Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Aptamer recognition-trigged label-free homogeneous electrochemical strategy for ultrasensitive cancer-derived exosome assay

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Experimental section

Reagents

Tris(hydroxymethyl)aminomethane (Tris), hydrochloric acid (HCl), sodium chloride (NaCl), magnesium chloride ($MgCl_2 \cdot 6H_2O$), methylene blue and doxorubicin hydrochloride were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Exo-spin™ kit was supplied by Shanghai XP Biomed Ltd. (Shanghai, China). Cell medium RPMI 1640 and fetal bovine serum (FBS) were purchased from Biological Industries Co., Ltd. (Beit-Haemek, Israel). The human breast cancer cell line MCF-7 and human hepatocyte cell line HL-7702 were bought from Procell Life Science Co., Ltd. (Wuhan, China). All of the chemicals were of analytical reagent grade. Ultrapure water (18.2 MΩ) produced by a Milli-Q water purification system (Millipore) was applied to prepare all aqueous solutions. All DNA probes used in this work were provided by Sangon Biotechnology Co., Ltd. (Shanghai, China) and all DNA sequences were shown in Table S1.

Apparatus

Transmission electron microscopy (TEM) was measured using the HT7700 microscope (Hitachi, Japan). The collected exosomes were loaded on the TEM grid and stained with phosphotungstic acid before TEM characterization. Polyacrylamide gel electrophoresis (PAGE) was carried out on a Bio-Rad electrophoresis system (Bio-Rad, USA), and imaged on a Gel Doc XR + Imaging System (Bio-Rad, USA). PAGE (12%) was carried out in 1 × TBE buffer (tris borate-EDTA) with 95 V for 1.5 h and stained for 30 min in a GelRed solution. Differential pulse voltammetric (DPV) tests were recorded on an Autolab electrochemical workstation (Metrohm, Netherlands) using a three-electrode system: an indium tin oxide (ITO) working electrode, an Ag/AgCl reference electrode and a platinum counter electrode. The potential swept from -0.7 V to -0.4 V, and the modulation amplitude, the modulation time, and the interval time were set to values of 0.025 V, 0.05 s, and 0.5 s. Before measurement, the ITO electrode was treated with ethanol and water for 30 min repeated and then dipped into NaOH (1 mM) for 6 h. Nanoparticle tracking analysis (NTA) was performed with Zetaview (Particle Metrix, Germany).

Cell culture and exosomes isolation

MCF-7 and HL-7702 cells were grown in PRMI 1640 and DMEM culture medium, respectively. To produce exosomes, cells were maintained in culture medium containing 10% exosome depleted FBS. After 48 h, cell and cell debris were first removed by centrifugation (300 × g, 10 min). Then, the exosomes were isolated using the Exo-spin™ kit (Promega) on the basis of the manufacturer's instructions. Finally, the purified exosomes were carefully resuspended in PBS and stored at -80 °C before use. The hydrodynamic radius and the concentration of the exosomes were tested by using NTA.

Exo III-aided homogeneous electrochemical exosomes detection

First, the P1-P2 was synthesized by mixing the P1 and P2 with the same concentration in buffer solution. The above mixture was heated to 95 °C for 5 min and then cooled to room temperature to make sure the formation of dsDNA P1-P2. The hairpin oligonucleotide HP was treated by the same procedures to make it form a hairpin structure. To detect exosomes, a specified concentration of exosomes was added into reaction buffer (10 mM Tris, 250 mM NaCl, 10 mM $MgCl_2$, pH 7.4) containing a certain amount of P1-P2. The obtained mixture was incubated at 37 °C for 30 min. Subsequently, 5 μL of hairpin DNA HP probe (0.02 μM) and 5 μL of Exo III (20 U/mL) were introduced into the above solution and further incubated at 37 °C for about 1 h. Finally, 10 μL of Dox (0.1 μM) was added to the above solution for 20 min. The total volume of the reaction system was 100 μL.

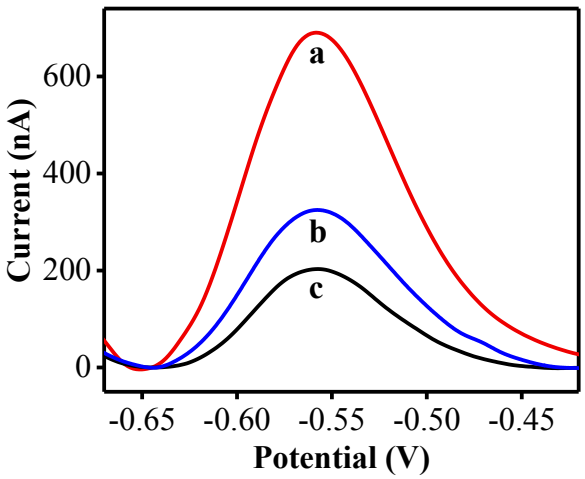

Fig. S1 DPV responses of DOX under different conditions: (a) DOX; (b) DOX + HP1; (c) DOX + HP2. The concentrations of DOX, HP1 and HP2 were 0.1 μM, 0.02 μM and 0.02 μM respectively.

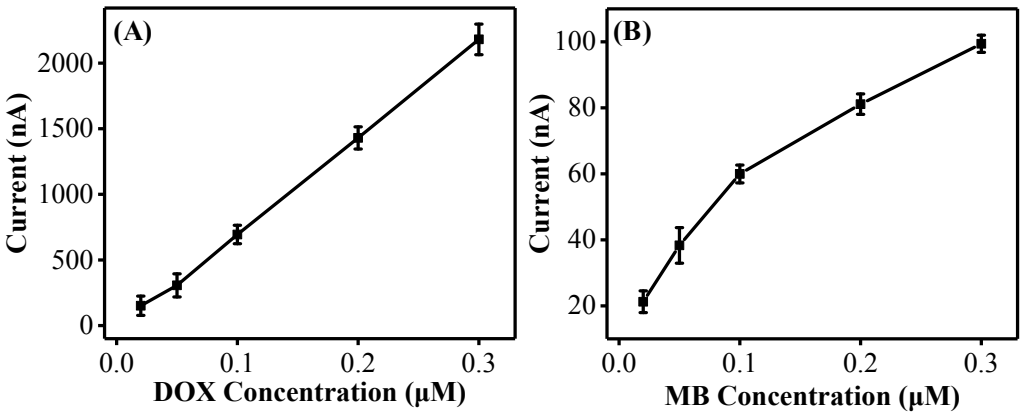

Fig. S2 DPV peak current of DOX (A) and MB (B) under different concentrations.

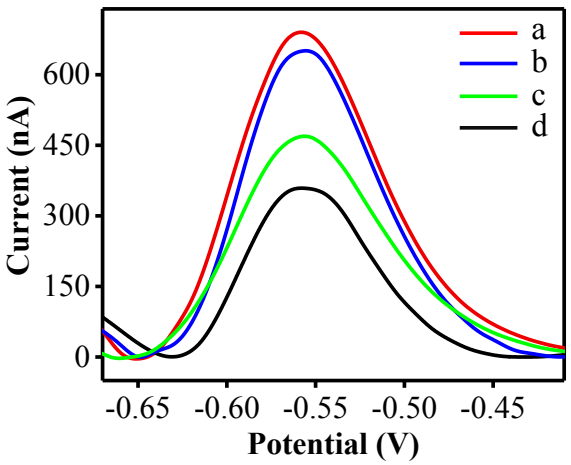

Fig. S3 DPV responses of DOX under different kinds of buffer solutions: (a) Tris-HCl; (b) MES; (c) PBS; (d) HEPES. The concentration of DOX was 0.1 μM.

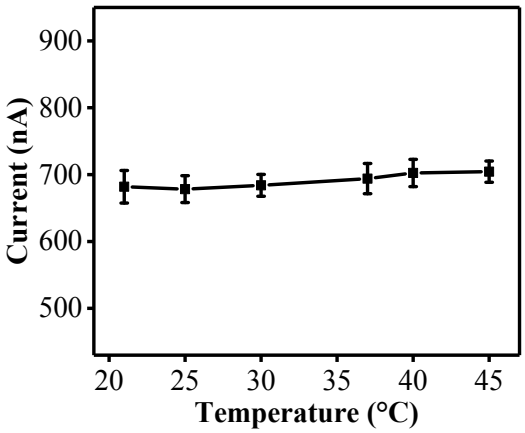

Fig. S4 The DPV peak current of DOX at different temperatures ranging from 20 to 45°C.

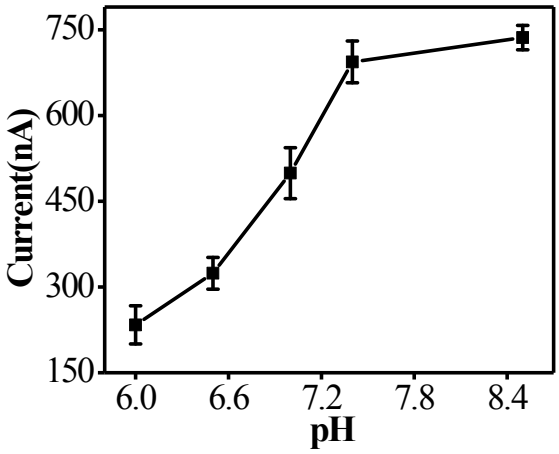

Fig. S5 The DPV peak current of DOX at different pH values ranging from 6.0 to 8.4.

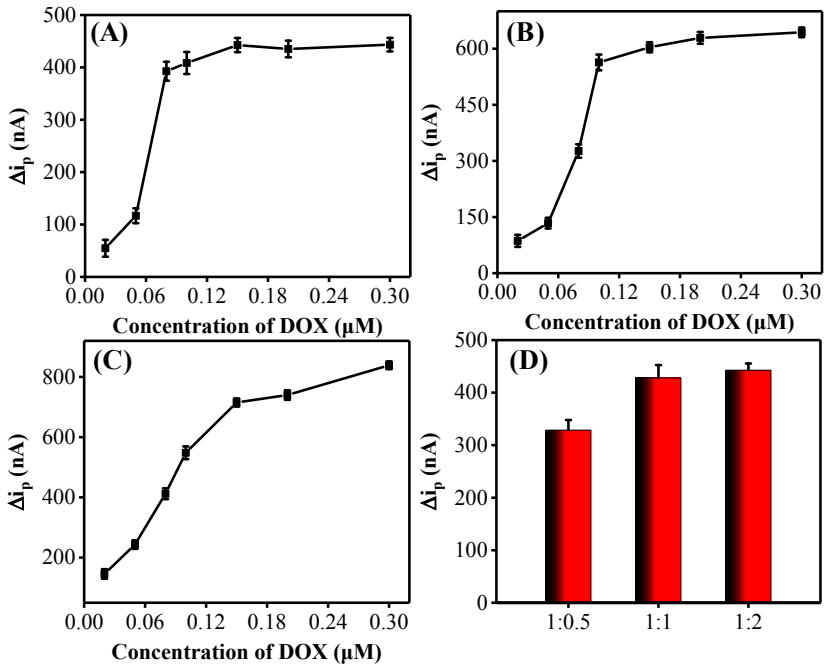

Fig. S6 DPV peak current change of DOX ($\Delta i_p = i_p - i_{p,0}$, in which i_p and $i_{p,0}$ are the DPV peak currents in the presence and absence of P1-P2 and HP, respectively) under the different ratios of P1-P2 and HP: (A) P1-P2 : HP = 1 : 0.5, (B) P1-P2 : HP = 1 : 1, (C) P1-P2 : HP = 1 : 2. The concentration of P1-P2 was 0.02 μM. (D) DPV peak current change ($\Delta i_p = i_p - i_{p,0}$, in which i_p and $i_{p,0}$ are the DPV peak current in the presence and absence of exosomes, respectively) vs the different ratios of P1-P2 and HP. The concentrations of P1-P2 and exosomes were 0.02 μM and 3.4×10^8 particles/mL, respectively.

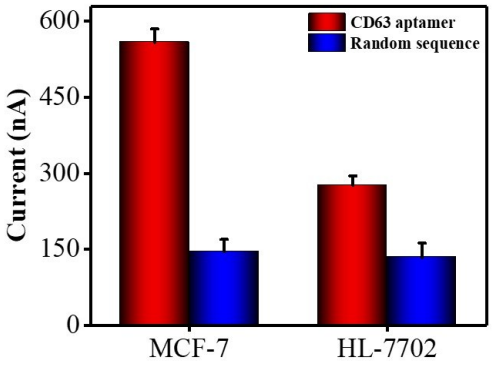

Fig. S7 Comparison of the DPV peak current in the presence of MCF-7 cells-derived exosomes and HL-7702 cells-derived exosomes respectively.

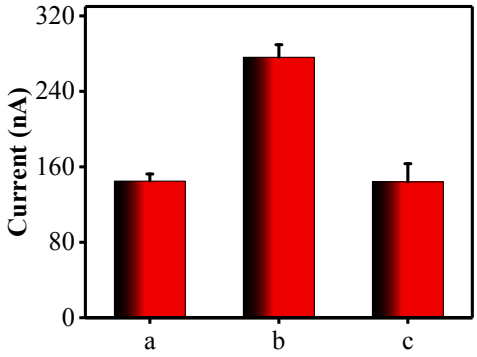

Fig. S8 DPV peak current of the proposed method in response to HL-7702 cells-derived exosomes in the culture medium. (a) culture medium, (b) HL-7702 cells culture medium, (c) HL-7702 cells culture medium filtrate.

Table S1. Oligonucleotide sequences used in this work

Oligo DNA	Sequence 5'-3'
P1	CACCCCACCTCGCTCCCGTGACACTAATGCTA
P2	AGGTGGGGTGAATTTTTT
HP	CGACGACGTGCTTGCTTTTTTTTTTTTGCAAGCACGTCG TCGTTCACCCCACCT
P3	CACCCCACCTACGTGCTCAATTCGATCTGCAT
HP1	CGACGACGTGCTTGCTTTTTTTTTTTTGCAAGCACGTCG TCG
HP2	TTAATGAATTACTTACTTTTTTTTTTTTGTAAGTAATTC ATTAA

Table S2 Comparison of the as-proposed strategy with other reported methods

Method	LOD (particles/μL)	Reference
Electrochemical sandwich immunosensor for determination of exosomes based on surface marker-mediated signal amplification	2×10^2	1
Ultrasensitive and reversible nanoplatform of urinary exosomes for prostate cancer diagnosis	50	2
Ultrasensitive microfluidic analysis of circulating exosomes using a nanostructured graphene oxide/ polydopamine coating	50	3
Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor	3×10^3	4
Direct exosome quantification via bivalent-cholesterol-labeled DNA anchor for signal amplification	2.2×10^3	5
Aptasensor with expanded nucleotide using DNA nanotetrahedra for electrochemical detection of cancerous exosomes	20.9	6
A paper-supported aptasensor based on upconversion luminescence resonance energy transfer for the accessible determination of exosomes	1.1×10^3	7
Concentration-normalized electroanalytical assaying of exosome markers	190	8
Highly sensitive electrochemical detection of tumor exosomes based on aptamer recognition-induced multi-DNA release and cyclic enzymatic amplification	70	9
Ti_3C_2 MXenes nanosheets catalyzed highly efficient electrogenerated chemiluminescence biosensor for the detection of exosomes	125	10
Quantification of exosome based on a copper-mediated signal amplification strategy	4.8×10^4	11

Enhancement of the intrinsic peroxidase-like activity of graphitic carbon nitride nanosheets by ssDNAs and its application for detection of exosomes	13.52×10^5	12
Molecular-recognition-based DNA nanodevices for enhancing the direct visualization and quantification of single vesicles of tumor exosomes in plasma microsamples	10^3	13
Bridging exosome and liposome through zirconium–phosphate coordination chemistry: a new method for exosome detection	7.6×10^3	14
Aptamer recognition-trigged label-free homogeneous electrochemical strategy for ultrasensitive cancer-derived exosome assay	12	This work

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