Electronic Supplementary Information

Oxygen electrocatalysis-driven electrochemiluminescence by hierarchical carbon nanoflowers-supported copper-modulated cobalt sulfide for cytosensing

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Reagents and materials

Copper (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$, AR), cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$, AR), urea (99.5%), dopamine hydrochloride (DA, 98%), thiourea (CH_4N_2S, AR), ethanol (99.0%), 6-mercapto-1-hexanol (MCH), urea (99%), melamine (99%), tris-(2-carboxyethyl)-phosphine (TCEP, 98%), and hydrochloric acid (HCl, 37%) were purchased from Aladdin Reagent Co., Ltd (China). Malonamide (98%) was purchased from Macklin Biochemical Co., Ltd. (China). Pure water was prepared by Millipore Elix 5 Pure Water System from Purelab Classic Corp (USA). Cell lines and reagents for the cells experiments were all provided by KeyGEN Biotech Co., Ltd. (China). All the DNA sequences (Table S1) were obtained from Sangon Biotech. Co., Ltd. (China). TE buffer was prepared with 10 mM Tris-HCl solution containing 1 mM EDTA, and 12.5 mM $MgCl_2$.

Table S1. Oligonucleotide sequences of all the utilized DNAs.

Name	Sequence (5' to 3')
Aptamer	GCAGTTGATCCTTTGGATACCCTGGAAATTTTTTT
Primer	GGGTATCCAAAGGATCAACTGC
Fc-H1	Fc-CCAGCAGTTGATCCTTTGGATACCCTGTACAGACATCCAAAGGAT
H2	GTAGTCTGTACAGGGTATCCAAAGGATCAACTGCGTGATTTGGATAC CCTGT
Capture DNA	SH-ACAGGGTATCCAAATCAC

Apparatus

UV-visible absorption spectra were measured with a Lambda 1050+ UV-Vis-NIR spectrophotometer (PerkinElmer, USA). X-ray powder diffraction (XRD) measurements were performed on a Shimadzu XRD-6000 X-ray powder diffractometer

(Shimadzu Co., Japan). Fourier transform infrared (FT-IR) spectra were measured by a Nicolet 6700 spectrophotometer (Nicolet Co., USA). Transmission electron microscopy and elements mapping images were obtained by a JEOL-2010 electron microscope (JEOL, Ltd., Japan). Electrochemical impedance spectroscopy (EIS) was measured by an Autolab electrochemical workstation (Metrohm, Switzerland). X-ray photoelectron spectroscopy were analyzed by an ESCALAB250Xi spectrometer (Thermo Fisher Scientific Co., USA). ORR-related data were collected by rotating ring disk electrode (RRDE) (Pine, USA) and a CHI 660D electrochemical workstation (Shanghai CH Instruments Co., ltd., China). Zeta potential analysis was conducted with a 90Plus Dynamic Light Scattering Instrument (Brookhaven Instruments Co., USA). ECL signals were collected by a home-built ECL analyzer mainly containing a CHI 660D electrochemical workstation (Shanghai CH Instruments Co., ltd., China) and full-spectrum photomultiplier tube.

Synthesis of Cu_xCo@PDA

$CuCl_2 \cdot 2H_2O$ and $CoCl_2 \cdot 6H_2O$ were dissolved in 20 mL of deionized water with a total cation concentration of 50 mM. Then, 0.08 g DA and 0.24 g urea were further added with continuous stirring. The resulting homogeneous mixture was then transferred into a Teflon vessel inside a stainless-steel reactor and subjected to a hydrothermal process at 100 ℃ for 12 h. The final products were centrifuged, washed, and dried to obtain the Cu_xCo@PDA products, where x represents the concentration molar ratio of Cu^{2+} and Co^{2+} at 0, 0.05, 0.10, 0.15 and 0.20, respectively.

Synthesis of Cu_x-Co_9S_8@CNFs

The Cu_xCo@PDA products were treated at 500 ℃ for 1 h at 200 sccm N_2 flow. Then, the products were etched in 60 mL HCl (2 M) at 60 ℃ for 24 h and washed completely with deionized water. Finally, the products of Cu_x-Co_9S_8@CNFs were prepared by pyrolyzing 0.1 g of the above powder and 12.0 g of thiourea at 800 ℃ for 1 h with a heating rate of 5 ℃ s^{-1} at 100 sccm N_2 flow.

Synthesis $Cu_{0.15}$-Co_9S_8@CNFs/CNNS

Bulk g-C_3N_4 was first prepared by a classical thermal polycondensation. 1.5 g of malonamide, 3.5 g of urea, and 0.04 g of malonamide was heated to 550 °C with a heating rate of 5.5 °C min^{-1} and maintained at this temperature for 5 h. Next, 0.20 g of the obtained g-C_3N_4 powder was subjected to protonation with 2.0 mL of concentrated HCl (37%) under continuous stirring for one day. The mixture was further washed by centrifugation until the suspension became neutral, and the centrifuged solid was dispersed in 40.0 mL of deionized water for ultrasonication (150 W, 40 KHz) for 12 h to obtain homogeneous CNNS suspension. At last, $Cu_{0.15}$-Co_9S_8@CNFs and CNNS were mixed in a certain mass ratio, and due to electrostatic interactions, CNNS was prone to the self-assembly on the surface of catalysts. The final nanocomposites were obtained by centrifugation, washing, and vacuum drying at 60 °C overnight.

ORR measurement

The electrochemical oxygen reduction reaction (ORR) was carried out in O_2-saturated 0.1 M KOH aqueous solution in a three-electrode configuration. The ring potential was at 0.50 V vs Ag/AgCl in the rotating ring disk electrode (RRDE) experiments. The percentage of H_2O_2 yield (H_2O_2 %) and the electron transfer number (n) were determined by the following equations[1] :

$H_2O_2\ \% = (200 \times I_r/N)/(I_d + I_r/N)$

$n = (4 \times I_d)/(I_d + I_r/N)$

where I_d and I_r are the disk and ring currents respectively, and N is the collection efficiency (3.6).

Cell Culture and Treatment

MCF-7, MCF-10A, and HeLa cells all cultured in Dulbecoo's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, glucose (4.5 g L^{-1}), L-glutamine (0.584 g L^{-1})), sodium bicarbonate (3.7 g L^{-1})), sodium pyruvate (0.584 g L^{-}

^{1})), penicillin (80 U mL^{-1}), and streptomycin (80 mg mL^{-1}) in an incubator, which were maintained at 37 °C in a humid air atmosphere containing 5% CO_2. When the cells were in the logarithmic growth phase, they were digested with trypsin (0.1% m/v) and then centrifuged at 1000 rpm for 5 min twice. The cells sediment was finally uniformly resuspended in cells culture for the experiments or the next round of culture.

Profiling of the Cells Surface MUC1

The cells were incubated with 0.5 μM DNA probe in an incubator (37 °C) for 40 min, after which they were centrifuged at 1000 rpm for 5 min. The supernatant was then collected and then added with hairpin DNAs of Fc-H_1 and H_2 (2.0 μM) to trigger the CHA reaction at 37 °C for 45 min.

The GCE was pre-treated following the general method, and the freshly cleaned GCE was dropped with 10 μL of the prepared $Cu_{0.15}$-Co_9S_8@CNFs/CNNS water dispersion. After drying naturally, a solid-state film was self-assembled on the electrode surface. TCEP (100 mM) was used to treat the capture DNA to break the S-S bonds. Then, 10.0 μL of the capture DNA (2.0 μM) was employed to functionalize the solid film, and 10.0 μL of MCH (2.0 μM) was subsequently used to block the remaining active sites. The sensing electrode was obtained after rinsing with distilled water and drying in a nitrogen atmosphere.

Subsequently, 10.0 μL of the above CHA products solution was incubated with the sensing platform for 30 min. Following the incubation period, the electrode was washed with distilled water and subsequently used to output the ECL signal with a counter electrode (Pt wire) and a reference electrode (Ag/AgCl) in phosphate buffer solution (PBS, pH 8.0) under ambient conditions.

Native Polyacrylamide Gel Electrophoresis (PAGE)

The native PAGE (8%) was fabricated with a mixture solution containing distilled water (4.0 mL), acrylamide/bisacrylamide (29:1) gel solution (30%), 5×TBE buffer (1.6

mL), ammonium persulfate (APS, 10%, 56 μL), and N, N, N′, N′-tetramethylethylenediamine (TEMED, 8 μL), which was transferred into a glue frame and kept still at room temperature until the PAGE was formed. Then, 10 μL of each sample mixed with 2.0 μL of 6×loading buffer was added into the corresponding channel, and the electrophoresis was conducted at 200 V for 40 min. The resultant gels were scanned and imaged with a gel imaging analysis system (WD-9413A, Beijing, China).

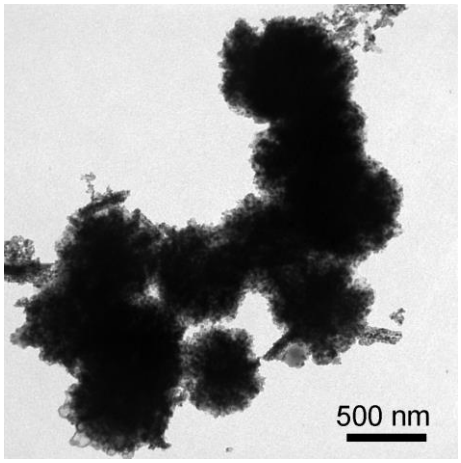

Fig. S1 TEM image of $Cu_{0.20}$-Co_9S_8@CNFs with the collapsed structures and CuS formation.

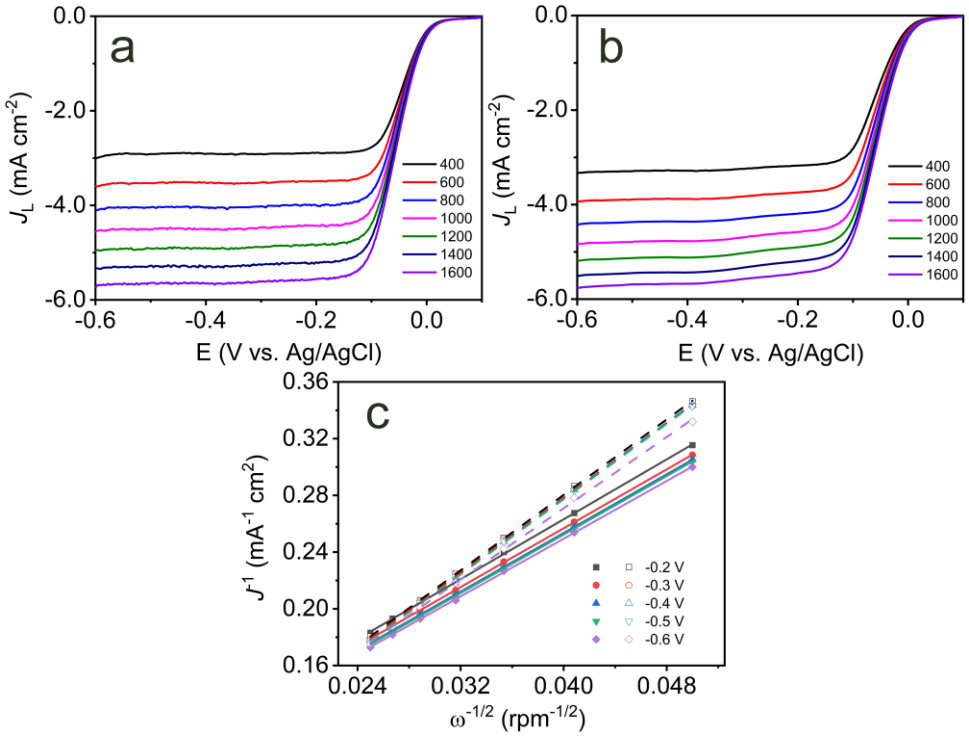

Fig. S2 LSV curves of (a) $Cu_{0.15}$-Co_9S_8@CNFs and (b) Pt/C measured in O_2-saturated 0.1 M KOH solution *via* RRDE at the rotating speeds of 400-1600 rpm, and (c) the corresponding Koutecky-Levich plots at the potentials of -0.20V~-0.60 V (vs. Ag/AgCl) (hollow: $Cu_{0.15}$-Co_9S_8@CNFs; solid: Pt/C).

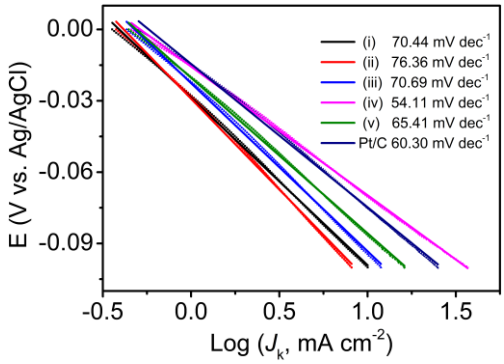

Fig. S3 Tafel plots (E vs. Log(J_k)) of Cu_x-Co_9S_8@CNFs (x=0 (i), 0.05 (ii), 0.10 (iii), 0.15 (iv), and 0.20 (v)) and Pt/C via LSV at 1600 rpm.

The kinetic current density (J_k) was calculated from the mass transport correction of RRDE according to the following equation[2]: $J_K=(J\times J_L)/(J_L-J)$, where J is the measured current density and J_L is the diffusion-limited current density.

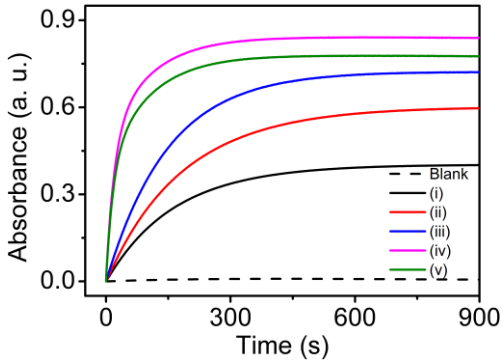

Fig. S4 Time-dependent absorbance changes of oxTMB at 652 nm in the presence of Cu_x-Co_9S_8@CNFs.

The peroxidase-catalyzed capability of the products is evaluated using the substrate of 3,3,5,5-tetramethylbenzidine (TMB) and H_2O_2 (Fig. S4, ESI†). $Cu_{0.15}$-Co_9S_8@CNFs also exhibits the fast catalytic kinetics and the highest activity, which is 2.14 times stronger than that of the monometallic ones (roughly estimated by the absorbance values at 10 min).

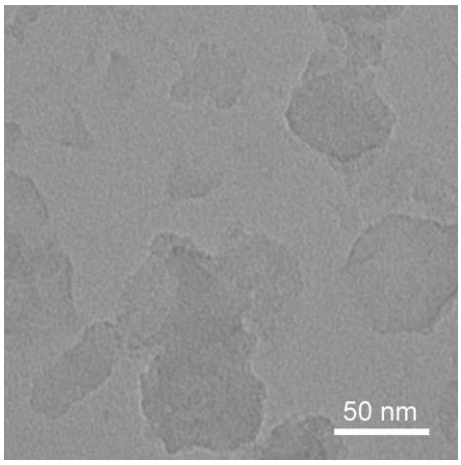

Fig. S5 TEM image of CNNS.

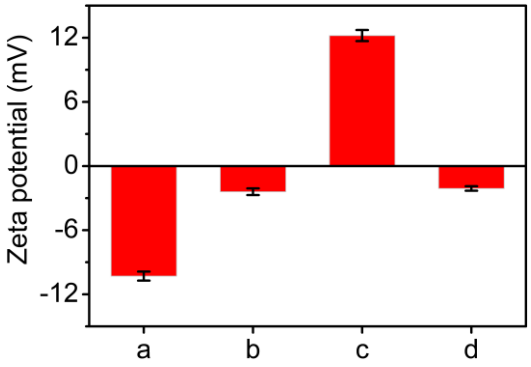

Fig. S6 Zeta potential values of $Cu_{0.15}$-Co_9S_8@CNFs, g-C_3N_4, CNNS, and $Cu_{0.15}$-Co_9S_8@CNFs/CNNS.

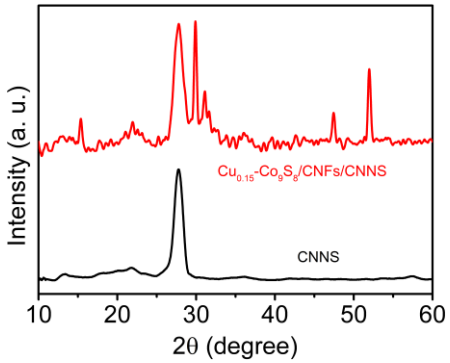

Fig. S7 XRD patterns of CNNS and $Cu_{0.15}$-Co_9S_8@CNFs/CNNS.

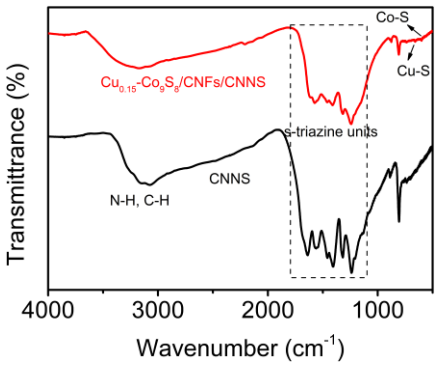

Fig. S8 FT-IR spectra of CNNS and $Cu_{0.15}$-Co_9S_8@CNFs/CNNS.

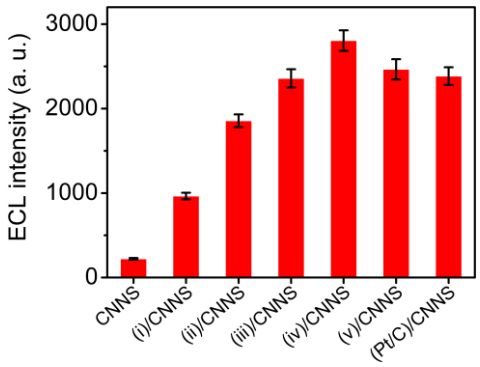

Fig. S9 ECL responses of Cu_x-Co_9S_8@CNFs/CNNS (x=0 (i), 0.05 (ii), 0.10 (iii), 0.15 (iv), and 0.20 (v)) and (Pt/C)/CNNS in air-saturated PBS.

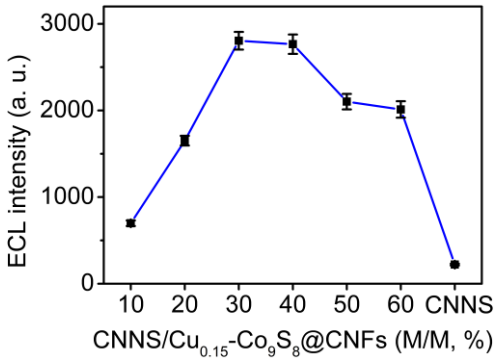

Fig. S10 Optimization of the mass ratio of CNNS and $Cu_{0.15}$-Co_9S_8@CNFs.

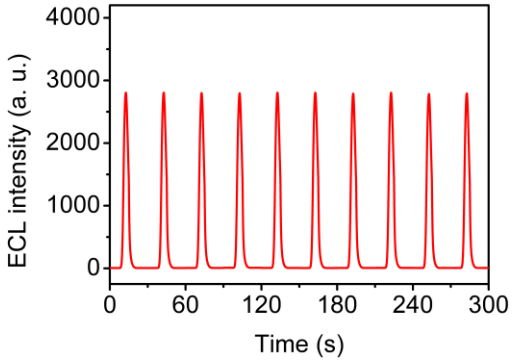

Fig. S11 ECL signal (10 cycles) stability evaluation in air-saturated PBS.

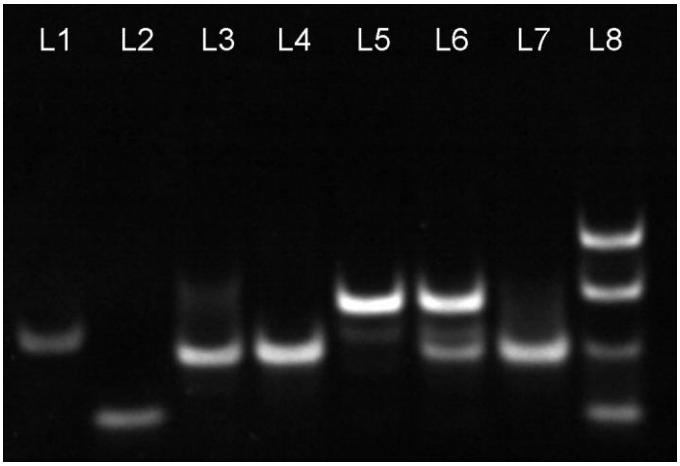

Fig. S12 PAGE testify of the DNA-related processes: L1, aptamer; L2, pDNA; L3, H1; L4, H2; L5, aptamer/pDNA duplexes; L6, aptamer/pDNA duplexes+H1; L7, H1+H2; L8 pDNA+H1+H2.

The DNA-related process in this strategy are validated by the polyacrylamide gel electrophoresis (PAGE) in Fig. S12 (ESI†). Lanes 1-4 represent the aptamer, primer ssDNA (pDNA), H1, and H2, respectively. The appearance of a new band in lane 5 indicates the formation of aptamer/pDNA duplexes. With regard to the annealed assembly products of pDNA, H1, and H2 in lane 8, the four bands (top-bottom) represent the following: H1/H2 hybrids, pDNA/H1 hybrids, residual H2, and regenerated pDNA, respectively. Control annealed products in lane 6 (aptamer/pDNA hybrids+H1) and lane 7 (H1+H2) have no obvious assembly reaction. These results reveal the feasibility of the targeting and CHA process.

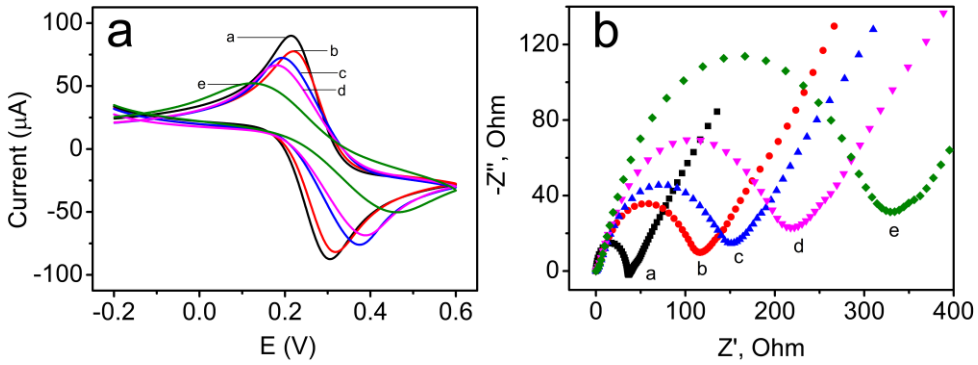

Fig. S13 (a) CV and (b) EIS characterizations of bare GCE (a), $Cu_{0.15}$-Co_9S_8@CNFs/CNNS/a (b), capture DNA/b (c), MCH/c (d), and CHA products/d (e) in 5 mM $[Fe(CN)_6]^{3-/4-}$ solution.

The fabrication process of the ECL platform on glassy carbon electrode (GCE) is examined by CV and electrochemical impedance spectroscopy (EIS) in Fig. S13 (ESI†). The successive modifications of the prepared ECL nanocomposites, capture DNA, MCH (blocking agent), and the obtained CHA products result in a gradually decreasing redox peak current and increasing interfacial impedance, demonstrating the successful construction of the sensing platform.

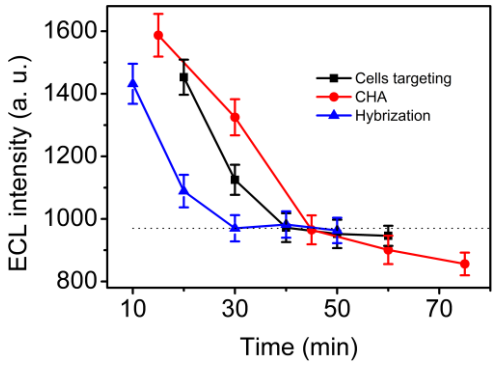

Fig. S14 Dependence of ECL intensity on incubation time for DNA probe targeting to cells (black), CHA reaction time (red), and hybridization time for electrode capturing (blue).

Table S2. Comparison of the as-proposed cytosensing strategy with other reported ones.

Method	Linear range Cell mL^{-1}	LOD Cell mL^{-1}	Reference
Photoelectrochemistry	5.0×10^{2}-5.0×10^{6}	198	3
Photoelectrochemistry	1.0×10^{3}-1.0×10^{5}	400	4
Electrochemistry	1.0×10^{5}-1.0×10^{8}	1.0×10^{5}	5
ECL	4.5×10^{2}-1.0×10^{7}	250	6
Surface plasmon resonance	5.0×10^{2}-1.0×10^{4}	500	7
Fluorescence	2.5×10^{2}-1.0×10^{4}	201	8
ECL	5.0×10^{2}-5.0×10^{5}	152	This work

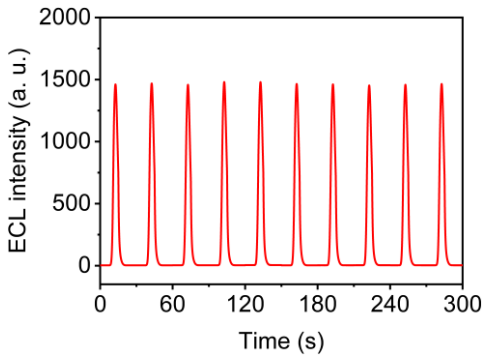

Fig. S15 The ECL stability of the proposed cytosensing strategy with MCF-7 cells of 1.0×10^{3} under 300 s of continuous CV scanning.

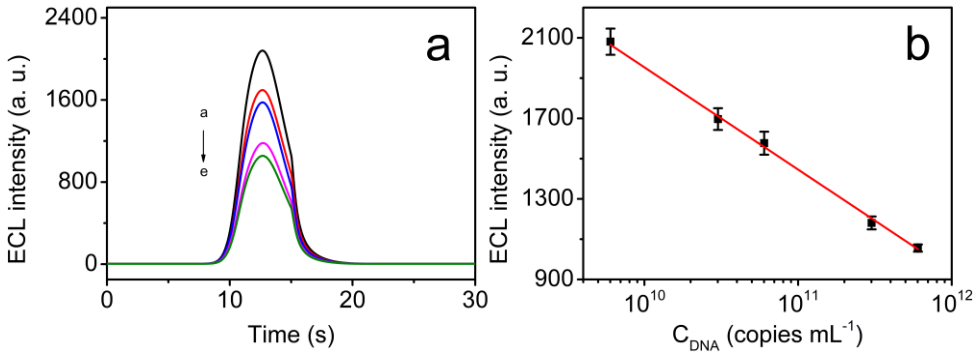

Fig. S16 (a) ECL intensity-time curves of the sensing platform by directly using the primer DNA concentrations of 6.02×10^{9}, 3.01×10^{10}, 6.02×10^{10}, 3.01×10^{11}, 6.02×10^{11} copies mL^{-1}, and (b) the corresponding calibration curve.

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