Supporting Information

A multipedal DNA walker for amplified detection of tumor exosomes

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Experimental

Materials and instruments

Iron(III) chloride hexahydrate ($FeCl_3$•$6H_2O$), gold(III) chloride trihydrate ($HAuCl_4$•$3H_2O$), sodium borohydride ($NaBH_4$), tris (2-carboxyethyl)-phosphine hydrochloride (TCEP), ethylenediaminetetraacetic acid (EDTA), and mercaptohexanol (MCH) were purchased from Sigma (United States). Nb.BbvCI and Nb.BssSI nicking endonucleases (NEases) were purchased from New England Biolabs Ltd. (United States). Sodium acetate (NaAc) and ethylene glycol were purchased from Shanghai Aladdin Reagent Company (Shanghai, China). Dulbecco's Modified Eagle's medium (DMEM) was supplied by Gibco (Gaithersburg, United States). Fetal bovine serum (FBS) was purchased from Hangzhou Sijiqing Biological Engineering Material Co., Ltd. (Hangzhou, China). HeLa cells and MCF-10A cells were from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). All other reagents were of analytical grade and were used as received. Water used was purified by a Millipore system with the resistivity of 18 MΩ·cm. All DNA sequences were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). The detailed sequences and modifications were shown in Table S2.

Ultracentrifuge Optima L-100XP (Beckman Coulter, USA) was used to isolate exosomes. JEM-1200EX transmission electron microscopy (JEOL Ltd., Japan) was used for the capture of TEM image. Hitachi S4800 scanning electron microscope (Hitachi, Japan) was used for the capture of SEM image. NanoSight LM10 (Malvern Instruments, UK) was applied for the determination of the concentration and diameter of extracted exosomes. CHI 660D electrochemical workstation (CH Instruments, China) was used for all electrochemical measurements.

Extraction and characterizations of exosomes

Cells were cultured in DMEM containing 10% FBS, 1% penicillin and streptomycin at 37°C in 5% CO_2 atmosphere. After reached a confluency over 90%, the cells were washed for three times with PBS. Then, 10^8 cells were further incubated with FBS-free DMEM for another 48 h. The culture supernatant was collected for the isolation of exosomes. Generally, it was centrifuged at 2,000 g for 20 min and then at 10,000 g for 30 min to remove cells and proteins. Next, the supernatant was further centrifuged at 110,000 g for 2 h to obtain the precipitated exosomes sediments, which were resuspended in 1 mL of PBS and stored at -80 ℃ before downstream analysis.

The obtained exosomes were firstly analyzed by negative staining TEM utilizing a 2% solution of phosphotungstic acid. Briefly, 10 μL of the sample solution was absorbed on carbon-coated copper grids for 5 min. Excess fluid was blotted gently by filter paper from the edge. PBS was used to wash the copper grids and phosphotungstic acid solution was then dropped. After removing the stain, the prepared copper grids were dried at room temperature and were visualized by TEM. NanoSight LM10 was then applied to analyze the concentration of obtained exosomes according to the manipulation.

Fabrication of multipedal DNA walker

Fe_3O_4@Au nanoparticles were prepared according to our previous report.[1] Thiolated DNA Probe A dissolved in 10 mM Tris–HCl (1 mM EDTA, 10 mM TCEP, and 0.1 M NaCl, pH 7.4) was mixed with Fe_3O_4@Au nanoparticles (0.8 mg/mL). The final concentration of Probe A was 5 μM. After reacting for 16 h, the mixture was “aged” in salts (10 mM phosphate, 0.1 M KNO_3, pH 7.0) for 24 h. Subsequently, Probe A modified Fe_3O_4@Au nanoparticles were purified by magnetic separation and washed by 10 mM phosphate buffer (0.25 M NaCl, pH 7.4). The pretreated cell

culture supernatant which contained exosomes was blended with Probe A modified Fe_3O_4@Au nanoparticles and Probe B (5 μM) for 1 h. Then, magnetic separation was performed and the collected nanoconjugates of exosomes and nanoparticles were resuspended in the buffer solution (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/ml BSA, pH 7.9). 0.5 μM Probe C and 0.5 units/μL Nb.BbvCI were further added. After reacted at 37°C for 1 h, exosomes loaded with a number of Probe B as multipedal walker strands were obtained by magnetic separation.

Modification of working electrode

The substrate gold working electrode was firstly treated with piranha solution (*Caution: highly corrosive*) for 5 min in order to remove any adsorbed materials. Then, the rinsed electrode was polished with silicon carbide paper (P5000) and alumina powders (1.0, 0.3, 0.05 μm), successively. Afterward, it was sonicated in ethanol and double-distilled water for 5 min, respectively. Next, the electrode was electrochemically cleaned using 0.5 M H_2SO_4 and rinsed carefully. After dried with nitrogen, the electrode was incubated with DNA probe D for 10 h. It was further treated with 1 mM MCH for 0.5 h to obtain the well aligned DNA monolayer. Probe D modified electrode was then immersed in the multipedal DNA walker solution obtained previously coupled with probe E (0.5 μM) and Nb.BssSI (0.5 units/μL). The walking reaction was carried out at 37°C for 1.5 h. Afterward, the electrode was carefully rinsed before the following measurements.

Electrochemical measurements

A traditional three-electrode system was applied, which was consisted of a platinum auxiliary electrode, a saturated calomel reference electrode and the gold working electrode. Cyclic voltammetry (CV) was scanned from 0.7 to -0.1 V with the scan rate of 50 mV/s. Electrochemical impedance spectroscopy (EIS) was performed with the parameters as follows: biasing potential, 0.217 V; amplitude, 5 mV; frequency range, 0.1 Hz to 100 kHz. Square wave voltammetry (SWV)

was carried out with a step potential of 4 mV, a frequency of 70 Hz, and an amplitude of 25 mV. The scan range was from -0.05 V to −0.55 V.

References

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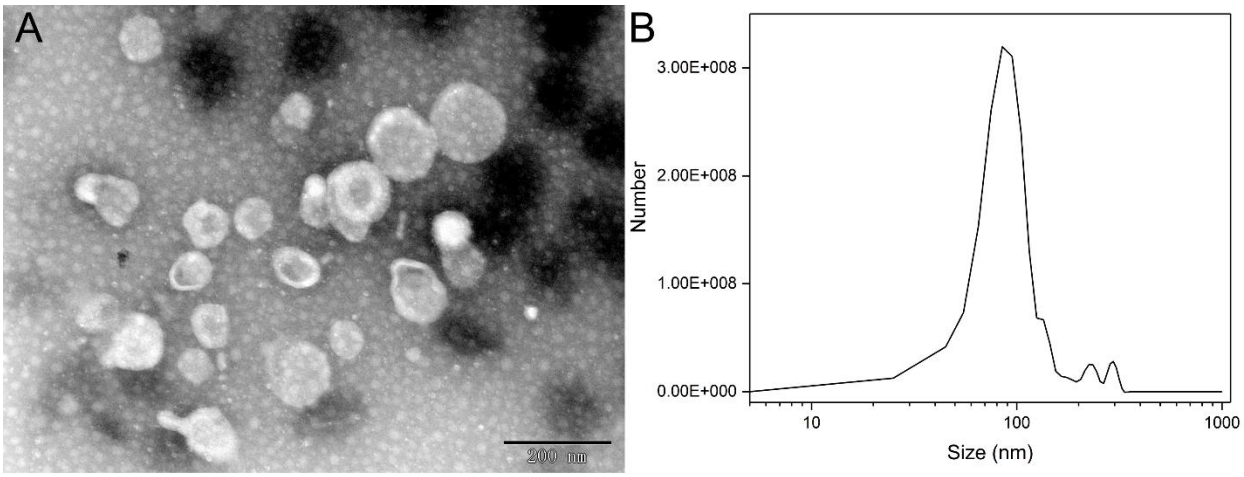

Figure S1. (A) TEM image of HeLa cell-derived exosomes. (B) Nanoparticle Tracking Analysis (NTA) of HeLa cell-derived exosomes.

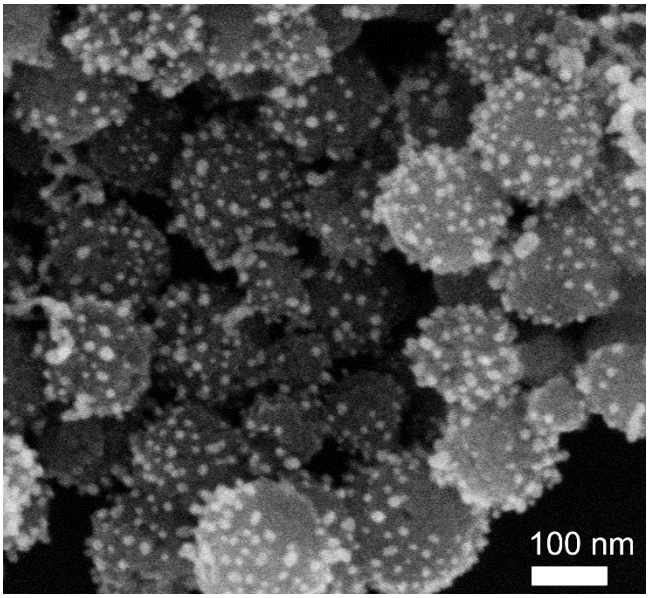

Figure S2. SEM image of Fe_3O_4@Au nanoparticles.

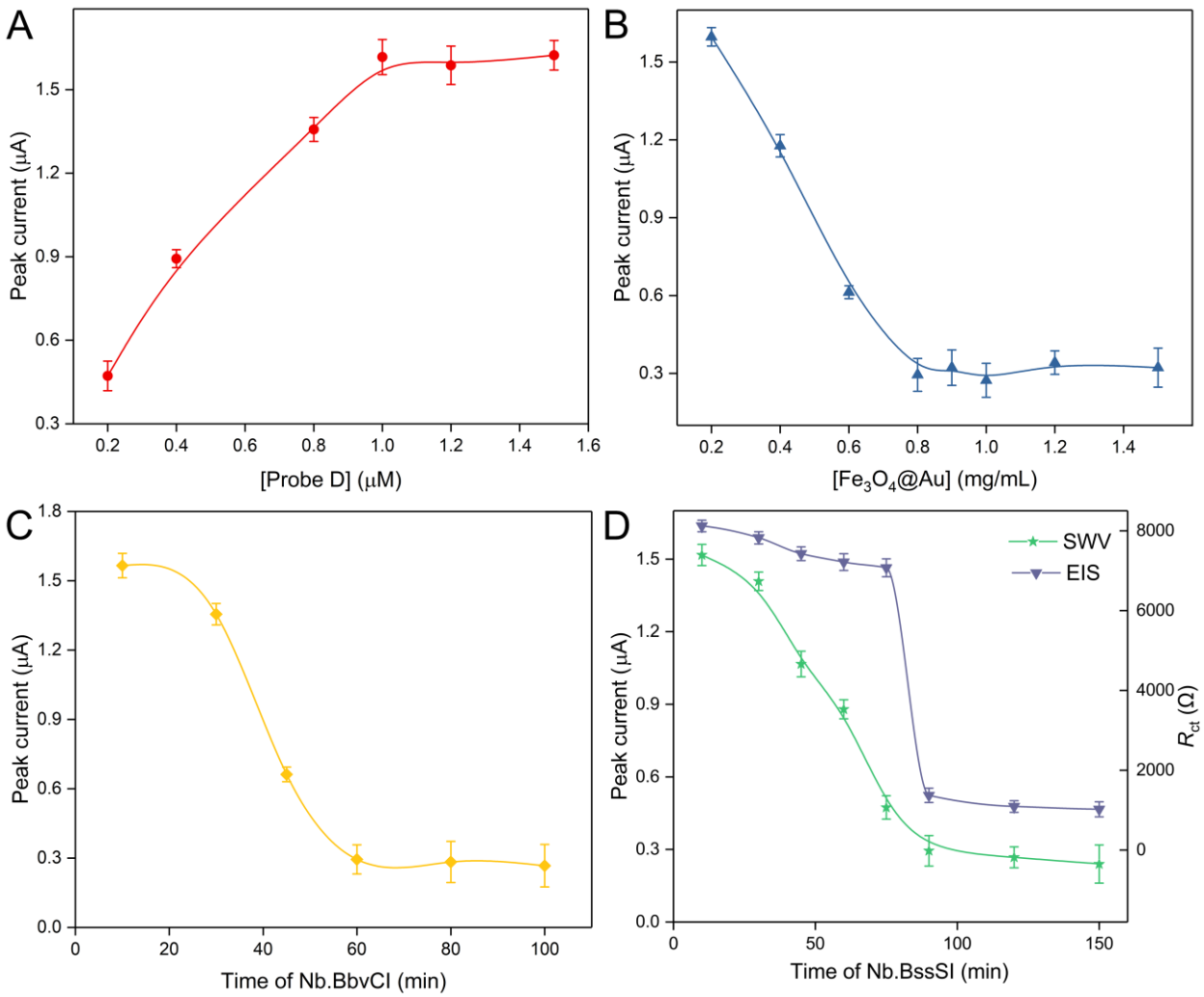

Figure S3. Optimization of (A) concentration of Probe D, (B) concentration of Fe_3O_4@Au nanoparticles, (C) Nb.BbvCI cleavage time, (D) Nb.BssSI cleavage time.

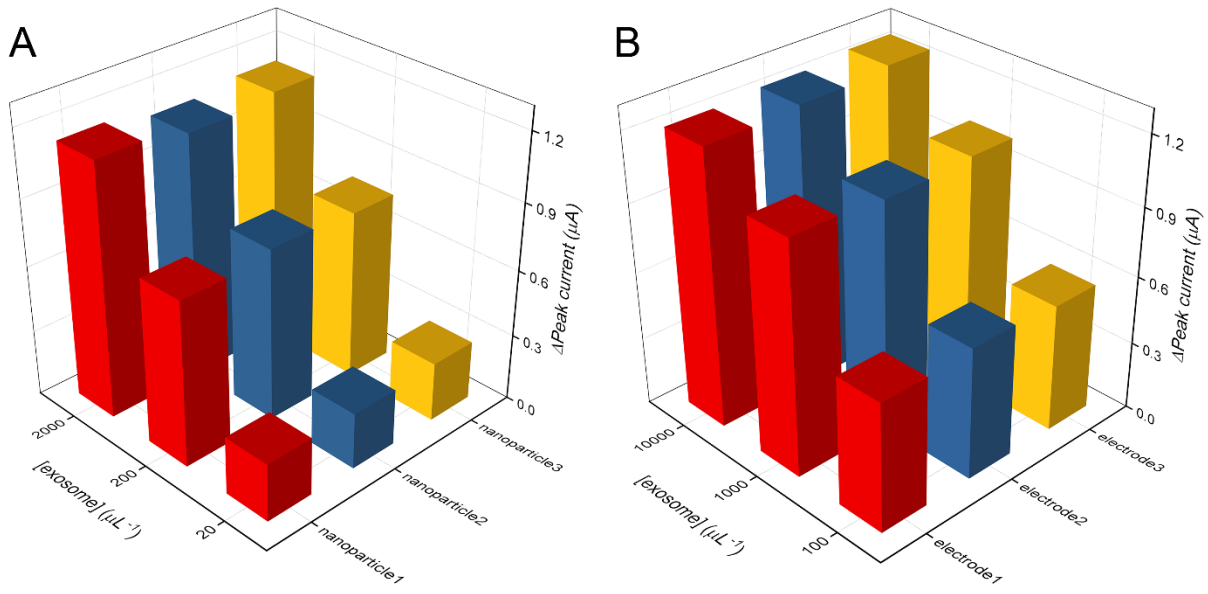

Figure S4. Reproducibility investigation: SWV peak intensities for the detection of exosomes using (A) three sets of Fe_3O_4@Au nanoparticles; (B) three independent gold electrodes.

Table S1. Comparison of analytical performances of the assays of tumor exosomes.

Technique	Strategy	Detection range (/μL)	LOD (/μL)	Ref.
colorimetry	graphitic carbon nitride nanosheets with enhanced peroxidase-like activity	1.9×10^6 to 3.38×10^7	1.352×10^6	2
fluorescence	polydiacetylene liposome	10^5 to 10^7	3×10^5	3
lateral flow assay	double gold nanoparticles conjugates	7.4×10^3 to 5.55×10^4	1.3×10^3	4
SWASV	quantum dots based immunoassay	10^2 to 10^7	10^2	5
ECL	aptamer-binding DNA walking machine	2×10^2 to 7.5×10^4	60	6
SERS	gold nanostar@Raman reporter@nanoshell structures	40 to 4×10^7	27	7
DPV	DNA walking and exonuclease III-assisted digestion	50 to 10^7	13	8
giant magnetoresistance sensor	2D magnetic MoS_2-Fe_3O_4 hybrid nanostructures	10 to 10^6	10	9
SWV	multipedal DNA walker	10 to 2×10^3	6	this method

Table S2. DNA sequences used in this study.

Name	Sequence (5'-3')
Probe A	**CACCCCACCTCGCTCCCGTGACACTAATGCTA**TTTTATACGC▼TGAGGATTTT-$(CH_2)_6$-SH
Probe B	**CACCCCACCTCGCTCCCGTGACACTAATGCTA**TTTTCTGCAGTTATTCCGGCTAGT
Probe C	TCCTCAGCGTAT
Probe D	MB-ACTAGCCGGGACTC▼TCGTGCTTTT-$(CH_2)_6$-SH
Probe E	GCACGAGAGTCTAACTGCAG
Probe B*	TCAAGTCGACTCAAGGTCTGAGTCAACTCCAATTTTCTGCAGTTATTCCGGCTAGT