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Aptamer-binding DNA walking machine for sensitive electrochemiluminescence detection of tumor exosomes

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1. Experimental Section

Chemicals and reagents. Tris (2, 2-bipyridyl) dichlororuthenium (II) hexahydrate, tripropylamine (TPrA), (3-aminopropyl) triethoxysilane (APTES), maleimidobenzoic acid N-hydroxy-succinimide ester (MBS), triton X-100, tetraethyl orthosilicate (TEOS), N-hydroxysuccinimide (NHS), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), N-(3-dimethylaminopropyl)-N-ethyl-carbodiimide hydrochloride (EDC), chitosan (CS) and glucose oxidase (GOD) were applied by Sigma-Aldrich (USA). Glucose was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Nb. BbvCI (10 000 U mL^{-1}) and 10× NEB buffer were obtained from New England Biolabs. Dulbecco's Modified Eagles Medium (DMEM), fetal bovine serum (FBS), and trypsin were bought from Invitrogen. Hepatocellular carcinoma cell line (HepG2) was purchased from Key GEN Biotech. All the other chemicals were of analytical reagent grade and used without further purification. Millipore ultrapure water (≥18.2 MΩ. cm resistivity) was applied throughout the entire experiment. Phosphate buffer solution (0.1 M, KH_2PO_4-Na_2HPO_4, pH 7.4) containing 30 mM TPrA and 10 mM glucose was employed as ECL detection solution. All oligonucleotides were synthesized by Sangon Biological Engineering Technology & Services Co. (Shanghai China). Detailed DNA sequences and modifications were shown in Table S1.

Table S1. DNA sequences used in this work

name	sequence (5′-3′)
anchor DNA	NH_2-$(CH_2)_6$-TTTCGGTACGCCGAGTTTTTTTTTTGCTAGCTGAGGACGGCGTAC
swing arm	TCCTCAGCTAGCATTAGTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTT-$(CH_2)_6$-NH_2
aptamer	CACCCCACCTCGCTCCCGTGACACTAATGCTAGCTG
probe DNA	CTCGGCGTACCGTTT-$(CH_2)_6$-SH

Apparatus. A MPI-A multifunctional electrochemical and chemiluminescent analytical system with 350-650 nm spectral width of photomultiplier tube (PMT) were applied to record all the ECL emission measurements. In the process of detection, the voltage of PMT was set to -700 V and potential scan was carried out from 0 to 1.0 V at a scan rate of 100 mV s^{-1}. A conventional three-electrode cell consisting of a Pt wire as counter electrode, Ag/AgCl electrode as reference electrode, and the modified glassy carbon electrode (GCE) (3 mm in diameter) as working electrode was applied for all electrochemical experiments. The morphology characterization of synthetic RuSi NPs was tested by scanning electron microscope (SEM, JSM-6330F microanalyzer, Japan). UV-visible spectrum of RuSi NPs was recorded on an UV-3150 UV-vis-NIR spectrophotometer (Shimadzu, Japan). Transmission electron microscope (TEM) image of exosomes was observed from a Tecnai F20 TEM (FEI, USA). Size distribution of exosomes was performed on a dynamic light scattering (DLS) with a 90 Plus/BI-MAS equipment (Brook haven, USA).

Synthesis of RuSi NPs. 1.8 mL of 1-hexanol, 1.77 mL of Triton-X-100, and 7.5 mL of cyclohexane were slowly mixed under constant magnetic stirring. Subsequently, 340 μL of $Ru(bpy)_3^{2+}$ (40 mM) was injected into the above mixture to form an orange water-in-oil microemulsion. Next, 60 μL of $NH_3 \cdot H_2O$ and 100 μL of TEOS were successively added and kept constantly stirring for 24 h at room temperature. When acetone was slowly injected, this emulsion was destroyed. Followed by washing with ethanol and water, RuSi NPs were collected via centrifugation. To form amino functionalized RuSi NPs, 10% APTES solution was added to RuSi NPs and incubated for 2 h. Ultimately, loosely bound APTES was removed by ethanol rinsing and the amino functionalized RuSi NPs were dispersed in 1 mL of PBS for the further use.

Cells culture and exosomes extraction. As the source of exosomes, HepG2 cells were inoculated in DMEM supplemented with 1% penicillin-streptomycin and 10% FBS. Then, all cells were kept in a 37 °C incubator with 5% CO_2 humidity atmosphere. After growing to about 50% confluence, HepG2 cells were transferred into serum-free

medium for 48 h. Through two-step centrifugation (1200 g for 5 min and 1500 g for 10 min at 4 °C) and subsequent filtration of 0.22 μm filter, the supernatant without cell debris was collected. For the isolation of exosomes, the obtained filtrates were further subjected to two-step ultracentrifugation (17 000 g for 90 min and 120 000 for 1 h). Followed by washing, the acquired exosome pellets were resuspended in PBS for the later utilization.[1]

Fabrication of probe DNA-GOD conjugation. The conjugation of GOD to probe DNA (abbreviated as probe DNA-GOD) was fabricated through bifunctional linking of MBS.[2] In brief, 25 μL of MBS (6.4 mM) and 1 mL of GOD (1 mg/mL) were mixed, followed by shaking for 1 h at room temperature. The maleimidobenzoic GOD amide was obtained and purified with 100 kDa Millipore. Meanwhile, to reduce the formation of disulfide bonds, thiolated probe DNA was first treated by 10 mM TCEP for 1 h before use. After the purification of 10 kDa Millipore, 1 mL of probe DNA (100 μM) was added to the MBS-activated GOD solution and the resultant mixture reacted continually for 1 h. Finally, the obtained probe DNA-GOD conjugation was purified again with 100 kDa Millipore and resuspended in PBS for the later utilization.

Preparation of ECL sensor and exosomes detection. Prior to surface modification, the 3 mm diameter GCE was cleaned according to the literature.[3] Then, to immobilize RuSi NPs, a CS film was prepared on the cleaned GCE surface (CS/GCE) by electrode position in 2.0 mg mL^{-1} CS solution at -2.0 V for 300 s. Then, 10 μL of RuSi NPs solution was dispersed onto CS/GCE surface, evaporating at room temperature. After soaking in PBS for 6 h, unstable RuSi NPs were peeled off. Subsequently, the resultant RuSi NPs/CS/GCE was immersed into 2.5% (v/v) glutaraldehyde solution for 2 h. After that, the obtained electrode was further conjugated with 100 μL of mixture containing anchor DNA and swing arm:aptamer duplex at a certain concentration ratio. Before use, anchor DNA was treated at 95 °C for 5 min, and then slowly annealed to room temperature. Swing arm:aptamer duplex was the hybridization product of swing arm and aptamer at 37 °C for 1.5 h. After

rinsing, the nonspecific binding sites of glutaraldehyde on the electrode was blocked by 2% BSA solution and then BSA/anchor DNA+swing arm:aptamer/RuSi NPs/CS/GCE as DNA walker substrate was achieved.

Next, the above electrode was submerged into 100 μL of reaction solution consisting of suspended exosomes with various concentrations and 1 U Nb. BbvCI for 2 h. Followed by rinsing, probe DNA-GOD was introduced to the electrode surface through incubating. Finally, ECL detection of the sensing system was accomplished in 4 mL of PBS containing 30 mM TPrA and 10 mM glucose.

2. The feasibility of DNA walking machine-based amplification strategy

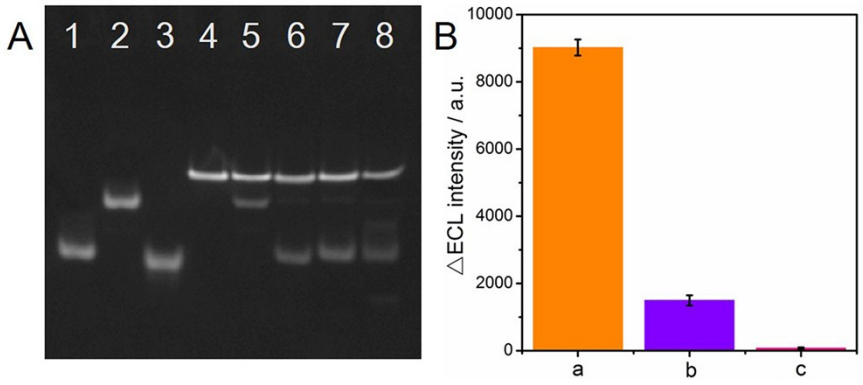

Fig. S1 (A) Polyacrylamide gel electrophoresis analysis of anchor DNA (lane 1), swing arm (lane 2), aptamer (lane 3), swing arm:aptamer (lane 4), swing arm:aptamer after treating with exosomes (lane 5), anchor DNA+swing arm:aptamer (lane 6), anchor DNA+swing arm:aptamer after treating with exosomes (lane 7), anchor DNA+swing arm:aptamer after treating with exosomes and Nb. BbvCI (lane 8). (B) Bar graph of ΔECL intensity of ECL aptasensor obtained upon analyzing 25000 particles/μL exosomes in the presence of Nb. BbvCI (bar a) and control experiments performed with no Nb. BbvCI (bar b) and no exosomes or Nb. BbvCI (bar c).

3. Optimization of concentration ratio between anchor DNA and swing arm

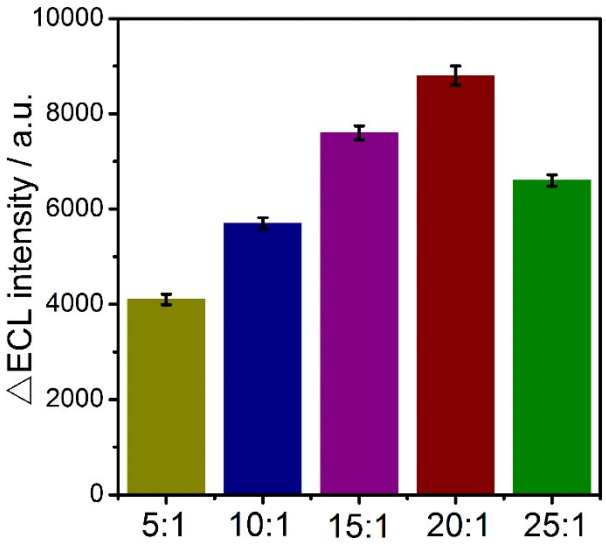

Fig. S2 Bar graph of ΔECL intensity of ECL aptasensor with different concentration ratios between anchor DNA and swing arm.

4. Comparison with other reported exosomes detection methods

Table S2. Comparison of analytical parameters of different detection methods for the assay of exosomes

Analytical method	Linear range (particles/μL)	Detection limit (particles/μL)	References
SERS	1.0×10^3 – 1.0×10^7	100	4
colorimetric	2.2×10^5 – 2.4×10^7	2.2×10^4	5
colorimetric	1.84×10^6 – 2.21×10^7	5.2×10^5	6
fluorescent	1.0×10^3 – 1.0×10^8	1.0×10^3	7
fluorescent	1.0×10^4 – 1.0×10^9	1.4×10^3	8
electrochemical	1.0×10^3 – 1.2×10^5	70	1
electrochemical	1.12×10^2 – 1.12×10^8	96	9
ECL	5.0×10^1 – 1.0×10^5	31	10
ECL	5.0×10^2 – 5.0×10^6	125	11
ECL	2.0×10^2 – 7.5×10^4	60	this work

5. The stability of ECL aptasensor

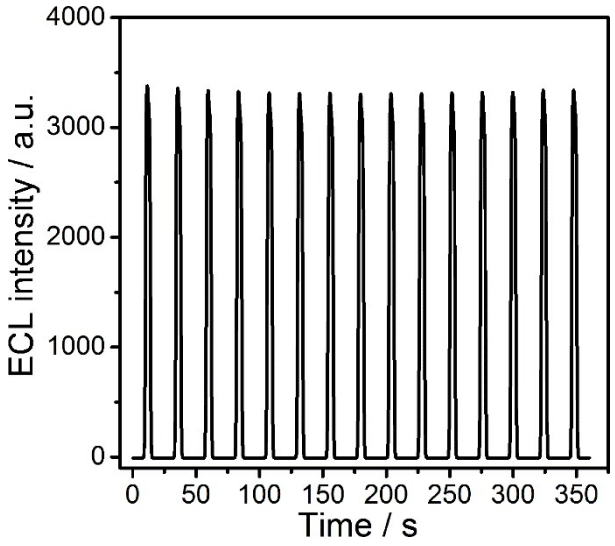

Fig. S3 Stabilization of ECL signals from BSA/anchor DNA+swing arm:aptamer/RuSi NPs/CS/GCE with the successive incubation of the reaction solution consisting of 25000 particle/μL exosomes and Nb. BbvCI, and probe DNA-GOD conjugation.

6. Recovery testing

Table S3. Recovery results for the assay of exosomes in 30% fetal bovine serum

Added (particles/μL)	Found (particles/μL)	Recovery (%)	R.S.D (%, n=3)
5000	5200	104	4.4
25000	24250	97	3.2

7. References

(1) H. Dong, H. Chen, J. Jiang, H. Zhang, C. Cai and Q. Shen, *Anal. Chem.*, 2018, **90**, 4507.

(2) Q. M. Feng, Y. H. Guo, J. J. Xu and H. Y. Chen, *Biosens. Bioelectron.*, 2018, **100**, 571.

(3) T. T. Han, H. Dong, L. L. Ren, N. Bao, W. Wu, S. N. Ding, *Chem. Commun.*, 2017, **53**, 5388. T. T. Han, H. Dong, L. L. Ren, N. Bao, W. Wu, S. N. Ding, *Chem. Commun.*, 2017, **53**, 5388.

(4) X. Zhang, C. Liu, Y. Pei, W. Song, S. Zhang, *ACS Appl. Mater. Interfaces* 2019, **11**, 28671.

(5) Z. Chen, S. B. Cheng, P. Cao, Q. F. Qiu, Y. Chen, M. Xie, Y. Xu, W. H. Huang, *Biosens. Bioelectron.*, 2018, **122**, 211.

(6) Y. Xia, M. Liu, L. Wang, A. Yan, W. He, M. Chen, J. Lan, J. Xu, L. Guan, J. Chen, *Biosens. Bioelectron.*, 2017, **92**, 8.

(7) D. He, S. L. Ho, H. N. Chan, H. Wang, L. Hai, X. He, K. Wang, H. W. Li, *Anal. Chem.*, 2019, **91**, 2768.

(8) Q. Zhang, F. Wang, H. Zhang, Y. Zhang, M. Liu, Y. Liu, *Anal. Chem.*, 2018, **90**, 12737.

(9) Y. An, T. Jin, Y. Zhu, F. Zhang, P. He, *Biosens. Bioelectron.*, 2019, **142**, 111503.

(10) Y. Zhang, F. Wang, H. Zhang, H. Wang, Y. Liu, *Anal. Chem.*, 2019, **91**, 12100.

(11) H. Zhang, Z. Wang, Q. Zhang, F. Wang, Y. Liu, *Biosens. Bioelectron.*, 2019, **124**, 184.