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Electronic Supplementary Information

Cascade Signal Amplification Strategy for the Detection of Cancer Cells by Rolling Circle Amplification and Nanoparticles Tagging

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1. Reagents and Apparatus

Reagents. All the reagents were analytical grade and used without further purification. Doubly distilled water (DDW) was used throughout this work. 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and mercaptoacetic acid(MAA) were purchased from Sigma. Carboxyl-modified polystyrene microsphere(PSM diameter0.2-0.5μm) and carboxyl-modified magnetic beads (MB, diameter 1.0 μm) were ordered from Tianjin BaseLine Chromtech Research Centre. *E. coli* DNA ligase and dNTPs were purchased from TAKARA Biotechnology (Dalian) Co.,Ltd. Phi29 DNA polymerase (10 U μL^{-1})were purchased from Fermentas. 0.01 M PBS buffer (pH=7.4) 5 mM Tris-HCI (0.17 M NaCl, pH 8.0, 0.05% Tween 20) were prepared by standard methods, The oligonucleotides used in this study were purchased from SBS Genetech Co.,Ltd. (China) with the following sequences:

aptamer: 3' - NH_2 - G TGG CCC ACC TCT GGG ACT TGT CGG TGG CTT GAT AGG AGG GCC ACA AGA CAT - 5';

primer: 3' - NH_2 - CCC ACC TCT GGG ACT TTT TTT TTT GAG GTG GGC CAC AGC TAT GTC TTG TGG– 5';

padlock probe: 3' – T CGA TAC AGA ACA CCG GGA GGA TAG TTC GGT GGC TGT TCA GGG TCT CCA CCC GGT G - phosphate - 5';

probe DNA: 3'- NH_2 - TT TTT TTT TTT TTT TTT TTT TTT TTT ATG TCT TGT GGC - 5';

signal DNA: 3' - NH_2 – AGT CCC AGA GGT GGG - 5'

Apparatus. The electrochemical measurements was carried out on a CHI660 electrochemical working station (Shanghai CH Instruments Co., China) using a three-electrode system. The electrodes include an Glassy carbon disk (4-mm-diameter) working electrode, an Ag/AgCl (sat.

KCl) reference electrode, and a Pt counter electrode. A Glassy carbon electrode with 4 mm diameter was polished carefully with 0.3 and 0.05 μm α-Al_2O_3 powder on fine abrasive paper and washed ultrasonically with water.

2. Cancer Cell Culture[1]

Ramos cells (CRL-1596, B-cell, human Burkitt's lymphoma) were obtained from Chinese Academy of Medical Sciences. All of the cells were grown in RPMI-1640 containing 10% fetal bovine serum (FBS) and 100 IU/mL penicillin-Streptomycin at 37°C in a humid atmosphere with 5% CO_2. The cell density was determined using a hemocytomer prior to each experiment. And then, a 3.0 mL suspension of ~1.0 × 10 6 cells dispersed in RPMI 1640 cell media buffer was centrifuged at 3000 rpm for 5 min and washed with PBS buffer (pH=7.4) three times and resuspended in 1.0 mL cell media buffer.

CdS NPs were prepared according to the method reported previously with a slight modification.[2] Briefly, 2μL MAA was added to 100 mL of 1 mM $CdCl_2$ solution and adjust the pH to 11.0 with 0.5M NaOH. Oxygen was removed by bubbling with nitrogen throughout this procedure. Add 50ml 1.34mM Na_2S solution drop by drop and stirring slowly with anaerobic conditions for 24 h. The final CdS NPs prepared by this method have an average diameter of approximately 10 nm. The prepared CdS NPs were stored in brown glass bottles at 4 °C.

3. Preparing the CdS Nanoprobe.

The CdS nanoprobes were prepared according to the refernece with a slight modification. [3] Briefly, 100 μL of 0.1 mol/L EDC was added to 600μL of 1.0×10^{-5} M signal DNA to activate amido of signal DNAand then mixed with 2mL of CdS NPs that have been adjusted pH to 4 by hydrochloric acid. The mixture was stirred for 12 hous at room temperature. Free oligonucleotides

were removed by centrifuging at 10 000 rpm for 30 min. Then, the precipitate was rinsed with 10 mM PBS (pH 7.4) twice. The supernatant was removed, and the precipitate at the bottom of the tube was dispersed in 2.0 mL of PBS (pH 7.4) stored at 4 °C.

4. Fabrication of the MB-DNA-PSM Biocomplex

Firstly, 50 μL of carboxyl-modified PSMs were added to 150 μL of 0.2M EDC solution and stirred for 30 min. The activated PSMs was then centrifuged and incubated with the mixture of 30 μL of 1.0 μM probe DNA and 10 μL of 1.0 μM primer DNA. The PSMs modified with probe DNA and primer DNA was incubated for 12 hours at 37 °C and re-centrifuged before use.

Secondly, 20 μL of carboxyl-modified MBs were washed three times and then activated in imidazole buffer (100 mL, 0.1M , pH 6.8) containing EDC (0.1 m) with gentle shaking for 40 min. 30 μL of 1.0 μM aptamer DNA was added into the activated MBs, and the resultant mixture was incubated for 12 hours at 37 °C with gentle shaking and magnetically separated before use.

Thirdly, the modified PSMs and the modified MBs were dispersed in 200μL of PBS (0.01 M， pH 7.4) and incubated for 2 hours at 37 °C with gentle shaking to form the MB-DNA-PSM biocomplexes. The mixture was magnetically separated, rinsed three times with imidazole buffer (100 mL, 0.1M , pH 6.8), and then resuspended in 100μL of PBS(pH 7.4) before use.

5. Rolling Circle Amplification and CdS Nanoprobes Tagging

20 μL of 1.0 μM padlock DNA and 10 U of *E*. coli DNA ligase were added to the solution of MB-DNA-PSM biocomplexes. The padlock DNA can hybridize partly with the primer DNA modified on the PSM. The mixture was incubated for 1 hour at 37 °C with gentle shaking. The MB-DNA-PSM biocomplexes anchored with padlock DNA were magnetically separated followed by the addition of 10 U Phi29 DNA polymerase and 100 μM dNTPs to start the rolling circle

process for 2 hours at 37 °C.

100 μL of CdS nanoprobes was added into the as-prepared RCA product solution. After 12 hours incubating at 37 °C with gentle shaking, the long single strand DNA of RCA product tagged with numerous nanoprobes trough the hybridization between the tandem-repeat of the RCA product and the probe DNA modified on the CdS NPs.

6. Specific Recognition of Ramos Cells

MB-DNA-PSMs biocomplexes with RCA product and CdS nanoprobes were firstly washed with PBS buffer (pH=7.4) three times and then 200μL of solutions of Ramos cells at different concentrations were added. The supernatant can be collected by magnetically separated and used for electrochemical detection.

7. Electrochemical Detection of Cadmic Cation

With 200 μL of 1.0 M nitric acid added into the supernatant, cadmic cation released from tagged CdS NPs. The obtained solutions of Cd^{2+} were mixed with 2.5 mL of 0.1 M, pH 4.7 HAc-NaAc buffer to perform ASV analysis with a mercury film modified glassy carbon electrode. The working electrode was prepared at 0.6 V for 1 min and scan from -0.8 to -0.4 at 0.05 V/s in 0.1 M, pH 4.7 HAc-NaAc buffer containing 1.8×10^{-4} M Hg^{2+} . The ASV detection was carried out by electrodepositing cadmium at -1.4 V for 300 s and stripping from -0.8 to -0.4 V using a square wave with 50 mV amplitude.

8. Effect of RCA Time on Electrochemical Signal

To improve the sensitivity of this assay, the influence of RCA time on electrochemistry signal was investigated, as shown in Figure S1. The electrochemical signal intensities increased almost linearly with the increase of RCA time to 2 h, and a plateau effect was reached after this time.

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Therefore, the RCA time reaction time was controlled at 2 h all through the experiment.

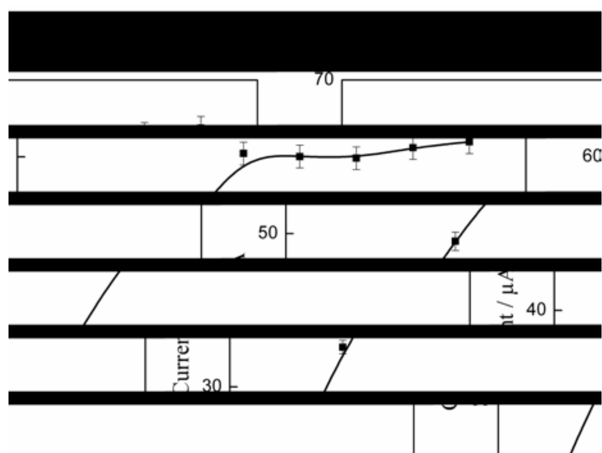

Fig.S1 Effect of RCA time on electrochemistry signal. The concentration of Ramos cell was 5000 cells mL^{-1}

9. Optimize Ratio between Probe DNA and Aptamer DNA

To improve the sensitivity of electrochemical signal, the ratio between probe DNA and aptamer DNA modified on PSM was also optimized. The signals were highest when the radio of probe DNA and aptamer DNA was 30:1 as shown in Figure S2. Therefore, this radio was used as optimal conditions.

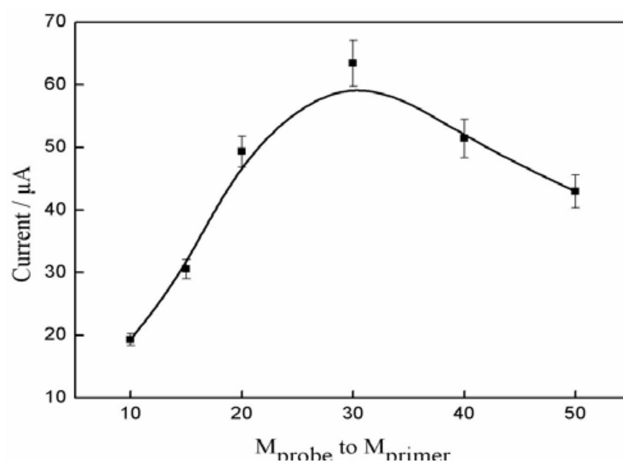

Fig.S2 The mole ratio of signal probe DNA to aptamer.DNA on the electrochemical signal. The concentration of Ramos cells was 5000 cells mL^{-1}

10. Optimize the reaction time between cells and aptamer

The incubation time was optimized for the Ramos cell and aptamer modified on MB-DNA-PSM biocomplexes with RCA product and CdS nanoprobes. With the incubation time increasing, the signals were increased first and the decreased. Thus, 120 min was chosen as the optimal incubation time which was shown in Figure S3.

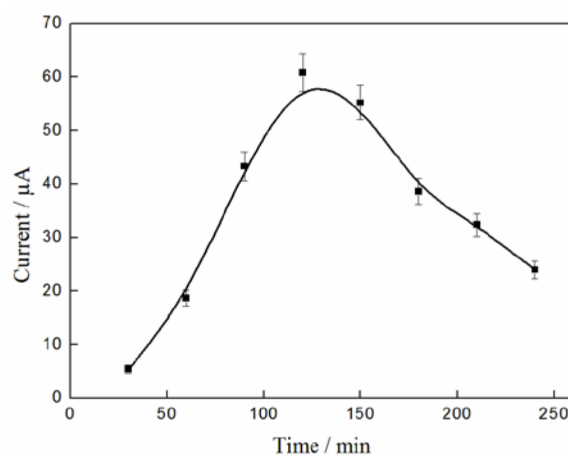

Fig.S3. Effect of incubation time with the Ramos cell. The concentration of Ramos cells was 5000 cells mL^{-1}

11. Optimize Volume of Nanoprobes

As shown in Figure S4, the electrochemical signal intensities increased almost linearly with the increase of volume of nanoprobes 100 μL, and a little decrease was found after this time. Therefore, 100 μL of nanoprobes was used as optimal conditions.

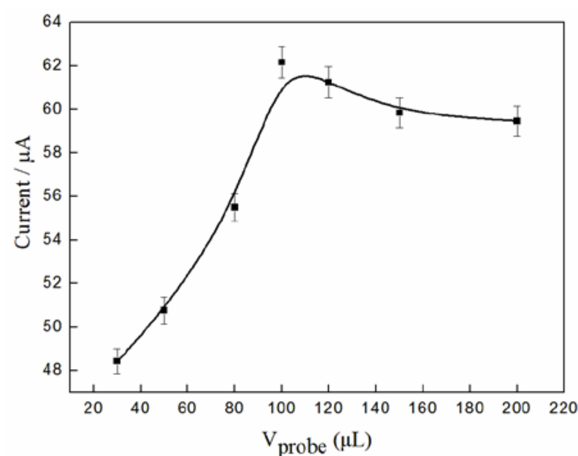

Figure S5. Effect of volume of nanoprobs on electrochemical signal. The concentration of Ramos

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cells was 5000 cells mL^{-1}

12. Optimization of the pH of the Buffer Solution

The pH of the buffer solution was another influence condition for the electrochemical signal. The electrochemical signal response of pH ranging from 4.2 to 5.2 was shown in Fig.S6. With the highest signal, we choose pH 4.7 as optimal conditions.

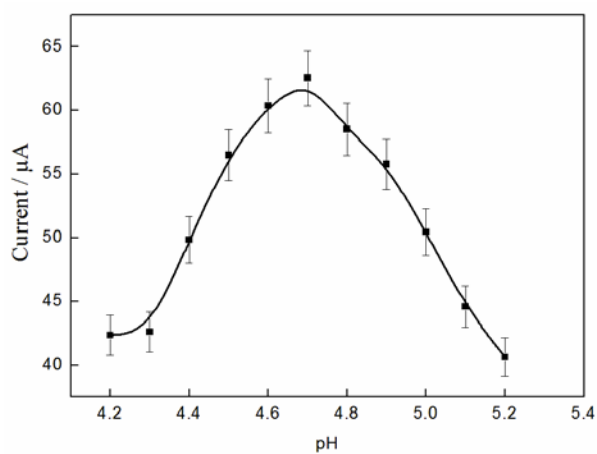

Figure S6. Effect of the pH of the buffer solution on the electrochemical signal. The concentration of Ramos cells was 5000 cells mL^{-1}

References

[1] Colin, D. M.; Joshua, E. S.; Tang, Z.-W.; Wu, Y.-R.; Suwussa, B.; Tan, W.-H. *Anal. Chem.* **2008**, *80*, 1067-1072.

[2] Milica, T. N., Mirjana, I. C., Veana, V., Olga, I. M., J. *Phys.Chem.*, **1990,** 94, 6390-6396.

[3] Xu, Y., Cai, Y., He, P.G., Fang, Y.Z., *Electroanalysis*, **2004**, 16, 150-155.