Supporting Information

A AuNPs modified Cu-g-C_3N_4 light-responsive nanozyme: Improved peroxidase-like activity for amaranth dual-mode fluorescence-SERS sensors

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1. Materials and methods

1.1 Materials and chemicals

Melamine, $HAuCl_4$, citric acid (CA), copper sulphate ($CuSO_4$), and amaranth (AT) were purchased from the Aladdin Industrial Corporation. Hydrogen peroxide (H_2O_2), isopropanol (IPA), and dimethyl sulfoxide (DMSO) were purchased by Shantou Xi Long Chemical Factory Co. Ltd. 3,3',5,5'-Tetramethylbenzidine (TMB, 99%), 1,2-diaminobenzene (OPD, 99%), 2,2′-azino-bis(3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS, 99%), horseradish peroxidase (HRP), tert-butanol (TBA), sodium oxalate ($Na_2C_2O_4$), 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy (TEMPO), methanol

(MeOH), benzoquinone (BQ) and p-phthalic acid (PTA) were bought from Shanghai Macklin Biochemical Co., Ltd. Drinks (grape juice, orange juice, Phenda soda) were purchased from market.

All chemicals in all the experiments utilized were of analytical reagent and deionized water (18.2 MΩ) was used throughout the preparation of all solutions.

1.2 Synthesis of Cu-CDs/AuNPs

Cu-CDs nanozymes were synthesized via a one-step hydrothermal approach. In a typical procedure, $CuCl_2$ and citric acid (molar ratio=5:4) were mixed in a polytetrafluoroethylene (PTFE)-lined stainless steel autoclave, followed by heating at 180°C for 8 h. After natural cooling to room temperature, the resulting product was filtered through a 0.22 μm membrane filter. The filtrate was stored at 4°C for subsequent experimental use.

First, 80 μL of 1% $HAuCl_4$ aqueous solution and 20 mL of deionized water were mixed in a beaker and subjected to vigorous magnetic stirring. Subsequently, 200 μL of Cu-CDs aqueous solution (1.948 mg/mL) was added, and the mixture was stirred vigorously at room temperature for 30 min, yielding a purple solution. Finally, the solution color immediately transitioned to purple, affording Cu-CDs/AuNPs.

1.3 Characterization

The transmission electron microscopy (TEM) images were performed on a JEM-2100F microscope (JEOL, Japan) working at 200 kV. X-ray photoelectron spectroscopy (XPS) was conducted on a Thermo Scientific ESCALab 250Xi equipped with 200 W of monochromatic Al Kα radiation. The crystal structure of nanozymes was characterized by the XRD relied on an X-ray diffractometer (Bruker, D8-advance). Fourier transform infrared (FTIR) was carried out utilizing a Bruker FTIR spectrometer (ALPHA) equipped with a MCT (HgCdTe) detector cooled with liquid nitrogen. Zeta potential analysis of materials was performed on a Zeta sizer-Nano ZSZEN3600 apparatus (Malvern Instruments, UK). The UV-vis absorption spectrum was recorded by a UV-2600 UV-vis spectrophotometer (Shimadzu, Japan). Fluorescence spectra measurements of reaction solution were performed using Agilent Technologies' CaryEclipse fluorescence spectrophotometer. SERS measurements were carried out

using a portable Raman spectrometer (BWS465, B&W Tek Inc., USA). The pH of solution was measured using a Mettler-Toledo pH meter. Electron spin resonance (ESR) spectra were recorded at room temperature using a JEOLJESFA200 spectrometer at 9.8 G Hz, X-band, with100 Hz field modulation.

1.4 Enzymatic properties of Cu-g-C_3N_4/AuNPs

The peroxidase-like activity was evaluated thoroughly by typically chromogenic reactions using the TMB, ABTS and OPD as the peroxidase substrate with the presence of H_2O_2. The total volume was 4 mL, which contains 100 μL of Cu-g-C_3N_4/AuNPs (1 mg/mL), 100 μL of the substrate (5 mM), 100 μL of H_2O_2 and 3.7 mL of phosphate buffer (PBS, pH 7).

Kinetic experiments, the kinetic assays of Cu-g-C_3N_4/AuNPs with TMB as the substrate, were performed by adding different concentrations of TMB by immobilization the concentration of H_2O_2 (100 μL, 50 mM) at 1.25 mM. The absorbances were measured (654 nm for oxTMB) at different reaction times (within 300 s). The kinetic parameters (K_m and V_{max}) were calculated from Lineweaver-Burk plot:

$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

where v is the reaction rate, [S] is the concentration of the substrate (TMB), K_m is the Michaelis constant, and V_{max} is the maximal reaction rate.

1.2 SERS measurement conditions

A Raman-SSR 3000 spectrometer (Simple & Smart Instrument (Nanjing) Co., Ltd., China) was used to detect the SERS signal. Before Raman spectra acquisition, the parameters were set as follows: a 785 nm laser light source at a 12 cm^{-1} spectral resolution, a 10 s accumulation time, and an average spectral value of triplicates. The range of Raman shift was from 300 to 1500 cm^{-1} in the mode.

1.5 AT analysis in beverages samples

To further evaluate the practicability and accuracy of FL–SERS nano-sensor, the AT content was detected in grape juice drinks (including main ingredients: water, sugar, citric, vitamin C, sodium carboxymethyl cellulose, aspartame, acesulfame potassium,

essence, grape juice and amaranth). As shown in **Table 1**, for FL sensor, the recovery values ranged from 90.4% to 107.2% and the relative standard deviation (RSD) (n =3) was less than 5.73%. For SERS sensor, the recoveries found values (for five replicates) for juice samples ranges from 87.9% to 105.1%. The RSD value for the SERS modes was less than 6.89%, indicating good precision. The above results show that the proposed FL–SERS sensor for AT detection has a good application prospect in actual samples.

2. Figures

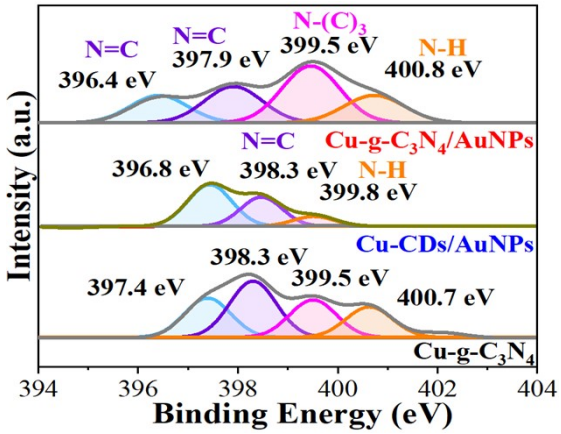
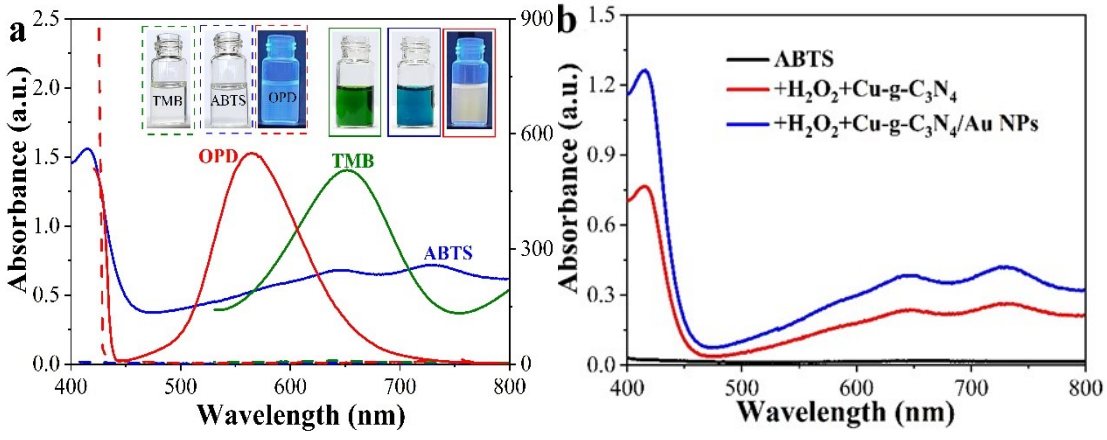


Fig. S1. N 1s high-resolution XPS spectra of Cu-g-C_3N_4, Cu-CDs/AuNPs and Cu-g-C_3N_4/AuNPs.



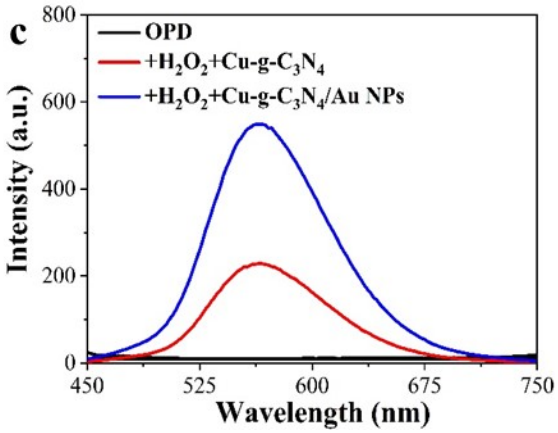


Fig. S2. (a) Absorption spectra of the oxidation reaction of different substrates in the presence of Cu-g-C_3N_4/AuNPs; the oxidation of (b) ABTS and (c) OPD in the presence of Cu-g-C_3N_4 or Cu-g-C_3N_4/AuNPs.

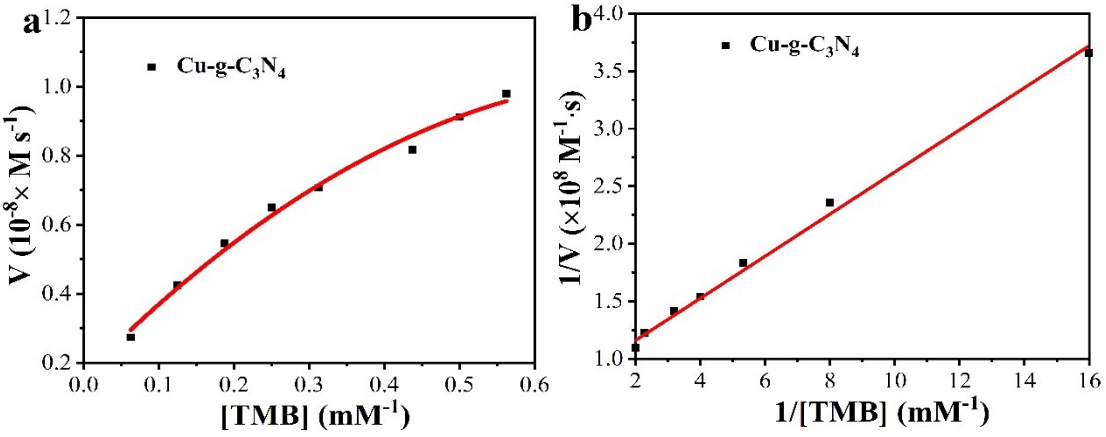
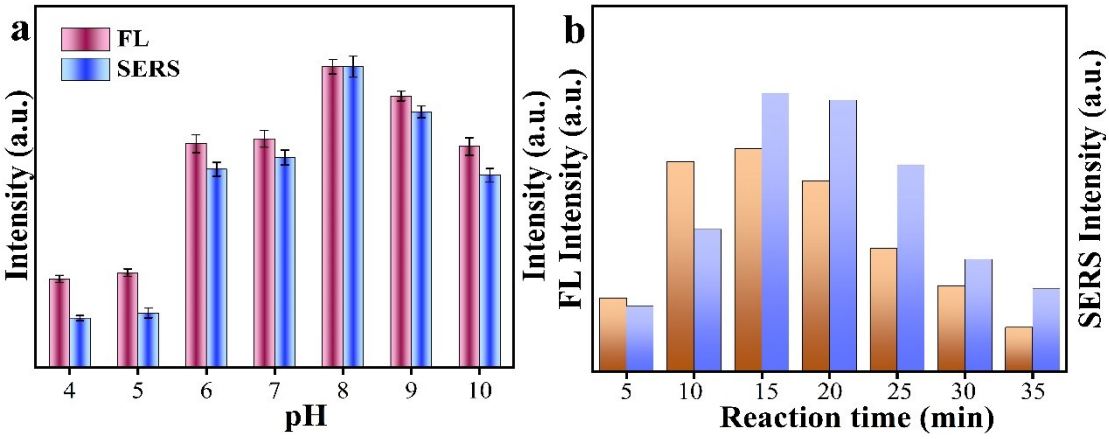


Fig. S3. (a and b) plots of initial rate versus concentration of TMB for the catalytic oxidation of TMB under the catalysis of Cu-g-C_3N_4.



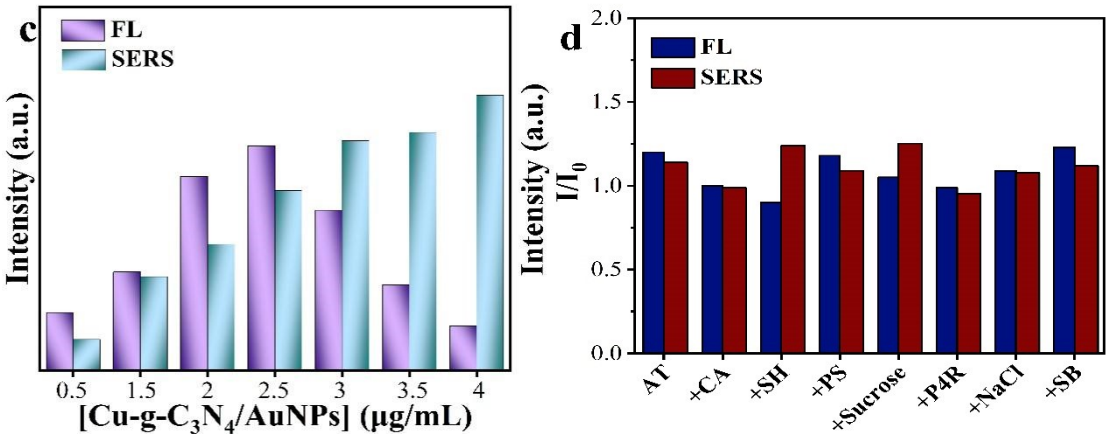


Fig. S4. Performance of Cu-g-C_3N_4/AuNPs nanozyme after incubation at different (a) pH, (b) reaction time, (c) different concentration of Cu-g-C_3N_4/AuNPs and (d) different coexisting substances.

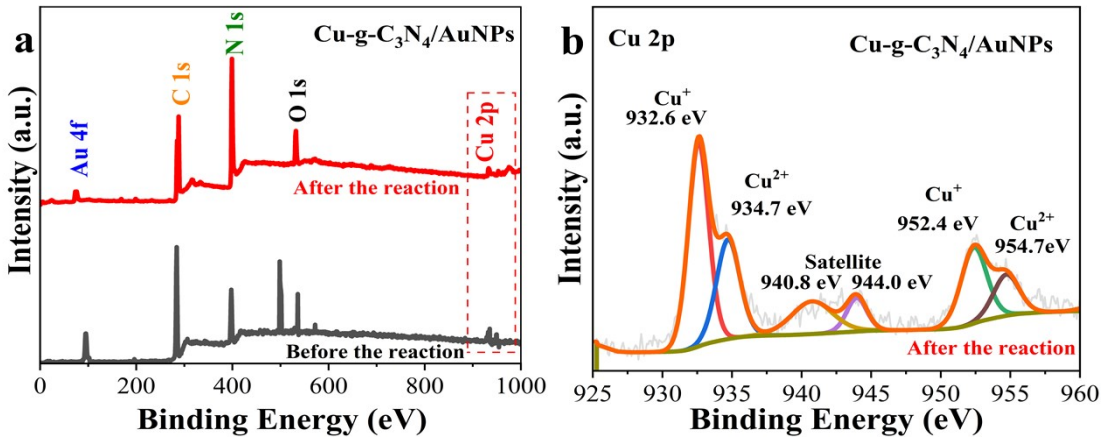


Fig. S5. (a) The full X-ray photoelectron spectroscopy (XPS) spectrum and (b) the high-resolution Cu 2p spectrum of Cu-g-C_3N_4/AuNPs after catalytic reaction.

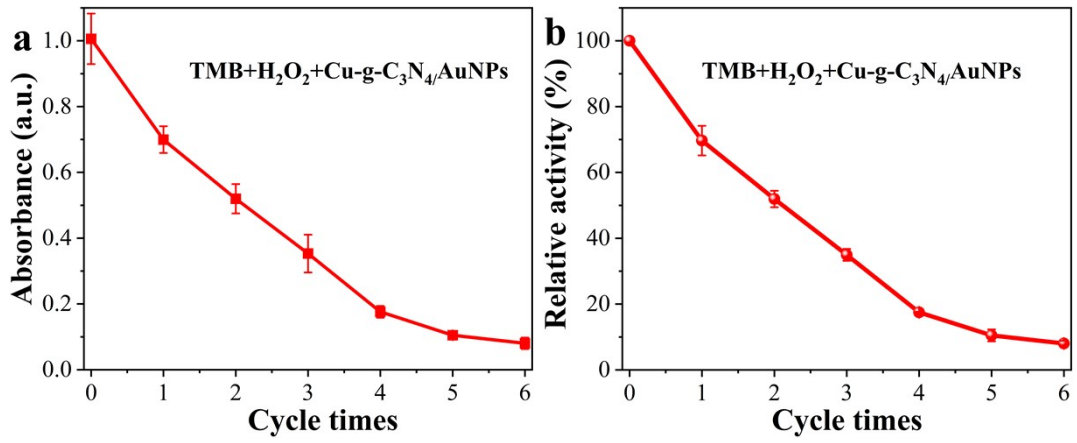


Fig. S6. (a) The absorbance and (b) corresponding relative activity variations of the Cu-g-C_3N_4/AuNPs system over six consecutive cycles of reuse.

3. Table

Table S1. Comparison of the stability of nanozymes

Elements	Atomic %
C 1s	57.7
N 1s	22.57
O 1s	18.47
Cu 2p	0.43
Au 4f	0.83

Table S2. Comparison of the stability of nanozymes

Nanozymes	Chemical stability	Ref.
PB@Au	24 h	[1]
BSA-Au NPs	30 day	[2]
AuCIT	6 h	[3]
Cu-g-C_3N_4/AuNPs	7 day	This study

Table S3. Comparison of the Michaelis–Menten constant (K_m) and maximum reaction rate (V_{max}) for TMB as substrate.

Catalyst	K_m (mM)	V_{max} (10^{-8} M^{-1} s)	Reference
Au/Cu_2O	0.21	6.08	[4]
CuO-Au nanoalloys	3.54	0.0111	[5]
C-Dots	0.039	3.61	[6]
HRP	0.434	10	[7]
AuNPs	0.023	2.15	[8]
Au/g-C_3N_4	0.27	1.27	[9]
Cu-g-C_3N_4	0.298	1.423	This work
Cu-CDs/AuNPs	0.409	5.033	This work
Cu-g-C_3N_4/AuNPs	0.158	7.482	This work

Table S4. Determination of AT in beverages samples.

sample	Added (mg/kg)	FL mode			SERS mode		
		detected (mg/kg)	Recovery (%)	RSD (%)	detected (mg/kg)	Recovery (%)	RSD (%)
grape	1.00	0.95	95.3	3.22	0.98	97.5	4.77
juice	10.00	11.36	113.6	2.58	10.51	105.1	5.21
orange	1.00	1.02	102.0	5.73	1.04	104.2	2.29
juice	10.00	10.72	107.2	4.24	8.79	87.9	3.40
Phenda	1.00	0.90	90.4	4.79	0.92	91.5	3.52
soda	10.00	10.43	104.3	2.42	9.53	95.3	6.89

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