Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Gas pressure-assisted ratiometric atomic flame assay for visual point-of-care testing of tumor cell-derived exosomes

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Materials and reagents. Copper chloride dihydrate ($CuCl_2·2H_2O$), strontium chloride hexahydrate ($SrCl_2·6H_2O$), sodium chloride (NaCl), calcium chloride dihydrate ($CaCl_2·2H_2O$) and manganese chloride dihydrate ($MnCl_2·2H_2O$) were purchased from Shanghai Titan Scientific Co.Ltd. (Shanghai, China). Sodium citrate, chloroplatinic acid hexahydrate (H_2PtCl_6 $6H_2O$), chloroauric acid ($HAuCl_4$) and bovine serum albumin (BSA) were acquired from Aladdin (Shanghai, China). L-ascorbic acid (L-AA), hydrochloric acid (HCl), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2，30 wt %) were obtained from Xilong Scientific Co., Ltd. (Guangzhou, China). Carboxyl-modified magnetic nanoparticles (MNPs) were purchased from BaseLine (Tianjing, China). Phosphate buffered saline (PBS, pH 7.4, 10 mM) and trypsin were obtained from Solarbio Life Sciences (Beijing, China). Dulbecco's modified essential medium (DMEM) cell culture medium and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific Inc. (MA, USA). ExoQuick-Tc Isolation Kit and exosome-free serum were acquired from System Biosciences (CA, USA). Human HeLa cervical cancer cells, human breast carcinoma MCF-7 cells, and mouse fibroblast L929 cells were obtained from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The human serum sample was kindly provided from a healthy donor and all related experiments were approved by the Institutional Ethical Committee (IEC) of Guangxi Normal University. All the DNAs were synthesized by Sangon Biotechnology Co., Ltd (Shanghai, China). The DNA sequences used were listed as follow (from 5′ to 3′): HS-TTT TTT TTT CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG (Apt_{EpCAM}), CAC CCC ACC TCG CTC CCG TGA CAC TAA TGC TAT TTT TTT TT-NH_2 (Apt_{CD63}), CAG ACT /Cy3/ GAT GTT GAC CAT GTG TAG ATC AAC ATC AGT CTG ATA AGC TA (H1), CCA TGT GTA GAC AGA CTG ATG TTG ATC TAC ACA TGG TCA ACA TC-Cy5 (H2), TAG CTT ATC AGA CTG ATG TTG A (catalytic probe).

Instrumentation. Transmission electron microscopy (TEM) characterization was performed using a Talos F200S microscope (Thermo Fisher Scientific, USA). Zeta potential analysis was carried out with a Malvern Zetasizer (Nano ZS-90, Malvern, UK). Ultraviolet–visible (UV–vis) absorption spectra and fluorescence spectra were

measured on a Cary 60 spectrophotometer (Agilent, USA) and a RF-6000 spectrofluorometer (Shimadzu, Japan), respectively. The concentration of exosomes was analyzed with a Pmx110 nanoparticle tracking analysis (NTA) device (Particle Metrix, Germany).

Synthesis of gold nanoparticles. Gold nanoparticles (AuNPs) were synthesized using a reported method with some modifications.[1] Firstly, the glassware was soaked in a newly prepared aqua regia overnight. Subsequently, 98 mL of water and $HAuCl_4$ (2 mL, 50 mM) were mixed in the flask and heated under stirring. When the mixture began to reflux, sodium citrate (10 mL, 38.8 mM) was quickly added. After a 20-minute reflux, the heating was turned off and the solution was naturally cooled down to room temperature under stirring. Finally, 13-nm AuNPs were synthesized.

Synthesis and functionalization of platinum nanoparticles. Platinum nanoparticles (PtNPs) were synthesized according to a previous report with minor modifications.[2] In general, H_2PtCl_6 $6H_2O$ (2 mL, 18.9 mM), NaOH (30 μL, 5 M), L-AA (0.2 mL, 1 M) and 18 mL of H_2O was added in sequential and then mixed in a beaker. After heating at 60 ℃ for 10 min, the color of the solution turned to be brown black. Then, the PtNPs solution was cooled down to room temperature for further use.

The PtNPs functionalization was performed with incubating 5 μM thiolated Apt_{EpCAM} with PtNPs overnight at room temperature. Then, 100 μL of NaCl (1 M) was added dropwise to the mixture over a period of 12 h, and stirred for another 24 h. Subsequently, free Apt_{EpCAM} was removed by centrifuging at 14000 rpm for 30 min. Finally, the formed Apt_{EpCAM} modified PtNPs (Apt_{EpCAM}-PtNPs) were suspended in 10 mM PBS containing 0.1 M NaCl.

Functionalization of magnetic nanoparticles. Typically, 500 μL of carboxyl-modified MNPs were magnetically washed with PBS (10 mM, pH 7.4), followed by the addition of 5 mg EDC and 2.5 mg NHS to activate the carboxyl group. Then, 2 μM aminated Apt_{CD63} was added and reacted for 12 h with a gentle shaking. After blocking with 1% BSA, Apt_{CD63} functionalized MNPs (Apt_{CD63}-MNPs) were obtained.

Cell culture, exosomes isolation and quantification. Human breast cancer MCF-7

cells, human cervical cancer HeLa cells and mouse fibroblast L929 cells were cultured in DMEM containing 10% exosomes-depleted FBS at 37 ℃ in a humidified 5% CO_2 incubator, respectively. Once 80 % confluence was reached, the cell culture medium was harvested. To remove cells and cell debris, the obtained medium was centrifuged at 300 g for 10 min and at 2000 g for 15 min, respectively, followed by filtering with a 0.22 μm filter to remove large contaminating vesicles and protein aggregates. Finally, the supernatant was treated with ExoQuick-Tc Isolation Kit following the manufacturer's instructions. The purified exosomes were re-suspended in 200 μL of PBS (10 mM, pH 7.4) for further use. The exosome concentration was measured by NTA.

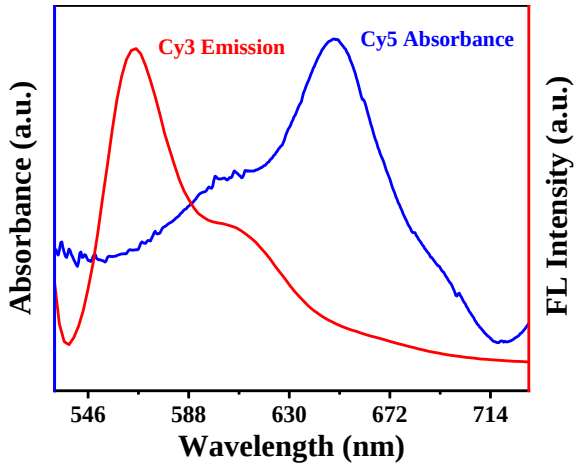

Figure S1. The spectrum of Cy3 and the absorption spectrum of Cy5.

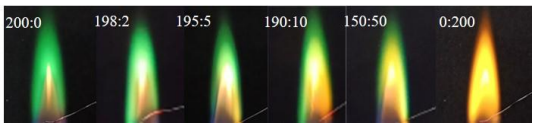

Figure S2. The atomic flame photos of the mixture of Cu^{2+} (20 mM) and Na^{+} (5 mM) with different molar ratios.

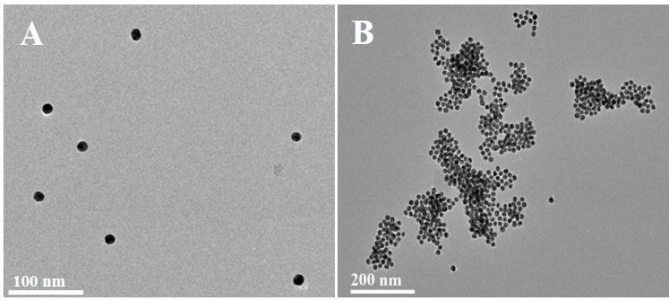

Figure S3. TEM micrographs of AuNPs in the (A) absence and (B) presence of 11.8 mM NaCl.

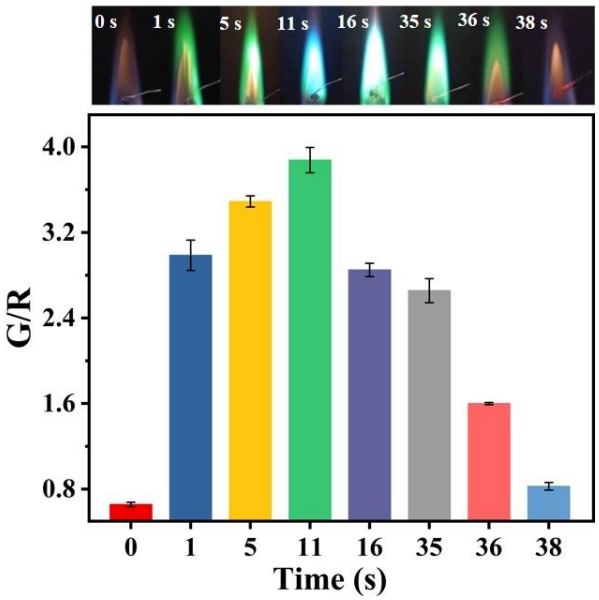

Figure S4. Time-dependent atomic flame reaction of saturated Cu^{2+} (about 4.35 M) solution.

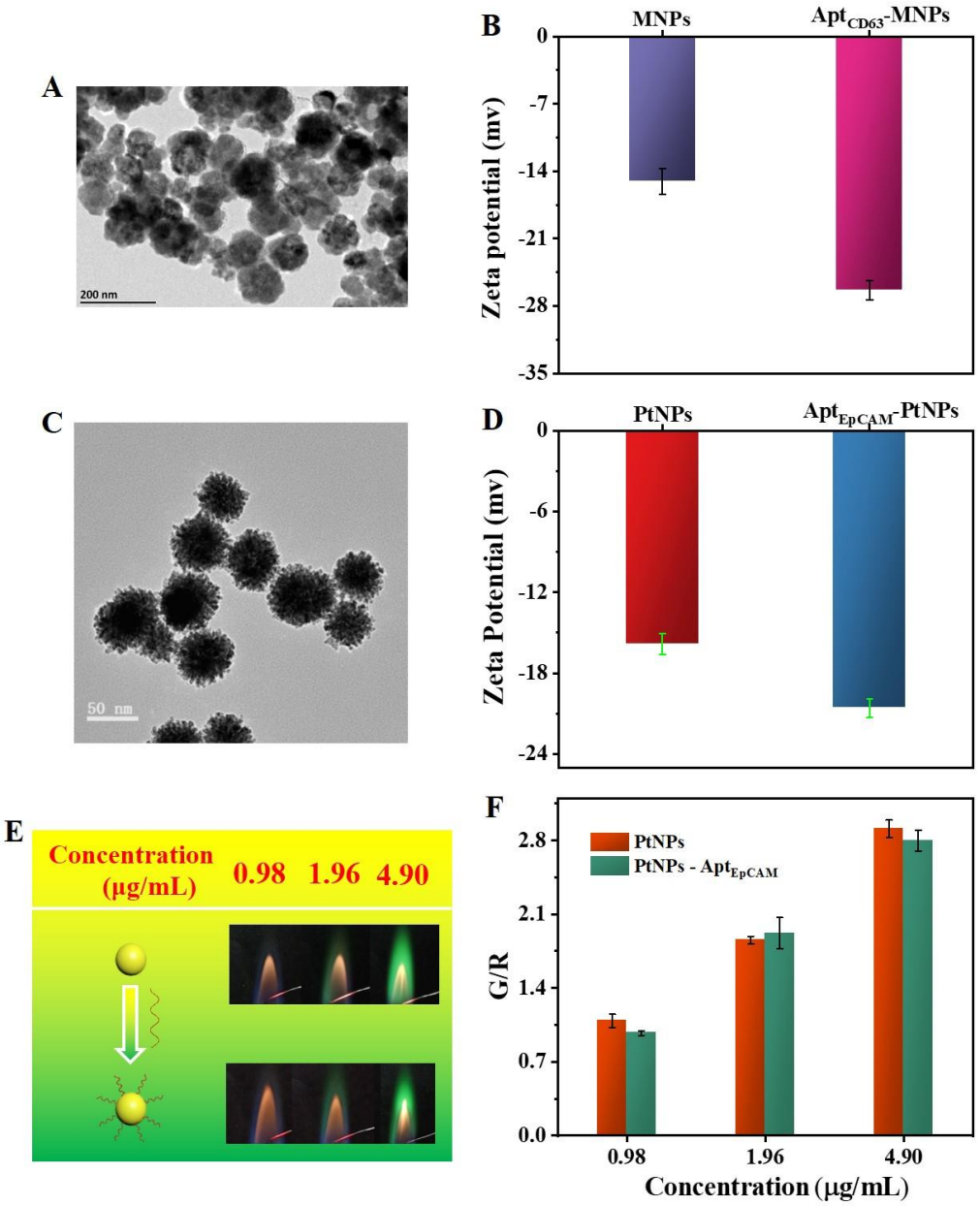

Figure S5. TEM images of (A) MNPs and (C) PtNPs, respectively. Zeta potential investigation of (B) MNPs and (D) PtNPs functionalization. (E) Visual and (F) quantitative comparison of the catalytic efficiency between PtNPs and Apt$_{EpCAM}$-PtNPs, respectively.

Additional reference

1. J. Liu, Y. Lu, *Nat. Protoc.* 2006, **1**, 246-252.

2. X. Fang, X. Zhang, Z. Zhang, S. Hu, F. Ye, S. Zhao, *Chem. Commun.* 2021, **57**, 3327-3330.