Supplementary Information

Highly Stable ECL Sensor Based on Self-Supplied H_2O_2 Probe and MOF Nanozyme for Ultrasensitive Environmental Mycotoxin ZEA Detection

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S1. Apparatus

The ECL measurements were performed using an MPI-E ECL analyzer (Xi'an Remax Electronic Science Tech. Co. Ltd., China) combined with an ECL spectrum analyzer comprising an Acton SP2300i mono-chromator equipped with a liquid N_2-cooled PyLoN 400BReXcelon digital charge-coupled device (CCD) detector (Princeton Instruments) and a VersaSTAT 3 electrochemical analyzer (Princeton Applied Research). All electrochemical data were recorded with a CHI760D electrochemical workstation (Chenhua, China). Surface morphology was characterized by scanning electron microscopy (SEM) (Zeiss, Germany). The immunosensing system was tested using a standard three-electrode configuration, which contains working electrode (modified glassy carbon electrode), a reference electrode (Ag/AgCl electrode), and a counter electrode (platinum electrode). X-ray diffraction (XRD) patterns were obtained with a D8 Focus diffractometer (Bruker AXS, Germany), while X-ray photoelectron spectroscopy (XPS) patterns were acquired using an X-ray photoelectron spectrometer (Thermo Scoemtific K-Alpha).

S2. Chemicals.

All chemicals used in the entire experiment were commercially available and used without further purification. Copper sulfate ($CuSO_4$), phthalic acid (PA), sodium citrate ($Na_3C_6H_5O_7$), sodium hydroxide (NaOH), ascorbic acid (AA), terbium nitrate ($Tb(NO_3)_3$), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), copper nitrate ($Cu(NO_3)_2$), luminol, and cysteine (Cys) were purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide trihydrate ($K_4[Fe(CN)_6]\cdot 3H_2O$), and N,N-dimethylformamide (DMF) were obtained from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). N-hydroxysuccinimide (NHS) was acquired from Aladdin Biotechnology Co., Ltd. (Shanghai, China). The 0.1 M phosphate-buffered saline (PBS) was prepared by mixing Na_2HPO_4 and KH_2PO_4 solutions at appropriate ratios. All experimental procedures utilized ultrapure water (resistivity ≥18 MΩ cm^{-1}) supplied by a Millipore water purification system. All chemical reagents were of analytical grade. Deionized (DI) water with a resistivity of 18.25 MΩ cm^{-1} was employed throughout the study. The nucleic acid sequence were as follows：

DNA-1: 5’ − NH_2 − C_6 − TCA TCT ATC TAT GGT ACA TTA CTA TCT GTA ATG TGA G − 3’.

DNA-2: 5’ − SH − C_6 − TAT GGT ACA TTA CTA TCT GTA ATG TGA TAT G − 3’.

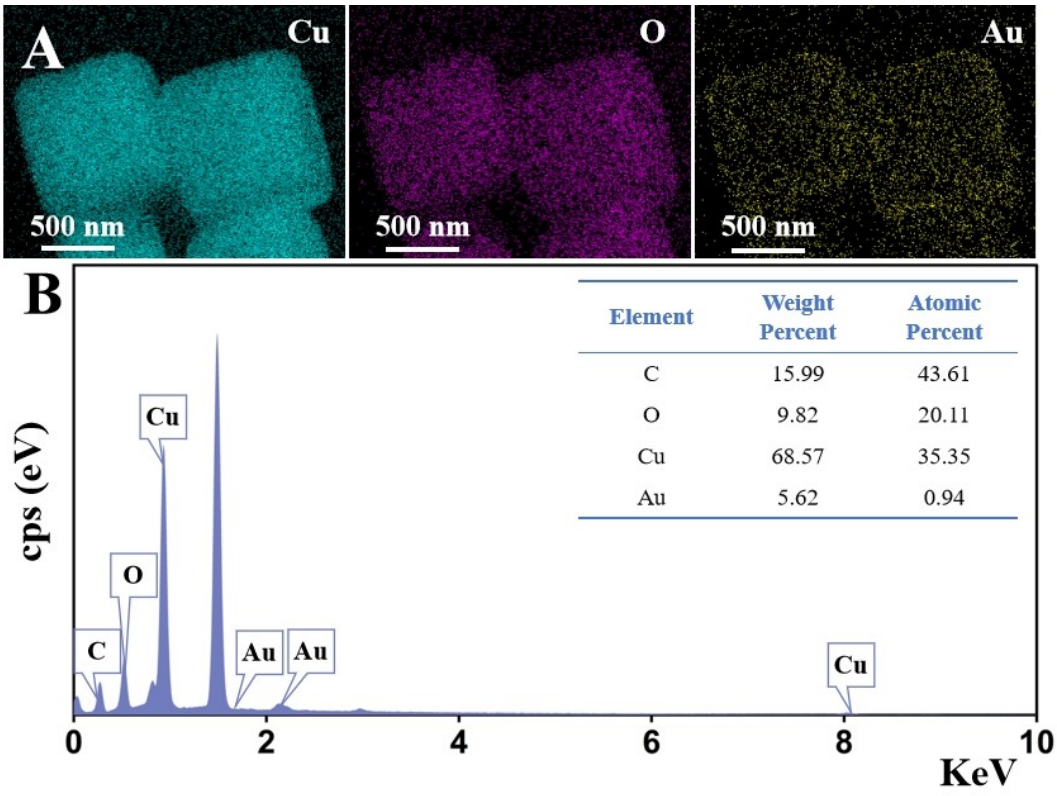
Preparation of Cu_2O@Au NPs. Cu_2O@Au NPs were synthesized according to a previously reported method with slight modifications[15]. Briefly, 375 mg $CuSO_4 \cdot 5H_2O$ was dissolved in 100 mL of deionized water under continuous stirring. Then, 174 mg of sodium citrate and 1.0 g of sodium hydroxide were added, and the mixture was stirred for an additional 30 min. Subsequently, 25 mL of 0.06 M ascorbic acid (AA) solution was added dropwise under constant stirring, followed by stirring for another 60 min. After centrifugation, the precipitate was washed and dried to obtain Cu_2O. To prepare Cu_2O@Au NPs, 5 mg of the as-prepared Cu_2O was dissolved in 30 mL of ultra-pure water. Then, 50 μL of $HAuCl_4$ solution (1 wt%) was added dropwise under stirring, and the reaction was allowed to proceed for 3 h.[16] The final product, Cu_2O@Au NPs, was obtained after centrifugal washing and drying.

Preparation of Tb-Cu MOF@CS-Au. Tb-Cu MOF@CS-Au nanocomposites were prepared by mixing the Tb-Cu MOF suspension with 0.5% chitosan (CS) solution in a volume ratio of 3:1. The mixture was vortexed or stirred until uniformly blended, resulting in the Tb-Cu MOF@CS complex. Then, 1 mL of Au NPs solution was introduced into the aforementioned solution, and the mixture was incubated at 4 ℃ with continuous oscillation overnight. This procedure ultimately resulted in the preparation of the Tb-Cu MOF@CS-Au solution.

Preparation of Cu_2O@Au NPs-cys-Luminol-DNA 2. To prepare the final probe, 5 mg of Cu_2O@Au NPs-cys-Luminol were mixed with 0.75 mL of PBS solution and 0.25 mL of 10 mM DNA 2 solution. The mixture was thoroughly homogenized and incubated at 4 °C for 24 h to allow for DNA attachmen. Following incubation, the probe was purified by centrifugation and redispersed in 1 mL of PBS buffer (pH 7.4), yielding Cu_2O@Au NPs-cys-Luminol-DNA 2 for further application.

S3. ECL and Electrochemical Measurements

The ECL and electrochemical measurements were conducted using a three-electrode system consisting of a modified glassy carbon electrode (GCE) as the working electrode, a platinum wire as the counter electrode, and a saturated Ag/AgCl electrode as the reference electrode, in 10 mL of PBS (0.1 M, pH 7.4). The ECL measurements were performed in the potential range of 0 to +0.6 V at a scan rate of 150 mV/s, with the photomultiplier tube (PMT) voltage set at 600 V. The differential pulse voltammetry (DPV) measurements were conducted within the same potential range, with a pulse amplitude of 0.05 V and a pulse width of 0.05 s.



Element	Weight Percent	Atomic Percent
C	15.99	43.61
O	9.82	20.11
Cu	68.57	35.35
Au	5.62	0.94

Figure. S1 Element mapping (A) and EDS (B) of Cu_2O@Au.

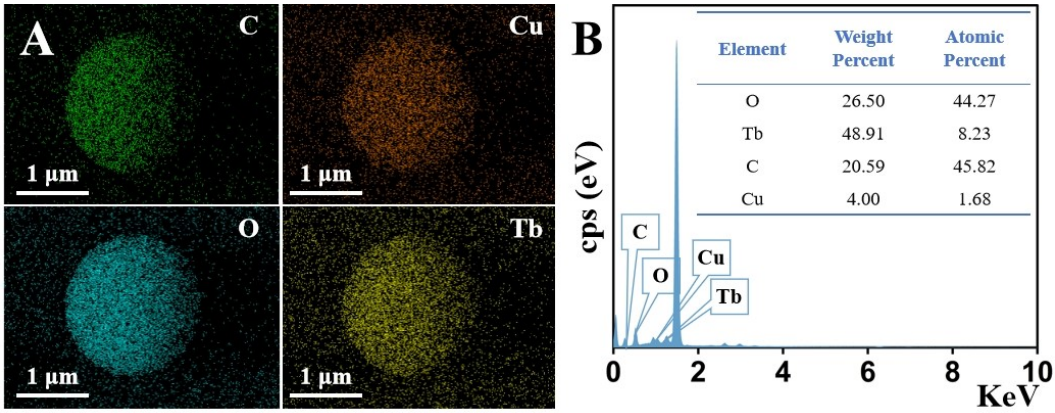


Figure. S2 Element mapping (A) and EDS (B) of Tb-Cu MOF.

S4. ECL sensor luminescence mechanism

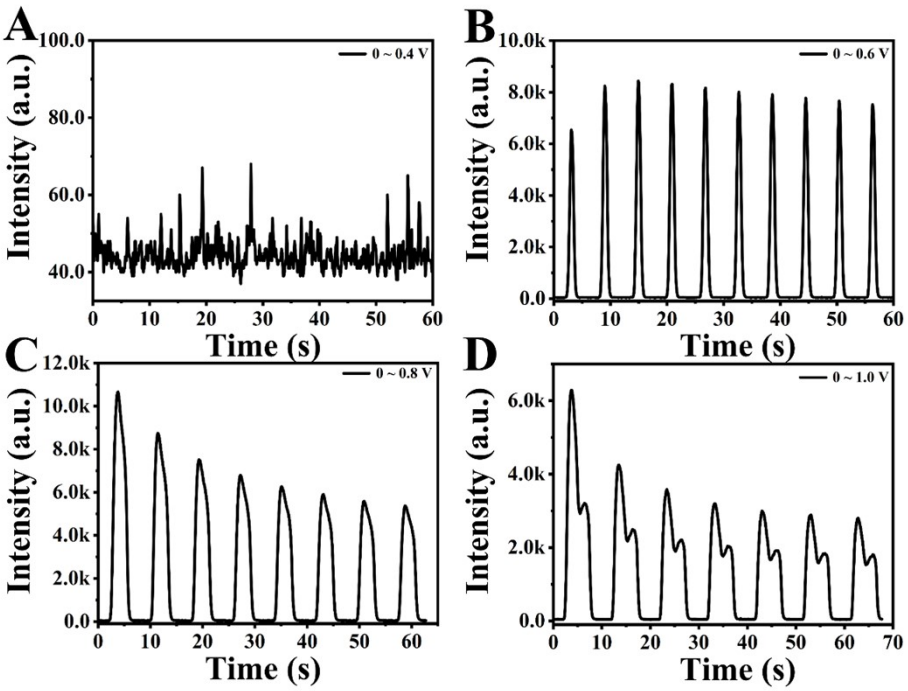


Figure S3. The ECL signals obtained under different applied potential ranges were evaluated as follows: (A) 0−0.4 V, (B) 0−0.6 V, (C) 0−0.8 V, and (D) 0−1.0 V.

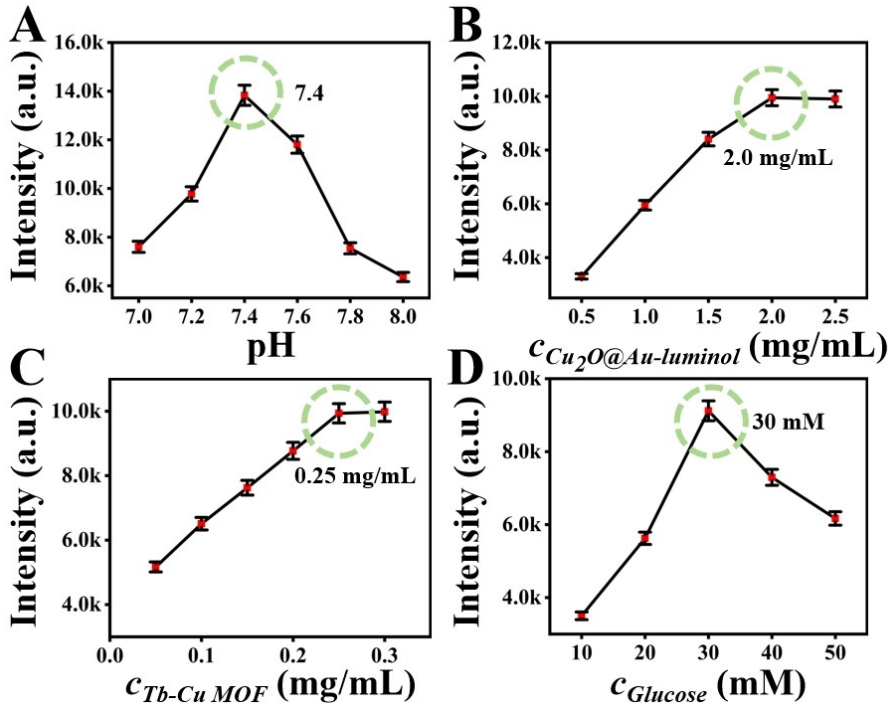


Figure S4. Optimization of conditions for (A) pH, (B) Glucose concentration, (C) luminescent material concentration, (D) substrate material.

S5. Comparison with other detection methods

Table S1 Comparison with Other Detection Methods

method	linear range（ng mL^{-1}）	detection limit（ng mL^{-1}）	references
ULISA	50 pM – 0.5 nM	2×10^{-4} nM	[1]
EC	0.01 nM – 50 nM	5×10^{-3} nM	[2]
iSPR	0 nM – 20 nM	24 μM	[3]
EC	159.2 nM – 2865.2 nM	23.14 nM	[4]
ECL	0.1 pM – 1 nM	1.7×10^{-8}nM	This work

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