Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Carbon-Nitrogen Conjugates composited $Cu_{1.8}S$ with Enhanced Peroxidase-like Activity in Colorimetric Detection of Hydrogen Peroxide and Glutathione

Nianlu Li, Mingquan Zhu, Zhenyu Feng, Wenhui Lu, Jing Chen*, Jinhua Zhan*

Key Laboratory for Colloid & Interface Chemistry of Education Ministry, Department of Chemistry, Shandong University, Jinan 250100, P.R. China

* Corresponding author: E-mail: jhzhan@sdu.edu.cn, jingchen@sdu.edu.cn

Tel: +86-0531-8836-5017; Fax: +86-0531-8836-6280

Text S1

The preparation of bare $Cu_{1.8}S$

CuCl (0.05 mol L^{-1}) was mixed with Na_2S aqueous solution at 75 ℃ with a molar ratio of n(Cu^+): n(S^{2-}) = 2:1. After stirring for 1 h, the obtained black precipitate was separated by centrifugation at 6000 rpm for 5 min, and washed with deionized water and ethanol three times.

Text S2

Free Radical Analysis

To verify hydroxyl radicals (•OH), the spin trap DMPO was used to verify the formation of spin adduct DMPO/•OH during the degradation of H_2O_2 in the presence of $Cu_{1.8}S$-cgmc in acid solution. Briefly, $Cu_{1.8}S$-cgmc (30 μg/mL) was dissolved in phosphate buffer (pH 5.0, 0.01 M), and the dispersion solution was mixed with H_2O_2 (0.5 mM) and DMPO (0.01 M) and incubated at room temperature for 5 min. Then, 50 μL sample solution was sealed in a glass capillary tube with internal diameter of 0.5 mm. All electron spin resonance measurements were conducted using a Bruker electron spin resonance spectrometer (A300-10/12, Germany) under ambient conditions.

Text S3

Standard analysis method for H_2O_2

GB 22216-2008 (China) was used as standard analysis method in H_2O_2 analysis. One gram of spiked sample was dissolved in 250 mL deionized water and then was added

with 100 mL H_2SO_4 (10%) under vigorous stirring. Potassium hypermanganate solution was used to titrate the above solution until the solution turn to pink and did not change within 30 s.

Text S4

Standard analysis method for glutathione

High performance liquid chromatography method according to the Pharmacopoeia of the People's Republic of China[S1] were used as standard method. Spiked homogenates were centrifuged, and the collected supernatants were filter by 0.22 μm membrane filter for analysis.

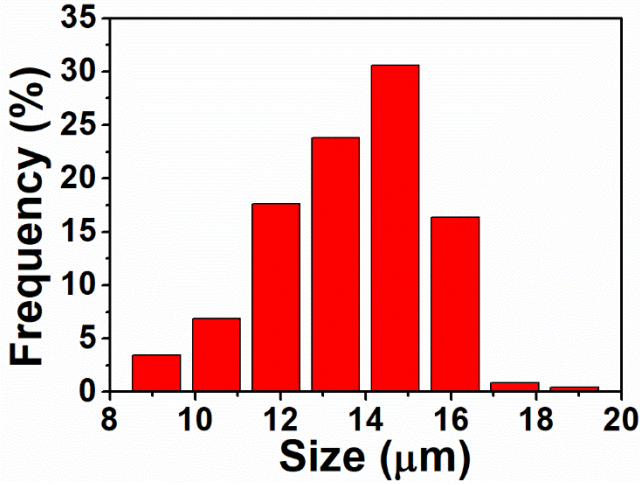

Fig. S1 Size distribution of $Cu_{1.8}S$-cgmc composites with C% at 1.83%.

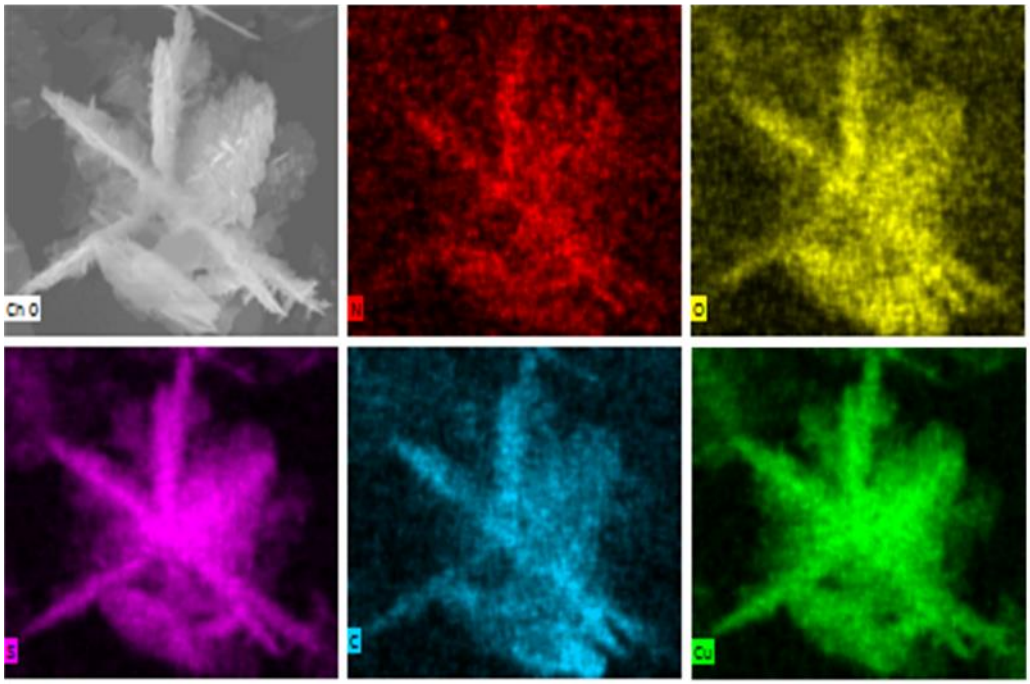

Fig. S2 EDS elements mapping of N, O, S, C and Cu in $Cu_{1.8}S$-cgmc composites.

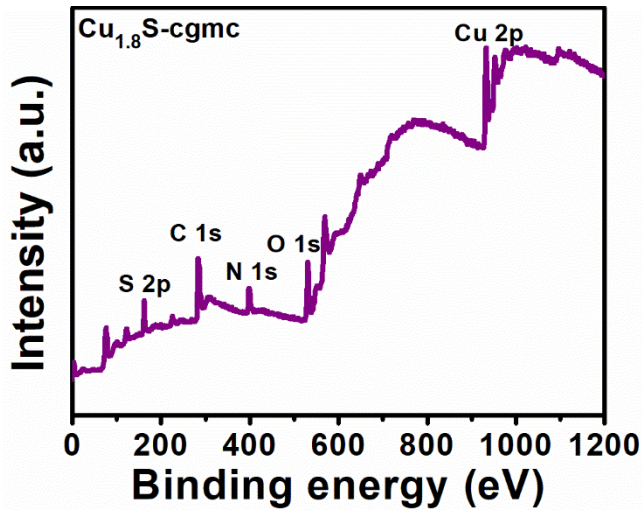

Fig. S3 XPS spectrum of $Cu_{1.8}$S-cgmc.

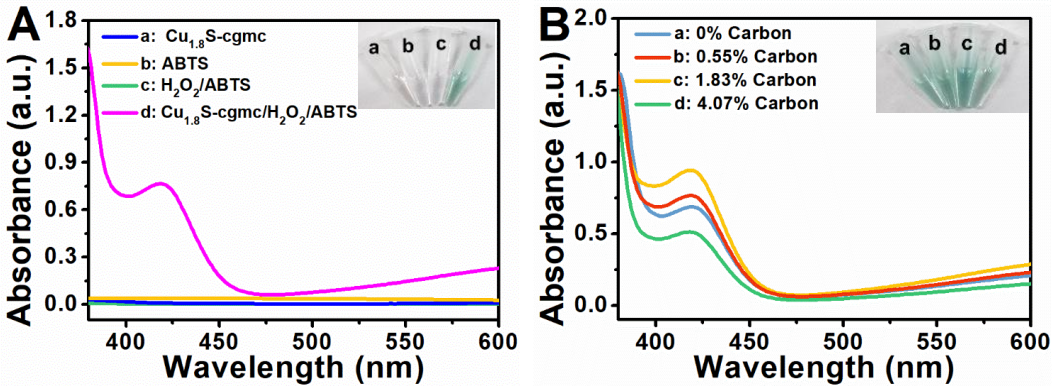

Fig. S4 (A) UV-vis absorption of ABTSox; (B) UV-vis absorption of ABTSox based on $Cu_{1.8}$S-cgmc with different amount of carbon (%).

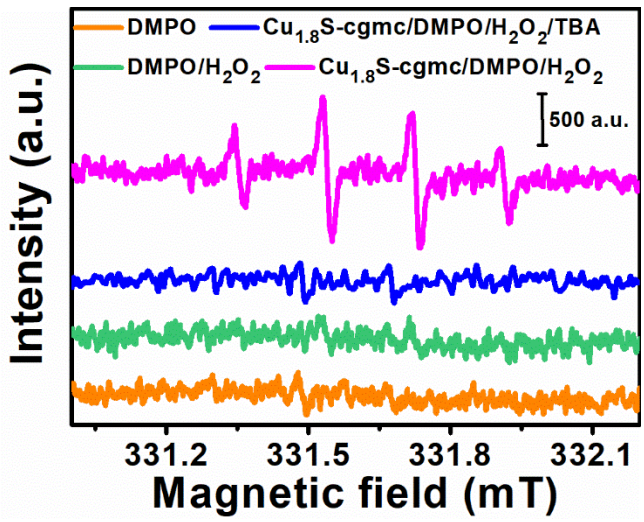

Fig. S5 ESR spectra of ROS spin adducts in the $Cu_{1.8}$S-cgmc catalysis.

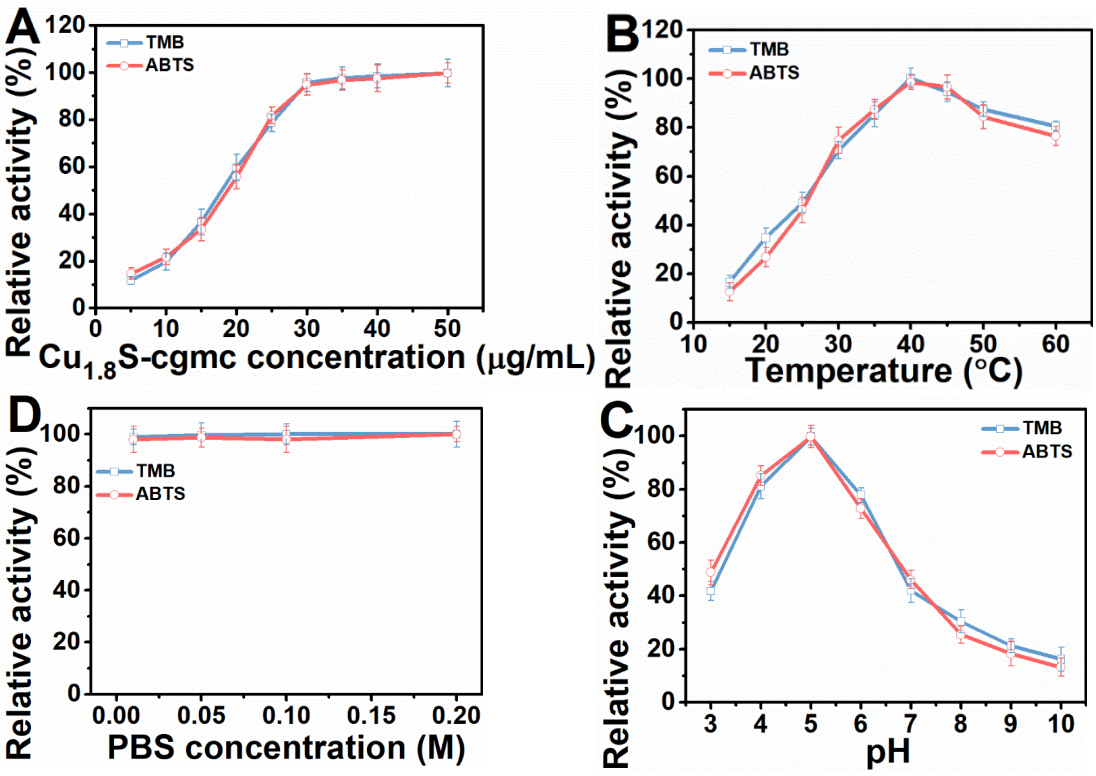

Fig. S6 Optimization on peroxidase-like activity of $Cu_{1.8}S$-cgmc on (A) $Cu_{1.8}S$-cgmc concentration, (B) temperature, (C) pH and (D) phosphate buffer concentration.

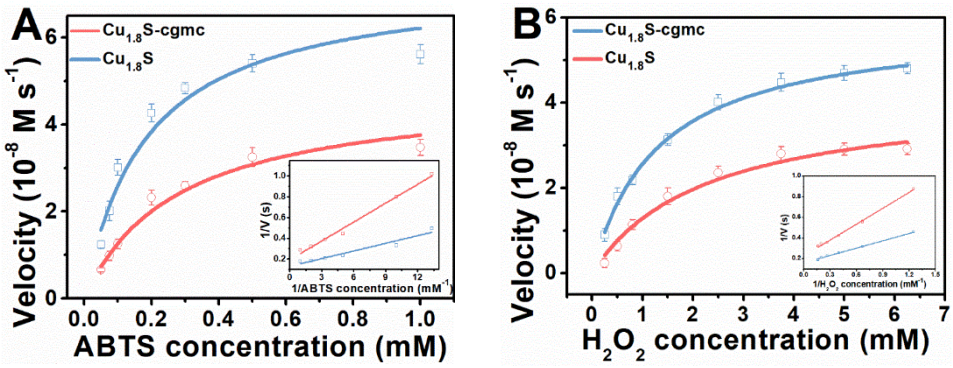

Fig. S7 Reactive velocity measured using (A) ABTS and (B) H_2O_2 as substrates, and (C) K_m values of $Cu_{1.8}S$-cgmc and $Cu_{1.8}S$.

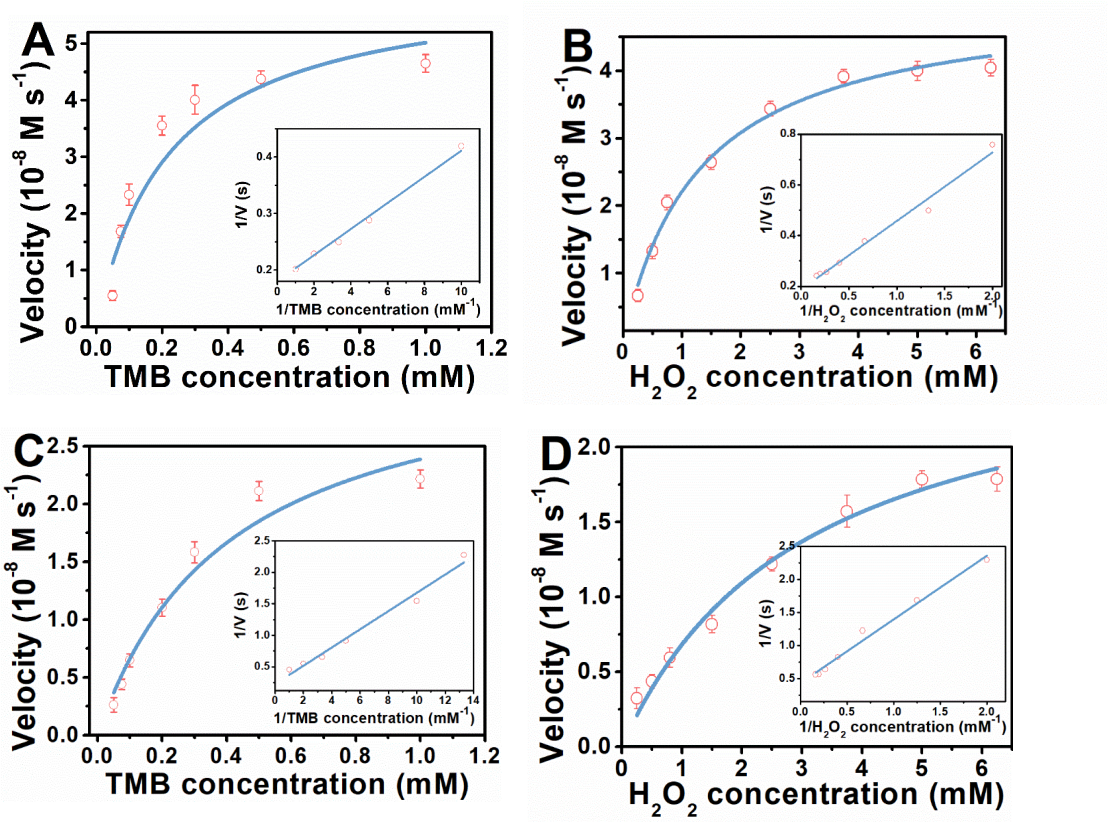

Fig. S8 Reactive velocities using TMB and H_2O_2 as substrates from $Cu_{1.8}S$-cgmc with 0.55% carbon (A-B) and $Cu_{1.8}S$-cgmc with 4.07% carbon (C-D). Inserts were double-reciprocal plots.

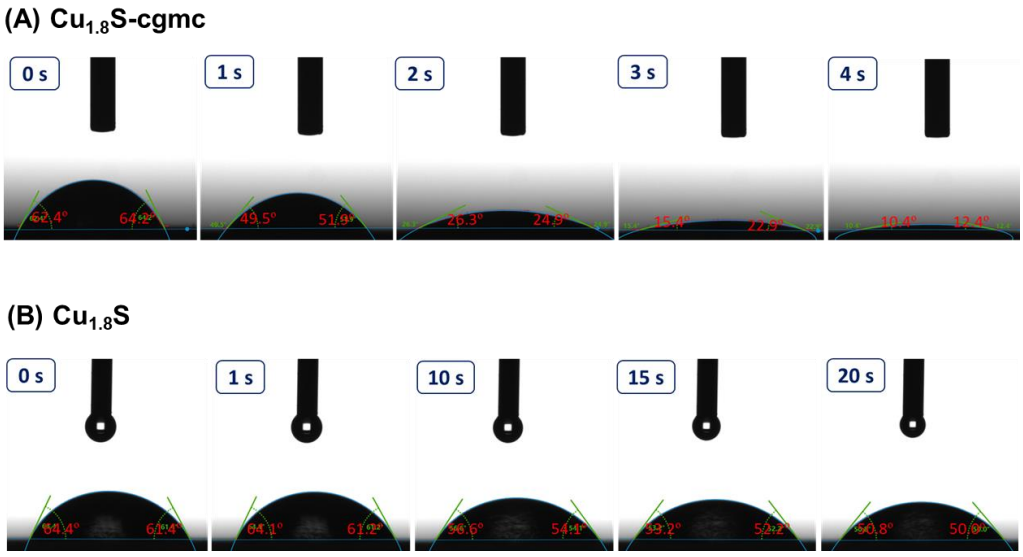

Fig. S9 Dynamic wetting of water droplet on the bottom of (A) $Cu_{1.8}S$-cgmc and (B) bare $Cu_{1.8}S$.

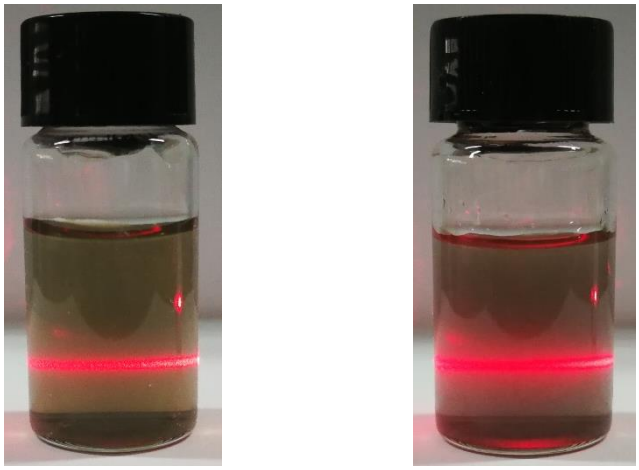

Fig. S10 Tyndall effect images obtained from $Cu_{1.8}S$-cgmc and bare $Cu_{1.8}S$ dispersed in aqueous solution.

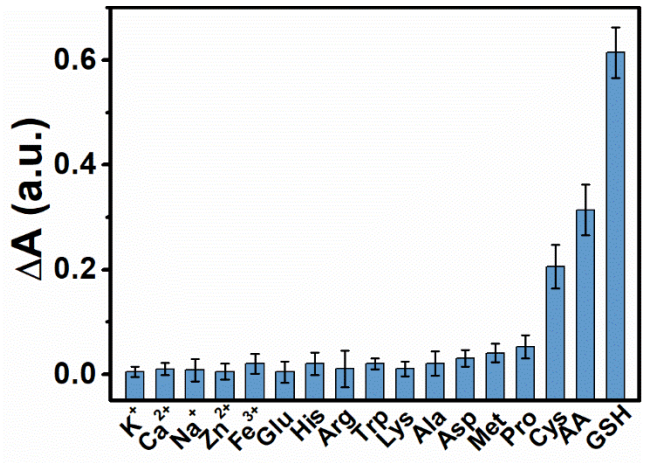

Fig. S11 Selectivity of glutathione over potential interferences. (glucose: Glu, histidine: His, arginine: Arg, tryptophan: Trp, lysine: Lys, alanine: Ala, asparaginate: Asp, methionine: Met, proline: Pro, cysteine: Cys, ascorbic acid: AA, glutathione: GSH). The concentrations of most interfering substances were set as 2 mmol/L and glutathione, cysteine and ascorbic acid were set as 0.1 mmol/L.

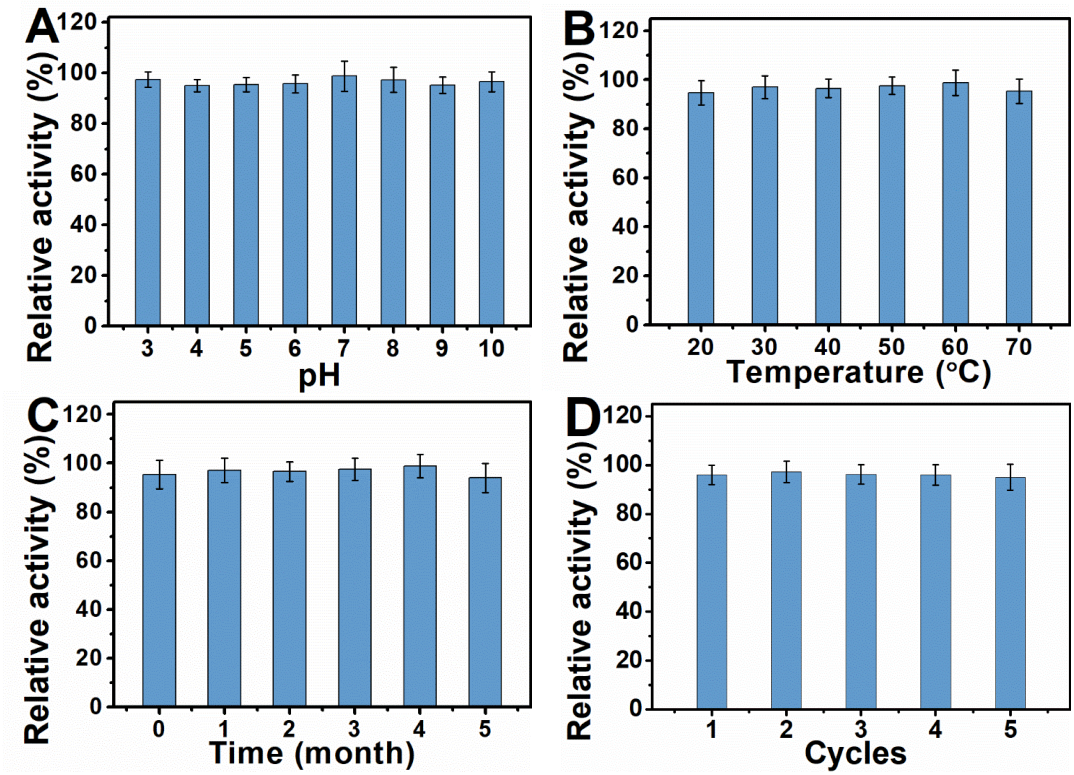

Fig. S12 Peroxidase-like activity of $Cu_{1.8}S$-cgmc stored at (A) pH 3.0-10.0 for 2 h, (B) 20-70 ℃ for 2 h and (C) room temperature for 0-5 months; (D) the stability of $Cu_{1.8}S$-cgmc after 5 regeneration cycles.

Table S1. Amount of ingredient and elemental composition of C, N and S in $Cu_{1.8}S$-cgmc composites

$Cu_{1.8}S$-cgmc			Ingredients (g)		
C%	N%	S%	glucose	cystine	$CuCl_2$ $2H_2O$
0.55%	0.75%	16.0%	0.01	0.60	0.46
1.83%	0.78%	16.3%	0.05	0.60	0.46
4.07%	0.80%	15.3%	0.10	0.60	0.46

Table S2. Comparison the steady-state kinetic parameters for the peroxidase-like activity of $Cu_{1.8}S$ at different carbon elemental ratio.

marterials	TMB			H_2O_2		
	K_m (mM)	V_{max} (10^{-8} M s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})	K_m (mM)	V_{max} (10^{-8} M s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
$Cu_{1.8}S$	0.33	5.59	2.11	2.14	3.91	0.22
$Cu_{1.8}S$-cgmc (0.55% carbon)	0.22	6.13	6.97	1.31	5.10	0.97
$Cu_{1.8}S$-cgmc (1.83% carbon)	0.16	7.53	11.75	0.99	6.24	1.57
$Cu_{1.8}S$-cgmc (4.07% carbon)	0.49	3.36	1.71	2.98	2.73	0.22

Table S3. Comparison the steady-state kinetic parameters for the peroxidase-like activity of $Cu_{1.8}S$-cgmc (1.83% carbon) and $Cu_{1.8}S$ using TMB and ABTS as substrates.

marterials	TMB			ABTS		
	K_m (mM)	V_{max} (10^{-8} M s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})	K_m (mM)	V_{max} (10^{-8} M s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
$Cu_{1.8}S$	0.33	5.59	2.11	0.34	4.8	1.99
$Cu_{1.8}S$-cgmc (1.83% carbon)	0.16	7.53	11.75	0.18	7.34	10.1

Table S4 Comparison of $Cu_{1.8}S$-cgmc and other nanomaterials in K_m and LOD for H_2O_2 detection.

Catalysts	K_m (mM)		LOD (μM)	Ref.
	TMB	H_2O_2		
HRP	0.434	3.7	---	S2
CuS-montmorillonite	0.0212	2.27	24.7	S3
MoS_2/PPy NMs	0.41	12.8	45	S4
CoS	0.41	7.15	20	S5
VO_2 nanofibers	0.518	1.043	18	S6
VO_2 nanosheets	0.11	2.924	266	S7
VO_2 nanorods	0.8	6.469	41	S7
Co_3O_4	0.012	0.026	---	S7
NiFe-LDH	0.5	2.4	23	S8
CeO_2NPs	0.274	0.278	19	S9
SiO_2/Imi/Pt	---	5.85	75	S10
Cu_9S_5	1.72	37.1	---	S11
$Cu_{1.8}S$	0.33	2.14	510	This work
$Cu_{1.8}S$-cgmc	0.16	0.99	20	This work

Table S5 Linear range and LOD of glutathione analysis obtained from different nanomaterials based colorimetric methods.

materials	linear range (μM)	LOD (μM)	reference
Co_3O_4	0.1-20	0.088	S12
Graphene dots	0.5-100	0.5	S13
MnO_2 nanosheets	1-25	0.3	S14
V_6O_{13} nanotextiles	2.5-30	0.63	S15
$Cu_{1.8}S$	7.5-300	0.79	This work
$Cu_{1.8}S$-cgmc	0.4-800	0.04	This work

References

S1. Pharmacopoeia of the People's Republic of China, People's Medical Publishing House, Beijing, China, 2020, **2**, 629.

S2. L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett and X. Yan, *Nat. Nanotechnol.*, 2007, **2**, 577-583.

S3. L. Zhang, M. Chen, Y. Jiang, M. Chen, Y. Ding and Q. Liu, *Sensor. Actuat. B Chem.*, 2017, **239**, 28-35.

S4. J. Lei, X. Lu, G. Nie, Z. Jiang and C. Wang, *Part. Part. Syst. Char.*, 2015, **32**, 886-892.

S5. H. Yang, J. Zha, P. Zhang, Y. Xiong, L. Su and F. Ye, *RSC Adv.*, 2016, **6**, 66963-66970.

S6. R. Tian, J. Sun, Y. qi, B. Zhang, S. Guo and M. Zhao, *Nanomaterials*, 2017, **7**, 347.

S7. H. Sun and W. Zhu, *Appl. Surf. Sci.*, 2017, **399**, 298-304.

S8. T. Zhan, J. Kang, X. Li, L. Pan, G. Li and W. Hou, *Sensor. Actuator. B Chem.*, 2018, **255**, 2635-2642.

S9. Q. Liu, Y. Yang, X. Lv, Y. Ding, Y. Zhang, J. Jing and C. Xu, *Sensor. Actuator. B Chem.*, 2017, **240**, 726-734.

S10. C. M. Maroneze, G. P. dos Santos, V. B. de Moraes, L. P. da Costa and L. T. Kubota, *Biosens. Bioelectron.*, 2016, **77**, 746-751.

S11. H. Zou, T. Yang, J. Lan and C. Huang, *Anal. Meth.*, 2017, **9**, 841-846.

S12. Y. Gao, K. Wu, H. Li, W. Chen, M. Fu, K. Yue, X. Zhu and Q. Liu, *Sensor. Actuator. B Chem.*, 2018, **273**, 1635-1639.

S13. A.-X. Zheng, Z.-X. Cong, J.-R. Wang, J. Li, H.-H. Yang and G.-N. Chen, *Biosens. Bioelectron.*, 2013, **49**, 519-524.

S14. J. Liu, L. Meng, Z. Fei, P. J. Dyson, X. Jing and X. Liu, *Biosens. Bioelectron.*, 2017, **90**, 69-74.

S15. H. Li, T. Wang, Y. Wang, S. Wang, P. Su and Y. Yang, *Ind. Eng. Chem. Res.*, 2018, **57**, 2416-2425.