Supplementary Material for

Quantification of EGFR and EGFR-overexpressed cancer cells based on carbon dots@bimetallic CuCo Prussian blue analogue

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S1. Experimental section

S1.1 Chemicals and regents

Copper nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$), potassium hexacyanocobaltate(III) ($K_3[Co(CN)_6]$), potassium dihydrogen phosphate ($KH_2PO_4 \cdot 12H_2O$), disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$) and potassium gold chloride ($HAuCl_4 \cdot 3H_2O$) were purchased from Aladdin Technology Co., Ltd., (Beijing, China). Dimethyl sulfoxide (DMSO) was obtained from Solarbio Life Sciences Reagent Co. Ltd. (Shanghai, China). The epidermal growth factor receptor (EGFR), human epidermal growth factor receptor-2, mouse immunoglobulin G, immunoglobulin E, prostate specific antigen, bovine serum albumin, and carcino embryonic antigen were purchased from Solarbio Life Sciences Reagent Co. Ltd. (Shanghai, China). All of reagents were of analytical grade and used directly without further purification and prepared using Millli-Q water with a resistivity of greater than 18 MΩ·cm throughout all experiments. The EGFR-targeted aptamer sequence is listed as 5'-TGA ATG TTG TTT TTT CTC TTT TCT ATA GTA-3'.[1]

S1.2 Preparation of phosphate buffered, aptamer, EGFR and real sample solutions

Phosphate buffered solution (PBS, 0.01 M, pH 7.4) was prepared by mixing 0.24 g KH_2PO_4, 1.44 g $Na_2HPO_4 \cdot 12H_2O$, 0.20 g KCl, and 8.0 g NaCl. The stock solution of aptamer (50 nM), EGFR (1 fg·mL^{-1}–1 ng·mL^{-1}), and all of the interferences (10 pg·mL^{-1}) were prepared using PBS which were filtered with a 0.22 μm membrane and adjusted to neutral pH before use. PBS containing $K_3[Fe(CN)_6]$ (1.65 g) and $K_4[Fe(CN)_6]$ (2.11 g) was used for the electrolyte solution. All solutions were prepared immediately before each experiment and stored at 4 ℃ until use.

To analyze the real application of the fabricated aptasensors, the human serum was diluted with 0.01 M PBS (pH 7.4) to 200 times in prior to analysis. Afterwards, EGFR with certain concentration was added into the diluted PBS. The solution of real samples were stored at 4 ℃ for further detection by the proposed method.

S1.3 Preparation of carbon dots

Carbon dots (CDs) were synthesized in according to the literature.[2]

S1.4 Preparation of CuCo Prussian blue analogue (CuCoPBA)

According to the reported literature,[3] CuCo PBA was prepared using a slightly modified method. In brief, 0.133 g of $K_3[Co(CN)_6]$ and 0.145 g of $Cu(NO_3)_2$ were dissolved in 15 mL of Milli-Q water to obtain Solution **A**, whereas 0.265 g of sodium citrate was dissolved in 15 mL of Milli-Q water to obtain Solution **B**. Afterward, Solution **A** was added into Solution **B** under magnetic stirring and aged for further 24 h at room temperature. The resultant precipitate was collected by centrifuging at 8000 rpm for 5 min, following by washing with water several times, and drying under vacuum at 60 ℃ for 6 h. Finally, the CuCo PBA powder was obtained.

S1.5 Pre-treatment of the bare gold electrode

Bare Au electrode (AE) with 3 mm diameter was polished into a smooth surface by using 0.05 μm alumina powder and ultrasonically washed in piranha solution (*v/v*, H_2SO_4:H_2O_2 = 7:3) for 15 min. The electrode was electrochemically cleaned through oxidation and reduction cycling in 0.5 M H_2SO_4 from −0.2 V to 1.6 V (*vs.* Ag/AgCl), rinsed with Milli-Q water, and dried under nitrogen.

S1.6 Characterizations

X-ray photoelectron spectroscopy (XPS) analysis was determined using an AXIS HIS 165 spectrometer (Kratos Analytical, Manchester, U.K.) with a monochromatized Al K_α X-ray source (1486.71 eV photons). X-ray diffraction measurements (XRD) were recorded on a Rigaku D/Max-2500 X-ray diffractometer using Cu K_α radiation. Fourier transform infrared spectroscopy (FT-IR) spectra were characterized by using a Bruker Tensor 27 OPUS FT-IR spectrometer (with KBr pellet) within the 4000−400 cm^{-1} range. The surface morphology of the synthesized samples was investigated through scanning electron microscope (SEM, JSM-6490LV, Japan) and high-resolution transmission electron microscopy (HR-TEM, FEI Talos F200S) with a field emission gun of 200 kV.

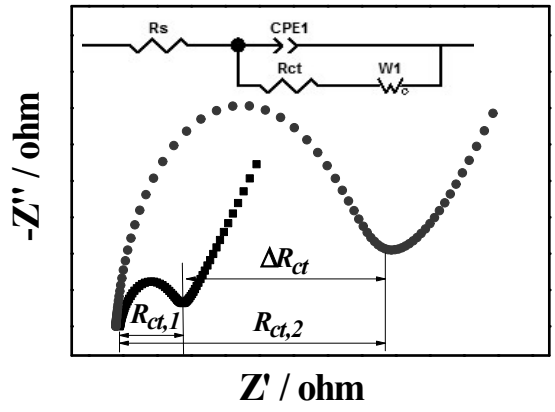

Fig. S1 EIS Nyquist plots and equivalent circuit.

S1.7 Cell culture

MCF-7 cancer cells were obtained from American Type Culture Collection and grown in Dulbecco's modified Eagle medium medium with 10% heat inactivated fetal bovine serum and antibiotics (50 units·mL^{-1} penicillin and 50 units·mL^{-1} streptomycin). The cells were maintained at 37 °C in 5% CO_2 until use.

S1.8 Cytotoxicity in vitro

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was

employed to evaluate the in vitro cytotoxicity of CD@CuCoPBA to MCF-7 cells. Cells were seeded in 96-well plates with a density of 8000 cells·well^{-1} and incubated prior to treatment with nanocomposite. After 24 h, the medium was replaced by fresh medium containing different concentrations of CD@CuCoPBA, and incubated for another 24 h. Then, the medium was discarded, and cells were washed twice with PBS. After 24 h of incubation in medium, MTT (5 mg·mL^{-1}, 20 μL) was added and the cells were cultured for another 4 h. Finally, 150 μL of DMSO was added to the plate wells, following by shaking the plates for 15 min. The absorbance values at 488 nm were measured with a microplate reader.

S1.9 Cell imaging

MCF-7 cells were seeded in a laser confocal culture dish with a density of 1×10^5 cells·well^{-1} and incubated at 37 °C and 5% CO_2. After 8 h, the media was replaced with media containing the CD@CuCoPBA nanocomposite (50 μg·mL^{-1}). After treatment, the cells were washed with PBS and fixed with 4% paraformaldehyde for 10 min at 37 °C. The fixed cells were then washed with PBS. All images were obtained with a Zeiss 710 LSM using a 420 nm HeNe laser excitation source.

S2. Chemical structure and components of CuCo PBA and CD@CuCoPBA

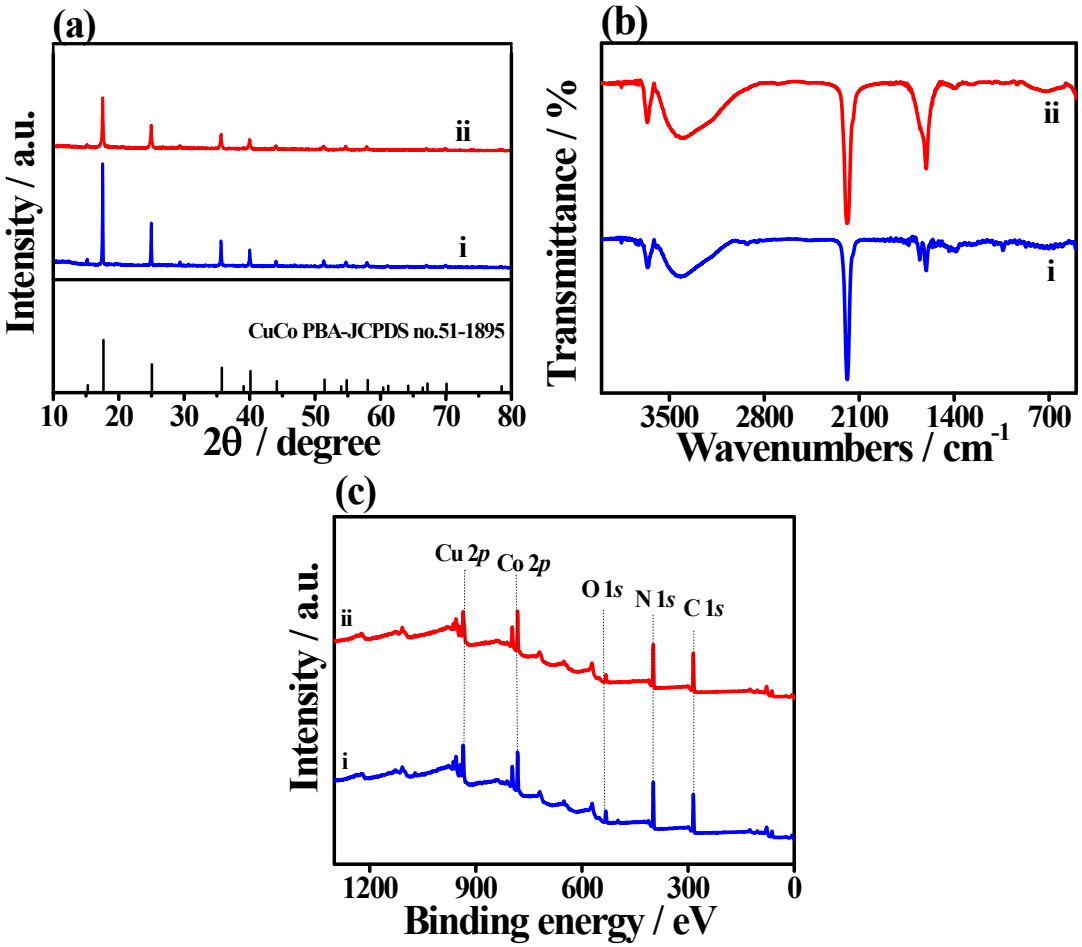

Fig. S2 (a) XRD patterns, (b) FTIR, and (c) XPS survey spectra of (i) CuCo PBA and (ii) CD@CuCoPBA nanocomposite.

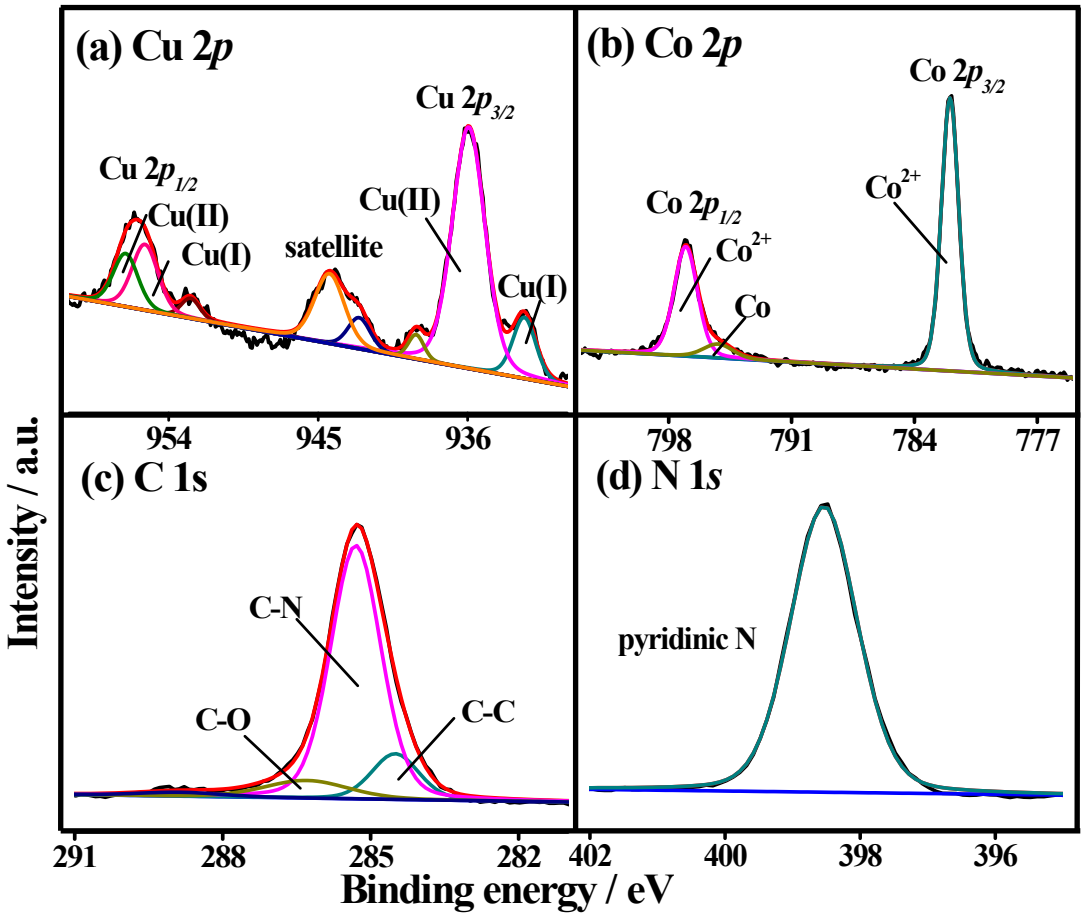

Fig. S3 The high-resolution Co 2*p*, Cu 2*p*, C 1*s*, and N 1*s* XPS spectra of the CuCo PBA nanocubes.

S3. Surface morphology of CuCo PBA

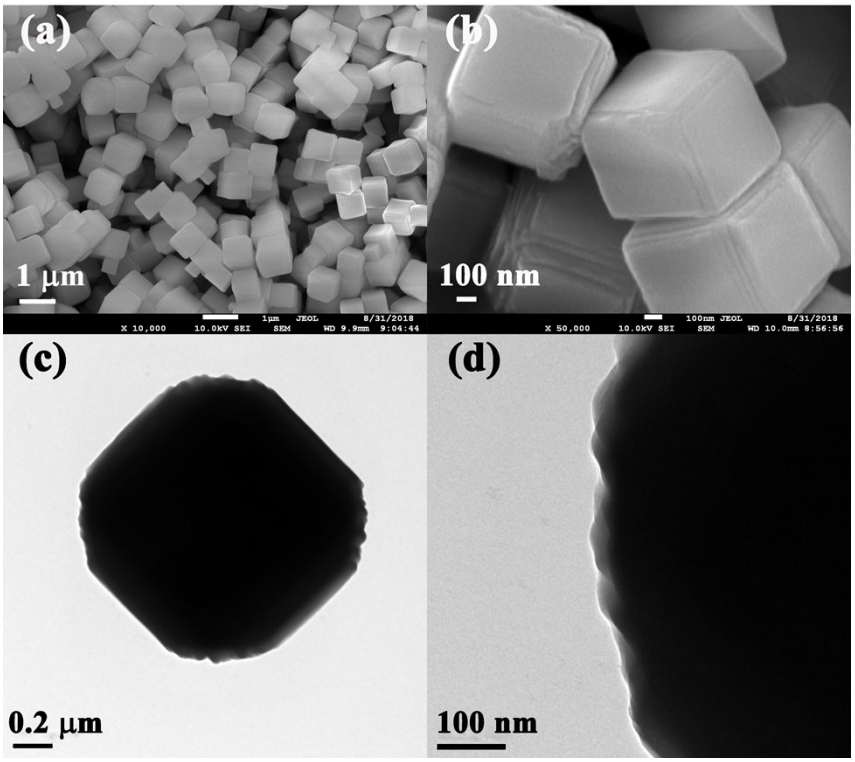

Fig. S4 (a, b) Low- and high-magnification SEM images and (c, d) TEM and HRTEM images of CuCo PBA nanocubes.

S4. Electrochemical performance of CuCo PBA and CD@CuCoPBA

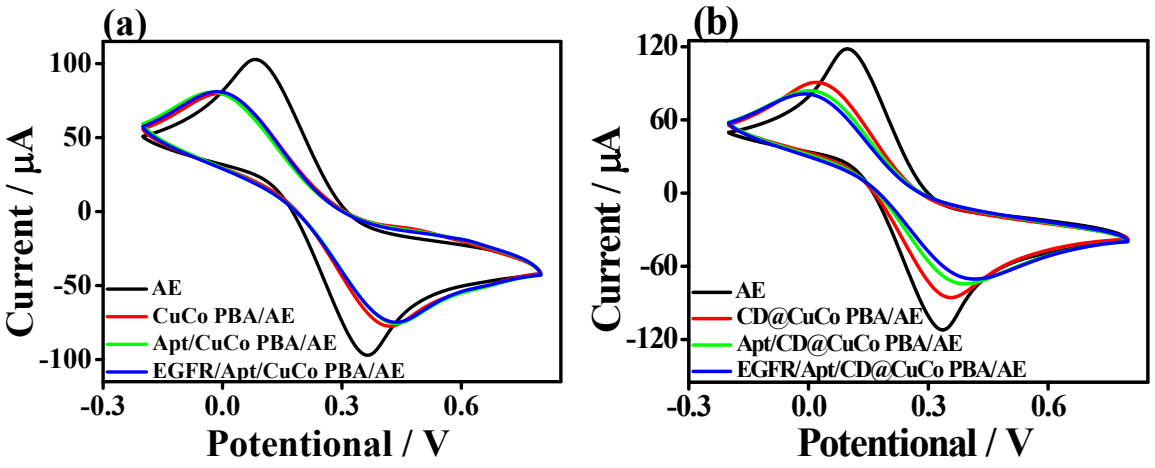

Fig. S5 CV curves for tracing the whole procedure for detecting EGFR using the developed aptasensor based on (b) the pristine CuCo PBA and (b) CD@CuCoPBA nanocomposite in 0.01 M PBS (pH 7.4) containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ redox.

Table S1 Detection of EGFR in human serum samples with the developed aptasensor.

Amount added ($pg \cdot mL^{-1}$)	ΔR_{ct} (ohm)	Found ($pg \cdot mL^{-1}$)	Recovery (%)	RSD (%)
0.005	300	0.00472	94.4	2.6
0.05	612	0.0513	102.6	3.7
0.5	905	0.479	95.7	1.9
5	1219	5.20	104.0	4.5
50	1519	51.4	102.8	4.1
500	1823	498.88	99.8	3.1

Table S2 Comparison with other published work for the detection of MCF-7 cells.

Materials	Methods	Detection range ($cell \cdot mL^{-1}$)	LOD ($cell \cdot mL^{-1}$)	Refs
Ag–PAMAM–luminol nanocomposits	Electrochemiluminescence	2×10^2–9×10^3	150	[4]
Aptamer-functionalized gold nanorods	Localized surface plasmon resonance	1×10^1–1×10^5	100	[5]
AuNPs@graphene	Electrochemiluminescence	5×10^2–2×10^7	230	[6]
$NaYF_4$:Yb,Er/TiO_2/CdTe	Photoelectrochemical	1×10^3–1×10^5	400	[7]
Platinum nanoparticles /graphene oxide	Colorimetric assay	0–8×10^3	125	[8]
CD@CuCoPBA	**EIS**	**5×10^2–1×10^5**	**80**	**This work**

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