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**Au bipyramids@CuZn MOF core-shell nanozyme enables
universal SERS and colorimetric dual-model bioassay**

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Experimental

Materials and Reagents. Chloroauric acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, $\geq 99.9\%$) was purchased from Aladdin Co., Ltd. (Shanghai). Silver nitrate (AgNO_3 , $\geq 99.8\%$), Sodium borohydride (NaBH_4 , $\geq 95\%$), sodium citrate dihydrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$, $\geq 99\%$), ascorbic acid (AA, 99%), cetyltriethylammonium bromide (CTAB, 99%), cetyltrimethylammonium chloride (CTAC, 99%), 1,2-Dimethylimidazole (2-MI, 98%), copper nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\geq 99\%$), zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\geq 99\%$), hydrochloric acid (HCl, 36.5%-38%), methanol (AR), nitric acid (AR), hydrogen peroxide (H_2O_2 , 30%), acetic acid ($\text{C}_2\text{H}_4\text{O}_2$, $\geq 99.8\%$), sodium acetate anhydrous (CH_3COONa , 99%) were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai). Reduced glutathione (GSH, 99%), 3,3',5,5'-tetramethylbenzidine (TMB) was purchased from America Acros.

All chemicals were used as received without further purification. Ultrapure water was applied in all experimenting sections. All vials were rinsed with aqua regia, and then treated ultrapure water at least three times with ultrasound, and being dried before use.

Apparatus. Transmission electron micrographs (TEM) was obtained via utilizing a JEM-2100 Emission transmission electron microscope (Japan) at an acceleration

voltage of 15 kV. Ultraviolet-visible (UV-vis) experiments were completed with a UV-vis-2500 spectrophotometer (Beijing General Instrument Co., Ltd). Scanning electron micrographs (SEM) was observed via using a Zeiss-Supra55 scanning electron microscope (Carl Zeiss AG, Germany) at an acceleration voltage of 15 kV. Experiments were completed with a A300-10/12 electron spin resonance spectrometer (EPR) (Bruker, Germany). SERS experiments were completed with Laser confocal Raman spectrometer (INVIA REFLEX, Renishaw, UK).

Synthesis of AuNBPs. Au bipyramids (AuNBPs) were synthesized by silver ion assisted seed-mediated method, and some modifications were made on this basis^{S1}. In short, the synthesis is divided into two steps. The production of decahedral gold seeds is the first step. 2.5 mL of 200 mM CTAC was mixed with 3.6 mL of 0.5 mM HAuCl₄ solution. Under vigorous agitation, 20 mM trisodium citrate and 25 mM cold and fresh NaBH₄ solution were added to the above mixture. When the solution turned brown, the solution was then stirred slowly at 85 °C for 6 h. With the successful synthesis of decahedral gold nanoparticle seed solution, the solution gradually turned pale pink. The seed solution was then collected and stored at room temperature.

The second step was to prepare the growth solution. 9 mL of 100 mM CTAB solution was fully mixed with 0.45 mL of 10 mM HAuCl₄ solution. 200 μL of 1 M HCl and 100 μL of 10 mM AgNO₃ were added to above solution during stirring. Next, 100 μL of 10 mM AA as a reducing agent was added to the solution, then quickly addition of 100 μL seed solution. After heating at 30 °C for 100 min, the reaction solution turned purple, indicating the synthesis of gold bipyramids. The product was centrifuged at 6500 rpm for 15 min, washed twice, and dispersed in 1 mM CTAB solution for preservation.

Preparation of AuNBPs@CuZn MOF nanozyme. 1 mL of 1.32 M 1,2-dimethylimidazole and 140 μL of 1 mM CTAB were added to a 20 mL vial, and mixed evenly by stirring for 50 min. 0.5 mL of 0.37 mM Zn(NO₃)₂, and 0.5 mL of 0.12 mM Cu(NO₃)₂ was added to above solution under stirring. Next, 100 μL of 15 mM CTAB and 1 mL AuNBPs were added to the mixed solution and stirred quickly for 10 min, and then kept completely undisturbed at 25 °C for 1 h. The obtained product was

centrifuged at 8500 rpm for 5 min, washed with ethanol twice, and dispersed in 1 mL ethanol to obtain AuNBPs@CuZn MOF.

SERS/colorimetric detection of GSH. 20 μ L GSH solution of different concentrations, 20 μ L AuNBPs@CuZn MOF (0.5 mg/mL) , 50 μ L of 0.4 mM TMB and 20 μ L of 10 mM H₂O₂ were added successively to the centrifuge tube. Subsequently, the pH of the mixture was adjusted to 4.0 with 0.1 M HAc-NaAc solution to maintain the final reaction volume at 2 mL, and then incubated at 35 °C for 25 min. The UV-vis absorbance spectrum of the obtained solution was recorded. For performing SERS detection of GSH, the resulting solution of 20 μ L was dripped onto the silicon wafer, and after natural drying, the SERS detection of GSH was carried out. The SERS spectrum was recorded by Raman microscope.

The characterizations of AuNBPs under different conditions.

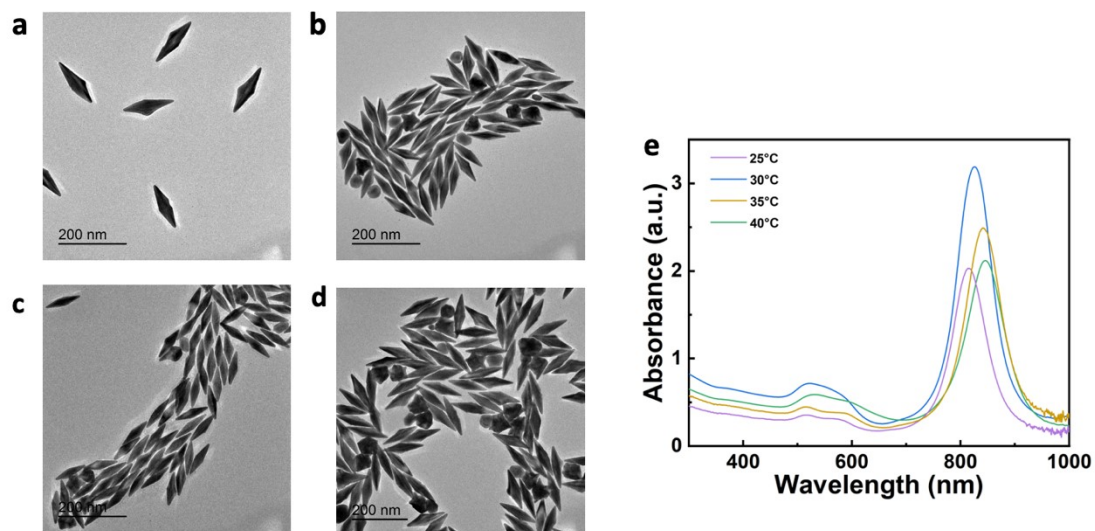


Fig. S1. TEM images of AuNBPs synthesized at various temperatures: (a) 25 °C, (b) 30 °C, (c) 35 °C, (d) 40 °C, (e) UV-vis spectra of AuNBPs synthesized at various temperatures.

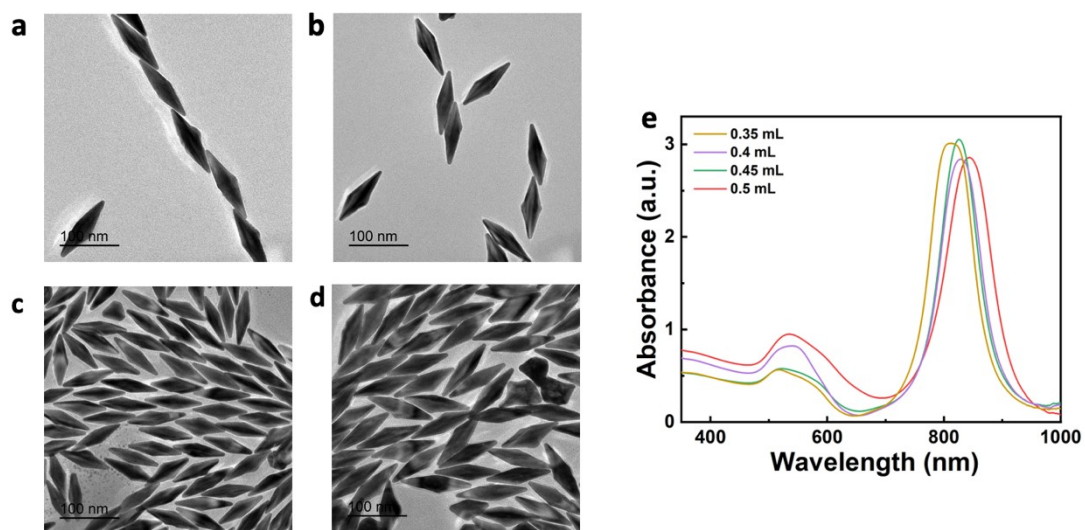


Fig. S2. TEM images of AuNBPs synthesized with different amounts of HAuCl₄: (a) 0.35 mL, (b) 0.4 mL, (c) 0.45 mL, (d) 0.5 mL, (e) UV-vis spectra of AuNBPs synthesized with different amounts of HAuCl₄.

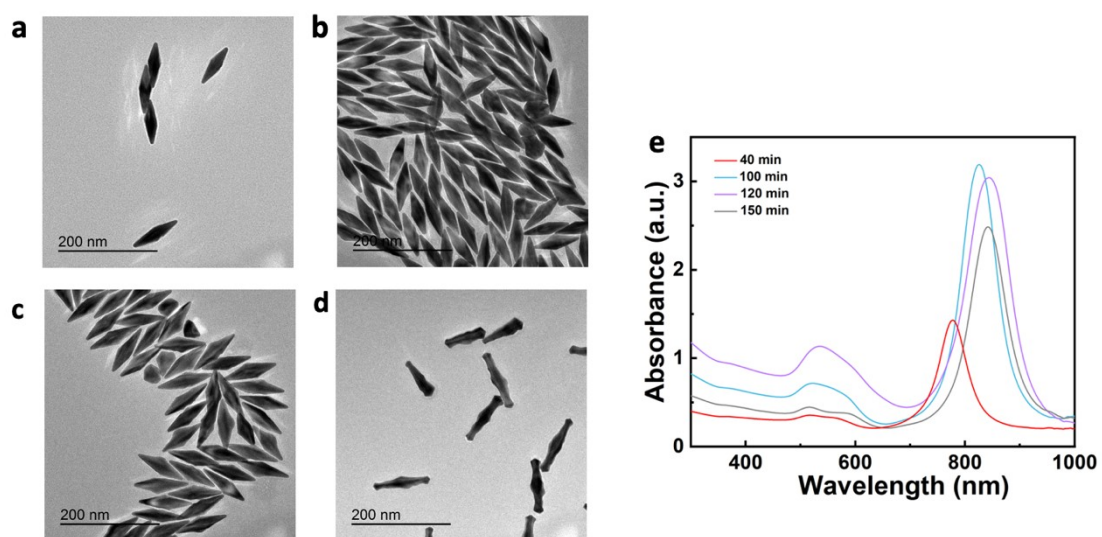


Fig. S3. TEM images of AuNBPs synthesized at different times: (a) 40 min, (b) 100 min, (c) 120 min, (d) 150 min, (e) UV-vis spectra of Au NBPs synthesized at different times.

The characterizations of AuNBPs@CuZn MOF nanozyme under different conditions.

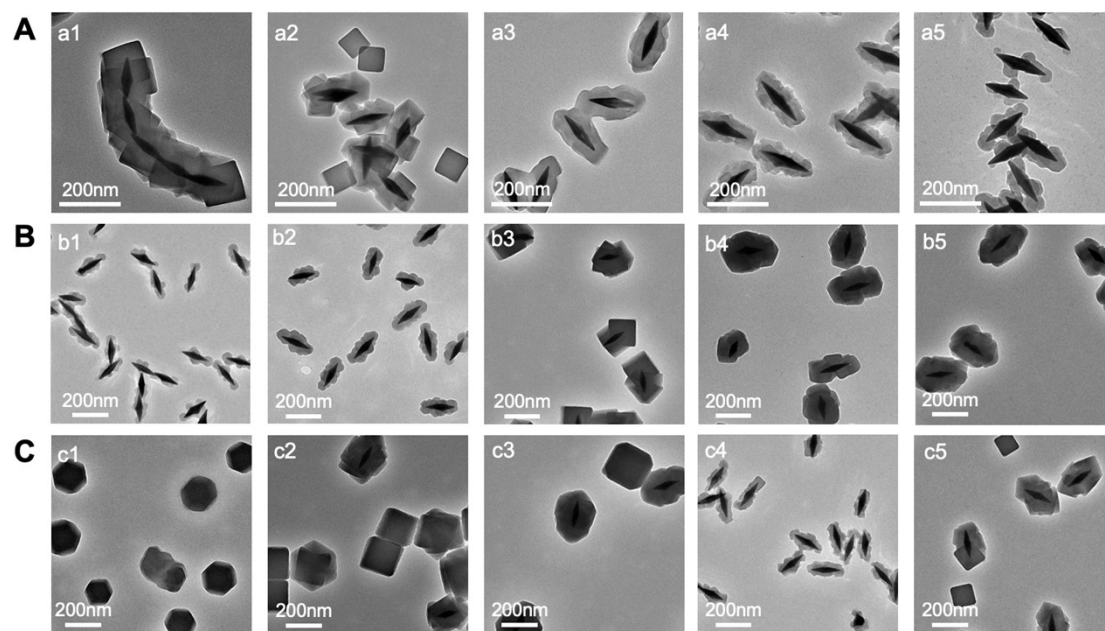


Fig. S4. Factors influencing the synthesis of AuNBPs@CuZn MOF. (a) TEM images of AuNBPs@CuZn MOF with different amounts of CTAB: 0 μL , 40 μL , 60 μL , 100 μL , 160 μL . (b) TEM images of AuNBPs@CuZn MOF at different stirring time of reaction solution: 5 min, 10 min, 20 min, 30 min, 40 min. (c) TEM images of Au NBPs@CuZn MOF with different amounts of Au NBPs: 0 μL , 400 μL , 800 μL , 1000 μL , 1200 μL .

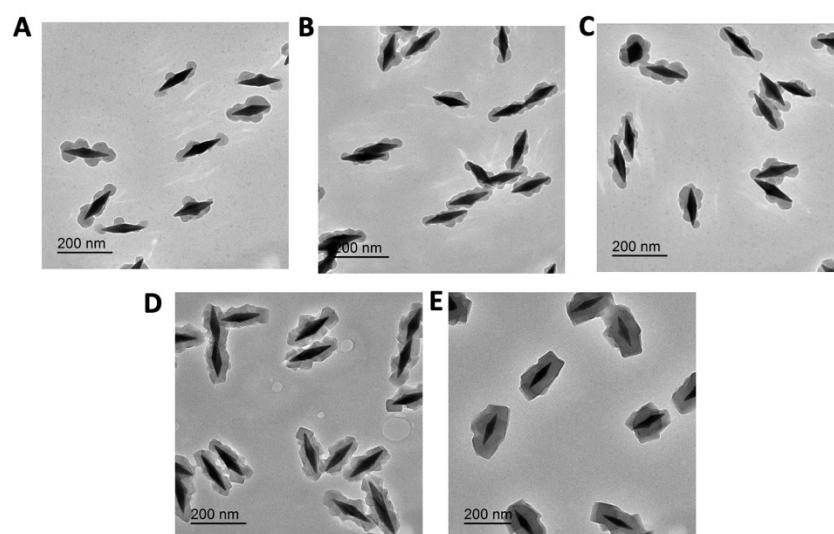


Fig. S5. TEM images of AuNBPs@CuZn MOF with different amounts of 2-MI. (A) 0.5 mL, (B) 1 mL, (C) 1.5 mL, (D) 2 mL, (E) 2.5 mL.

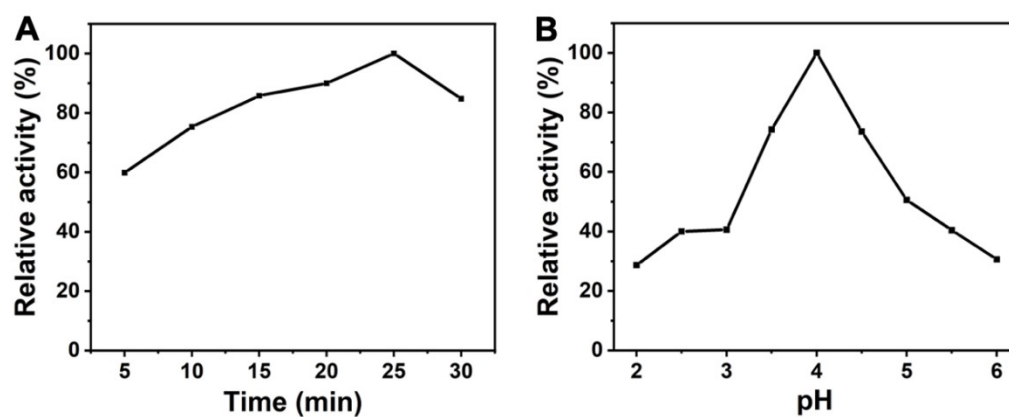


Fig. S6. (a) Effect of reaction time and (b) pH on POD-like activity of Au NBPs@CuZn MOF nanozyme. Fixing the maximum absorbance of the TMB solution at 652 nm to 100% (relative activity).

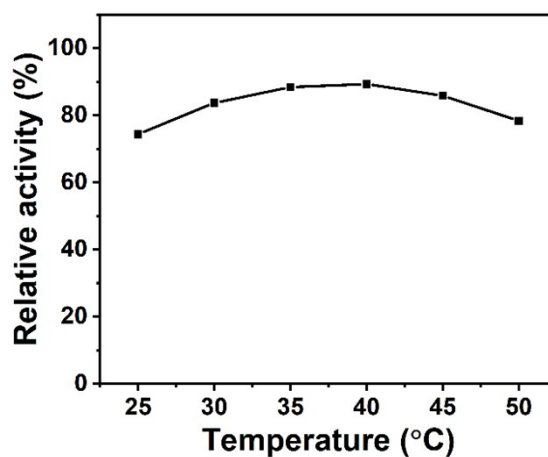


Fig. S7. Effect of temperature on POD-like activity of Au NBPs@CuZn MOF nanozyme.

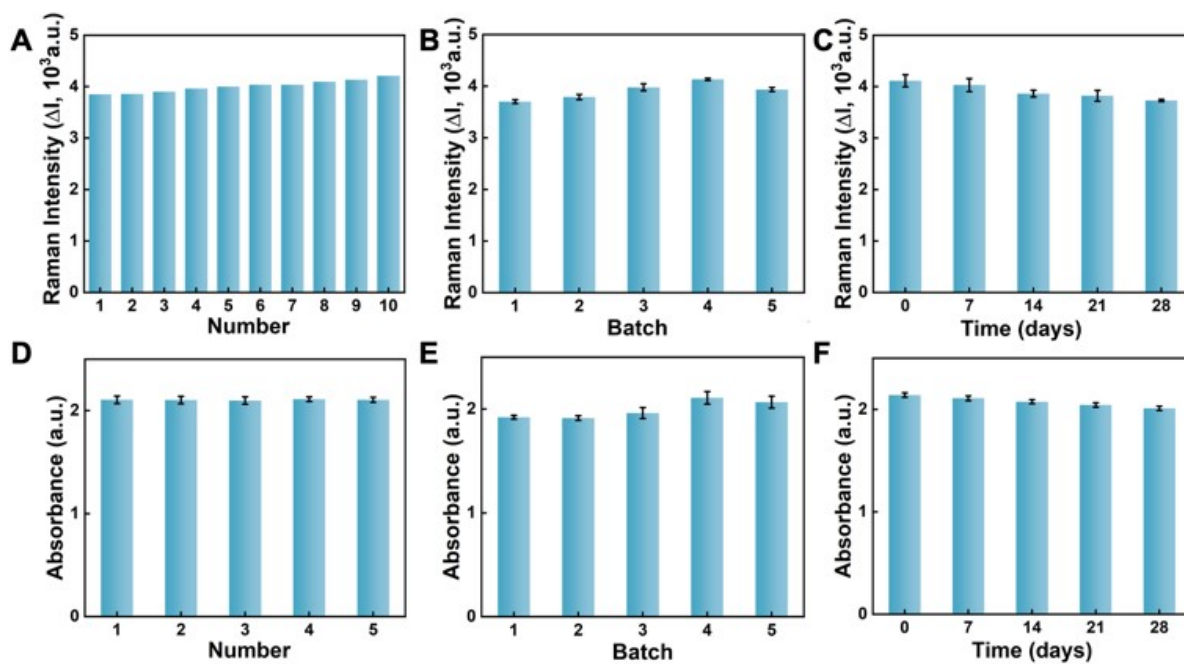


Fig. S8. The same-batch (A) and batch-to-batch (B) reproducibility and long-term stability (C) of the AuNBPs@CuZn MOF-based SERS assay; the same-batch (D) and batch-to-batch (E) reproducibility and long-term stability (F) of the AuNBPs@CuZn MOF-based colorimetric assay.

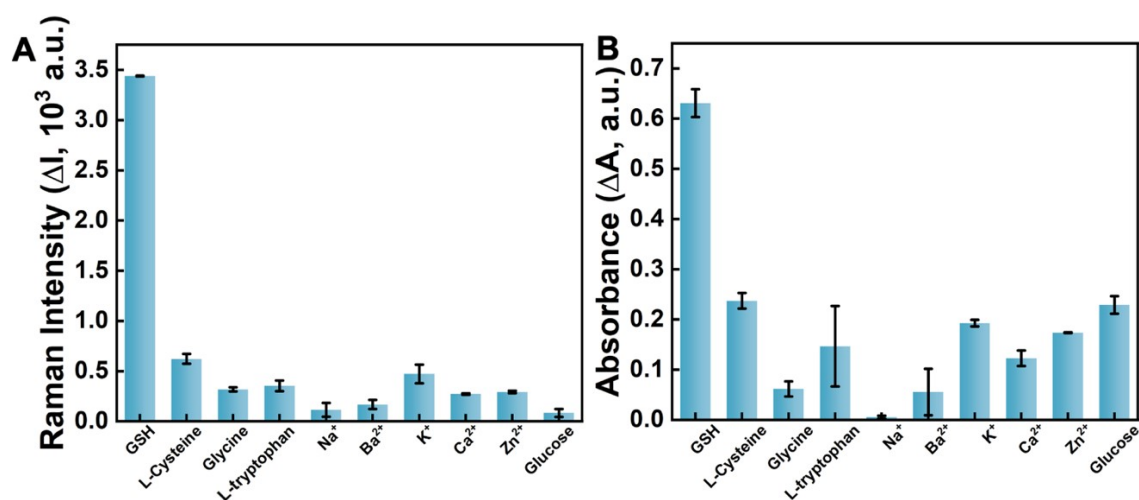


Fig. S9. Selectivity of SERS (a) and colorimetric (b) assay method for detection of GSH.

Table S1 Comparison of steady-state parameters of AuNBPs@CuZn MOF nanozyme and other nanozymes reported in the literatures

Samples	Substrate	K_m (mmol/L)	V_m ($\times 10^{-8}$ mol L ⁻¹ s ⁻¹)	Ref.
HRP	TMB	0.434	10	S2
	H ₂ O ₂	3.7	8.71	
Fe-MOF	TMB	2.6	5.6	S3
	H ₂ O ₂	1.3	2.5	
Ag ₁ Pd ₁	TMB	0.32	11.9	S4
	H ₂ O ₂	147	5.58	
U66-PV-Pep@AuNPs	TMB	0.54	0.95	S5
	H ₂ O ₂	0.18	0.71	
D-His@Au NCs	TMB	0.41	7.69	S6
	H ₂ O ₂	72.0	5.55	
AuNBPs@CuZn MOF	TMB	0.372	67.97	This work
	H ₂ O ₂	0.469	123.8	

PV: dimethylvinylloxazolinone; Pep: pepsin; D-His: D-distidine; NCs: nanoclusters.

Table S2 Comparison of nanozyme-mediated bioassays for GSH detection via SERS/colorimetric assay method.

Nanozyme	Method	Linear range (μM)	Detection limit (μM)	Ref.
Cy-Au NCs	Colorimetric	0-400	10	S7
Iron-copper MOF	Colorimetric	2-20	0.439	S8
ZnMn ₂ O ₄ -Mt	Colorimetric	10-700	0.52	S9
PMBA@Ag/Au@MnO ₂	SERS	1-100	2.95	S10
Mn-CDs/AgNPs	SERS	1-100	0.41	S11
	Colorimetric	1-300	0.53	
AuNBPs@CuZn MOF	SERS	1-150	0.091	This work
	Colorimetric	1-2000	0.17	

Cy-Au NCs: Cytidine-Au nanoclusters; Mt: Montmorillonite; CDs: carbon dots;
PMBA: P-mercaptophenylboronic acid.

Table S3 Recoveries for GSH by the proposed SERS-colorimetric assay (n = 3).

Sample	Added (μM)	Detection Method	Detected (μM)	Recovery (%)
1	5	colorimetric	5.17	103.3
2	10	colorimetric	9.78	97.8
3	20	colorimetric	20.16	100.8
4	5	SERS	5.1	102.0
5	10	SERS	10.58	105.8
6	15	SERS	14.66	97.7
7	20	SERS	19.48	97.4

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