Electronic Supplementary Information (ESI)

Sandwich-type surface-enhanced Raman scattering sensor using dual aptamers and gold nanoparticles for the detection of tumor extracellular vesicles

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EXPERIMENTAL SECTION

Materials

Trisodium citrate was obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Rhodamine 6G (R6G) were purchased from Alfa Aesar. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Shanghai Chemical Reagent Company (Shanghai, China). Bovine serum albumin (BSA) and phosphate-buffered saline (PBS) were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). Sodium chloride (NaCl), polyethylene glycol (PEG, MW 4000) and Sodium dodecyl sulphate (SDS) was purchased from Sinopharm Chemical Reagent Co., Ltd. Agarose beads modified with streptavidin were purchased from GE Healthcare Bio-Sciences AB. Antibodies used are rabbit polyclonal anti-CD63 antibody (Abcam, Catno. Ab59479). Ultrapure water (18.2 MΩ) obtained from a Milli-Q system was used through-out the study. All other chemicals were analytical reagent grade, unless otherwise stated. Human acute lymphoblastic leukemia CCRF–CEM (CEM) cells, human Burkitt's lymphoma cells (Ramos) were obtained from the American Type Culture Collection (Manassas, VA). Human blood was provided by volunteers. All oligonucleotides were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China) (See tableS1).

Apparatus

The transmission electron microscopic (TEM) images were obtained on a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). The UV-vis absorption spectrum was obtained with a Biospec-nano UV-vis spectrophotometer (Japan). The particles size distributions were determined by a dynamic light scattering (DLS) detector (Malvern Zetasizer 3000 HS, Worcestershire, England). Flow cytometric assays on the agarose beads were obtained on the flow cytometer (Gallios, Beckman Coulter, USA). SERS measurements were performed using a confocal microprobe Raman instrument (Ram Lab-3010, Horiba Jobin Yvon, France), and spectra were acquired using a 633 nm He-Ne laser and 50 × working objective lens (8 mm).

Preparation of gold nanoparticle

The AuNPs were synthesized using the sodium citrate reduction method. Before the experiments, all glassware was cleaned in aqua regia, rinsed with H_2O, and then oven-dried. Next, 100 mL of 0.01% $HAuCl_4$ was heated with vigorous stirring, 1 mL of trisodium citrate (1%) was quickly added under stirring when boiling. Then the solution color was observed to turn from pale yellow to colorless and then purple. Boiling was continued for an additional 20 min. After the heating, the colloid was stirred until the solution reached room temperature. Then it was filtered through a 0.22-μm Millipore membrane filter. The prepared AuNPs were stored at 4 °C.

Preparation of the SERS probes (SP)

3.0 μL of 1 mM R6G solution as a Raman dye was added to 50 nm AuNPs (1 mL, 0.1044 nM) , then incubated the mixture for 10 min at room temperature, following by centrifugation at the speed of 9000 rpm for 8 min to remove free R6G. Next, 30 μL of 10 μM DNA solution reduced by Tris (2-carboxyethyl) phosphine hydrochloride (TCEP•HCl) was added to the above solution overnight in 4 °C. After 16 hours, Phosphate buffer (0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4 pH = 7.4) containing 0.01 % BSA and 1% SDS was added to the mixture to achieve a 1 mM phosphate concentration, and aliquots of sodium chloride solution (2.0 M) were added to the mixture over an eight-hour period to achieve a final sodium chloride concentration of 0.3 M. The solution containing the functionalized particles was centrifuged (10,000 rpm, 10 min) and resuspended in phosphate buffered saline (PBS; 137 mM NaCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.7 mM KCl, pH 7.4) to produce the purified SERS probe used in all subsequent experiments.

Quantification of loading capacity of SH-Apt_{CD63} on each AuNP

We used SH-Apt_{CD63} to quantify the number of Raman dyes loaded on each AuNP. 30 μL of 10 μM DNA solution reduced by Tris (2-carboxyethyl) phosphine hydrochloride (TCEP•HCl) was added to the 200 μL of AuNP-R6G (0.522 nM) overnight in 4 °C. After incubation and centrifugation, the supernatant of free SH-Apt_{CD63} after centrifugation was collected, UV-vis spectroscopy was used to

determine the different concentration of SH-Apt_{CD63}, and the number of SH-Apt_{CD63} molecules loaded on each AuNPs was calculated through dividing the amount of DNA substance by the concentration of gold nanoparticles, the corresponding number of DNA loads can be obtained.

Preparation of agarose beads probe (AAP)

The modification was carried out according to the product manual of AAP. First, 1 μL of purchased agarose beads suspension was washed three times with 50 μL of PBS buffer. After centrifugal separation, AAP were resuspended in 200 μL of PBS buffer containing 50 μL of 2 μM Apt_{PTK7} for capture and then incubated for 0.5 h on a thermostatic metal shaker bath (25 °C, 500 rpm). Subsequently, the supernatant was saved to measure the absorbance. AAP were washed three times with 50 μL of PBS buffer and stored in 20 μL of phosphate buffered saline.

EVs extraction from CEM cells, Ramos cells

Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 100 U/mL 1% antibiotics penicillin/streptomycin and in a 100% humidified atmosphere containing 5% CO_2 at 37 °C. All cells were passaged and seeded at 70-80% confluence in flasks for at least 24 h before adding serum-free medium. The conditioned medium was removed after 2 days and was used to collect EVs. The EVs were collected using conventional untracentrifugation as described in the previous report. Briefly, media were collected and centrifuged at 2000 g for 20 min to remove cells, then, the supernatant was centrifuged at 12,000 g for 45 min to remove cell debris, followed by filtration a 0.22 μm filter. The filtered supernatant was then centrifuged at 100,000 rpm for 1 h to retain the precipitated pellets of EVs. The EVs pellets were pipetted, washed with PBS once, and precipitated by again centrifugation at 100,000 rpm for 1 h, and resuspended in PBS.

EVs detection using the GNP probes and AAP

To capture the EVs with the AAP and attach the corresponding probes, 20 μL of AAP probe solution were added to different concentration of EVs. After incubation for 30 min at 25 °C in a thermostatic shaker, the EVs were separated by centrifugation, the

pellet was suspended in 10 μL of PBS solution and 20 μL of Raman probe solution was added, The mixture was shaken for 30 min at room temperature, the SERS probes were separated by centrifugation at 500 rpm and the precipitate was subjected to SERS measurement.

Flow-cytometric analysis

To test the feasibility by using aptamers for recognition of specific tumor-derived EVs, 20 μL of 5 μM FAM-labeled Apt_{CD63} were mixed with 20 μL of AAP probe solution and 1240 μg/μL CEM EVs. After washing as described previously, fluorescence was determined with a FACScan cytometer.

TEM analysis

In brief, 1 μL of EVs suspension was diluted with 9 μL of PBS, and then adsorbed to copper-coated mesh-grids for 10 min. Redundant suspension was removed with filter paper, and the grids were allowed to dry. EVs were then negatively stained with 1% sodium phosphotungstate for 10 min. The samples were viewed with a JEOL-3010 transmission electron microscope.

Western blot

Western Blotting for CD63 proteins in EVs: In brief, the EVs were lysed by radioimmunoprecipitation assay (RIPA) lysis buffer along with phenylmethysulfonyl fluoride (PMSF) and a protease inhibitor cocktail. Then, the lysate was separated by SDS-gel electrophoresis (12%) and then transferred to polyvinylidene difluoride membrane. After blocking of non-specifc sites with 5% skim milk, the membrane was incubated with anti-CD63 primary antibody (dilution = 1:1000), followed by incubation with horseradish peroxidase-conjugated secondary antibodies (dilution =1:12000). Positive band was visualized via using enhanced chemiluminescence substrateand the Azure Biosystems Gel Documentation system.

Ethics statement

Clinical samples from one healthy donor were collected from Hunan Cancer Hospital. All experiments were performed in accordance with the Guidelines of Clinical Sample Management Rules of Hunan Cancer Hospital, which were reviewed and

approved by the Ethics Committee at Hunan Cancer Hospital. Informed consents were received from the blood donors of this project.

Table S1. Detailed sequence information of DNA.

Name	Sequence (5'-3')
Apt_{PTK7}	ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGATTTTTT-Biotin
Apt_{CD63}	SH C6-TTTTTTTTTTCACCCCACCTCGCTCCCGTGACACTAATGCTA
C-Apt_{PTK7}	CGATACATGGACCGAAGTTCCGCTTGCAACCGCAGGATCGCTTTTTT-Biotin
C-Apt_{CD63}	SH C6-TTTTTTTTTTACTACCGGCAGCCTAGCTCCAACCGGATCTGG

Table S2. Comparison of different methods for the detection of EVs.

Method	Sensitivity (LOD)	Dynamic range (orders of magnitude)	Reference
Electrochemical sensor	3×10^3/L	4	1
SERS	1.2×10^3/L	4	2
Nano-plasmonic sensor	3×10^3/L	2	3
Aptamer-based electrochemical	1×10^3/L	2	4
Lateral flow immunoassay	8.54×10^5/L	2	5
Fluorescence	15.6 ng/μL	3	6
LSPR	0.194 ng/μL	3	7
SERS	2.44 pg/μL	4	This work

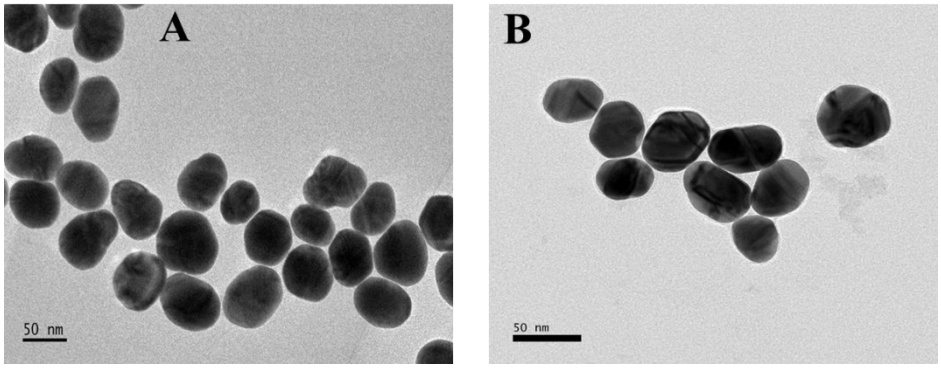

Fig S1. TEM image of (A) Au NPs,（B) SP.

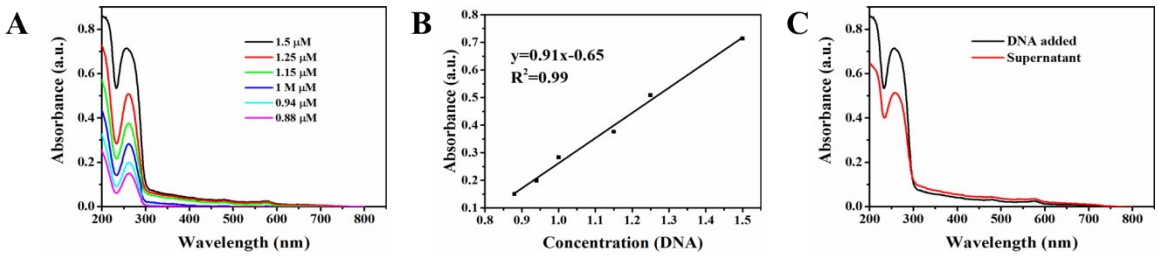

Fig S2. (A) Absorption spectra of different concentrations of SH-Apt_{CD63}; (B) Plot of absorbance as function of concentrations of SH-Apt_{CD63}; (C) Absorption spectrum of SH-AptCD63 Unbound on AuNPs and SH-Apt_{CD63} added before reaction.

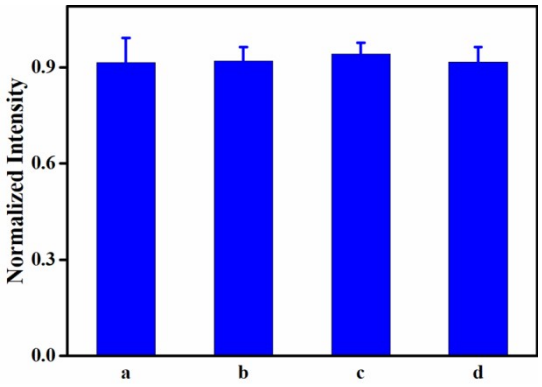

Fig S3. SERS stability of SP. The bars represent the Raman intensities of 0.1 nM SP upon different oxide addition. The Raman intensity of 0.1 nM SP without oxide addition were normalized to 1.0, (a) blank, (b) hydrogen peroxide, (c) sulfuric acid, (d) hypochlorite (the concentrations of hydrogen peroxide, superoxide anion, hypochlorite, were 10 mM, 46 μM and 37 μM, respectively). Error bars are the standard deviation of three repetitive experiments.

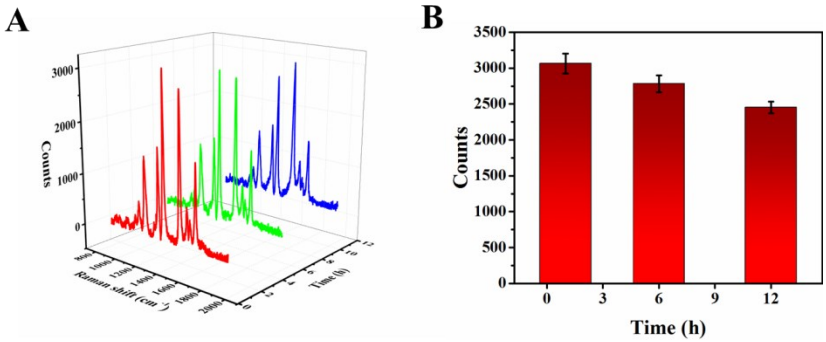

Fig S4. The SERS stability of SP in diluted serum over time. (A) The SERS spectra of SP within 12h. (B) Signal intensities of SP at 1359 cm^{-1} of data.

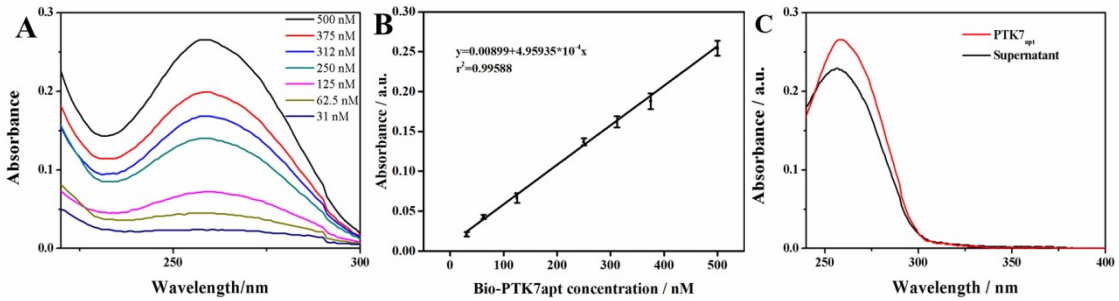

Fig S5. (A) UV absorption spectra of different concentrations of Bio-Apt_{PTK7}. (B) Plot of absorbance as function of concentrations of Bio-Apt_{PTK7}. (C) UV absorption spectrum of Bio-Apt_{PTK7} added (red), Bio-Apt_{PTK7} detected on supernatant (black).

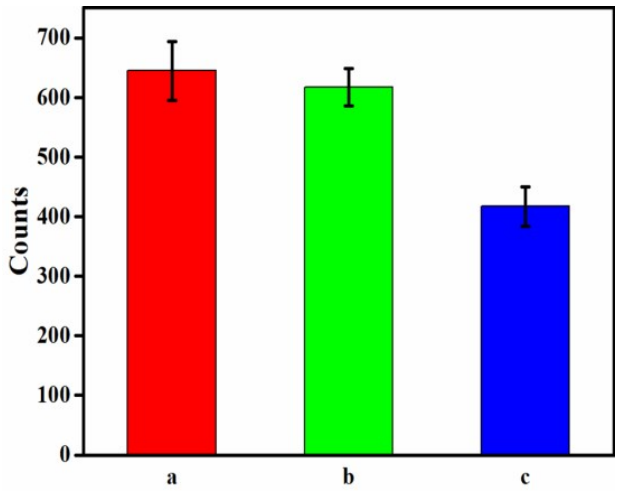

Fig S6. SERS spectra of SP (a), SP incubated with AAP in PBS buffer containing 0.1% PEG after centrifugation separation (b), SP incubated with AAP in PBS-buffer after centrifugation separation (c).

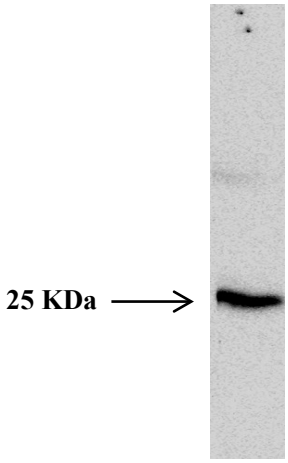

Fig S7. Western blot analysis of CD63 protein in EVs.

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