Electrochemical aptasensor based on Ce-MOF@COF to detect carcinoembryonic antigen

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Reagents

Sodium molybdate dehydrate ($Na_2MoO_4 \cdot 2H_2O$), cerium nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$), hydrogen peroxide (H_2O_2), trimellitic acid (H_3BTC), melamine ($C_3N_3(NH_2)_3$), Cyanuric acid($C_3H_3N_3O_3$), dimethyl sulfoxide (C_2H_6OS), chloroauric acid($H_2AuCl_4 \cdot 4H_2O$), Poly(ethylenimine) (C_2H_5N), sodium citrate($Na_3C_6H_5O_7 \cdot 2H_2O$), concentrated sulfuric acid (H_2SO_4), graphite powder, concentrated nitric acid (HNO_3), potassium permanganate ($KMnO_4$), hydrochloric acid (HCl), thiourea (NH_2CSNH_2), L-cysteine ($C_3H_7NO_2S$), ascorbic acid ($C_6H_8O_6$), were bought from Macklin Reagent Co. Ltd (Shanghai, China). Deionized water (DI) with an electrical resistance of 18.25 $M\Omega \cdot cm^{-1}$ was used in the whole study. Sangon Biotechnology Co. Ltd (Shanghai, China) purchased CEA, HRP, Apt1, and Apt2. The detailed sequences were shown below:

Apt1：5′-NH_2-$(CH_2)_6$-CTT TTA TAC CAG CTT ATT CAT TGG-3'

Apt2：5′-NH_2-$(CH_2)_6$-GAC CCA TAG GGA AGT GGG GGA TGT GTG TGT GTG TGT GTG TGT GTG-3′

Apparatus

All electrochemical measurements were performed on an RST5000 electrochemical workstation (Shanghai CH Instruments Co., China). Electrochemical impedance spectroscopy (EIS) was obtained from the impedance measurement unit (IM6e, ZAHNER Elektrik, Germany). Scanning electron microscopy (SEM) and energy dispersive spectrum (EDS) were carried out on a Gemini300 (Zeiss Co., Germany) with an acceleration voltage of 2.0 kV. X-ray diffraction (XRD) was performed on a D8 FOCUS (BRUKER AXS GMBH, Germany). X-ray Photoelectron Spectroscopy (XPS) was obtained on an X-ray Photoelectron Spectrometer (Thermo Scientific K-Alpha, United States). BET surface area (BET) was obtained with an Automatic surface area and porosity analyzer (Micromeritics ASAP 2460, United States).

Preparation of the Au@Ce-MOF@COF

Firstly, 0.50 g melamine and 0.51 g cyanuric acid were weighed, and 20 mL and 10 mL dimethyl sulfoxide were added respectively to form a transparent solution by magnetic stirring. The two solutions were mixed, stirred vigorously, and kept for 30 min, centrifuged and washed repeatedly with ethanol, and then dried at 60 °C to obtain the white product COF.

434 mg Ce(NO$_3$)$_3$ 6H$_2$O was fully dissolved in 40 mL ethanol-water solution (*V:V* = 1:1) by ultrasonic and stirring, and 210 mg H$_3$BTC was dispersed in 10 mL ethanol-water mixed solution (*V:V* = 1:1) by magnetic stirring for 1 h. Mix the two solutions, and then the mixture was refluxed at 90 °C for 2 h. After natural cooling to room temperature, it was centrifuged and washed with ethanol and water several times and then dried at 60°C. Get Ce-MOF.

For the synthesis of Ce-MOF@COF, 300 mg COF was added during the preparation of Ce-MOF to obtain Ce-MOF@COF.

Preparation of the Au@MoS$_2$@rGO

1.0 g graphite powder was added to mixed acid ($V_{H_2SO_4}$: V_{HNO_3}=9: 1) medium. Then, 6.0 g KMnO$_4$ was added to the mixed acid, and the reaction was stirred at 90 °C for 12 h, and 30% H$_2$O$_2$ was added until there were no bubbles. After centrifugal separation, it was washed with HCl and H$_2$O and dried at 60 °C. Successfully prepared to GO

500 mg GO was added to 20 mL H$_2$O and passed through 30 min of ultrasonication to obtain a homogeneous and transparent yellow-brown suspension. Subsequently, 0.1053 g Na$_2$MoO$_4$·2H$_2$O was weighed and added to the above suspension and sonicated for 1 h. The solution was adjusted to 80 mL by adding ultrapure water, and then 0.2710 g thiourea was added under constant stirring, and stirring was maintained until the solution was well dispersed. Afterward, it was transferred to a polytetrafluoroethylene liner and reacted in an oven at 220 °C for 24 h. After cooling to room temperature, it was centrifuged and washed several times to obtain a black precipitate, which was dried at 60 °C to obtain the product MoS$_2$@rGO.

Weighing 10 mg MoS$_2$@rGO composite was dispersed in 10 mL ultrasonic water, and after ultrasonic treatment for 30 min, 1 mL 5 mmol/L L-cysteine solution was added to the suspension, and then 1 mL 1% HAuCl$_4$ was added to the mixture, and after vigorous stirring for 30 min, 10 mL 5 mmol/L ascorbic acid was quickly added. L-ascorbic acid was stirred vigorously for 3 h, centrifuged and washed several times, and dried at 60 °C to obtain the black precipitate Au@MoS$_2$@rGO.

Real time photo images of the electrode and setup

A

Pt electrode →
Ag/AgCl electrode →
Working electrode →
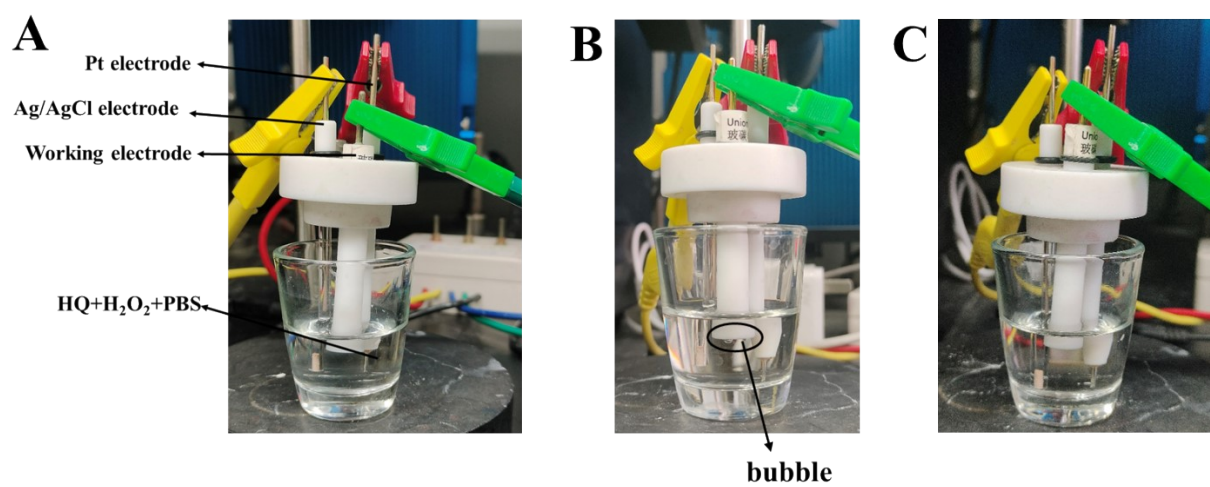
HQ+H$_2$O$_2$+PBS →

B

bubble

C

Fig. S1 Real time photo images of the electrode in DPV test (-0.4 V-0.4V): (A) before testing; (B) under testing; (C) after testing.
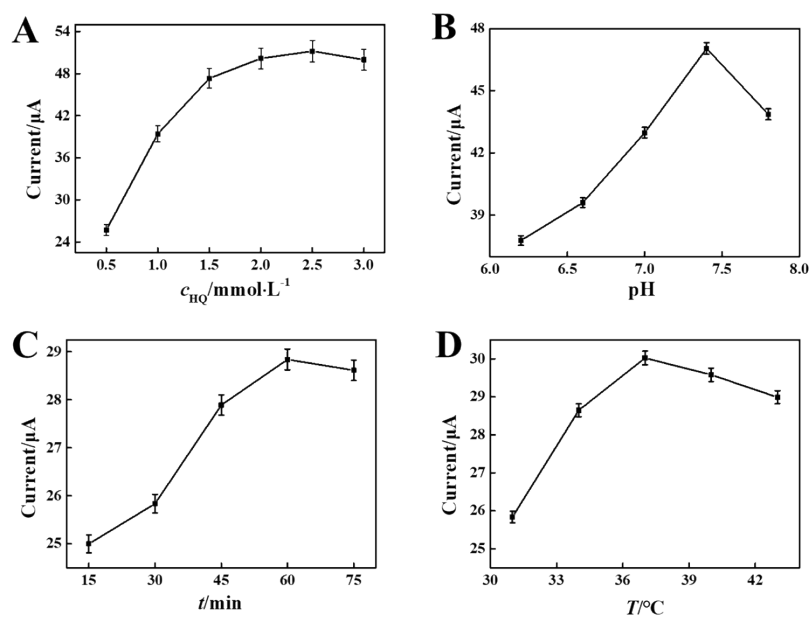
Optimizations of experimental conditions

Figure S2 (A) the optimizations of HQ concentration; (BC) pH of PBS, (C) incubation time, and (D) incubation temperatures on aptasensors' performance. (Error bars: ± standard deviation (SD), $n = 5$).

Performance comparison of this proposed method with previously reported methods for CEA detection.

Table S1. Performance comparisons of this proposed method with previously reported methods for CEA detection.

Method	Linear range	LOD	Ref.
Colorimetry	0.1 - 20 ng/mL	0.05 ng/mL	[1]
FL aptasensor	0.05 - 10 ng/mL	0.008 ng/mL	[2]
PEC immunosensor	0.02 - 50 ng/mL	15.0 pg/mL	[3]
FL sensor	0.5 pg/mL - 100 pg/mL	0.46 pg/mL	[4]
ECL immunosensor	0.0001 - 10 ng/mL	0.085 fg/mL	[5]
Electrochemical immunosensor	0.005 ng/mL - 4.0 ng/mL	1.9 pg/mL	[6]
Electrochemical aptasensor	0.1 pg/mL - 100 ng/mL	0.019 pg/mL	This work

Analytical performance comparisons of the proposed method and other reported methods for CEA detection.

Table S2. The analytical performance comparisons of the proposed method and other reported methods for CEA detection.

Method	Reproducibility [a] (%)	Stability [b] (%)	Ref.
Electrochemical immunosensor	3.2	16%	[7]
ECL immunosensor	3.8	12.2%	[8]
PEC immunosensor	2.13	12%	[9]
Electrochemical aptasensor	1.9	3.2%	This work

a, the relative standard deviation (RSD) of the detection signal between parallel samples;
b, the reduction rate of the signal in detection system for different storage days.

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