Supplementary Information for

Preparation of covalent organic frameworks coated with cell membrane for precise analysis of cancer exosomes

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Apparatus:

Transmission electron microscopy (TEM) images were carried out on Tecnai G2 F30 S-Twin (300 kV). The confocal images were acquired on LSM880 (Zeiss, Germany). Particle size (DLS)and potential (Zata) data were carried out on Tensor 27. The electrochemical measurements were conducted on a CHI660E electrochemical workstation (CH Instruments, USA) with a platinum electrode as the counter electrode, saturated calomel electrode electrode as the reference electrode, and a gold electrode as the working electrode.

Solution preparation

(1) Preparation of 1× PBS Buffer (pH 7.4): take one packet of 1× PBS powder and dissolve it in purified water. Transfer the solution to a 1000 mL volumetric flask and dilute to volume with purified water. Adjust the pH to 7.4 using hydrochloric acid (HCl) and sodium hydroxide (NaOH).

(2) Preparation of Impedance Detection Solution (10 mM Tris-HCl, 5 mM Potassium Ferrocyanide, 5 mM Potassium Ferricyanide, 1 M Potassium Chloride, pH 7.4): 0.4116 g potassium ferrocyanide [$K_4Fe(CN)_6$], 0.5280 g potassium ferricyanide [$K_3Fe(CN)_6$], 18.6375 g potassium chloride (KCl), and 0.3028 g Tris-HCl. Dissolve the reagents in purified water in a beaker, transfer to a 250 mL volumetric flask, and dilute to volume with purified water. Adjust the pH to 7.4 using hydrochloric acid (HCl) and potassium hydroxide (KOH).

(3) Preparation of Piranha Solution (98% H_2SO_4: 30% H_2O_2): Mix 98% sulfuric acid (H_2SO_4) and 30% hydrogen peroxide (H_2O_2) at a 3:1 volume ratio. CAUTION: Prepare immediately before use.

(4) Preparation of Low-Permeability Lysis Buffer (20 mM Tris, 2 mM Magnesium Chloride, 10 mM Potassium Chloride, pH 7.4): Weigh 1.211 g Tris, 0.203 g magnesium chloride ($MgCl_2$), and 0.303 g potassium chloride (KCl). Dissolve in purified water in a beaker, transfer to a 500 mL volumetric flask, and dilute to volume with purified water. Adjust the pH to 7.4 using hydrochloric acid (HCl) and potassium hydroxide (KOH).

Materials and apparatus

Aladdin Reagent Co., Ltd. (Shanghai, China) provided aniline, EDC-HCl, NHS, 3-mercaptopropionic acid (MPA), glacial acetic acid, DBCO, $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, EDTA·2Na, and Tris base. [1,1':4',1''-Terphenyl]-2',5'-dicarbaldehyde (PDA) and 1,3,5-tris(4-aminophenyl)benzene (TPB) were obtained from Bide Pharmatech Ltd. (Shanghai, China). Horseradish peroxidase (HRP) was a product of Meryer (Shanghai) Chemical Technology Co., Ltd. Nanjing Chemical Reagent Co., Ltd. (Nanjing, China) supplied tetrahydrofuran (THF), magnesium chloride hexahydrate ($MgCl_2·6H_2O$), sulfuric acid (H_2SO_4, 98%), and hydrogen peroxide (H_2O_2, 30%). Beyotime Biotechnology Co., Ltd. (Shanghai, China) furnished the cell membrane dyes (DiO and DiI) and TMB substrate. Potassium chloride (KCl) and sodium chloride (NaCl) were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals were of analytical grade and used without further purification. The oligonucleotide sequences employed in this study are listed in Table S1. A conventional three-electrode system was employed on a CHI660E workstation for the measurements, with a modified gold working electrode, a saturated calomel reference electrode, and a platinum wire counter electrode. Electrochemical impedance spectroscopy (EIS) was conducted in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl (pH 7.4) over a frequency range of 0.1-10^4 Hz. Differential pulse voltammetry (DPV) was used to evaluate the sensing performance. The current response of the biosensor was recorded at -0.1 V in TMB solution using the chronoamperometric (i-t) method.

Preparation of COFs

The synthesis of COFs was initiated by dissolving TPB (14 mg, 0.04 mmol) in 9 mL of ethanol. HRP solution (2 mL, 5 mg/mL) was then incorporated under stirring, succeeded by the addition of PDA (17 mg, 0.06 mmol in 1 mL THF). After dropwise addition of 80 μL glacial acetic acid, the reaction proceeded under stirring (200 rpm, 30 min). The resulting precipitate was isolated by centrifugation (10,000 rpm, 10 min), thoroughly washed with deionized water, and stored at 4 °C.

Preparation of Hairpin Probe H1 and H1-COFs

A total of 20 μL of H1 single-stranded DNA (100 μM) was mixed with 80 μL of annealing buffer and vortexed for 10 s to obtain 100 μL of a 20 μM H1 solution. The

mixture was then heated at 95 ℃ for 5 min in a thermostatic water bath and subsequently allowed to cool slowly to room temperature. This annealing process enabled the single-stranded DNA to self-assemble into a stable hairpin structure (H1) through complementary base pairing. To prepare H1-COFs, 800 μL of COFs dispersion (1 mg/mL) was mixed with 50 μL of EDC (0.4 M) and 50 μL of NHS (0.1 M). The mixture was vortexed in an ice bath for 10 min to activate the amino groups on the COF surface. Subsequently, 100 μL of H1 solution (10 μM) was added and incubated with gentle mixing for 2 h to enable covalent coupling through amide bond formation. The resulting product was centrifuged at 10,000 rpm for 10 min and washed three times with PBS to remove unbound DNA. The purified H1-COFs complex was resuspended in 1 mL of PBS and stored at 4 ℃ for further use.

Preparation of MCF-7 Cell Culture and Membrane Extraction

MCF-7 cells were cultured in high-glucose DMEM containing 10% (v/v) fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in a 5% CO_2 incubator. Following digestion and harvest of confluent cells, three PBS washes were performed. The cells were then lysed in hypotonic buffer (3 mL per T25 flask) at 4 °C for 12 h. Subsequent processing involved sonication (4 °C, 30% amplitude, 20 min) and initial centrifugation (3,000 rpm, 20 min, 4 °C) to remove debris. The supernatant was retained for protein analysis, and the pellet was subjected to high-speed centrifugation (14,000 rpm, 35 min, 4 °C) to isolate purified membranes. The final membrane fraction was quantified via BCA assay and stored at -20 °C.

Exosomes were isolated using a differential ultracentrifugation protocol. Briefly, the collected supernatant was sequentially centrifuged at 300 × g for 10 min, 2,000 × g for 10 min, and 10,000 × g for 30 min to remove cellular debris. The clarified supernatant was then ultracentrifuged at 100,000 × g for 70 min. The resulting pellet was resuspended in PBS and subjected to a second round of ultracentrifugation under identical conditions to obtain purified exosomes. Exosome concentration was quantified by nanoparticle tracking analysis (NTA).

Serum samples were obtained from Jiangning Hospital Affiliated to Nanjing Medical University. Written informed consent was obtained from all participants prior

to sample collection. This study was approved by the Ethics Committees of Jiangning Hospital and Nanjing Forestry University.

Preparation of CM@COFs and miR-375-H1-COFs

To prepare the functionalized exosomes, MCF-7 cell membranes (1 mg/mL in PBS) were combined with an equal volume of H1-COFs (1 mg/mL). The mixture was then subjected to sequential extrusion through 1000 nm and 200 nm polycarbonate membranes (12 cycles per membrane) on a microextruder maintained at 4 °C. The resulting CM@COFs were centrifuged at 10,000 g for 15 min at 4 °C to remove free membrane fragments and resuspended in PBS for storage.

For the preparation of miR-375-H1-COFs, CM@COFs were incubated with equal volumes of exosomes at different concentrations for 2 h. The mixture was subsequently processed by ice-bath sonication (4 °C, 30% amplitude, 10 min) and a subsequent centrifugation step (10,000 g, 15 min, 4 °C). The resulting precipitate (miR-375-H1-COFs) was collected and resuspended in PBS for storage.

Construction of biosensors

After pretreatment, the bare gold electrode was modified through a sequential surface functionalization process. The functionalization protocol commenced with the drop-casting of 10 μL of 1 mM MPA and incubation at 4 °C. Then, 10 μL of 1 mM 6-mercaptohexanol (MCH) was introduced onto the electrode surface and incubated for 30 min to block the unbound active sites, thereby minimizing non-specific adsorption. Unbound molecules were then rinsed away, and a subsequent 10 μL aliquot of 1 mM DBCO was introduced for 1 h at room temperature to conjugate with the MPA layer. Following a final wash, the assembly was completed by incubating the electrode with 10 μL of miR-375-H1-COFs solution for 2 h at room temperature. The azide-alkyne cycloaddition (SPAAC) reaction enabled directional immobilization of probe molecules. Finally, the electrode was washed with deionized water and used for electrochemical detection in TMB solution.

The treatment of clinical serum samples

Clinical serum samples were obtained from five healthy individuals, five breast cancer patients (BC), and five post-treatment patients at the Affiliated Jiangning

Hospital of Nanjing Medical University. Each participant with a confirmed clinical diagnosis provided a sample between 8:00 and 9:00 AM following a 12-hour fast. All serum specimens underwent stringent quality control screening to eliminate confounding analytical interferences including hemolysis, lipemia, and icterus. 100 μL aliquots of serum were subjected to ultrafiltration for 30 minutes at 14,000 g and 4 °C using Nanosep (30 k) and Nanosep (3 k) centrifugal devices, respectively. The resulting filtrates were collected and stored long-term at −80 °C in a cryogenic freezer. This study obtained approval from the Scientific Ethical Committee of the Affiliated Jiangning Hospital of Nanjing Medical University and Nanjing Forestry University, and informed consent was obtained from all participants.

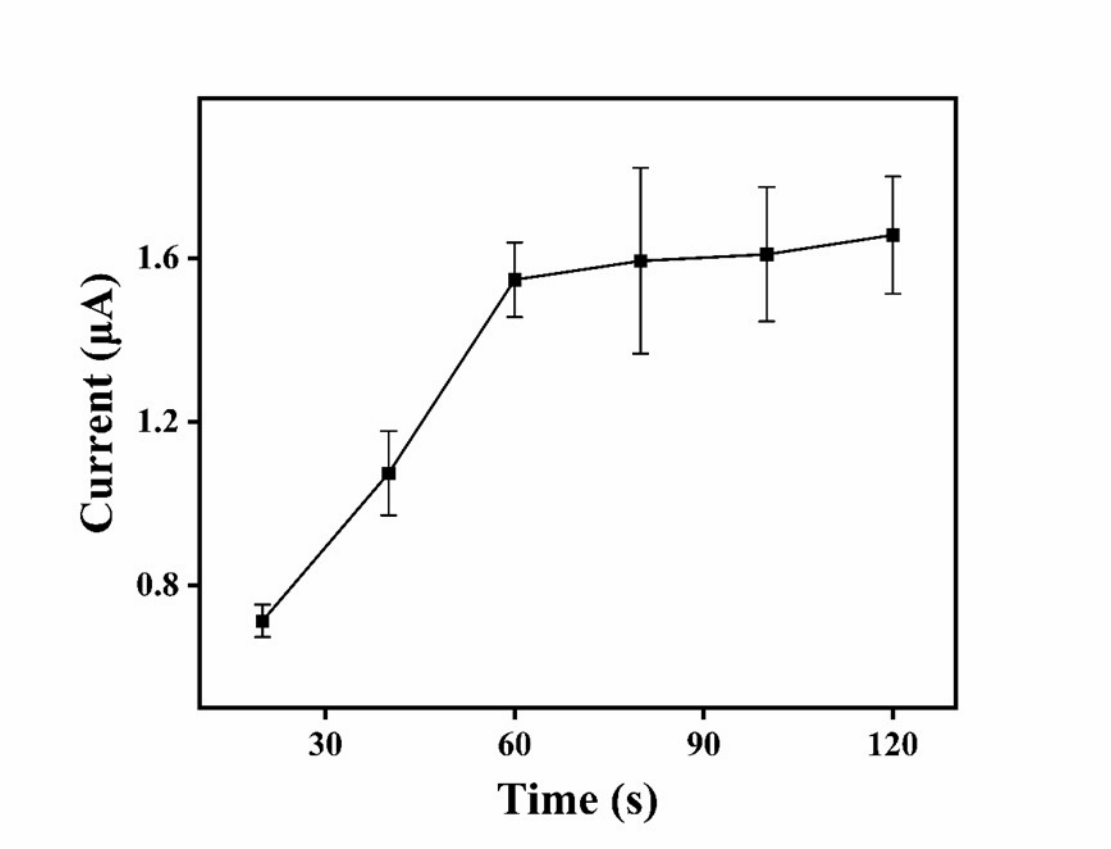

Figure S1. Impact of DBCO-NH_2 incubation duration on current response.

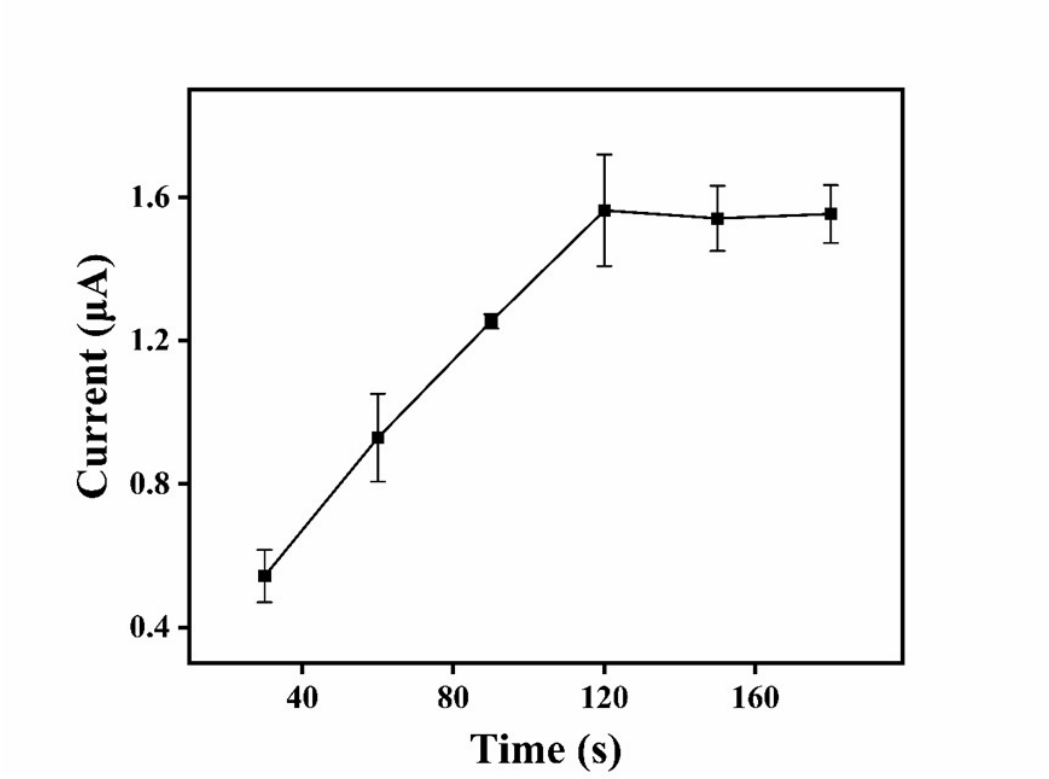

Figure S2. Exosomes and CMV-H1-HRP@COFs co-incubation duration on current response.

Table S1. Oligonucleotides used in this work.

Oligonucleotide	Sequence
miR-375	5'-UUU GUU CGU UCG GCU CGC GUG A-3'
H1	5'-COOH-TCA CGC GAG CCG AAC GAA CAA ATG TTC GTT CGG C-N_3-3'

Table S2. Comparison of the developed method with other methods.

Methods	Linear range	Detection limit	Subtype differentiation	References
Fluorescence	10^2 to 10^5 particles/μL	87 particles/μL	no	[1]
Electrochemiluminescence	10^2 to 10^6 particles/μL	31 particles/μL	no	[2]
Electrochemiluminescence	340 to 1.70×10^5 particles/μL	74 particles/μL	no	[3]
Electrochemistry	10^2 to 10^7 particles/μL	70 particles/μL	no	[4]
Electrochemistry	200 to 5.00×10^5 particles/μL	80 particles/μL	no	[5]
Electrochemistry	10^3 to 10^8 particles/μL	158 particles/μL	no	[6]
Electrochemistry	10^2 to 10^8 particles/μL	27 particles/μL	yes	This work

Reference

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