Electronic Supplementary Information

Bridging exosome and liposome through zirconium-phosphate coordination chemistry: a new method for exosome detection

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Experimental Section

Reagents and Materials. N-Hydroxysuccinimide modified magnetic beads (NHS-MB) is purchased from Beaver Biosciences Inc. Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) are purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). CD63 polyclonal antibody is obtained from Proteintech Group Inc. Zirconium dichloride oxide octahydrate ($ZrOCl_2$) and calcein are purchased from J&K Scientific Ltd. (Shanghai, China). 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-Dioleoyl-*sn*-glycero-3-phosphoglycerol sodium salt (DOPG) are purchased from Sigma-Aldrich. All of the chemicals are of at least analytical reagent grade and used without additional purification. The solution is prepared using ultrapure water (≥18.25 MΩ·cm) purified by a Millipore Milli-Q water purification system.

Instrumentation. HeLa cells are cultured in a constant temperature incubator (Thermo Fisher Scientific Inc., USA). The transmission electron microscopy (TEM) images of liposomes and exosomes are performed on a Hitachi H-7650. The scanning electron microscopy (SEM) images of magnetic beads (MB) are obtained via Hitachi SU-3500. The particle diameter and concentration

of exosomes are measured using NanoSight (NanoSight Ltd., Malvern, UK). Mixing operation is performed on a HulaMixer sample mixer. The fluorescence intensity is recorded by F-7000 spectrometer (Hitachi, Japan).

Preparation of Cell Culture Supernatant. The HeLa cells are cultured in DMEM supplemented with 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin and maintained at 37 °C in a humidified atmosphere with 5% CO_2. When the HeLa cells are achieved at 60% confluency, the supernatant is removed and the cells are rinsed twice with phosphate-buffered saline (PBS), followed by incubation for 48 h in exosomes-free medium. Then the medium of HeLa cells is collected for the preparation of exosomes.

Isolation and Quantification of Exosomes. Exosomes isolated from cell conditioned medium are collected and centrifuged at 1000 g for 5 min at 4 °C, and the medium is centrifuged at 2000g for 10 min at 4 °C to remove cellular debris and large particles. Then the microvesicles are discarded by centrifuged at 17000 g for 15 min at 4 °C. The resulting supernatant is centrifuged at 160000 g for 1h and the final pellets are resuspended with PBS. The acquired exosome sample is stored at −80 °C before use. The concentration of purified exosome is measured by nanoparticle tracking analysis (NTA).

Preparation of Calcein Entrapped Liposome. DOPC (10 mg) and DOPG-Na (10 mg) are dissolved in chloroform (1 mL). The solvent is then removed under nitrogen gas to leave a thin lipid film and dried overnight in vacuo. The lipid film is hydrated in 1 mL calcein buffer (10 mM Tris, 150 mM NaCl, 2 mM calcein, pH 7.4) and sonicated for 30 min. The suspension solution is extruded 31 cycles through polycarbonate membranes with a size of 100 nm using an Avanti Mini Extruder. The unencapsulated calcein is then separated from the calcein-loaded liposomes by G-50

SepharoseTM exclusion chromatography. Finally, the resulting calcein entrapped liposomes are stored in Tris buffer (pH 7.4) at 4 °C for further use. For simplicity, the concentration of the calcein entrapped liposome is designated as 1×.

Preparation of MB Probe. The CD63 antibody modified MB probe is prepared according to the manufacturer's instructions. Briefly, 100 μL of NHS-MB is pre-washed with 1 mM HCl and incubated with 100 μL CD63 antibody for 2 h at room temperature on a mixer. The MB is washed with blocking buffer (100 mM Tris-HCl, 150 mM NaCl, pH 8.0) and incubated with blocking buffer for 3 h at room temperature to block the unreacted NHS group of MBs. The MB probe is washed three times with PBS buffer and finally suspended in 500 μL PBS for further experiment.

Detection of Exosomes. The detailed procedure for exosomes assay is as follows. First, 20 μL of MB probe is used to capture exosomes in 200 uL PBS by incubating them at room temperature for 1 h with gentle shaking, followed by being washed with Tris buffer (10 mM Tris, 150 mM NaCl, pH 7.4) for three times. Afterwards, the MB probe is resuspended by 200 μL Tris buffer containing 10 mM $ZrOCl_2$ to form MB-exosomes-Zr^{4+} complex. After gentle shaking for 20 min, the complex is then washed for three times and 100 μL of calcein entrapped liposomes solution is added to form MB-exosomes-Zr^{4+}-liposomes complex after incubation for 30 min. The final complex is then washed for three times and treated with 1% Triton X-100 for 30 min to release calcein from liposomes. Finally, the supernatant is transferred to a new tube and measured by the F-7000 with an excitation at 485 nm.

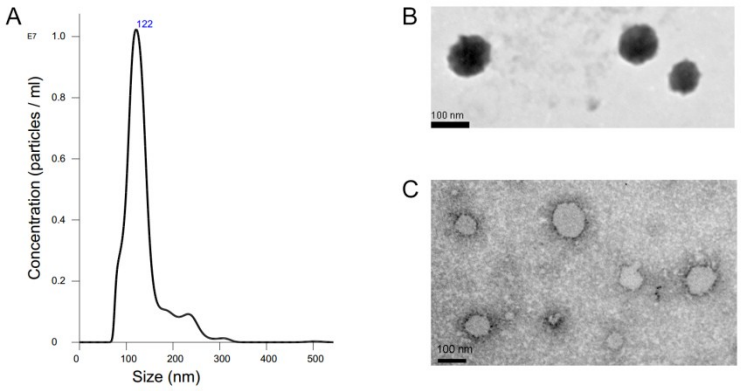

Figure S1. (A) The size distribution characterization of exosomes using NanoSight. (B) TEM images of exosomes purified by ultracentrifugation. (C) TEM images of liposomes.

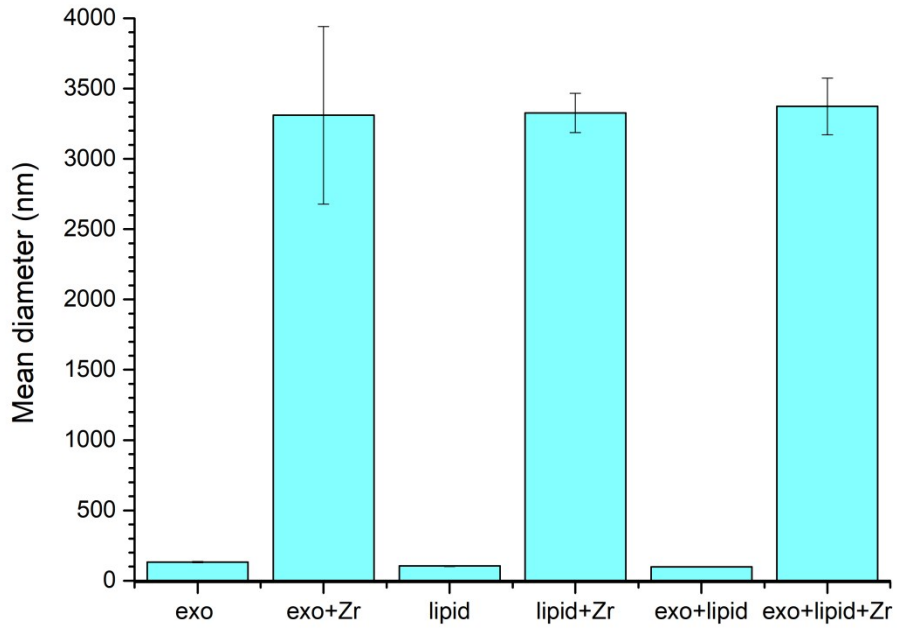

Figure S2. The DLS of exosomes, exosomes + Zr^{4+}, liposomes, liposomes + Zr^{4+}, exosomes + liposomes, exosomes + liposomes + Zr^{4+}. “exo” is the abbreviation of exosomes, and “lipid” is the abbreviation of liposomes. The concentration of Zr^{4+} ion is 5mM. Error bars represent the standard deviations of three repetitive measurements.

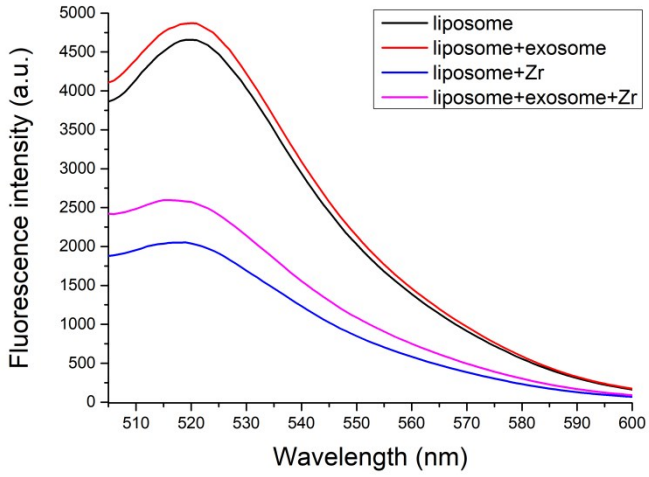

Figure S3. The fluorescence intensity of liposomes, liposomes + Zr^{4+}, exosomes + liposomes, exosomes + liposomes + Zr^{4+}. The concentration of Zr^{4+} ion is 5 mM.

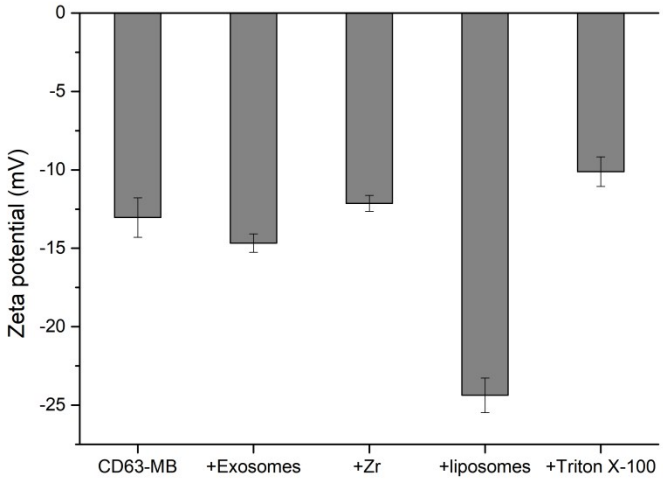

Figure S4. The zeta potential of each procedure of the assay. Error bars represent the standard deviations of three repetitive measurements.

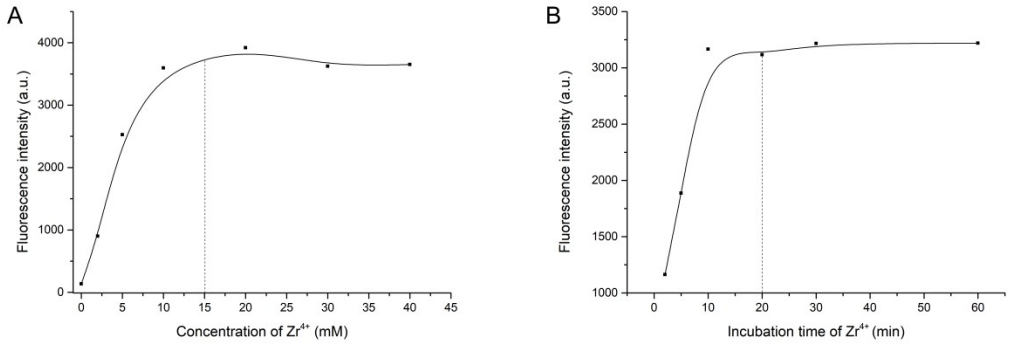

Figure S5. The optimization of (A) the concentration of Zr^{4+} and (B) the incubation time of Zr^{4+}. The concentration of exosomes is 4.2×10^6 particles/μL and the concentration of liposomes is 1mg/ml.

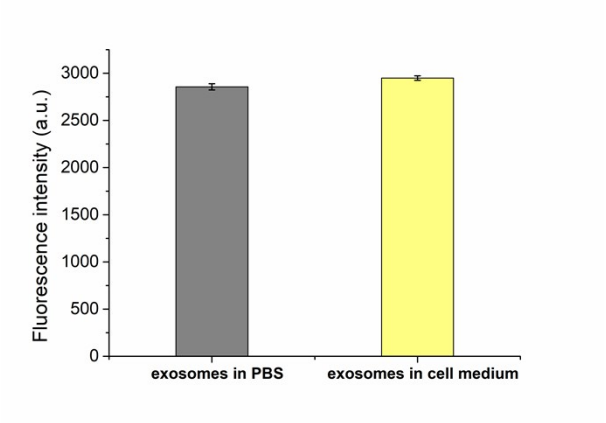

Figure S6. Comparison of our method for the detection of exosomes in PBS and in cell medium. Error bars represent the standard deviations of three measurements.

Table S1. Comparison of current assays for the detection of exosomes.

Number	Method	LOD (particles/μL)	signal output	signal label	reference
1	paper-based aptasensor	1.1×10^3	fluorescence	upconversion nanoparticles,	[1]

				gold nanorods	
2	alternating current electrohydrodynamic induced nanoshearing	2.76×10^3	colorimetric	HRP	2
3	integrated magneto - electrochemical sensor	3×10^4	electrochemistry	HRP	3
4	copper-mediated signal amplification	4.8×10^4	fluorescence	copper oxide nanoparticles	4
5	aptasensor DNA-capped single-walled carbon nanotubes	5.2×10^5	colorimetric	label-free	5
6	lateral flow immunoassay	8.54×10^5	colorimetric	HRP	6
7	zirconium-mediated signal amplification	7.6×10^3	fluorescence	label-free	this method

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