Electronic Supplementary Material (ESI) for Analytical Methods.

Covalent organic framework linked with amination luminol derivative as enhanced ECL luminophore for ultrasensitive analysis of cytochrome c

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Reagents and materials

Herein, 1,3,5-benzenetricarboxaldehyde (BTA) was purchased from Meryer (Shanghai) Chemical Technology Co., Ltd. (Shanghai, China). N-(4-aminobutyl)-N-ethylisoluminol (ABEI), oxalic acid, sodium bicarbonate ($NaHCO_3$), trifluoroacetic acid (TFA), acetonitrile, p-phenylenediamine (PDA) and n-(3-dimethylaminopropyl)-n-ethylcarbodiimide hydrochloride (EDC), streptavidin (SA), hemoglobin (Hb), sulphate ($ZnSO_4$) and ferric chloride ($FeCl_3$) were obtained from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Glucose injection and medical saline were acquired from Chimin Health Management Co., Ltd. Acetic Acid, and N,N-dimethylformamide (DMF) were acquired from J & K Chemical Technology. (Beijing, China). Human serum samples were gained from Jinhua City Central Hospital and stored at -20 °C. Absolute ethanol and dimethyl sulfoxide (DMSO) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Tri-n-propylamine (TPA), N-hydroxysuccinimide (NHS), streptavidin (SA), hemoglobin (Hb), thrombin from human plasma (TB), protein tyrosine kinase 7 (PTK-7), and cytochrome c (Cyt c) from equine heart were obtained from Sigma-Alrich (Shanghai, China).

Bovine serum albumin (BSA) and all of the oligonucleotides were purchased from Sangon Biotech Co, Ltd. (Shanghai, China), which were distilled with sterilization phosphate buffer solution (PBS, pH 7.4, 10X) from Sangon Biotechnology. The sequence of capture DNA (cDNA) was described as follows:

5'-NH_2-CCGTGTCTGGGGCCGACCGGCGCATTGGGTACGTTGTTGC-'3.

Phosphate buffer solution (PBS, pH 7.4, 0.1 M NaH_2PO_4/Na_2HPO_4) performed as the detection buffer. High-purity water was acquired from the Millipore water purification system (≥18MΩ, Milli-Q, Millipore) and used in all of the analysis.

Apparatus

Transmission electron microscopy (TEM) images were obtained by a JEM-2100 HR transmission electron microscope (Japan Electronics Co., Ltd, Japan). X-ray photoelectron spectroscopy (XPS) test was conducted at a K-Alpha XPS spectrometer excited with AlKα X-ray radiation (*hv* = 1486.6 eV, Thermo Fisher Scientific, America). X-ray diffraction (XRD) analysis was conducted from Bruker X-ray diffractometer D8 ADVANCE (Bruker (Beijing) Scientific Technology Co., Ltd. China). UV-visible (UV-vis) adsorption spectra were acquired at a Thermo nicolet evolution 500 UV-vis spectrophotometer (Beijing Puxi general instrument Co., Ltd., China) in a wavelength region of 200-800 nm. Fluorescence (FL) measurements were carried out on an RF-6000 spectrometer (Thermo Fisher Scientific, America). Fourier transform infrared spectroscopy (FT-IR) measurement was carried out at the Nicolet 670 spectrometer only in a mid-infrared range of 400-4000 cm^{-1} to fully disperse the sample in KBr pellet (Shimadzu Co., Japan).

Electrochemistry and electroluminescence (ECL) experiments were performed on the CHI 660D electrochemical workstation and MPI-E multi-function ECL analyzer (Xi'an Remax Analytical Instrument Co., Ltd., China). The ECL experiments were carried out by COFs-ABEI in the above buffer by applying a potential of 1.2 V.

Electrochemical impedance spectroscopy tests were conducted in a 0.1 M KCl solution containing 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in a frequency range of 0.1 ~ 100,000 Hz. Photoelectrochemical measurements were performed on the electrochemical workstation ZAHNER Zennium IM6 (ZAHNER, Germany) by using a white LED as the light source. Herein, the classic three-electrode system is used: A glassy carbon electrode (GCE, Φ = 5 mm) worked as the working electrode, an Ag/AgCl electrode (saturated KCl) as the reference electrode, and a platinum wire as the counter electrode.

The high performance liquid chromatography (HPLC) experiment were performed on the Shimadzu LC-20AD coupled with UV detector (λ = 214 nm) and C18 stationary column (Shimadzu, Japan), in which $NaHCO_3$ as a solvent for Cyt c detection, acetonitrile aqueous solution (90%) containing 0.1% TFA as eluent[1].

The ECL emission efficiency (ϕ_{ECL}) was evaluated exactly according to the follow-up equation:[2, 3]

$$\phi_{ECL} = \frac{\int_0^{t'} Idt}{\int_0^{t'} i_c dt} = \frac{\int_0^{t'} Idt}{Q_c}$$

where I is the total ECL intensity, t' refers to a finite period of time, i_c is divided by the integrated cathodic current, and Q_c is equal to the total charges over the same time period.

Fig. S1.

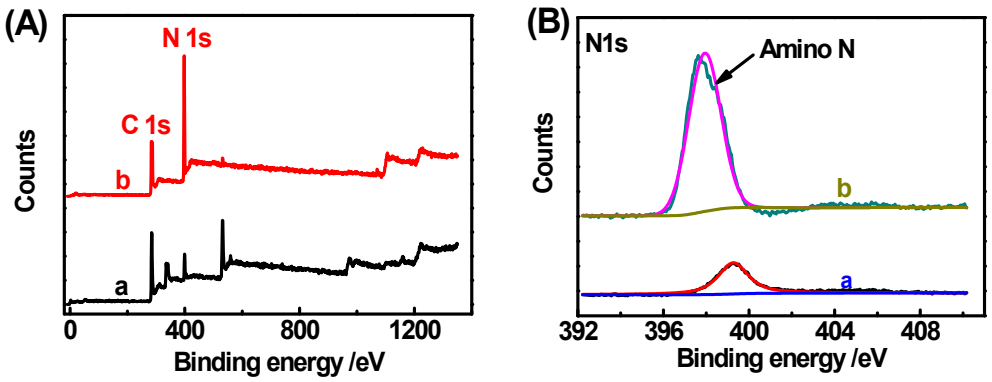

Figure S1. Survey (A) and high-resolution N 1s (B) XPS spectra of ABEI (curve a) and ABEI-COFs (curve b).

Fig. S2.

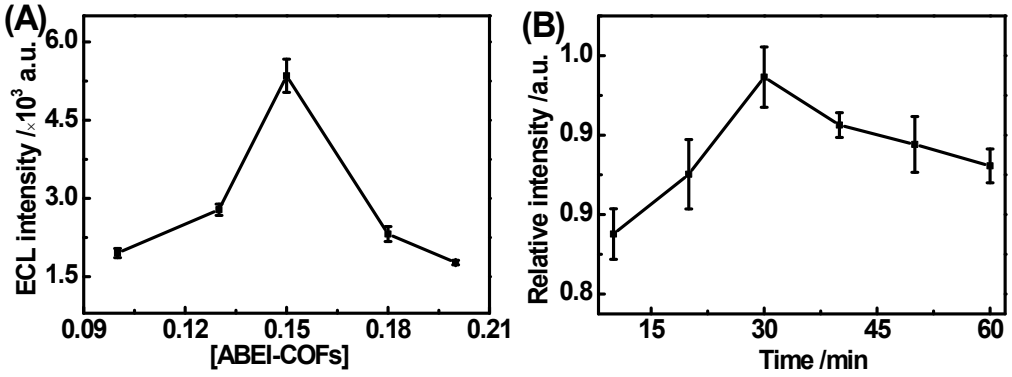

Figure S2. Influences of the ABEI-COFs (A) and incubation time (B) at 37 °C on the ECL responses of the built biosensor.

Fig. S3.

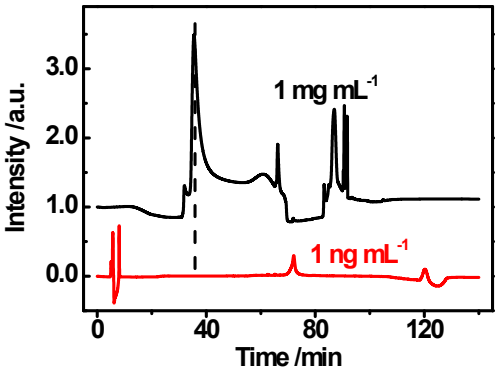

Figure S3. The HPLC plots of the Cyt c detection with different concentrations.

Table S1. Comparison of the analytical data of the as-fabricated biosensor for determination of Cyt c with those reported previously.

Methods	Linear ranges	Detection limits	References
SERS	12.38 ng mL^{-1}-12.38 μg mL^{-1}	12.38 ng mL^{-1}	[4]
FL	99.07 ng mL^{-1}-8.42 μg mL^{-1}	10.28 ng mL^{-1}	[5]
CE	12.38 μg mL^{-1}-7.43 mg mL^{-1}	42.11 μg mL^{-1}	[6]
CE	2.48 pg mL^{-1}-50.00 ng mL^{-1}	7.43 ng mL^{-1}	[7]
ECL	1.00 fg mL^{-1}-100.00 pg mL^{-1}	0.73 fg mL^{-1}	This work

SERS: Surface-Enhanced Raman Scattering.

Table S2. Detection results of Cyt c in the diluted serum samples (n = 3).

Samples	C_{added} (pg/mL)	C_{found}(pg/mL)	Recovery (%)	RSD (%)
1	1.0000	0.9069,1.0247,0.9910	97.42	6.23
2	0.1000	0.1058,0.0944,0.0984	99.54	5.85
3	0.0100	0.0094,0.0101,0.0092	94.66	5.16

Table S3. Detection results of Cyt c in the glucose injection samples (n = 3).

Samples	C_{added} (pg/mL)	C_{found}(pg/mL)	Recovery (%)	RSD (%)
1	1.0000	0.9969,1.0113,0.9719	99.33	5.17
2	0.1000	0.1049,0.0974,0.1034	101.90	5.05
3	0.0100	0.0093,0.0107,0.0097	99.00	4.93

Table S4. Detection results of Cyt c in the medical saline samples (n = 3).

Samples	C_{added} (pg/mL)	C_{found}(pg/mL)	Recovery (%)	RSD (%)
1	1.0000	0.9812,1.0367,1.0420	102.00	5.31
2	0.1000	0.1064,0.0964,0.0932	98.66	4.65
3	0.0100	0.0104,0.0098,0.0096	99.33	3.43

References

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