Supporting Information

A Fluorescence Assay for Exosome Detection Based on Bivalent Cholesterol-Anchor Triggered Target Conversion and Enzyme-Free Signal Amplification

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General Experimental Section

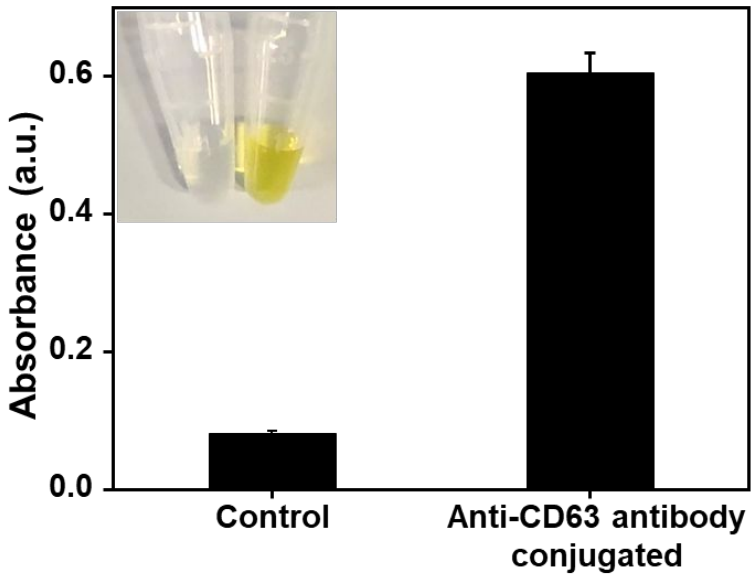
Instruments. Native polyacrylamide gel electrophoresis (PAGE) was carried out using vertical electrophoresis systems (Beijing, China). Confocal microscopy fluorescence images were obtained by an Olympus FV1000. Transmission electron microscopy (TEM) image was acquired using a JEM-1230 instrument (JEOL, Tokyo, Japan). DS-11 Series UV-Vis Spectrophotometer (Wilmington, USA) and fluorescence spectrophotometer (F-7000, HITACHI) were used to obtain absorption and fluorescence spectral data, respectively. The annealing process was carried out by using PCR (Framingham, USA). The size and concentration of exosomes were obtained by using nanoparticle tracking analysis (NTA) at VivaCell Biosceinces with ZetaView PMX 110 (Particle Metrix, Meerbusch, Germany) and corresponding software ZetaView 8.04.02.

PAGE analysis of enzyme-free DNA circuits. For the enzyme-free DNA circuits, the sequences were designed according to previous literature with a slight modification. [1] TNK buffer containing BC-anchor (5 nM), H1 (90 nM), H2 (135 nM), and RFQ (90 nM) was incubated for 2 h at room temperature. After different reaction products were prepared, 10 μL of each sample was mixed with 2 μL of 6×loading buffer, then 10 μL of the mixed solution was loaded into the lanes of a polyacrylamide gel. The gel was run at 180 V constant voltage for 2 min and then 135 V constant voltage for 70 min in 1×TAE buffer at room tem-perature. After the gel was stained for 1 min in EtBr solu-tion, it was photographed using the multifunctional imag-ing system.

Exosome quantification by enzyme-linked immunosorbent assay (ELISA). 30 μL Anti-CD63 MBs were mixed with 20 μL different concentrations of exosome and allowed to react under gentle shaking at room temperature. After 2 h, the mixture was washed three times with PBST and resuspended in PBS. 50 μL anti-CD 63 rabbit antibody and anti-rabbit IgG-HRP were sequentially added to the immunocomplexes at an interval of 2 h. After incubation for another 1 h, the mixture was washed three times. Subsequently, 3,3’,5,5’-tetramethylbenzidine (TMB) solution was added to induce the enzymatic reaction and TMB stop solution was added to terminate the reaction. Finally, the absorption spectra were measured by UV-Vis Spectrophotometer.

Table S1. Sequences of oligonucleotides used in this work.

Name	Sequence (5'-3')
I	CGACATCTAACCTAGCTCACTGACATAAGGCACGACGGCTTT-Cholesterol
I_c	Cholesterol-TTTGCCGTCGTGCCTTAT
I_{AF}	GTCAGTGAGCTAGGTTAGATGTCG-Alexa Fluor 488
H1	GTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAACCTAGCCCTTGTCATAGAGCAC
H2	AGATGTCGTCTACACATGGCGACATCTAACCTAGCCCATGTGTAGA
RF	FAM-CGAGTGCTCTATGACAAGGGCTAGGTT
RQ	CCCTTGTCATAGAGCACTCG-Dabcyl



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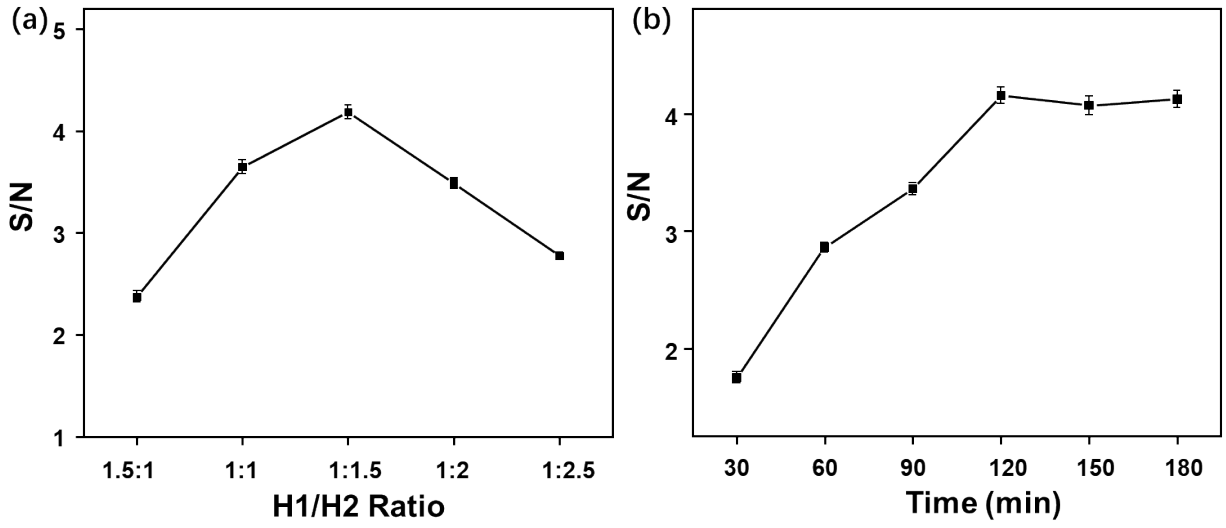

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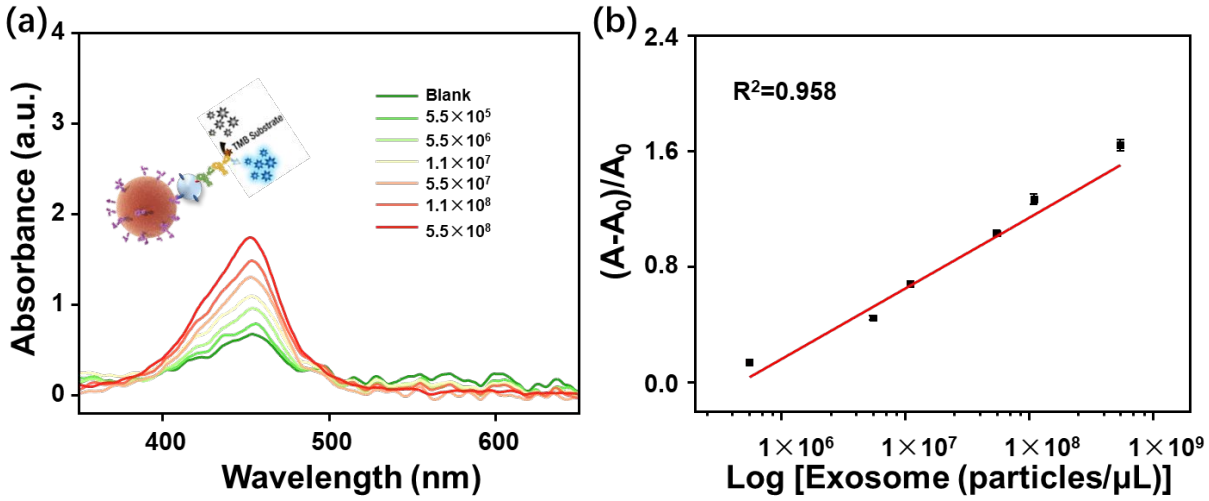

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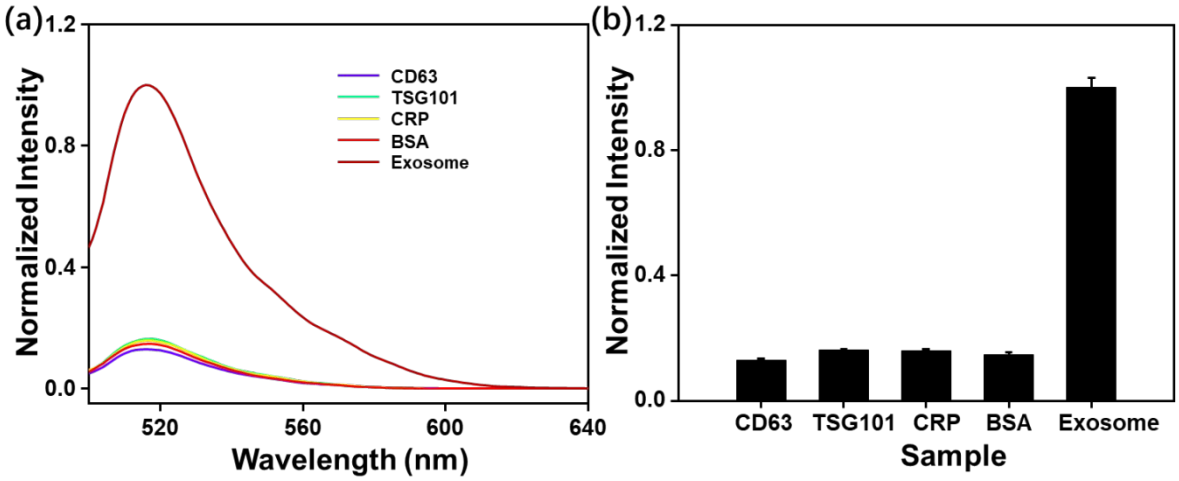

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Table S2. Comparison among exosome detection methods.

Detection Method	Identification Target	Linear Range (particles/μL)	Limit of Detection (particles/μL)	Real Sample	Reference
Colorimetry assay	CD63	1.8×10^6-2.2×10^7	5.2×10^3	Serum	2
Colorimetry assay	CD63 and CD9	2.2×10^5-2.4×10^7	—	Serum	3
Paper-based aptasensor with LRET	CD63	1.0×10^4-1.0×10^8	1.1×10^3	—	4
SPRi-based biosensor	CD63	3.1×10^4-1.0×10^6	2.37×10^4	Serum	5
Lateral flow aptasensor	CD63	1.0×10^4-1.0×10^8	1.4×10^4	Serum	6
Fluorescent nanosensor	GPC-1	7.8×10^4-3.9×10^9	6.56×10^4	Serum	7
Fluorescent aptasensor	CD63 and bilipid layer	7.5×10^4-1.5×10^7	4.8×10^4	Serum	8
Fluorescent aptasensor	CD63	1.0×10^5-1.0×10^9	1.0×10^5	Serum	9
Electrochemical aptasensor	CD63	1.12×10^2-1.12×10^8	96	Serum	10
Electrochemical enzymatic amplification	PSMA	1.0×10^3-1.2×10^5 (particles)	70	FBS	11
Fluorescence biosensor with enzyme-free amplification	CD63 and bilipid layer	5.5×10^3-1.1×10^7	1.29×10^3	FBS/serum/urine	This Work

Table S3. Recovery values of exosomes spiked into 20% diluted ED-FBS, 20% diluted UC-Serum, and 20% diluted UC-Urine by the proposed assay.

Samples	Added (Particles/μL)	Found (Particles/μL)	Recovery (%)	RSD (%)
20% Diluted ED-FBS	4.0×10^4	3.85×10^4	96.25	3.21
	4.5×10^5	4.21×10^5	93.56	4.41
	2.5×10^6	2.52×10^6	100.8	2.89
20% Diluted UC-Serum	4.0×10^4	3.94×10^4	98.50	3.28
	4.5×10^5	4.69×10^5	104.2	5.25
	2.5×10^6	2.67×10^6	106.8	2.84
20% Diluted UC-Urine	4.0×10^4	3.69×10^4	92.25	5.40
	4.5×10^5	4.38×10^5	97.33	3.13
	2.5×10^6	2.58×10^6	103.2	3.30

Table S4. Clinical information of healthy donors and cancer patients.

Clinical Condition	Number	Gender	Age
Healthy donors	H1	Male	33
	H2	Male	23
	H3	Female	33
	H4	Female	30
	H5	Male	59
	H6	Female	59
	H7	Male	34
Liver cancer	P1	Male	68
	P2	Female	69
	P3	Male	65
Cholangiocarcinoma	P4	Female	52
	P5	Male	70
	P6	Male	77
Breast cancer	P7	Female	49
	P8	Female	55

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