adsorption layers of AuNPs and Sem. When the adsorption layers reached 2, the ECL intensity of the aptasensor achieved the maximum and then tended to stability. Therefore, we selected the $(AuNPs\text{-}Sem)_2$-AuNCs as ECL signal label in this experiment.

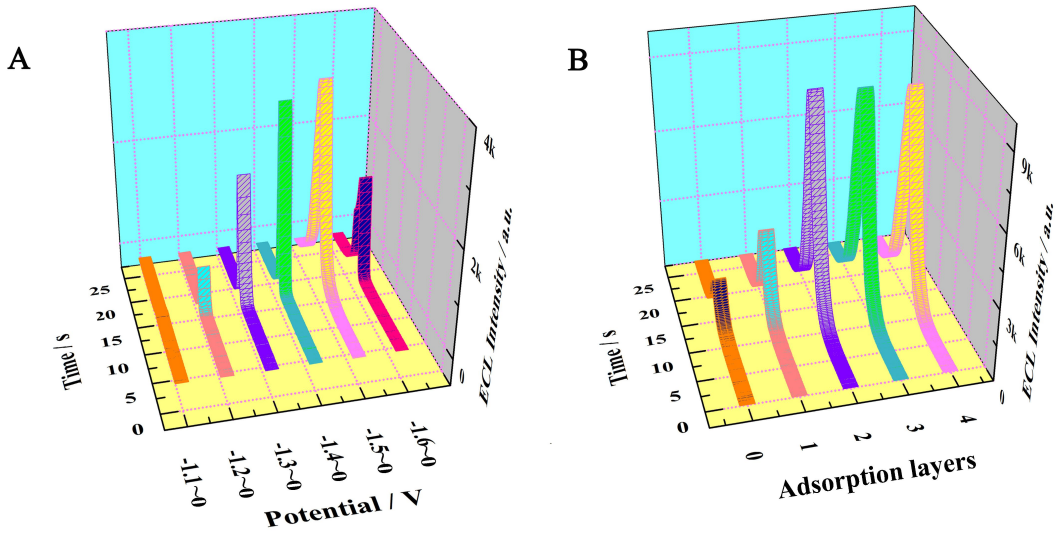

Figure S2. Optimization of (A) the working potential of CdTe QDs and (B) the adsorption layers of AuNPs and Sem in 0.1 M $S_2O_8^{2-}$ solution.

4. Preliminary analysis of real samples

To evaluate the reliability of the proposed ECL aptasensor for clinical analysis, recovery experiments were performed by measuring the TB of different concentrations in freshly human serum samples with the proposed ECL aptasensor. As shown in Table S1, the recoveries (94.4%-106%) were acceptable, indicating practical application for determining TB in real biological samples.

Table S1. Determination of TB in normal human serum with the proposed aptasensor.

Sample number	ECL responses /a. u	Add /nM	Found /nM	Recovery /%
1	10103.4	1	1.01	101
2	8871.40	0.1	0.106	106
3	7548.20	0.01	0.00944	94.4
4	6304.80	0.001	0.000971	97.1
5	5050.00	0.0001	0.0000979	97.9