Electronic Supplementary Information for:

Perylenetetracarboxylic Dianhydride and Aniline Assembled Supramolecular Nanomaterial with Multi-color Electrochemiluminescence for Highly Sensitive Label-free Immunoassay

Wei Zhang,[a] Yue Song,[a] Yunyun Wang,[a] Shuijian He,[b] Lei Shang,[a] Rongna Ma,[a] Liping Jia,[a] and Huaisheng Wang*[a]

[a]Chemistry of Department, Liaocheng University, Liaocheng, Shandong, 252059, China

[b]College of Materials Science and Engineering, Nanjing Forestry University, Nanjing, 210037, China

Corresponding Authors' Information:

***Email:** hswang@lcu.edu.cn

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1. Experimental details

1.1. Chemical reagents

Human colon CEA and CEA antibody (anti-CEA) were purchased from LincBio Science Co. Ltd. (Shanghai, China). $HAuCl_4 \cdot 4H_2O$, bovineserum albumin (BSA), 3, 4, 9, 10-perylenetetracarboxylic dianhydride (PTCDA, 97%) were obtained from Sigma–Aldrich. Aniline (An) and uric acid (UA) were purchased from Aladdin Industry Corporation. Thrombin (Thr) was purchased from Archie Chem Ltd. (Liverpool, England). Human serum samples were provided by Liaocheng People's Hospital. All chemical reagents were analytical grade and used as received. All aqueous solutions were prepared with ultrapure water (> 18 MΩ) from a Milli-Q Plus system (Millipore).

1.2. Instruments

ECL immunoassay measurements were carried out on a Model RFL-1 ECL analyzer (Xi'an Remex Instrument Co., Ltd., China) with the voltage of the photomultiplier tube (PMT) set at 900 V and with the auxiliary equipment of CHI 990B (Shanghai CHI Instruments Co., China) electrochemical workstation. The ECL spectra acquisition are accomplished in Professor Guizheng Zou's lab (Shandong University) on a homemade ECL spectrometer consisting of an Acton SP2300i monochromator equipped with a liquid N_2 cooled PyLoN 400BR-eXcelon digital CCD detector (Princeton Instruments, U.S.A) and a

VersaSTAT 3 electrochemical analyzer (Princeton Applied Research, U.S.A.). ECL emission at the electrode surface was collected with an objective lens before it is delivered to the ECL spectrum system. Electrochemical impedance spectroscopy (EIS) was performed using a CHI 760C electrochemical workstation. Scanning electron microscopy (SEM) was performed on a Zeiss Supra-40 scanning electron microscope (accelerating voltage: 5 kV). Ultraviolet–visible (UV–Vis) spectroscopy was obtained using Lambda 750 spectrophotometer (Perkin Elmer, USA). Fluorescent(FL) spectroscopy of PTCDA in solution were recorded on F-7000(HITACHI Ltd., Japan). The FL of PTCDA powder were recorded using FLS1000(Edinburgh, UK). X-ray photoelectron spectrum (XPS) was carried out on an ESCALAB MK II X-ray photoelectron spectrometer to evaluate the composition of the samples. SK type ultrasonic cleaning machine (Shanghai Keguo Ultrasonic instrument Co., Ltd), pHS-3C meter (Shanghai second analysis instrument factory), high-speed refrigerated centrifuge Z36HK-HERMLE (Germany), dry type thermostat (Hangzhou Aomori Instrument Co., Ltd).

1.3. Preparation of PTCDA-An

PTCDA-An was prepared by PTCDA self-assembling with An through hydrogen bonding. In brief, 10 mg PTCDA was dissolved into 5.0 mL acetone, then 1.0 mL 99.9% An was added into the above solution and stirred violently at room temperature for 7 days, to remove the residue An,

the mixture was centrifuged for four times at 12000 rpm, and washed with anhydrous ethanol and ultrapure water. The final red product PTCDA-An was dispersed into 20.0 mL ultra-pure water and placed in a refrigerator at 4 ℃. And 10 mg PTCDA was disolved into 20.0 mL ultra-pure water for control experiment.

1.4. Fabrication of the ECL immunosensor

It is more convenient to use disposable printed/screen printed electrode as working electrode to fabricate immunosensor for detecting CEA, no need complexed polishing work as that of glass carbon electrode (GCE) before use. However, in laboratory fundamental study, the cost of GCE was much cheaper than disposable printed/screen printed electrode and a common electrolytic cell is enough for GCE working electrode. It needs to order a special electrolytic cell for disposable printed/screen printed electrode. Thus, GCE was chosen as the working electrode in this work. First, GCE with a diameter of 3 mm was polished by Al_2O_3 powder (0.3 and 0.05 μm), then rinsed with ultra-pure water and ethanol, dry with nitrogen. The prepared ECL supramolecular nanomaterial PTCDA-An was dropped on the GCE surface and dried at room temperature. Then i-t curve was used to electrodeposit AuNPs for 60 s with 1 mM $HAuCl_4$ at initial potential -0.2 V and dried at room temperature. Subsequently, 5 μL of 18 μg mL^{-1} anti-CEA (Ab_1) was dropped on the AuNPs/PTCDA-An/GCE for 12 h at 4 ℃. Next, 5 μL of 5% BSA solution was dropped on

the Ab_1/AuNPs/PTCDA-An/GCE and incubated at 37 ℃ for 1 h. Finally, the Ab_1/AuNPs/PTCDA-An/GCE was incubated in different concentrations of CEA solution at 37 ℃ for 2 h. The prepared sensors are stored at 4 ℃. The ECL tests of fabricated immunosensor was performed during cycle potential scanning from -1.9 V to -0.8 V.

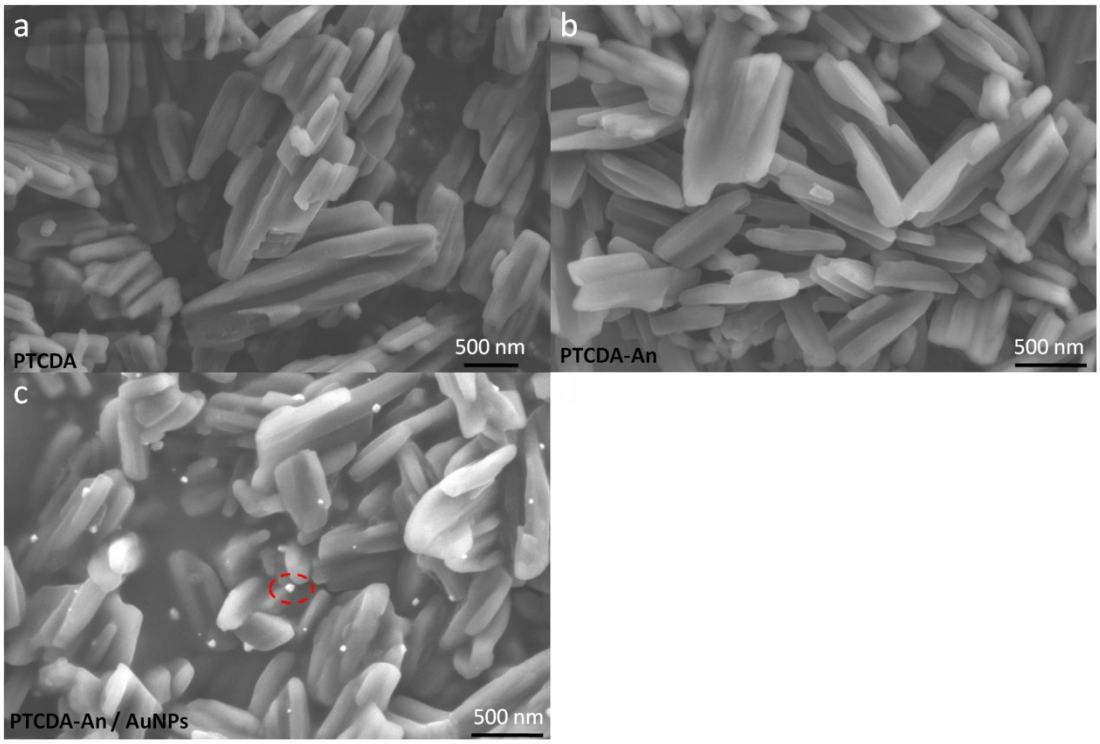

Fig. S1 the SEM images of PTCDA(a), PTCDA-An(b) and PTCDA-An/AuNPs(c)

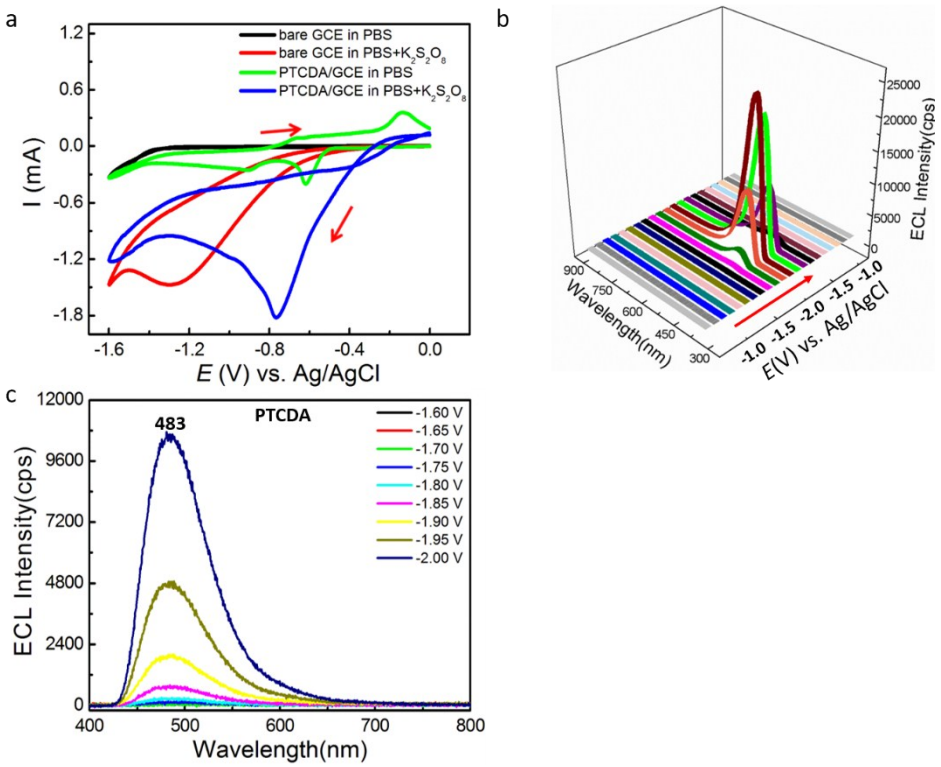

Fig. S2 a. The CV of bare GCE and PTCDA modified GCE in PBS in the absence and presence of $K_2S_2O_8$; b. The spooling spectroscopy of PTCDA modified GCE in PBS solution with $K_2S_2O_8$ as coreactant recorded at intervals of 4 s, during a cycle scanning potential range from -1 and -2.0 V with the scanning rate both at 25 mV/s; c. the stacked ECL spectroscopy of PTCDA during scanning potential range from -1.6 to -2.0.

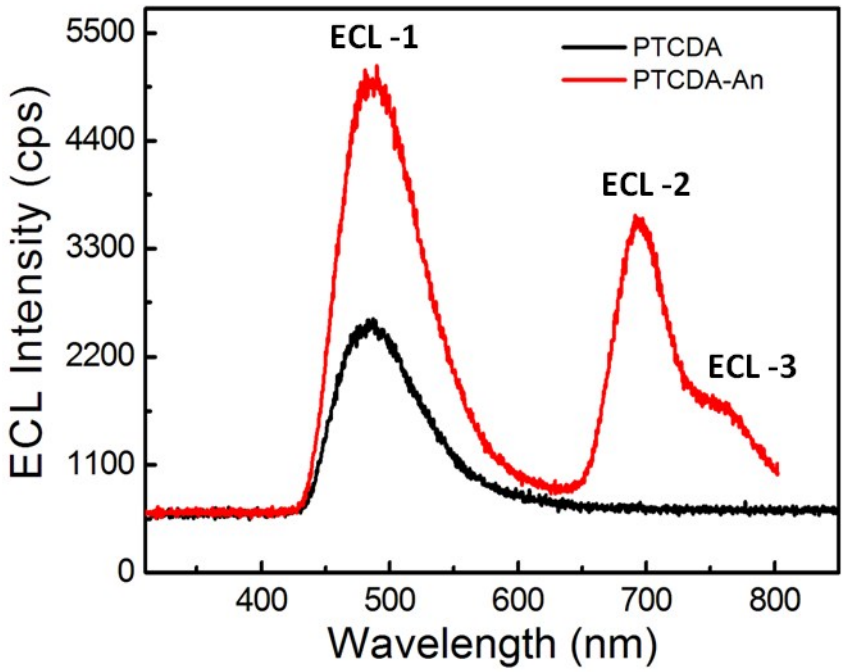

Fig. S3 the ECL spectroscopy of PTCDA(dark line) and PTCDA-An(red line) recorded at -1.9 V.

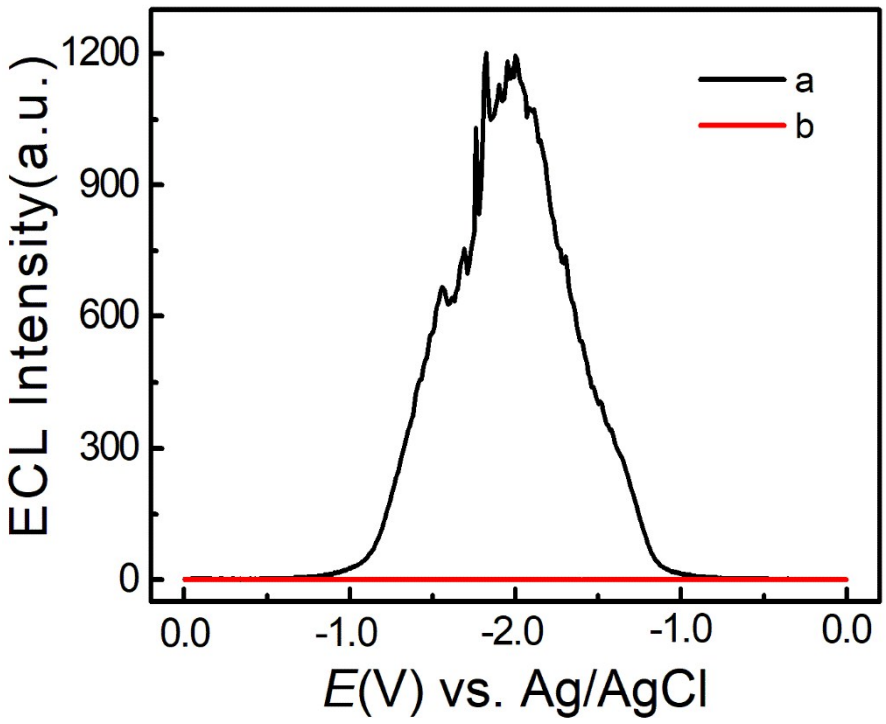

Fig. S4 a. the ECL of bare GCE in PBS with 0.1 M $K_2S_2O_8$ during a cycle scanning potential range from 0 to -2 V; b. the ECL of PTCDA modified GCE in PBS without $K_2S_2O_8$.

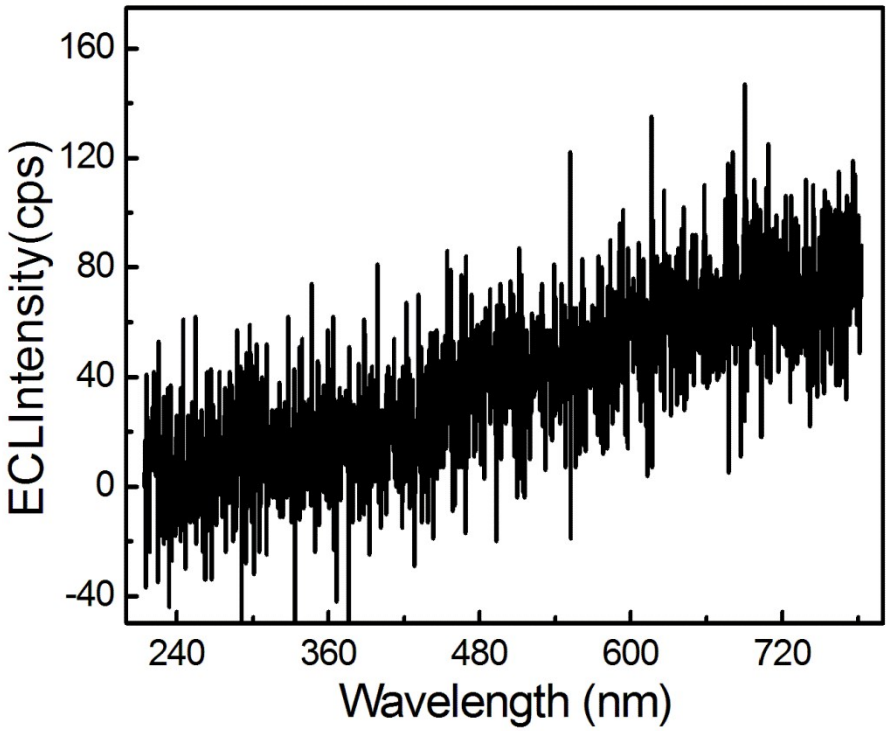

Fig. S5 The ECL spectroscopy of bare GCE in PBS with 0.1 M $K_2S_2O_8$ during cycle scanning potential range from 0 to -2 V.

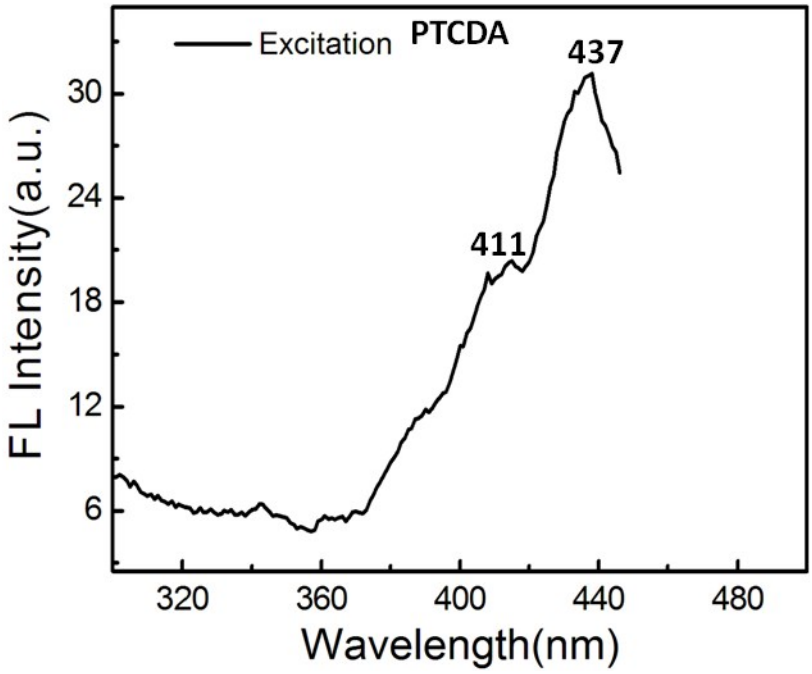

Fig. S6 the FL excitation spectra of PTCDA in aqueous solution.

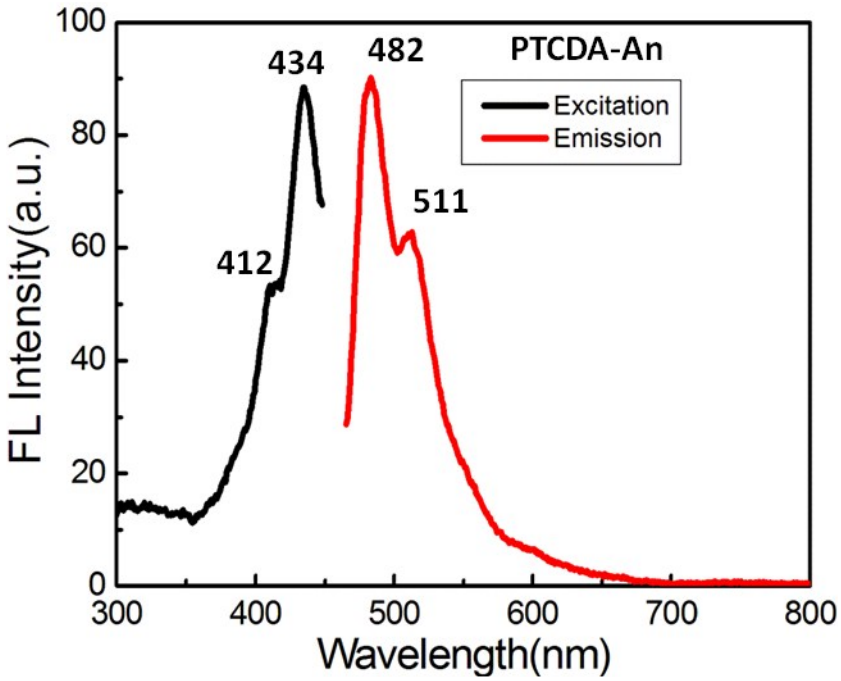

Fig. S7 the FL excitation(dark line) and emission(red line) spectra of PTCDA-An in aqueous solution. The excitation wavelength was hold at 434 nm.

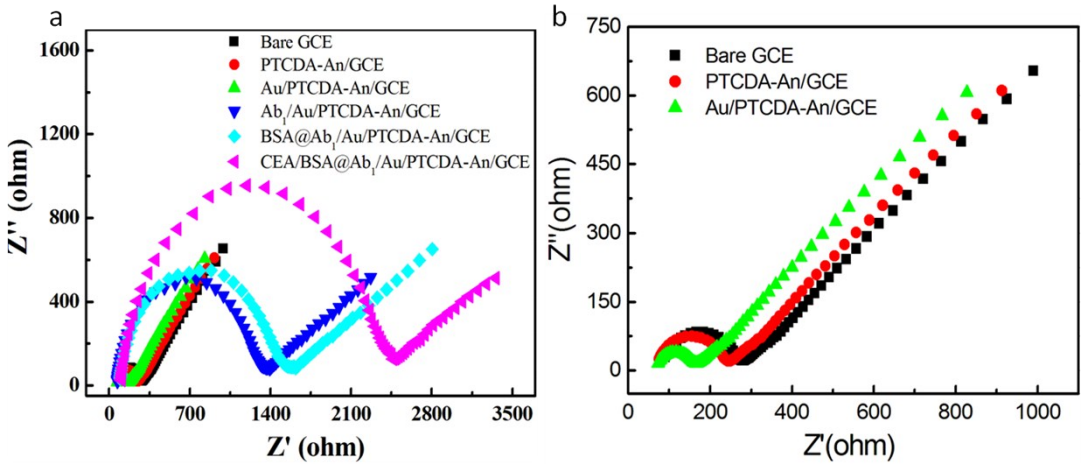

Fig. S8 a. the EIS of bare GCE, PTCDA-An modified GCE, Au/PTCDA-An modified GCE, Ab_1/Au/PTCDA-An modified GCE, BSA@Ab_1/Au/PTCDA-An modified GCE, CEA/BSA@Ab_1/Au/PTCDA-An modified GCE in PBS with 5 mM $K_2[Fe(CN)_6]$/ $K_3[Fe(CN)_6]$; b. the enlarged figure for EIS of bare GCE, PTCDA-An modified GCE, Au/PTCDA-An modified GCE in a.

Table S1 Comparison of the proposed ECL nanomaterial with other single-emission ECL nanomaterials for CEA detection using label-free ECL immunoassay.

sensor	Linear logarithmical range (ng mL^{-1})	Detection limit (ng mL^{-1})	Detection mechanism	References
Anti-CEA/AuNPs/C_3N_4	0.02-80	0.0068	ECL label-free	(Chen et al. 2014)[1]
Anti-CEA/AuNPs@CeO_2/MWCNTs/GO	0.05-100	0.02	ECL label-free	(Pang et al. 2015)[2]
NH_2-ssDNA/GLD/CS/ZnS–CdS/MoS_2	0.05-20	0.031	ECL label-free	(Wang et al. 2016)[3]
Anti-CEA/AuNPs/SWCNTs/CNOs/CS	0.0001–400	0.0001	EC label-free	(Rizwan etal. 2018)[4]
Anti-CEA/AuNPs/PDI-CH_3/GO	10^{-6} - 1000	2.9×10^{-7}	ECL sandwiched	(Zhang etal. 2019)[5]
Anti-CEA/AuNPs/PTCDA-An	0.001-10^4	0.00023	ECL label-free	This work

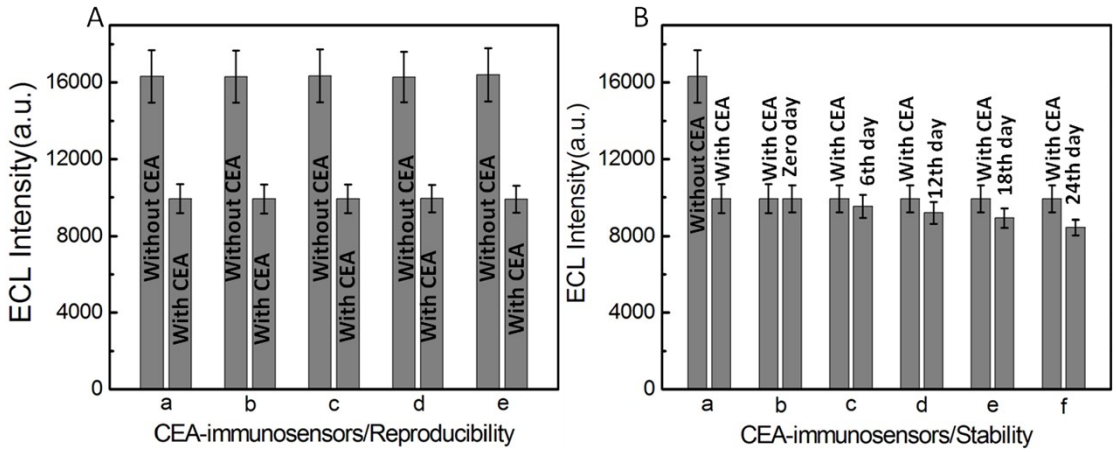

Fig. S9 A. Reproducibility, where pairs of bars from (a–e) without CEA (0 ng mL^{-1}) and with CEA (1 ng mL^{-1}) shows a consistent signal; and B. Long-term stability where pair of the bars shows ECL intensity: a. without CEA (0 ng mL^{-1}) and with CEA (1 ng mL^{-1}), b. with CEA (1 ng mL^{-1}) and with CEA (1 ng mL^{-1}) zero-day, c. with CEA (1 ng mL^{-1}) on zero-day and with CEA (1 ng mL^{-1}) on 6th day, d. with CEA (1 ng mL^{-1}) on zero-day and with CEA (1 ng mL^{-1}) on 12th day, e. with CEA (1 ng mL^{-1}) on zero-day and with CEA (1 ng mL^{-1}) on 18th day, and f. with CEA (1 ng mL^{-1}) on zero-day and with CEA (1 ng mL^{-1}) on 24th day.

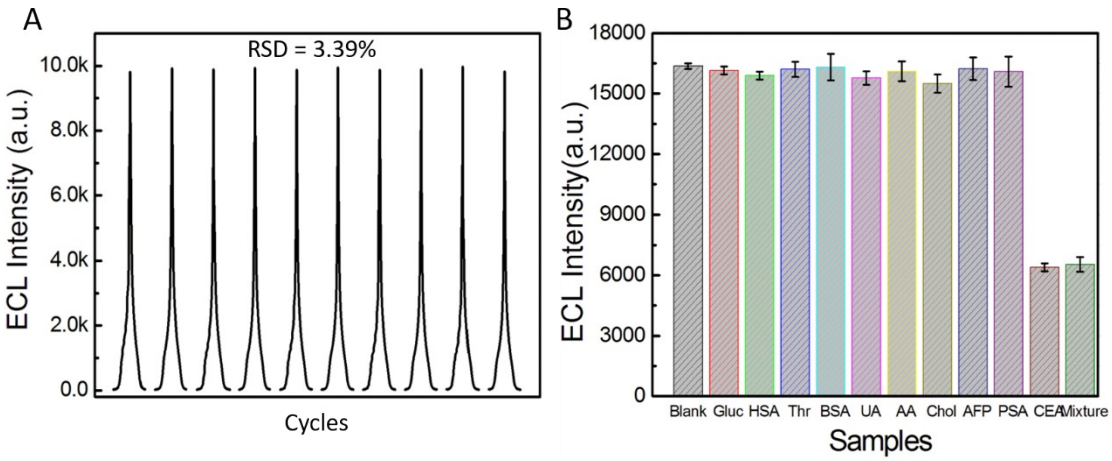

Fig. S10 A. The stability of immunosensor at C_{CEA} 1 ng mL^{-1}; B. Selectivity of the immunosensor toward 20 mg mL^{-1} glucose, 600 mg mL^{-1} HSA, 10 μg mL^{-1} thr, 10 μg mL^{-1} BSA, 700 mg mL^{-1} UA, 700 mg mL^{-1} AA, 20 mg mL^{-1} cholesterol, 10 μg mL^{-1} AFP, 10 μg mL^{-1} PSA, 1 μg mL^{-1} CEA and mixtures contained the above-mentioned analyte.

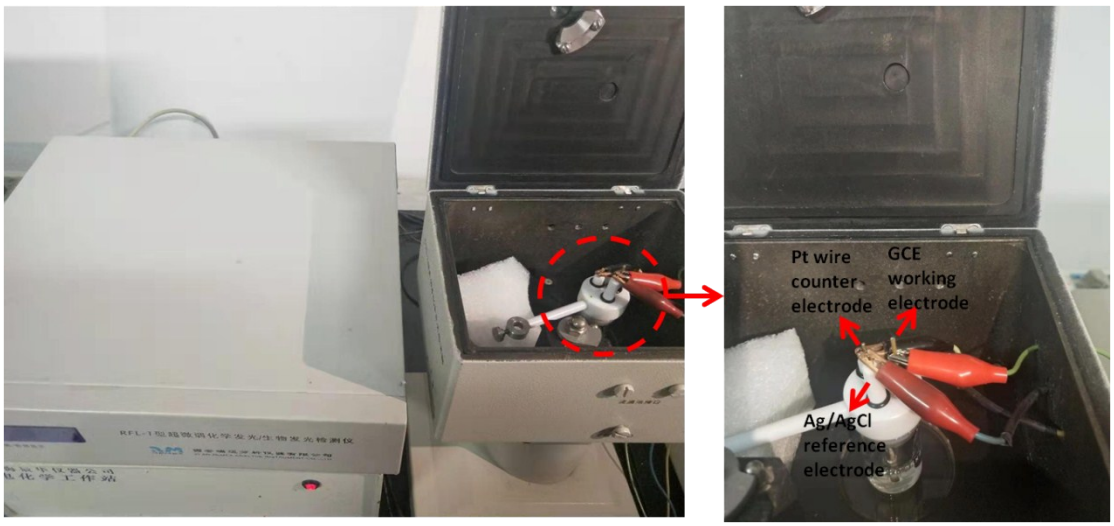

Fig. S11 The photo of experimental set-up for ECL assay

References

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