Supplementary material

"Gold-plated" PCN-222(Fe) and superconductive carbon black based sandwich-type immunosensor for detecting CYFRA21-1

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The main contents as follows:

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S1) Reagents and materials

Ketjen black (ECP-600JD) was purchased from Lion Co., Ltd. (Japan). Soluble fragment of cytokeratin 19 (CYFRA21-1, 1 mg mL^{-1}) was ordered from Wuhan Cloud Clone Technology Co., Ltd. (Wuhan, China). $ZrCl_4$ and Fe(III) meso-tetra(4-carboxyphenyl) porphyrin chloride (Fe-TCPP) were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Dimethylformamide (DMF), benzoic acid, Methylene blue (MB) were provided by Macklin Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA) was bought from J&K Scientific Co., Ltd. (Beijing, China). Gold chloride hydrate ($HAuCl_4$) was purchased from Sigma Aldrich Chemical Co., Ltd. (USA). Polyethyleneimine (PEI, Mw = 1800) was supplied by Alfa Aesar Co., Ltd. (USA). Anhydrous ethanol was achieved from Chongqing Chuandong Chemical Group Co., Ltd. (Chongqing, China). Hydrogen peroxide (30%) was ordered from Chengdu Kelong Chemical Co., Ltd. (Chengdu, China).

S2) Apparatus and measurements

A three-electrode system was used for the electrochemical detection, in which the working electrode was a modified glassy carbon electrode (GCE, 4 mm in diameter), the auxiliary electrode was platinum wire, and the reference electrode was saturated calomel electrode (SCE). Cyclic voltammetry (CV) performed with an electrochemical workstation (Chenhua CHI 660E, China). Differential pulse voltammetry (DPV) measurements were carried out using an electrochemical workstation (Autolab PGSTSAT 302N, Netherlands). The pH was monitored with a pH meter (MP 230, Mettler-Toledo, Switzerland). Transmission electron microscopy (TEM) image was taken with Talos F200X instrument (Thermos Fischer, USA). Fourier transform infrared (FTIR) spectroscopy was performed with a Nicolet 6700 FTIR spectrometer (Thermos Nicole, USA). Zeta potential of particles was measured on ZS90 zeta potential analyzer (Malvern Instruments, UK). Scanning electron microscope (SEM) was used for quanta FEG 450, (Merlin Compact, Germany). Atomic force microscope (AFM) was performed with Dimension Edge (Brock, Germany). N_2 adsorption-desorption isotherms were recorded with BELSORP-max (MicrotracBEL, Japan) surface area and porosity analyzer using liquid nitrogen. UV-vis absorption spectrophotometer (UV-2600, Shimadzu, Japan), X-ray diffraction (XRD, RIGAKU smartlab, Japan) and fluorescence spectrometer (FS5, Edinburgh Instrument, UK).

S3) The pretreatment process of the GCE

After mirror-polishing the bare glassy carbon electrode (GCE) with Al_2O_3 powder (0.3, 0.05 μm), in order to prepare the electrode for usage, it was ultrasonically treated with deionized water, absolute ethanol, and deionized water and then being dried for use before.

S4) Calculation of effective specific surface area

According to the well-known equation: $I = 2.69 \times 10^5 \times n^{2/3} \times A \times D_o^{1/2} \times C_o^* \times v_1^{1/2}$, Where n is the transferred electron number of electron transfer (n = 1), D_0 is the diffusion coefficient of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution (D = 7.6×10^{-6} cm^2 s^{-1} at 25 °C), C_0 is the concentration of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution (5 mM).

S5) Testing cost of this method compared with commercial ELISA

Firstly, we used the human cytokeratin 19 fragment antigen 21-1 (CYFRA21-1) ELISA kit to assay 18 samples (repeated three times) using a 96-well plate at a total cost of 1600 RMB. The cost of one serum test was around **29.63 RMB.**

Based on the prices of all the reagents used, the cost of testing one serum sample using the immunosensor is estimated to be **1 RMB** (labor costs are not included). In detail, it takes about 9.46 RMB to synthesize 1 mg mL^{-1} PCN-222(Fe)/AuNPs-Ab_2. The raw materials for the synthesis of PCN-222(Fe) were mainly Fe-TCPP and $ZrCl_4$, which were synthetized to approximately 16.5 mg. The price of Fe-TCPP (250 mg) and $ZrCl_4$ (25 g) is 374 RMB and 166.9 RMB respectively. The price of polyclonal antibody to cytokeratin fragment Antigen 21-1 (CYFRA21-1) (200 μL, 1 mg mL^{-1}) is 1693 RMB. The price of H_4AuCl_4 is 396.9 RMB. The price of bovine serum protein (5 g) was 205.9 RMB. Other materials have negligible costs due to their cheapness, low concentration, and small drops. The reagent cost is estimated to be 1 RMB according to the amount of each reagent and the corresponding concentration. (CYFRA21-1: 10 ug mL^{-1}, BSA: 10 mg mL^{-1}, H_4AuCl_4: 1%)

S6) SEM image of KB/PEI-AuNPs

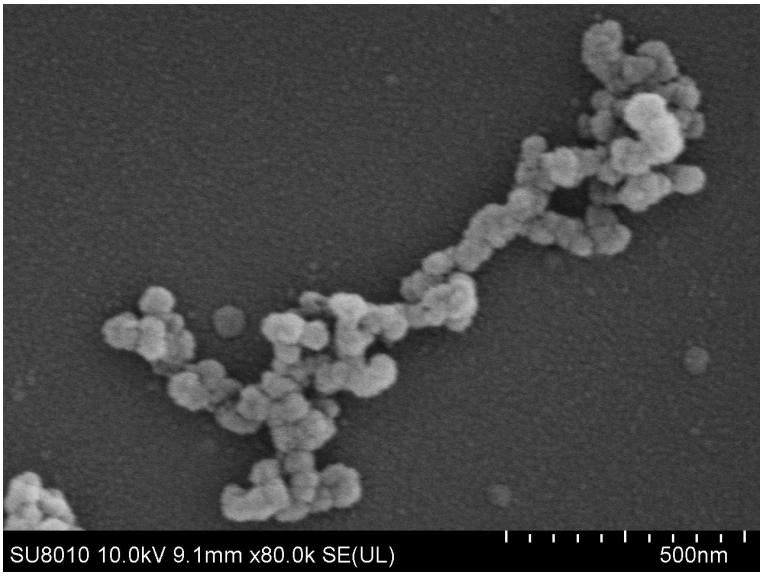

Fig. S1. SEM image of KB/PEI-AuNPs. (500 nm)

S7) AFM images of KB/PEI and KB/PEI-AuNPs

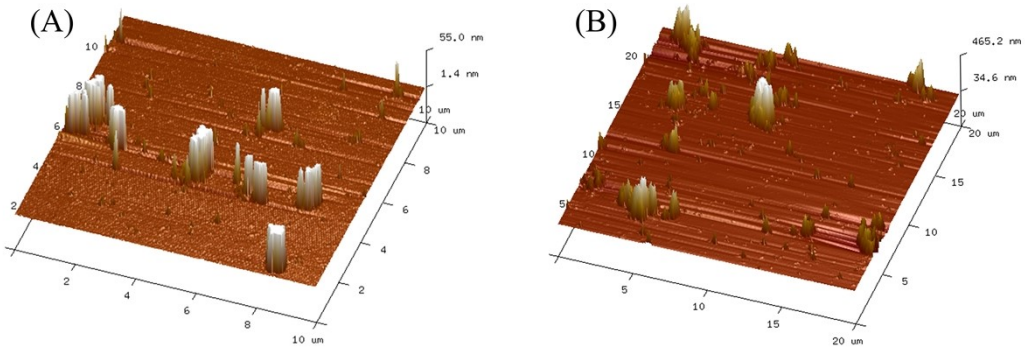

Fig. S2. AFM images of electrodes modified with (A) KB/PEI and (B) KB/PEI-AuNPs.

S8) The stability of KB/PEI-AuNPs

Repeated CV measurements were conducted to explore the stability of KB/PEI-AuNPs. Modified GCE (Fig. S4). After 50 cycles, the oxidation peak current value had dropped by only 8.9% compared with the initial oxidation peak current, revealing the high electrochemical stability of KB/PEI-AuNPs modified GCE.

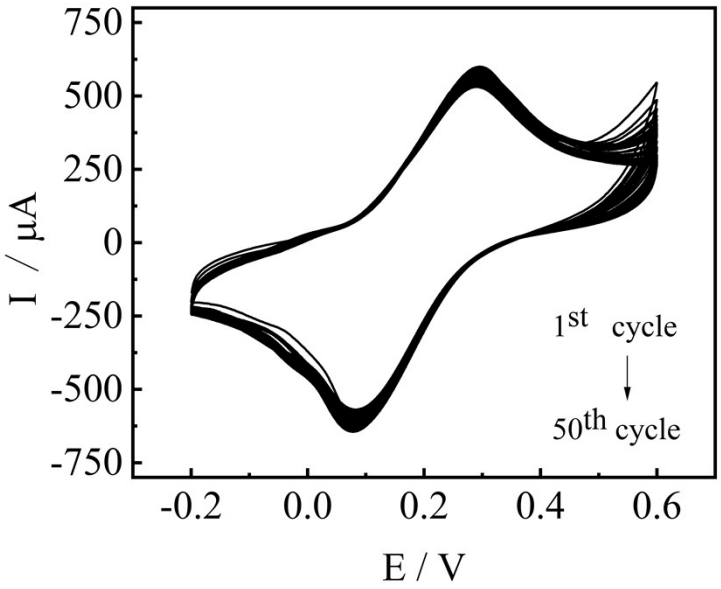

Fig. S3. The stability of KB/PEI-AuNPs.

S9) Characterization of charge transfer rate by CV and EIS

Compared with the bare electrodes, modification with KB/PEI-AuNPs had significantly higher peak current and less electron transfer impedance hindrance. It clearly demonstrates the faster charge transfer on the electrode surface after material modification.

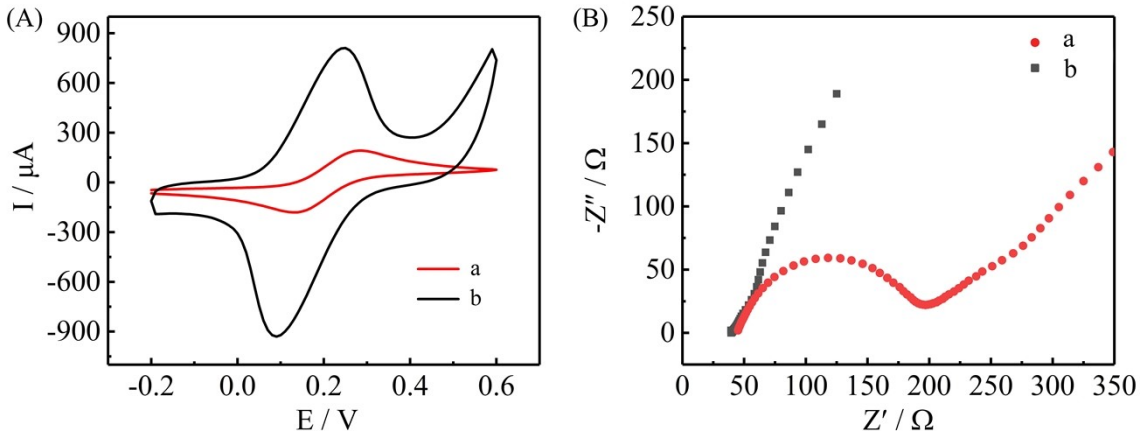

Fig. S4. The CV (A) and EIS (B) experiments of (a) GCE and (b) KB/PEI-AuNPs/GCE.

S10) UV-vis spectra of PCN-222(Fe) degrading MB molecules

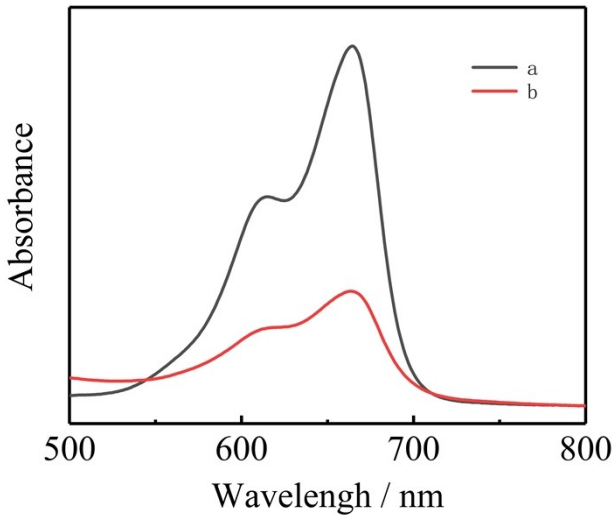

Fig. S5. UV-vis spectra of PCN-222(Fe) degrading MB molecules:(a) adsorption curve of MB solution; (b) adsorption curve of MB solution after being degraded by PCN-222(Fe).

S11) XRD patterns of PCN-222(Fe)

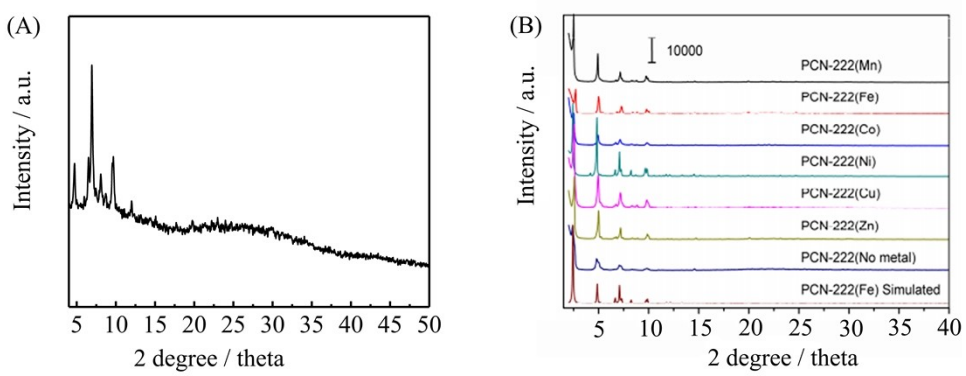

Fig. S6. XRD patterns of PCN-222(Fe) (A) synthesized in our experiment and (B) reported by literature[2].

S12) Optimization of experimental condition

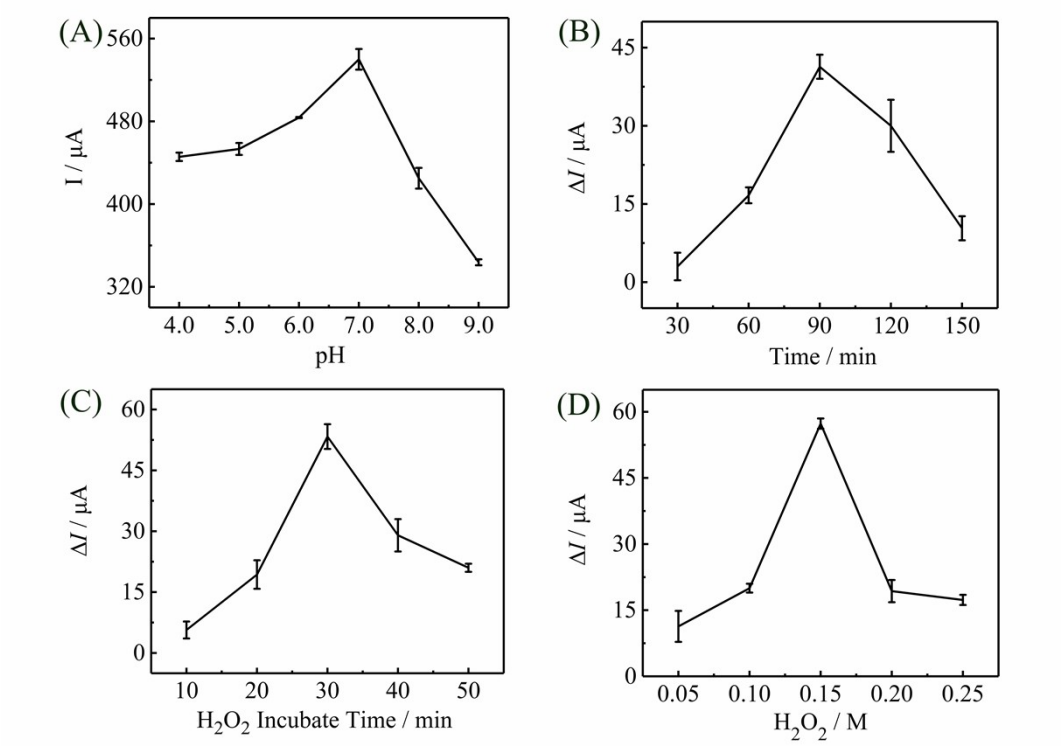

Fig. S7. pH value of incubation (A), incubation time of immunoprobe (B), reaction time of H_2O_2 (C) and concentration (D) about DPV response to current (The error bars represent standard deviations for n=3).

S13) Comparison of analytical performance of different strategies for CYFRA21-1 detection

Table S1 Comparison of the recently reported immunoassay for detection of CYFRA21-1

Material	Method	LRD ($ng\ mL^{-1}$)	LOD ($pg\ mL^{-1}$)	Ref.
TB-Au-COF	SWV	0.0005 ~ 10	0.1	[3]
AuNPs@MoS_2@Ti_3C_2Tx	SWV	0.0005 ~ 50	0.03	[4]
3D-G@Au	DPV	0.25 ~ 800	100	[5]
TPAPCN	ECL	0.05 ~ 1	0.016	[6]
AuNPs/Thi/$MWCNTNH_2$	DPV	0.1 ~ 150	43	[7]
carboxyl-MoS_2	SPR	0.00005 ~ 100	0.05	[8]
PCN222(Fe)/AuNPs	DPV	0.00001 ~ 100	6.15×10^{-4}	This work

SWV: square wave pulse voltammetry, DPV: differential pulse voltammetry, SPR: surface plasmon resonance, ECL: electrochemiluminescence.

Reference

1. A. J. Bard and L. R. Faulkner, *Electrochemical methods*, 2001, **2**, 580-632.
2. D. Feng, Z.-Y. Gu, J.-R. Li, H.-L. Jiang, Z. Wei and H.-C. Zhou, *Angewandte Chemie International Edition*, 2012, **51**, 10307-10310.
3. J. Cheng, K. Hu, Q. Liu, Y. Liu, H. Yang and J. Kong, *Analytical and Bioanalytical Chemistry*, 2021, **413**, 2543-2551.
4. K. Hu, J. Cheng, K. Wang, Y. Zhao, Y. Liu, H. Yang and Z. Zhang, *Talanta*, 2022, **238**, 122987.
5. Y. Zeng, J. Bao, Y. Zhao, D. Huo, M. Chen, M. Yang, H. Fa and C. Hou, *Talanta*, 2018, **178**, 122-128.
6. X. Lv, M. Bi, X. Xu, Y. Li, C. Geng, B. Cui and Y. Fang, *Analytical and Bioanalytical Chemistry*, 2022, **414**, 1389-1402.
7. Y. Zeng, J. Bao, Y. Zhao, D. Huo, M. Chen, Y. Qi, M. Yang, H. Fa and C. Hou, *Bioelectrochemistry*, 2018, **120**, 183-189.
8. N.-F. Chiu and H.-T. Yang, *Frontiers in Bioengineering and Biotechnology*, 2020, **8**.