**Supplementary Information**

# Ultrasensitive electrochemical biosensor for detection of circulating tumor cells based on highly efficient enzymatic cascade reaction

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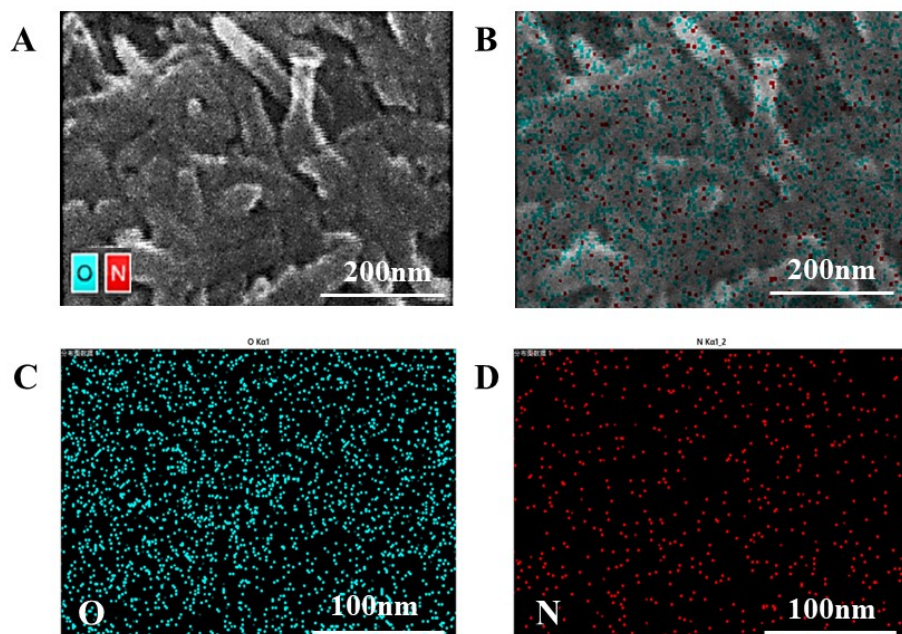
Fig S1. SEM images of (A) MWCNTs-CS, EDX elemental mapping images of N and O elements in MWCNTs-CS(B): O (green, C), N (red, D).
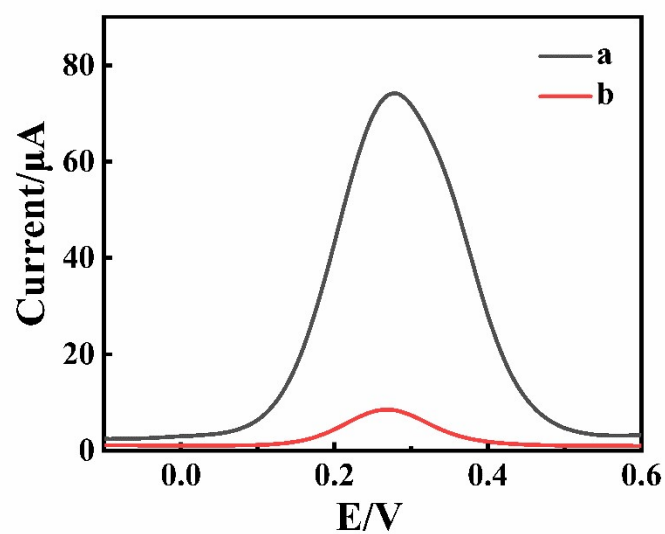
Fig S2. DPV response of GCE/CNTs-CS/GOD/aptamer/CTCs/anti-EpCAM-PS-H RP with (a) and without (b) glucose in the PBS solution containing $K_3Fe(CN)_6$ /$K_4Fe(CN)_6$.
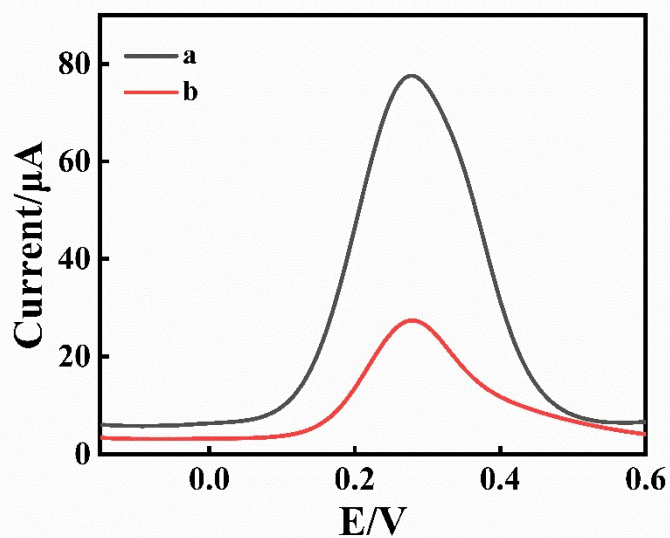


Fig S3. DPV response of GCE/CNTs-CS/GOD/aptamer/CTCs/anti-EpCAM-PS-H RP with (a) and without (b) $K_3Fe(CN)_6$/$K_4Fe(CN)_6$ in the PBS solution containing glucose.

## Optimization of experimental conditions

As shown in Fig S4, to obtain the excellent performance of electrochemical

biosensing detection, the concentration of MWCNTs-CS, the enrichment time of DNA, the concentration of HRP, and the concentration of glucose were optimized by DPV. In Fig S4A, the DPV peak current showed a significant increase with the MWCNTs-CS composite concentration increased, while after 10 mg/ml, there was a slight decrease in the DPV peak current. Hence, the optimal concentration relationship between MWCNTs and CS was 10 mg/ml. In Fig S4B, the DPV peak current increased with DNA enrichment time, but there were almost no changes after 20 h, indicating that 20 h is the optimal DNA enrichment time. Moreover, the DPV peak current increased with the increase of HRP concentration, and there were almost no changes after 10 mg/ml, which indicated the optimal concentration of HRP was 10 mg/ml in Fig S4C. The glucose concentration in the $K_3Fe(CN)_6/K_4Fe(CN)_6$ also affected the performance of electrochemical biosensing, and we found the DPV peak current was optimal at 1mM in Fig S4D.
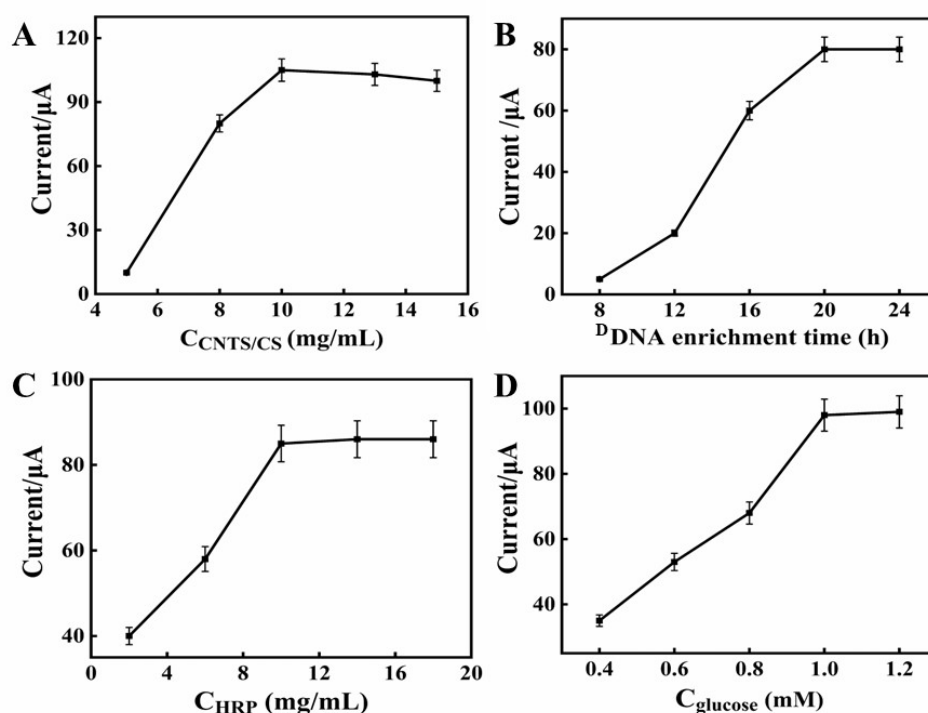


Fig S4. Optimization of the concentration of MWCNTs/CS (A), the enrichment time of DNA (B), the concentration of HRP (C), and the concentration of glucose (D).
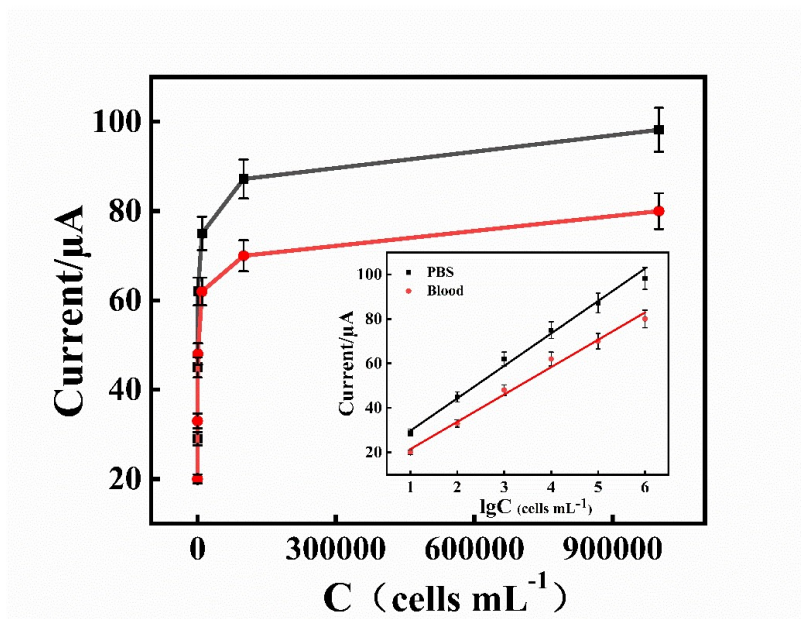
Fig S5. The relationship between the peak current of DPV and CTCs concentration under PBS and whole blood conditions.

Table S1. The relative standard deviations (RSD) at different concentrations of CTCs.

| CTCs concentration (cells mL$^{-1}$) | The relative standard deviations (RSD) (%) |
|---|---|
| 10 | 1.45 |
| 100 | 2.25 |
| 1000 | 3.1 |
| 10000 | 3.75 |
| 100000 | 4.2 |
| 1000000 | 4.91 |

Table S2. The RSD of CTCs in whole blood samples.

| CTCs concentration (cells mL$^{-1}$) | The relative standard deviations (RSD) (%) |
|---|---|
| 10 | 1 |
| 100 | 1.65 |
| 1000 | 2.4 |
| 10000 | 3.1 |
| 100000 | 3.5 |
| 1000000 | 4 |