Supporting Information

An Electrochemical Biosensor Designed by Using Zr-Based Metal−Organic Frameworks for the Detection of Glioblastoma-Derived Exosomes with Practical Application

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This material includes experimental sections; fourier transform infrared spectroscopy of Zr-MOFs; square wave voltammograms recorded with the different concentrations, and different incubation time of peptide; square wave voltammograms recorded with different incubation time of exosomes; the comparison of exosomes diluted in PBS and serum; the selectivity and reproducibility of electrochemical biosensors; the amount of MB in the MOF; comparisons of this biosensor with previous methods and the recovery results of exosomes at different concentrations spiked in human serum.

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Experimental Section

Materials. EGFR/EGFRvIII-binding peptide (H-C-acp-acp-FALGEA-NH_2) (6-aminocaproic acid, acp) is custom-synthesized by Bank Peptide Biotech (Hefei, China). Aminoterephthalic acid (NH2-BDC), N, N-dimethylformamide (DMF), benzoic acid, ZrCl4, tris (2-carboxyethyl) phosphine (TCEP), 6-mercapto-1-hexanol (MCH), sodium citrate, glutaraldehyde, phosphotungstic acid, methylene blue (MB) are ordered from Sigma-Aldrich (Shanghai, China). Dulbecco's modified eagle medium (DMEM), exosome-depleted fetal bovine serum (FBS) and penicillin-streptomycin are purchased from Thermo Fisher Scientific Inc. (Waltham, MA). Other commercially available reagents are of analytical grade and were directly used without additional purification. Human blood samples of healthy individuals, patients with GBM, and patients after surgery are obtained from the Nanjing First Hospital.

Instrumentation. Cells were cultured in a constant temperature incubator (Eppendorf Galaxy 170S, Germany). The diameter and concentration of exosomes were measured by Nanoparticle Tracking Analysis (NTA) using a NS 300 instrument (Malvern Instruments, U.K.). Transmission electron microscopy (TEM) images were obtained by Tecnai G2 F20 S-TWIN (FEI). Scanning electron microscopy (SEM) images were obtained by using FEI Nova Nano SEM (FEI) at an accelerating voltage of 15.0 kV. Powder X-ray diffraction patterns (PXRD) data of the samples were recorded by a D8 Advance diffractometer with a Cu Kα anode (λ= 0.15406 nm) at 40 kV and 40 mA (Bruker, Germany). The nitrogen isotherms of MOFs were measured on a Micropore & Chemisorption Analyzer (Micromeritics ASAP2020) at 77 K. Fourier transform infrared spectroscopy (FT-IR) were obtained by a NEXUS640 infrared pectrometer system (NICOLET). Electrochemical measurements were carried out on a CHI660D Potentiostat (CH Instruments). The ultrapure water used in this work was obtained from a Milli-Q system (Millipore, Bedford, MA).

Cell Culture. Human L-02, Hela and U-87 cells were cultured in DMEM supplemented with 10% (V/V) exosome-depleted fetal bovine serum (FBS), 1% (V/V) penicillin-streptomycin (Invitrogen), and maintained at 37 °C in a humidified incubator with 5% CO_2 atmosphere. Conditioned cell medium was harvested after the cell was grown to 70% confluency.

Isolation of Exosomes. Exosomes from cell supernatant were isolated according to the standard ultracentrifugation method with slight modification.[1] Firstly, the culture medium was centrifuged at 5000g for 10 min and 10000g for 30 min to discard intact cells, cellular debris and large granular

vesicles. Secondly, the supernatant was filtered through a 0.22 μm filter (Millipore). Then, exosome pellets were obtained by ultracentrifugation at 120000g for 70 min, washed once with sterile phosphate-buffered saline (PBS). Finally, the sediment was resuspended in PBS and stored at -80 °C for further use. Exosome extraction from human blood was carried out with the similar procedure: first, the human blood was collected in a tube and centrifuged twice at 3000g for 10 min; then, the supernatant was filtered with a 0.22 μm membrane to obtain clean serum. Last, the exosome pellets were obtained by ultracentrifugation at 120000g for 70 min and stored at -80 °C for use.

Synthesis of UiO-66-NH_2. First, 120 mg of ZrCl4 was added in the mixture of aminoterephthalic acid (110 mg), benzoic acid (1.9 g) and DMF (10 mL). After sonication for a few minutes, the mixture solution was transferred to a Teflon liner and reacted at 120 °C for 24 h under static conditions. Then, the resulting product was obtained by centrifugation at 8000 rpm for 10 min, and washed with methanol repeatedly. Finally, the nanoparticles were dried at 65 °C overnight under vacuum oven.

Characterization of MOFs. The morphology and particle size of UiO-66-NH_2 was conducted by SEM. The formation of Zr-MOFs and MB@MOF was carried out with XRD technique. The nitrogen isotherms of Zr-MOFs were obtained on a Micropore & Chemisorption Analyzer at 77 K. FT-IR was obtained by a NEXUS640 infrared pectrometer system.

Characterization and Quantification of Exosomes. TEM was used to characterize the morphology and particle size of exosomes. 10 μL exosome pellets were loaded on a mesh carbon-coated copper grid and fixed for 20 min with 2% glutaraldehyde. After removing the excess solution with paper, the grids were negatively stained with 2% phosphotungstic acid for 1 min, and then rinsed with Tris-HCl and left to dry at room temperature. The sample was observed under a Tecnai G2 F20 S-TWIN. The size and concentration of the purified exosomes were quantified by NTA. 20 μL exosome pellets were diluted 50-fold with PBS after filtrating through a 0.22-μm pore filter. With the optimal settings, both size distribution and concentration of exosome particles can be measured.

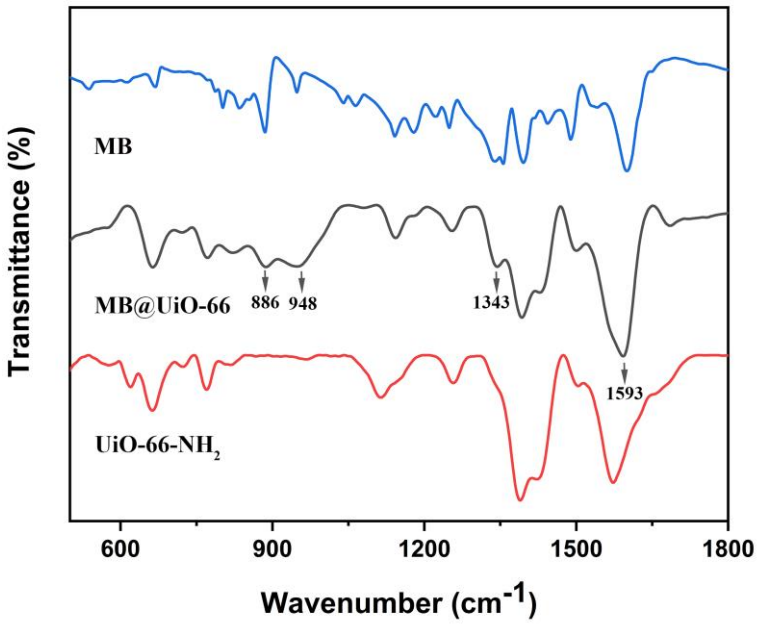

Figure S1. Fourier transform infrared spectroscopy of MB, MB@UiO-66 and UiO-66-NH_2. The new peaks in MB@UiO-66 have been pointed out.

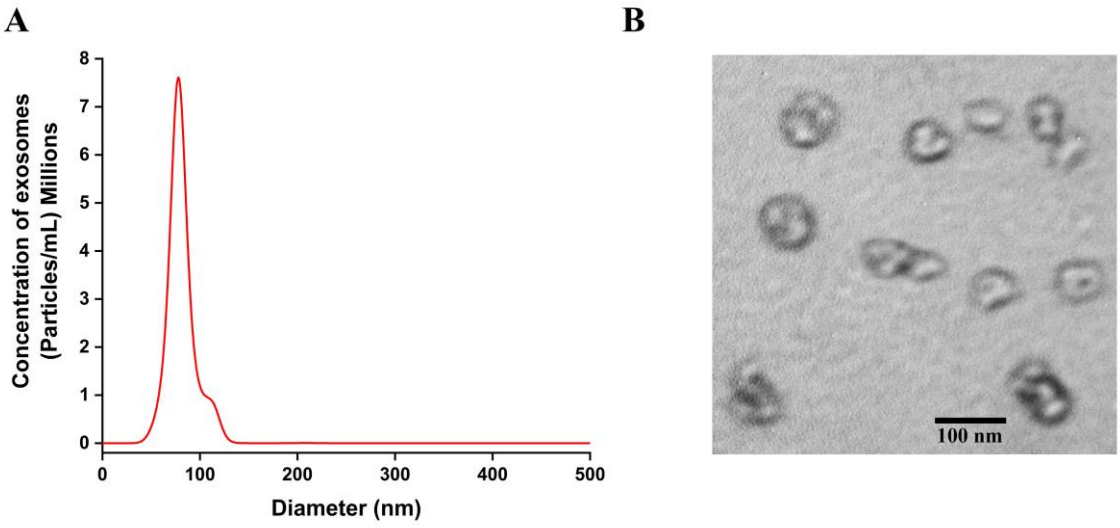

Figure S2. (A) Dynamic light scattering analysis and (B) TEM characterization of exosomes derived from U-87 cells.

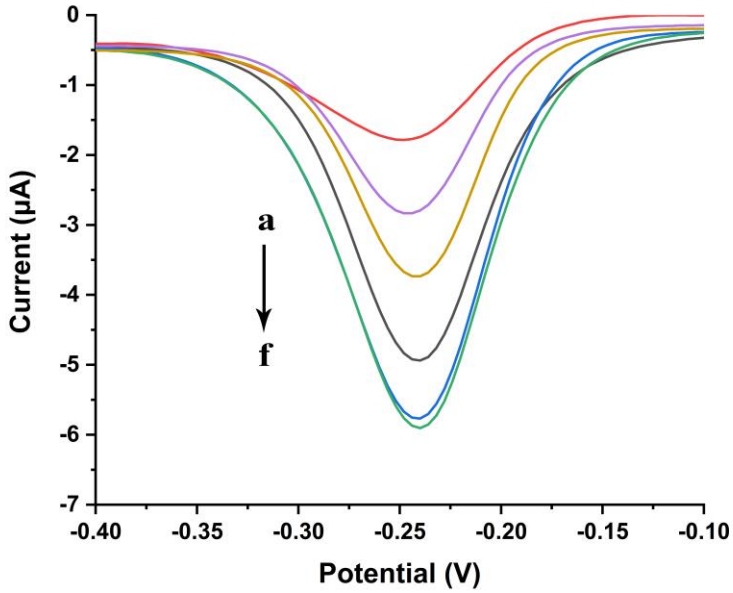

Figure S3. Square wave voltammograms recorded with the different concentrations of peptide, a–f (μM): 0.1, 0.5, 1, 2.5, 5, 10.

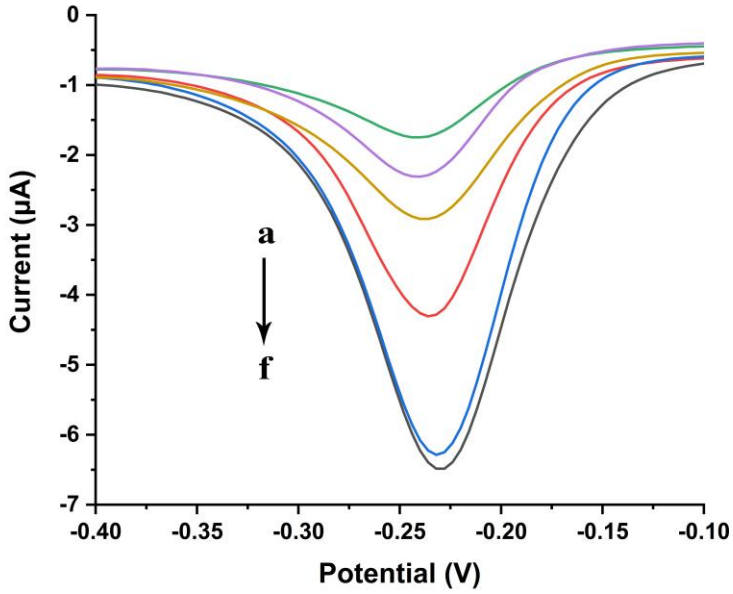

Figure S4. Square wave voltammograms recorded with the different incubation time of peptide, a–f (h): 2, 4, 8, 12, 16, 20.

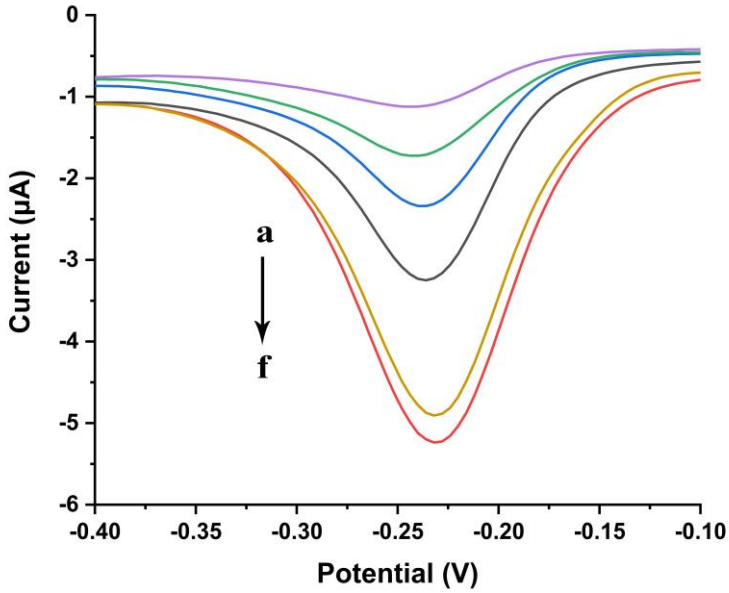

Figure S5. Square wave voltammograms recorded with the different incubation time of exosomes, a–f (min): 15, 30, 60, 90, 120, 180.

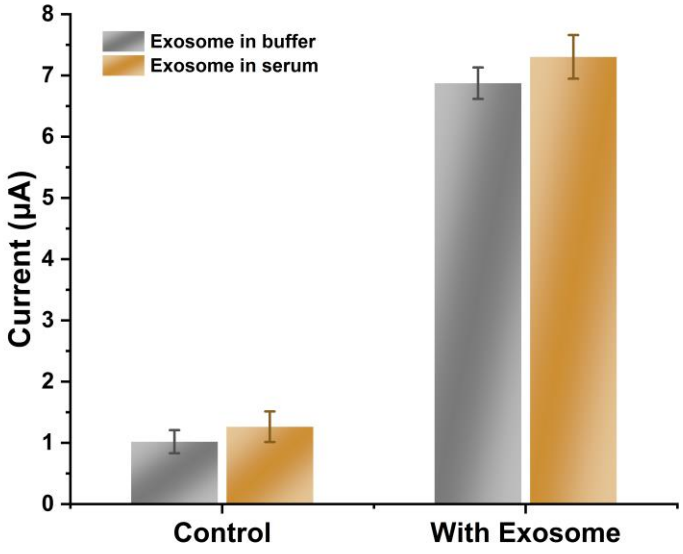

Figure S6. The peak currents obtained from square wave voltammetry of the exosomes diluted in PBS and in serum. Error bars represent the standard deviations of three independent experiments.

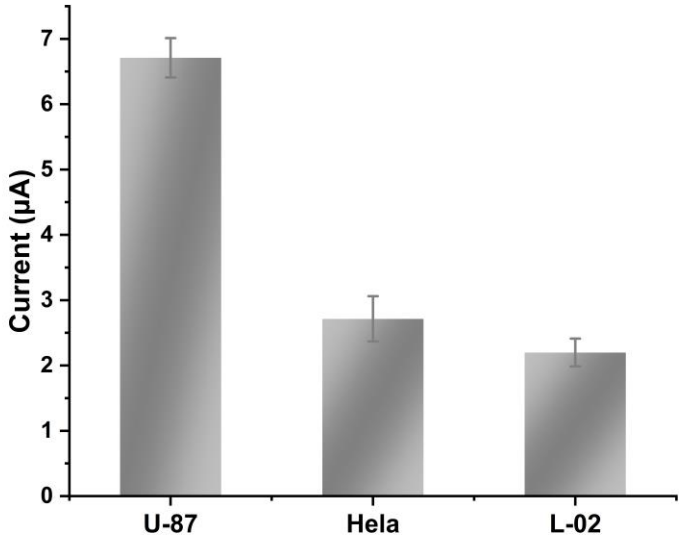

Figure S7. The peak currents obtained from square wave voltammetry of the exosomes derived from different cells. Error bars represent the standard deviations of three independent experiments.

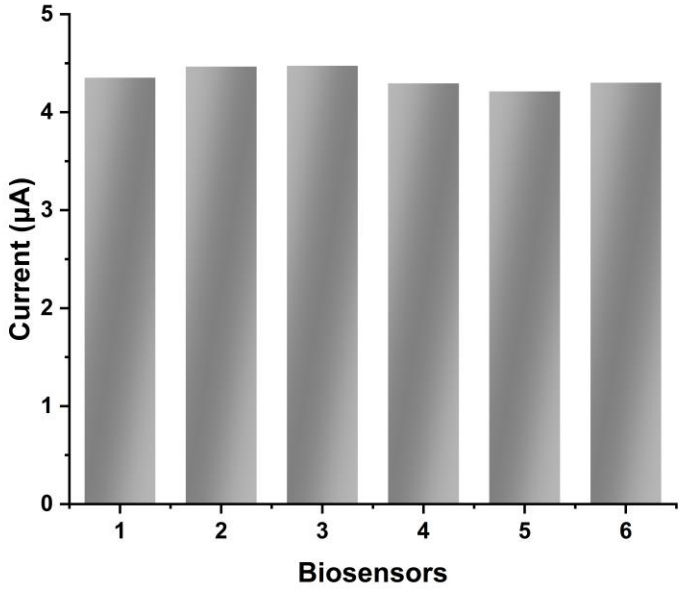

Figure S8. The reproducibility of electrochemical biosensors is conducted by detecting exosomes at the concentration of 9.5×10^5 particles/μL.

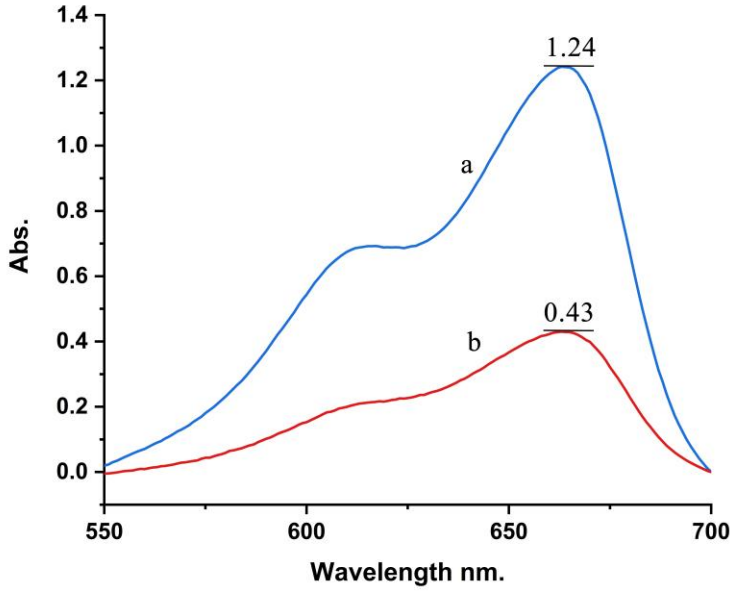

Figure S9. The supernatant of MB is determined by UV/vis spectroscopy after stirring without (a) and with (b) MOF for 24 h, the values of absorbance at the 665 nm are labeled.

Table S1. Comparisons of this biosensor with previous methods.

Method	Linear range (particles/μL)	LOD (particles/μL)
Alternating current electrohydrodynamic induced nanoshearing [2]	2.76×10^3 - 4.15×10^4	2.76×10^3
Integrated magneto - electrochemical sensor [3]	3×10^4 - 3×10^7	3×10^4
Aptasensor DNA-capped single-walled carbon nanotubes [4]	1.84×10^6 - 2.21×10^7	5.2×10^5
Zirconium-mediated signal amplification [5]	1.68×10^4 - 4.2×10^7	7.6×10^3
Copper-mediated exosome detection [6]	8×10^4 - 1.6×10^7	4.8×10^4

This proposed method	$9.5 \times 10^3 – 1.9 \times 10^7$	7.83×10^3

Table S2. Recovery results of exosomes at different concentrations spiked in human serum.

Added (particles)	Detected (particles)	RSD (%)	Recovery (%)
7.6×10^5	$8.1 \pm 0.19 \times 10^5$	2.34	106
9.5×10^6	$9.8 \pm 0.31 \times 10^6$	3.16	103
9.5×10^7	$9.1 \pm 0.27 \times 10^7$	2.96	95.7

References:

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