Supporting Information

An electrochemical biosensor to identify phenotype of aggressive breast cancer cells

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Experimental Section

Chemicals and Materials. The peptide sequence (Biotin-GPLGVRKKYCDGFYACYMDV) was compounded by Synthbio (Hefei, China). Polyvinylpyrrolidone (PVP MW: 8000) was obtained from Alfa Aesa Chemical (China). Streptavidin was brought from Heowns Opde Technologies Ltd (Tianjin, China). 10% of Brij-35 solution, acetone, 30% ammonia, 30% hydrogen peroxide solution, *N*, *N*-dimethylformamide (DMF) and (3-aminopropyl) triethoxysilane (APTES) were ordered from Nanjing Chemical Reagent (Chian). Toluene, $K_3(Fe(CN)_6)$, chloroauric acid ($HAuCl_4$), $Zn(OAc)_2$, $K_4(Fe(CN)_6)\cdot 3H_2O$, KCl, $CaCl_2$, tris(hydroxymethyl)aminomethane (tris-base) and tris(2-carboxyethyl)phosphine (TCEP) was obtained from Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Ethanol, bovine serum albumin (BSA), cysteine, hemoglobin (Hb), 2-methylimidazole, $NaBH_4$, lysozyme, immunoglobulin G (IgG) and protein kinase A (PKA) was brought from Shanghai Titan Technology Co., Ltd. The RIPA lysis buffer and TMB was obtained from Beyotime Biotechnology Co., Ltd. (Shanghai, China). HRP, MMP-9, MMP-2 and HER-2 were brought from Sino Biological Inc. (Beijing, China). Collected from BC cells, MMP-2 and HER-2 were qualitatively and quantitatively determined by a human MMP-2 and HER-2 ELISA kit which was brought from MultiSciences (Lianke) Biotech Co., Ltd. (Hangzhou, China). Indium tin oxide (ITO) glass (glass thickness: 1 mm, sheet resistance < 10 Ω/square) was obtained from Grish Technology Co., Ltd (Bejing, China). Peptide sequence was dissolved in peptide buffer containing 5 mM TCEP (pH 7.4). The 10 mM Bi-NHS solution was prepared in a solution which the proportion of PBS and DMF is nine (pH 7.4). HER-2 was dissolved in 1 × PBS. MMP-2 was dissolved in 1 × TCNB buffer.[1]

Instrumentation. Morphologies of synthesized materials were observed from transmission electron microscopy (TEM) and scanning TEM-energy-dispersive X-ray spectroscopy (STEM-EDS) (TEM, FEI Tecnai G2 F30, U.S.A.) respectively. Powder X-ray diffraction (PXRD) patterns were collected to record the crystal structure on Brucker D8 Advance (Germany). Fourier transform infrared spectrum (FT-IR) spectrum was recorded on Nicolet IS10 (U.S.A.). UV-vis absorption spectra were performed on UV-2450 (Japan). Zeta potential was measured by Zetasizer Nano ZS90 (Malvern, UK). The

electrochemical measurements data was measured by CHI660D electrochemical workstation (Chenhua, China).

Synthesis of HRP@ZIF-8 and AuNPs@HRP@ZIF-8. The HRP@ZIF-8 was synthesized based on the reported literature with some modifications. 2 mg HRP was dispersed in 4 mL deionized water, then adding 4 mg PVP (MW: 8000) for 30 s under the condition of magnetic stirring.[2] Then, 8 mg L-Cys was added. Finally, 4 mL 160 mM 2-mIM (pH=10.5) and 4 mL 40 mM $Zn(OAc)_2$ solution were separately added into the above solution dropwise. After that, the mixed solution aged at room temperature (RT) overnight. The produced white product was collected by centrifugation at rotate speed of 10000 rpm and washed with water, following by drying in vacuum at 37 °C for 10 h. AuNPs@HRP@ZIF-8 probe was prepared as following. 3 mg/mL HRP@ZIF-8 solution was stirred magnetically. Afterward, 3 mL 1 mg/mL $HAuCl_4$ was added into HRP@ZIF-8 with stirring. Then, 3 mL 2 mg/mL freshly prepared $NaBH_4$ solution was dripped into the solution and strongly stirred at RT. Then, the mixture was centrifuged at rotate speed of 5000 rpm and washed with water and dried at 37℃ to obtain AuNPs@HRP@ZIF-8 stored for further use.

Preparation of dual-trapping peptide electrochemical biosensing platform. The ITO electrode was washed and cleaned by successive sonication in sufficient acetone, ethanol, and water and dried with N_2. Then, ITO was dipped in the 3 mL solution (H_2O: 25% $NH_3 \cdot H_2O$: 30% H_2O_2 = 6:1:1, by volume ratio) at 85°C to form hydroxyl group on ITO. Then, we used water to rinse ITO and activated ITO electrode was dried with N_2 gas and incubated in 1.0% APTES toluene solution at RT on which of the amination monolayer was formed. Next, ITO electrode was washed by toluene quickly and annealed in tube at 110°C. Afterward, ITO electrode was dipped in 10 mM Bi-NHS solution by ultrasonication for 3 min and was resting at RT for 60 min and washed with doubled distilled water. For biotin-modified dual-recognized peptide immobilization, the 0.1 mg/mL streptavidin solution was dipped on conductive surface of NHS-treated ITO electrode at RT for 60 min. After rinsing the surface with 1 × PBS, 40 μL 1 mM biotin-peptide was added on ITO electrode for 60 min at 37℃. The peptide-modified ITO was rinsed by sufficient 1 × PBS to wipe off excessive peptide. Afterward, peptide-modified ITO electrode was incubated with 40 μL 0.5 mg/mL BSA for 30 min at RT to block ITO surface. After that, the electrode was rinsed with PBST once and PBS for two times before use.

Electrochemical assay of HER-2 and MMP-2 activity. Firstly, 20 μL of different concentrations of HER-2 were added on the surface of peptide/ITO electrode for 1 h at 37℃. After washing unbound HER-2 and dried with N_2 gas, HER-2/peptide/ITO electrode was immersed in $[Fe(CN)_6)]^{-3/-4}$ to record DPV. Secondly, 40 μL 1 mg/mL AuNPs@HRP@ZIF-8 solution was incubated with the peptide/ITO electrode overnight at room temperature. Afterward, the AuNPs@HRP@ZIF-8/peptide/ITO electrode was incubated with MMP-2 in TCNB buffer with 200 rpm for 30 min at 37℃. The electrode before and after MMP-2 incubation was immersed in TMB horseradish peroxidase color development solution to record i-t curve at -0.1 V. The electrochemical data were measured on a three-electrode system as following: ITO was working electrode, platinum electrode was counter electrode and a saturated calomel electrode (SCE) was reference electrode.

Electrochemical analysis of HER-2 and MMP-2 in BC cell lines. Four BC cell lines (BT474, SK-BR-3, MCF-7, and MDA-MB-231) and normal hepatocyte (LO2) were cultured in six-well plate in DMEM with 10% FBS at 37°C in 5% CO_2 atmosphere. HER-2 protein from cell surface was collected by RIPA lysis buffer. Firstly, five kinds of cells were washed with PBS for two times to avoid other interferents. Then, 250 μL RIPA lysis buffer was dipped into each cells culture bottle and incubated for

5 min at RT and blown with pipetting gun to make the lysate fully contact with cells. Then, the lysates were collected into tubes and centrifuged for 10 min at 4°C at a rotation speed of 12000 g and the supernatant was stored for further use. MMP-2 was collected from cell culture supernatant. The MMP-2 and HER-2 was qualitatively and quantitatively determined by a human MMP-2 and HER-2 ELISA kit, respectively. Afterwards, these BC cell samples were detected by the electrochemical biosensor following above electrochemical experimental procedures.

Additional Figures

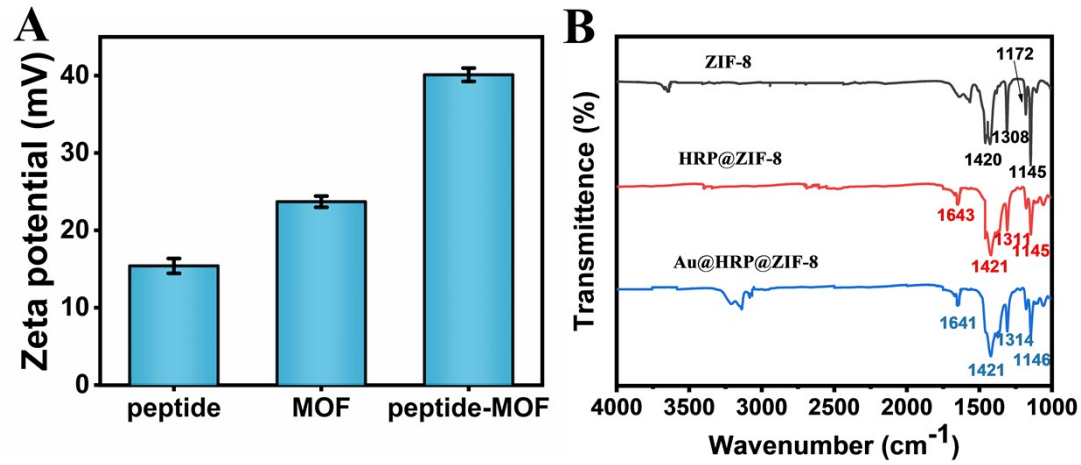

Figure S1. (A) Zeta potential of peptide, MOF (AuNPs@HRP@ZIF-8) and peptide-MOF. (B) FT-IR spectra of ZIF-8, HRP@ ZIF-8 and AuNPs@HRP@ZIF-8.

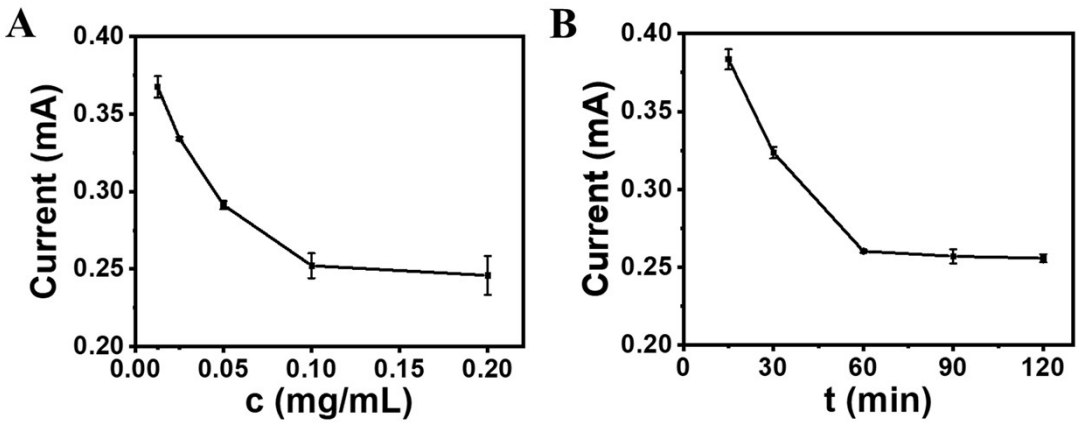

Figure S2. Effects of peptide-ITO electrode with (A) different concentrations (0.0125, 0.025, 0.05, 0.1 and 0.2 mg/mL) and (B) reaction time (15, 30, 60, 90 and 120 min) of streptavidin on the peak current.

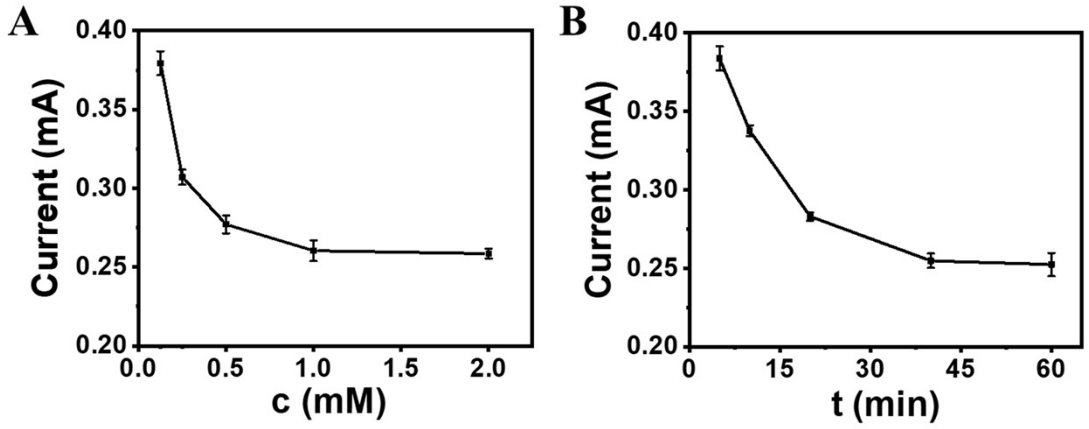

Figure S3. Effects of peptide-ITO electrode with (A) different concentrations (0.125, 0.25, 0.5, 1 and 2 mM) and (B) reaction time (5, 10, 20, 40 and 60 min) of peptide on the peak current.

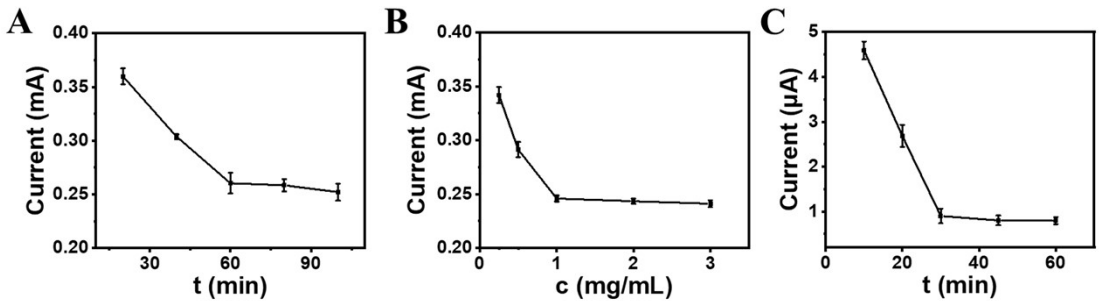

Figure S4. Effects of peptide-ITO electrode with (A) different reaction time (20, 40, 60, 80 and 100 min) of HER-2 and (B) different concentrations (0.25, 0.5, 1, 2 and 3 mg/mL) of AuNPs@HRP@ZIF-8 on the peak current. (C) Effects of peptide-ITO electrode with different reactioTGn time (10, 20, 30, 45 and 60 min) of MMP-2 on the current at 60 s.

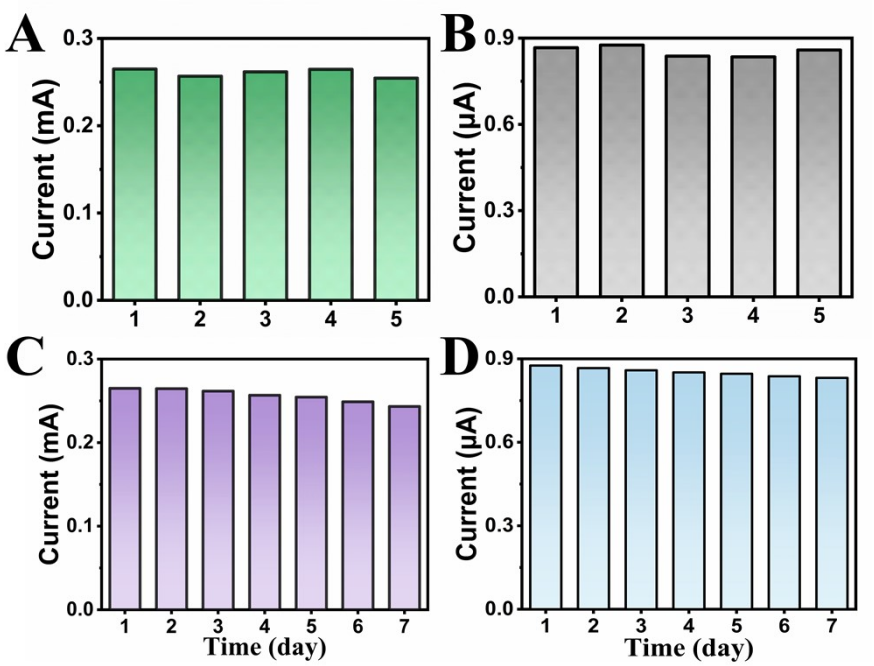

Figure S5. The reproducibility of the fabricated biosensor for the detection of (A) HER-2 (50 ng/mL) and (B) MMP-2 (10 ng/mL). The stability of the biosensor for the detection of (C) HER-2 and (D) MMP-2.

Table S1 Determination of HER-2 and MMP-2 from tumour cells by using ELISA as the contrast assay.

No.	Cells	Mean concentration (ng/mL)	
		ELISA	This work
HER-2			
1	SK-BR-3	8.524±0.67	7.954±0.51
2	BT-474	6.827±0.36	6.453±0.23
MMP-2			
1	LO2	0.467±0.21	0.414±0.18
2	MDA-MB-231	8.879±0.77	8.458±0.62
3	MCF-7	3.946±0.64	3.739±0.44
4	SK-BR-3	6.961±0.51	6.457±0.36
5	BT-474	7.725±0.43	7.222±0.31

References

1 L. Wang, H. Li, L. Shi, L. Li, F. Jia, T. Gao and G. Li, *Biosens. Bioelectron.*, 2022, **195**, 113671.

2 G. Chen, S. Huang, X. Kou, S. Wei, S. Huang, S. Jiang, J. Shen, F. Zhu and G. Ouyang, *Angew. Chem. Int. Ed.*, 2019, **58**, 1463-1467.