Signal–off electrochemiluminescence immunosensor based on AgS quantum dots quenching luminol modified $Ag/Cu_2O/Ti_3C_2$ nanocomposites for h–FABP detection

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Chemicals

Luminol, Ti_3C_2 nanosheets, polyvinylpyrrolidone (PVP), copper sulfate ($CuSO_4$), $FeCl_3 \cdot 6H_2O$, and 2-aminoterephthalic acid were obtained from Macklin Biochemical Co., Ltd. (Shanghai, China). Silver nitrate, hydrazine hydrate (80%), bovine serum albumin (BSA), and glutaraldehyde (GA) were all sourced from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, sodium chloride, N,N-dimethylformamide (DMF), potassium chloride, disodium hydrogen phosphate, and sodium dihydrogen phosphate were all acquired from Tianjin Chemical Reagent Company (Tianjin, China). The ultrapure water utilized in this study was produced by a water purification system with a resistivity of 18.2 MΩ. The electrolyte solution consisted of a phosphate-buffered saline (PBS, 0.1 M, pH 7.4) prepared by mixing 0.1 M sodium dihydrogen phosphate and disodium hydrogen phosphate.

Instruments

The ECL measurement was performed on the RFL-1 chemiluminescence analyzer (Xi'an Ruimai Analyzer Co., Ltd.). Electrochemical measurements were carried out using the CHI 760D electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd., China). Transmission electron microscopy (TEM) analysis was conducted with the JEM-200 kV 2100 instrument (JEOL). Ultraviolet-visible absorption spectra were recorded using the UH4150 spectrophotometer (Hitachi). X-ray diffraction (XRD) patterns were obtained using the D/Max2500 system (Rigaku Corporation, Japan). Scanning electron microscopy (SEM) images were acquired with the Hitachi SU-70 instrument (Carl Zeiss AG, Germany). Fourier transform infrared (FT-IR) spectroscopy

was performed using the Nicolet iS50 FT-infrared spectrophotometer (Thermo Fisher Scientific Co., Ltd.). X-ray photoelectron spectroscopy (XPS) data were collected using the Escalab 250Xi electron spectrometer (Thermo Fisher Scientific Co., Ltd.).

Synthesis of NH_2-MIL-101(Fe)

NH_2-MIL-101(Fe) was synthesized according to the described method[1]. In detail, 0.5 mM $FeCl_3 \cdot 6H_2O$ and 0.25 mM 2-aminoterephthalic acid were dissolved in 6 mL DMF, ultrasonicated for 30 minutes, and transferred to a reaction vessel. A hydrothermal reaction was conducted at 110 °C for 20 hours. Afterward, the product was washed with ethanol and dried at 60 °C for 8 hours.

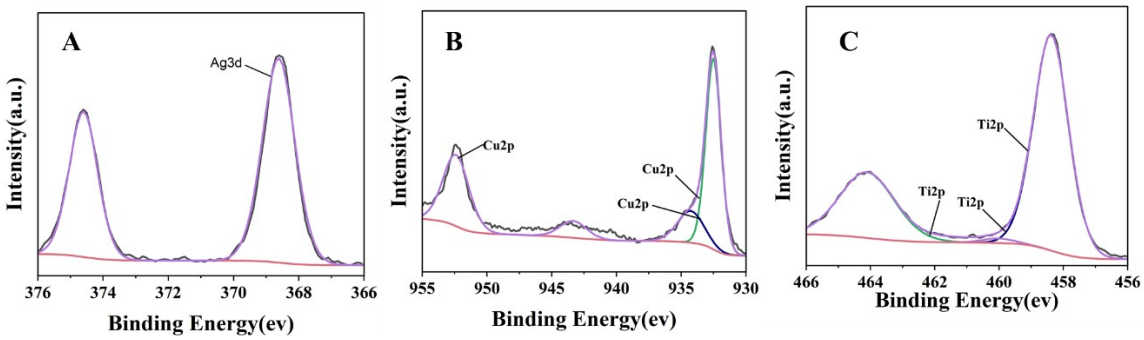

Fig. S1. XPS spectra of the Ag 3d (A), Cu 2p (B), and Ti 2p (C).

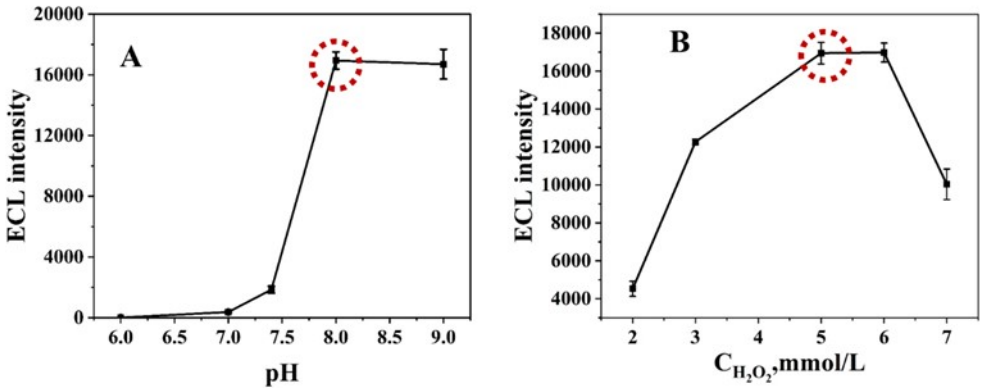

Fig. S2. Condition optimization: optimization of pH (A) and H_2O_2 concentration (B) for the reaction of Luminol@Ag/Cu_2O/Ti_3C_2.

Optimize the experimental conditions to enhance the efficiency of the ECL immunosensor. The influence of pH on the ECL system was investigated using PBS buffers ranging from pH 6.0 to 9.0. As shown in Fig. S2A, the ECL intensity increased with pH from 6.0 to 8.0 and then peaked. This is because higher pH values within this range promote the generation of superoxide radicals, enhancing the excited state AP^{2-*} and increasing the ECL signal. However, excessively high pH may reduce the activity of antigens and antibodies, affecting the ECL efficiency and detection performance. Therefore, the optimal pH was determined to be 8.0. Additionally, the hydrogen peroxide concentration was optimized, with a concentration of 5 mM yielding the highest ECL intensity (Fig. S2B). Thus, the optimal conditions were established as PBS buffer at pH 8.0 and 5 mM hydrogen peroxide.

Characterization of the prepared ECL immunosensor

The surface modifications of ITO were systematically evaluated through ECL and electrochemical impedance spectroscopy (EIS), aiming to investigate the performance variations of the developed ECL immunosensor across different fabrication stages. As illustrated in Fig. S3A, the unmodified ITO electrode exhibited a low electron transfer resistance (Ret) (curve a). Upon modification with the Luminol@Ag/Cu_2O/Ti_3C_2 nanocomposite (curve b), there was a substantial increase in Ret because of restricted electron transfer. Further steps, such as the attachment of Ab_1 (curve c), BSA blocking (curve d), recognition of h–FABP (curve e), and binding of Ab_2 (curve f), led to a gradual enhancement of Ret. This rise was caused by the insulating properties and steric hindrance of protein molecules, reducing electron transfer efficiency at the electrode surface. The significant differences in Ret values between steps confirmed specific antibody–antigen interactions, validating the biosensor's integrity and functionality. As shown in Fig. S3B, the bare electrode had negligible ECL signal intensity (curve a). In contrast, the Luminol@Ag/Cu_2O/Ti_3C_2–modified ITO electrode emitted an ECL signal of 17,000 arbitrary units (curve b). Sequential layer–by–layer modifications progressively reduced the ECL signal intensity (curves c–f). Finally, after modification with Ab_2–AgS QDs@NH_2–MIL–101(Fe), the sensor achieved the most significant quenching effect. These results confirmed the successful fabrication of the quenching–type ECL immunosensor.

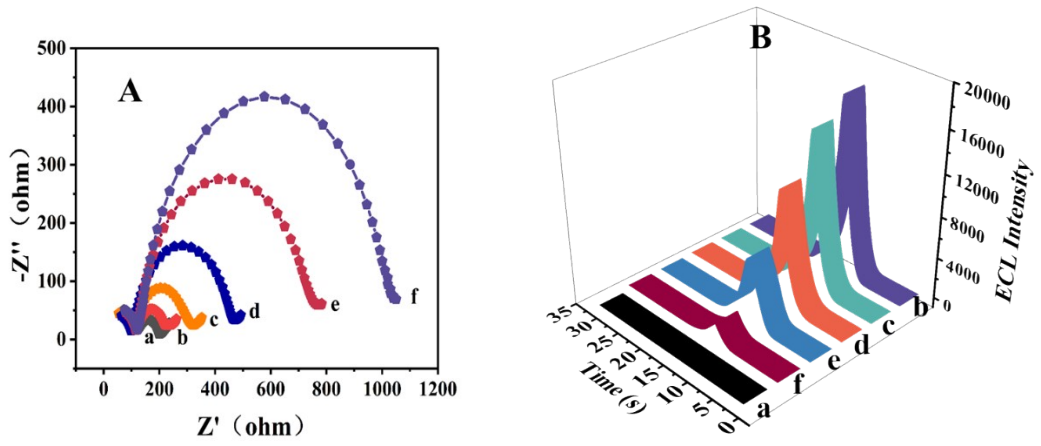

Fig. S3. EIS curves (A) and ECL–time curves (B) in different states: bare ITO (a), Luminol@Ag/Cu_2O/Ti_3C_2/ITO (b), Ab_1/Luminol@Ag/Cu_2O/Ti_3C_2/ITO (c), BSA/Ab_1/Luminol@Ag/Cu_2O/Ti_3C_2/ITO (d), h–FABP/BSA/Ab_1/Luminol@Ag/Cu_2O/Ti_3C_2/ITO (e) and Ab_2–AgS QDs@NH_2–MIL–101/h–FABP/BSA/Ab_1/Luminol@Ag/Cu_2O/Ti_3C_2/ITO (f).

Table S1 Comparison of different sensors for the determination of h–FABP.

Detecting Method	Linear Range (ng mL^{-1})	LOD	Reference
Microfluidic ECL Immunoassay	1 ~100	0.71 ng mL^{-1}	[2]
Fluorescence Immunoassay	0.5 ~100	100 pg mL^{-1}	[3]
Electrochemical Immunoassay	0.0001 ~100	1.47 ng mL^{-1}	[4]
ECL Immunoassay	0.0001 ~100	44.5 fg mL^{-1}	[4]
Photoelectric Immunoassay	0.1 ~100	10 pg mL^{-1}	[5]
ECL Immunoassay	$1.0*10^{-6}$ ~100	0.36 fg mL^{-1}	This work

Table S2. Determination results of h–FABP in human serums.

Samples (ng mL^{-1})	Addition (ng mL^{-1})	Average (ng mL^{-1}, n=5)	Recovery (%)	RSD (%)
0.08	0.01	0.093	103.71%	3.38%
	0.10	0.187	103.70%	9.22%
	1.00	1.126	104.25%	2.84%
	10.00	9.594	95.18%	8.38%

Reference

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