Supporting Information

For

Valence states defects mediated copper nanoclusters nanozyme with high peroxidase-like activity for multimodal colorimetric detection of mercury(II)

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2. Experimental section

2.1 Reagents and chemicals

2,4-Toluenediamine (2,4-TDA), 3,3',5,5'-tetramethylbenzidine (TMB), copper sulfate pentahydrate ($CuSO_4$•$5H_2O$), 1,4-benzoquinone (*p*-BQ) were obtained from Titan Scientific Co., Ltd. (Shanghai, China). Hydrogen peroxide (H_2O_2) was obtained from Jiangsu Tongsheng Chemical Reagent Co., Ltd. (Jiangsu, China). Mercuric nitrate ($Hg(NO_3)_2$) was purchased from Taixing Chemical Reagent Co. Ltd. (Jiangsu, China). Histidine (His), silver nitrate ($AgNO_3$), potassium chloride (KCl), calcium chloride ($CaCl_2$), nickel chloride ($NiCl_2$), barium nitrate ($Ba(NO_3)_2$), aluminum chloride ($AlCl_3$) and magnesium sulfate ($MgSO_4$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cysteine (Cys), glutathione (GSH), chromium(III) chloride ($CrCl_3$), zinc nitrate hexahydrate ($Zn(NO_3)_2$•$6H_2O$) and cadmium chloride ($CdCl_2$) were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Thiourea (TU) and cobaltous nitrate hexahydrate ($Co(NO_3)_2$) were obtained from Shanghai Shanpu Chemical Co., Ltd. (Shanghai, China). Lead(II) nitrate ($Pb(NO_3)_2$) was obtained Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). NaAc-HAc buffer solution (pH 3.6 - 5.6, 0.2 M) were prepared by varying the ratio of HAc to NaAc. Other solvents and chemicals used in the experiment were of analytical grade and required no further purification. Ultrapure water was used throughout the experimental process.

2.2 Instruments

The ultraviolet-visible (UV-vis) spectra were measured using a Shangfen L6S UV-visible spectrophotometer (Shanghai, China). Transmission electron microscopy (TEM) images were conducted on a JEOL JEM-2100 transmission electron microscope (Japan). The fourier transform infrared (FT-IR) spectrum was recorded on a Nicolet 5700-IR spectrometer (USA). X-ray photoelectron spectroscopy (XPS) was performed on a Thermo ESCALAB 250XI X-ray photoelectron spectrometer (USA). All the pictures involved were taken with the help of a smartphone.

2.3 Synthesis of copper nanoclusters (CuNCs)

For preparation of CuNCs, firstly, 2,4-TDA (500 μL, 75 mM) was added to 100 μL $CuSO_4$ solution (0.1 M), and the mixed solution was continuously stirred using a Vortex mixer at ~2000 rpm for 3 min, i.e., CuNCs. Finally, the synthesized CuNCs was

dialyzed for 10 min at 8,000 rpm through ultrafiltration centrifuge tubes (500 molecular weight cutoff) to obtain pure CuNCs.

2.4 Peroxidase (POD)-like catalytic activity assay

To evaluate the POD-like catalytic activity of CuNCs, 50 μL of CuNCs (0.7 mg/mL) was mixed with 10 μL of NaAc-HAc buffer (pH 4.0, 0.2 M). Subsequently, 100 μL of TMB (20 mM) and 30 μL of H_2O_2 (0.2 M) were added to the catalytic system, and the final volume was adjusted to 500 μL with deionized water. After incubation at room temperature for 15 min, photographs were captured to record the color change observed in the reaction solution, followed by detecting the UV-vis absorption changes at 652 nm (A_{652}). In addition, experiments were performed to optimize the reaction conditions using different CuNCs final concentrations (42 - 125 μg/mL), reaction times (5 - 30 min), pH (3.6 - 5.7), and temperatures (25 - 60 °C), all following the same procedures as above.

2.5 Calculation of the catalytic kinetics and specific activity

Under the optimal reaction conditions for POD-like activity, different concentrations of TMB, H_2O_2 and CuNCs were used to continuously record the changes in the absorption peak at 652 nm for 15 min, with a recording interval of 10 s. The enzymatic reaction velocity and substrate concentration were used to fit the *Michaelis-Menten* equation to calculate the typical catalytic kinetic constants and specific activity, these included the *Michaelis* constant (K_m), maximal reaction velocity (V_{max}) and specific activity (SA) values of the CuNCs.

2.6 Hg^{2+} colorimetric detection protocol

For Hg^{2+} analysis, 40 μL Cys (1.0 mM) was premixed with 1.0 mM Hg^{2+} (variable volume) for 5 min. Subsequently, 50 μL CuNCs (0.7 mg/mL), 100 μL TMB (20 mM), 30 μL H_2O_2 (0.2 M), and 10 μL HAc-NaAc buffer (0.2 M, pH 4.0) were added. The mixture was diluted to 500 μL with deionized water, incubated at 50 °C for 15 min, and analyzed via UV-vis spectroscopy and visual colorimetry.

2.7 Specificity assessment

To evaluate the specificity of this colorimetric detection method for Hg^{2+}, various common metal cations (including Pb^{2+}, Cd^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Ba^{2+}, Ca^{2+}, Zn^{2+}, Mg^{2+}, Ag^{+}, K^{+}, Cu^{2+} and Al^{3+}) found in aquatic solutions (with concentration 0.1 mM, but Ag^{+} of 0.03 mM) were introduced into the reaction system (with Hg^{2+} concentration

0.03 mM) as potential interfering factors.

2.8 Smartphone-assisted detection of Hg^{2+}

The image recognition function of smartphone software was used to convert color information into RGB values for the detection of Hg^{2+}. In brief, the 96-well plates were used as the test pools, and these sample pools were then filled with 200 μL of a mixture containing different concentrations of Hg^{2+} and Cys (80 μM). Next, CuNCs (70 μg/mL), TMB (4.0 mM), H_2O_2 (12 mM), and HAc-NaAc buffer (pH 4.0, 4 mM) were added dropwise onto the test pool. After incubation at 50 °C for 15 min, the test pools were photographed using an iphone12 smartphone. The average RGB value of the image was then recorded using Adobe Photoshop 2023 and fitted with the Hg^{2+} concentration to create a standard curve for Hg^{2+} analysis.

2.9 Real sample analysis in tap water and industrial wastewater

The real samples were collected by the Local Tap water and Industrial wastewater. Wherein the collected Industrial wastewater samples were filtered through a 0.22 μm membrane and then centrifuged at 12 000 rpm for 30 min, but Tap water samples. Then, the supernatants and primitive Tap water samples respectively were diluted 100-fold with 4.0 mM HAc-NaAc buffer (pH 4.0) for subsequent Hg^{2+} analysis. The applicability of the CuNCs sensing platform was evaluated using the conventional standard addition method. Standard Hg^{2+} solutions with concentrations of 2.0, 10.0 and 30.0 μM were added to 100 μL of the diluted real samples, along with 8.0 μL Cys (5 mM) for 10 min, then, 50 μL of CuNCs solution (0.7 mg/mL), 100 μL TMB (20 mM), 30 μL H_2O_2 (0.2 M), and 10 μL HAc-NaAc buffer (0.2 M, pH 4.0) were added. The mixture was diluted to 500 μL with deionized water, incubated at 50 °C for 15 min, and the UV-vis and Smartphone analysis were carried out, respectively. The performance of the CuNCs-based Hg^{2+} sensing was assessed by analyzing the tested results.

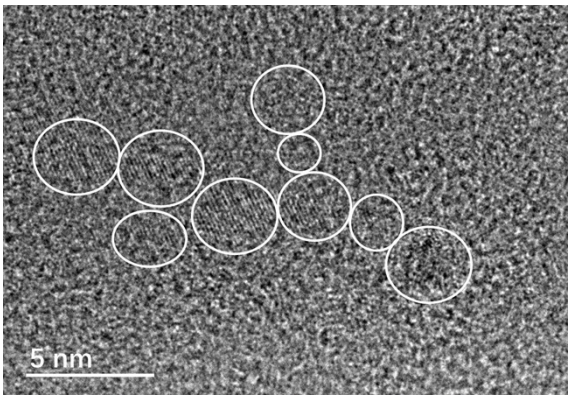

Fig. S1 High-resolution TEM image of the CuNCs.

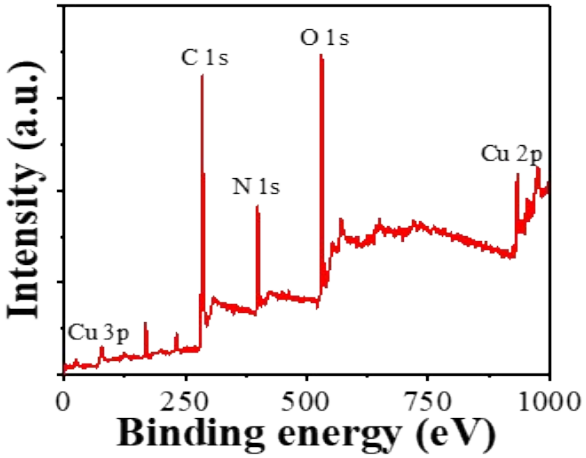

Fig. S2 The survey XPS spectrum of the CuNCs.

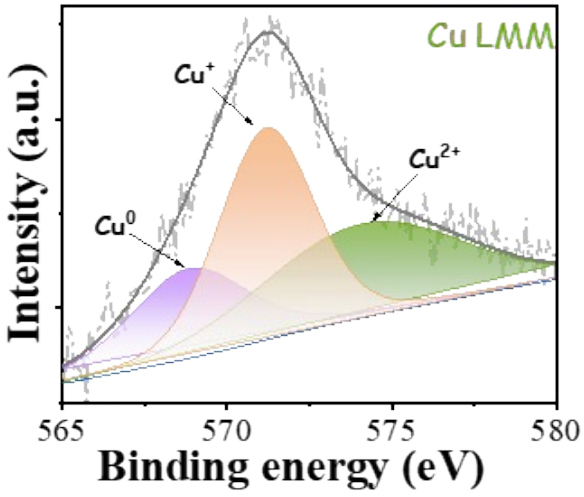

Fig. S3 The Cu LMM spectrum of the CuNCs.

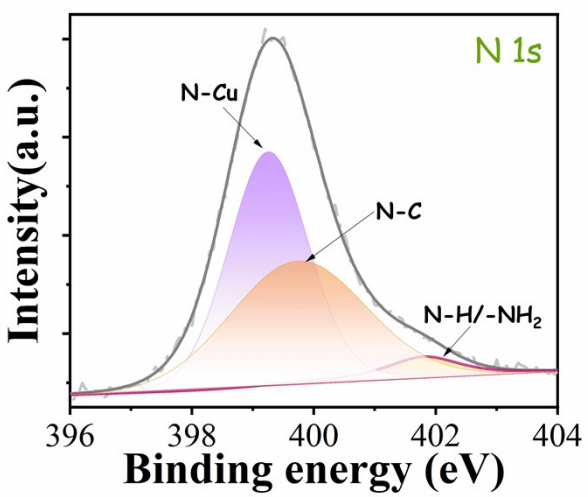

Fig. S4 The high-resolution N1s spectrum of the CuNCs.

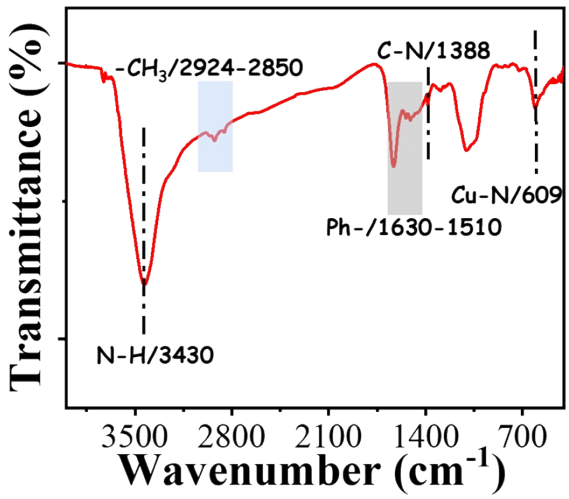

Fig. S5 The FT-IR spectrum of the prepared CuNCs.

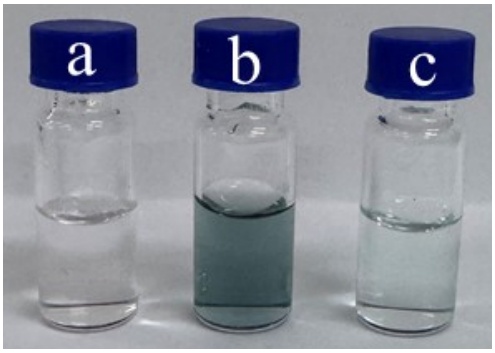

Fig. S6 The study of the POD-like activity of the 2,4-TDA + TMB + H_2O_2 system (a), ^{ox}CuNCs + TMB + H_2O_2 system (b) and reduced CuNCs + TMB + H_2O_2 system (c).

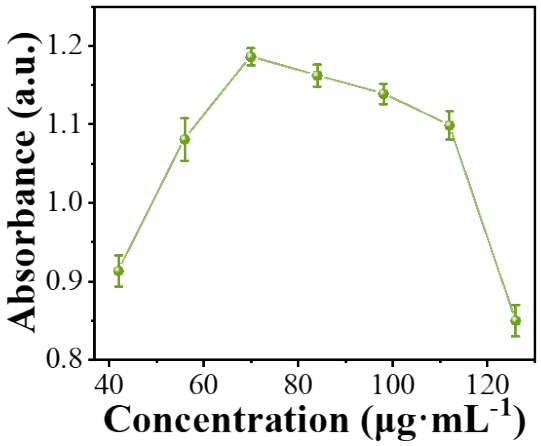

Fig. S7 The POD-like activity was affected by the content of CuNCs.

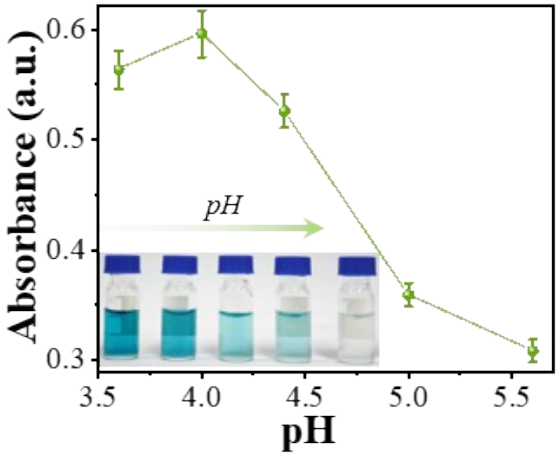

Fig. S8 The POD-like activity of the CuNCs was affected by pH value.

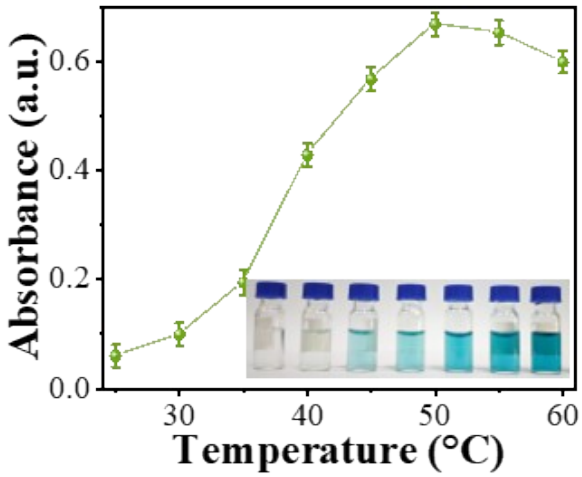

Fig. S9 The POD-like activity of the CuNCs was affected by reaction temperature.

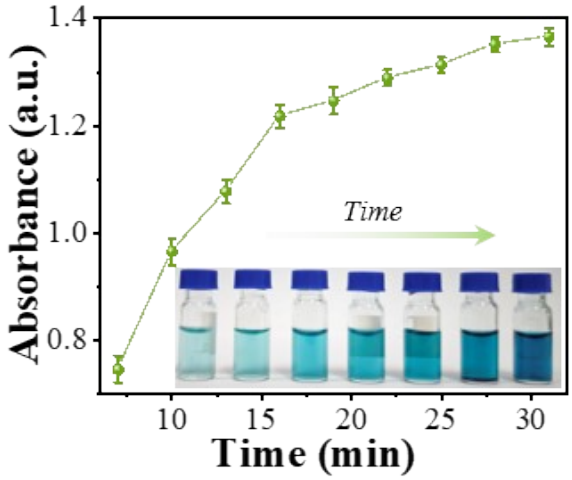

Fig. S10 The POD-like activity of the CuNCs was affected by reaction time.

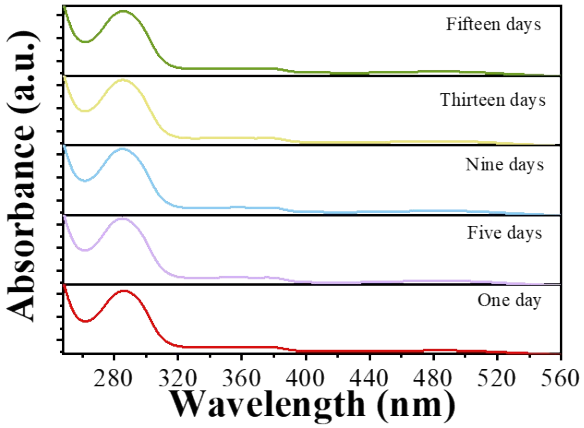

Fig. S11 The UV-vis absorption spectra of CuNCs as the storage time.

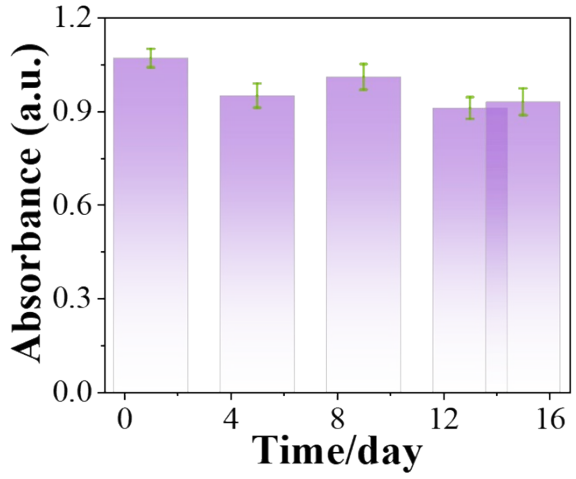

Fig. S12 The POD-like activity of the CuNCs as the storage time (Absorbance at 652 nm).

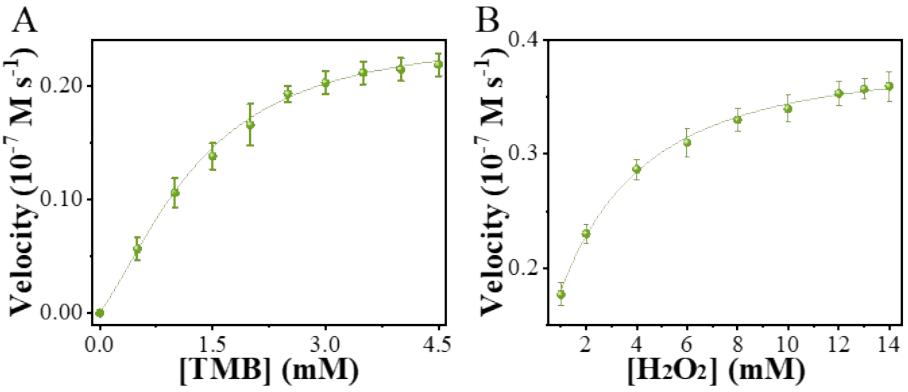

Fig. S13 Steady-state kinetic assay of CuNCs for TMB (A) and H_2O_2 (B).

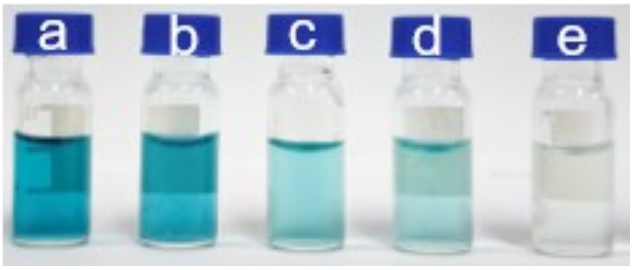

Fig. S14 The color change of CuNCs + TMB+ H_2O_2 (a) with the scavengers of TU (b), *p*-BQ (c), His (d) and the mixed three scavengers (e), respectively, and the concentration of the three scavengers was all 0.1 mM.

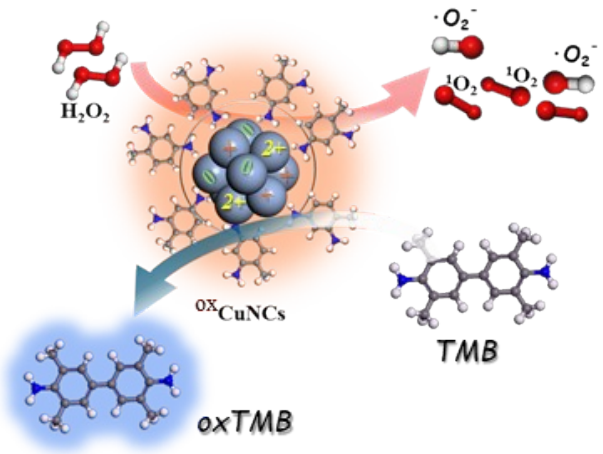

Fig. S15 Schematic representation of POD-like activity of the CuNCs.

Table S1 Determination of Hg^{2+} in real Tap water (T_w) and industrial wastewater (I_w) samples.

Sample	Original value	Standard value (μM)[a]	UV-vis spectrometer analysis			Smartphone analysis		
			Found (μM)[a]	Recovery (%)	RSD (%)	Found (μM)[a]	Recovery (%)	RSD (%)
T_w-1#	0	5	4.9	98.0	2.9	4.6	92.0	3.5
T_w-2#	0	15	14.6	97.3	3.2	16.0	106.7	5.0
T_w-3#	0	30	29.1	97.0	2.7	29.0	96.7	3.5
I_w-4#	0	5	5.1	102.0	4.2	5.5	110.0	4.7
I_w-5#	0	15	14.4	96.0	3.6	14.6	97.3	3.3
I_w-6#	0	30	31	103.3	4.5	31.5	105.0	3.1

The concentration of Hg^{2+} in the real samples were prepared to the linear range of 0.01 - 30 μM before determination.

[a]The data were obtained from three parallel samples.