Electronic Supplementary Material (ESI) for Analyst.

CuO nanorods decorated hemin-graphene with enhanced peroxidase-mimic performance for colorimetric and electrochemical determination of 4-aminophenol with a smartphone

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Contents

Chemicals and instruments

Chemicals

Graphite was provided by Alfa Aesar. Hemin, o-phenylenediamine (OPD), dimethyl sulfoxide (DMSO), polyvinyl pyrrolidone (PVP), ammonia solution ($NH_3 \cdot H_2O$, 25% wt), hydrazine hydrate ($N_2H_4 \cdot H_2O$, 80 wt%), NaOH, $Cu(NO_3)_2 \cdot 3H_2O$, meso-tetra(4-carboxyphenyl)porphine (TCPP), KCl, $AlCl_3$, tetrachlorobiphenyl (PCB72), atrazine (ATZ), terephthalic acid (TA), 4-aminophenol (4-AP), phenylamine (Pha), phenol (Ph), 4-chlorophenol (4-CP), 4-nitrophenol (4-NP), 2-aminophenol (2-AP), 3-aminophenol (3-AP), 3-nitrophenol (3-NP), 3-chlorophenol (3-CP), resorcinol (RC), bisphenol (BPA), KCl and $MgCl_2$ were bought from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). 3,3',5,5'- tetramethylbenzidine (TMB) was bought from Sigma-Aldrich (St. Louis, MO, U.S.A.). Hydrogen peroxide (H_2O_2, 30% wt) was bought from Tianli Chemical Reagent Co., Ltd. (Tianjin, China). Ethyl alcohol (C_2H_5OH) and 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), were bought from Innochem Co., Ltd. (Beijing, China). 5, 5-dimethyl-1-pyrroline-N-oxide (DMPO) were bought from Dojindo China Co., Ltd. (Shanghai: Subsidiary). All reactions were prepared with HAc-NaAc buffer (pH 4.0, 0.20 M).

Instruments

Ultraviolet-visible (UV-vis) spectrum was carried out on an Agilent Cary 8454 ultraviolet visible spectrophotometer (Agilent, U.S.A.). Scanning Electron Microscope (SEM) images were taken with merlin compact (Zeiss, Germany). Transmission electron microscopy (TEM) images were obtained with JEM 2100 (Japan). X-ray photoelectron spectroscopy (XPS) analysis of CuO/H-Gr nanocomposites was conducted on Escalab 250Xi (Thermo, U.S.A.). Electron spin resonance (ESR) measurements were performed via a Bruker EMXPLUS10/12 spectrometer. The electrochemical experiments were carried out an electrochemical workstation (CHI 660C, Shanghai Chenhua Instruments Company, China) with three-electrode system.

The synthesis of CuO, CuO/PVP-Gr and CuO/TCPP-Gr

PVP-Gr was prepared via the following approach. Briefly, 7.0 mg of PVP was dispersed in 10 mL of GO (0.50 $mg \cdot mL^{-1}$). Then 200 µL of $NH_3 \cdot H_2O$ (25 wt%) and 80 µL of $N_2H_4 \cdot H_2O$ (80 wt%) were successively added into the above-mentioned solution. After stirring for 15 min, the mixture was heated at 60°C in a water bath for 3.5 h. Finally, the obtained stable black suspension was centrifuged (12,000 rpm, 10 min) and washed for two times with distilled water to obtain PVP-Gr.

TCPP-Gr was prepared via the following approach. Briefly, 7.0 mg of TCPP was dispersed in 10 mL of GO (0.50 $mg \cdot mL^{-1}$). Then 200 µL of $NH_3 \cdot H_2O$ (25 wt%) and 80 µL of $N_2H_4 \cdot H_2O$ (80 wt%) were successively added into the above-mentioned solution. After stirring for 15 min, the mixture was heated at 60°C in a water bath for 3.5 h. Finally, the obtained stable black suspension was centrifuged (12,000 rpm, 10 min) and washed for two times with distilled water to obtain TCPP-Gr.

CuO was prepared through the following procedure: 1.0 mL of NaOH (0.50 M) was quickly added to 200 µL of $Cu(NO_3)_2 \cdot 3H_2O$ (0.020 M) stirring for 5 min at 100°C. The obtained suspension was centrifuged and wash with distilled water to gain CuO.

The CuO/PVP-Gr was synthesized according to the following method: 200 µL of $Cu(NO_3)_2 \cdot 3H_2O$ (0.020 M) was dissolved in 2.0 mL of PVP-Gr aqueous (0.50 $mg \cdot mL^{-1}$) with vigorous stirring at ambient temperature. Subsequently, the homogeneous dispersion was heated at 100°C with vigorous stirring. Then 1.0 mL of NaOH (0.50 M) was quickly mixed with the above solution, keeping for 5 min. Finally, the solution was washed and redispersed in distilled water. The CuO/TCPP-Gr was prepared by an analogous approach.

1. The CV curves of H-Gr

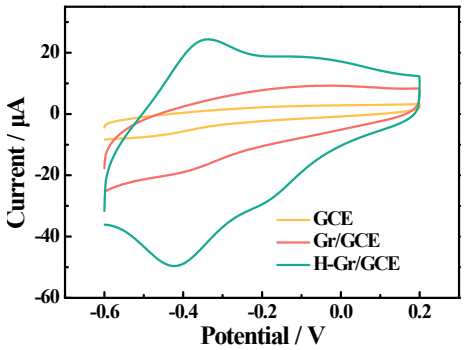


Fig. S1 CV curves of GCE, Gr/GCE and H-Gr/GCE in 0.1 M PBS (pH 7.0).

2. XPS spectrum

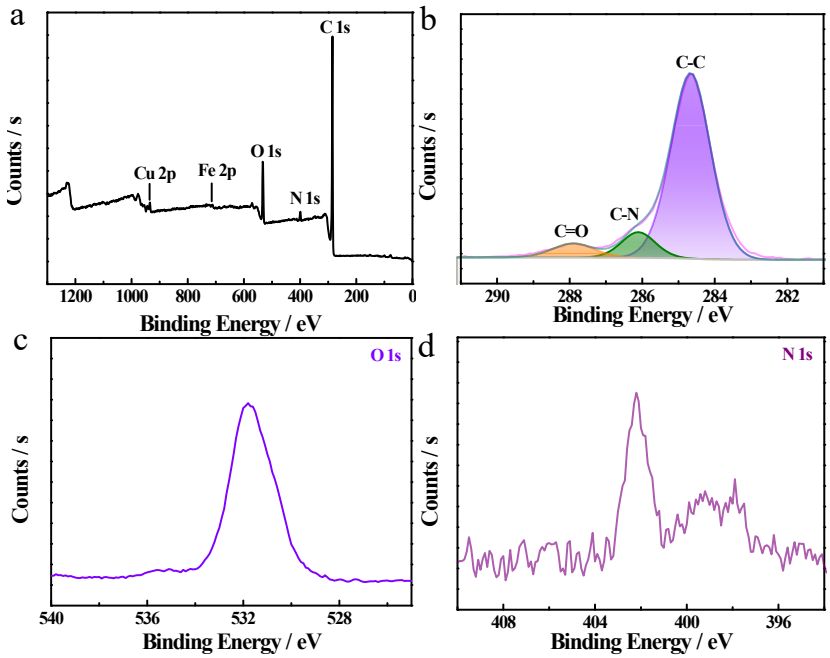
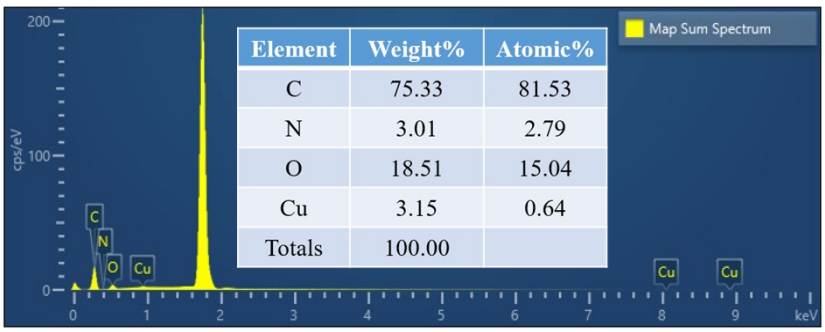


Fig. S2 (a) XPS spectrum of CuO/H-Gr nanomaterial; (b) XPS spectrum of C 1s; (c) XPS spectrum of O 1s; (d) XPS spectrum of N 1s.

3. EDX spectroscopy of CuO/H-Gr



Element	Weight%	Atomic%
C	75.33	81.53
N	3.01	2.79
O	18.51	15.04
Cu	3.15	0.64
Totals	100.00	

Fig. S3 EDX spectroscopy of CuO/H-Gr.

4. The CuO/H-Gr catalyze oxidation of various substrates

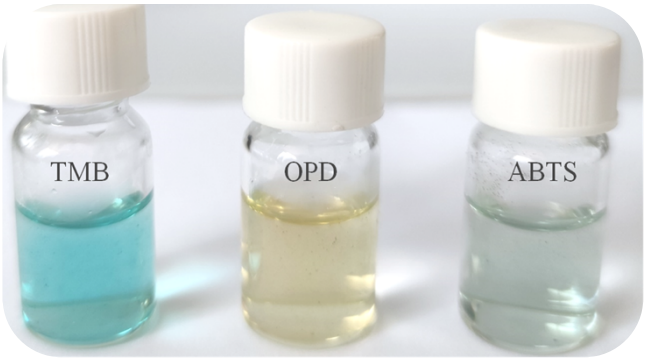


Fig. S4 The CuO/H-Gr catalyze oxidation of various peroxidase substrates in the presence of H_2O_2 to produce different color reactions.

5. The effects of different factors on the peroxidase-like activity of CuO/H-Gr

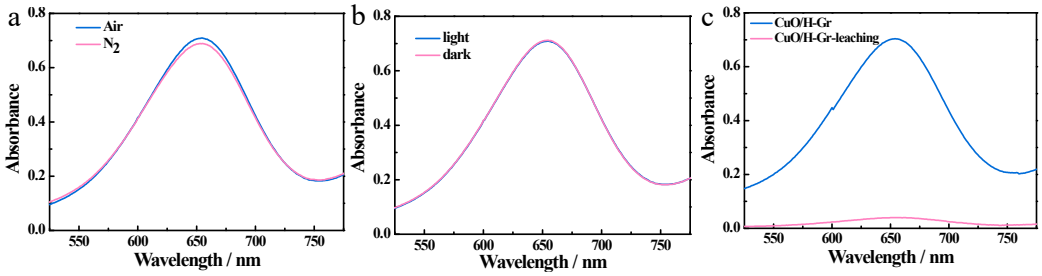


Fig. S5 The effects of different factors on the peroxidase-like activity of CuO/H-Gr.

6. The peroxidase-like activity of different nanomaterials

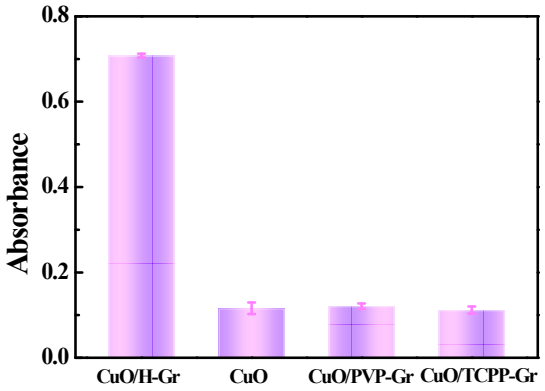


Fig. S6 The peroxidase-like activity of different nanomaterials.

7. Steady-state kinetic assays and corresponding double reciprocal (Lineweaver-Burk) plots of CuO/H-Gr

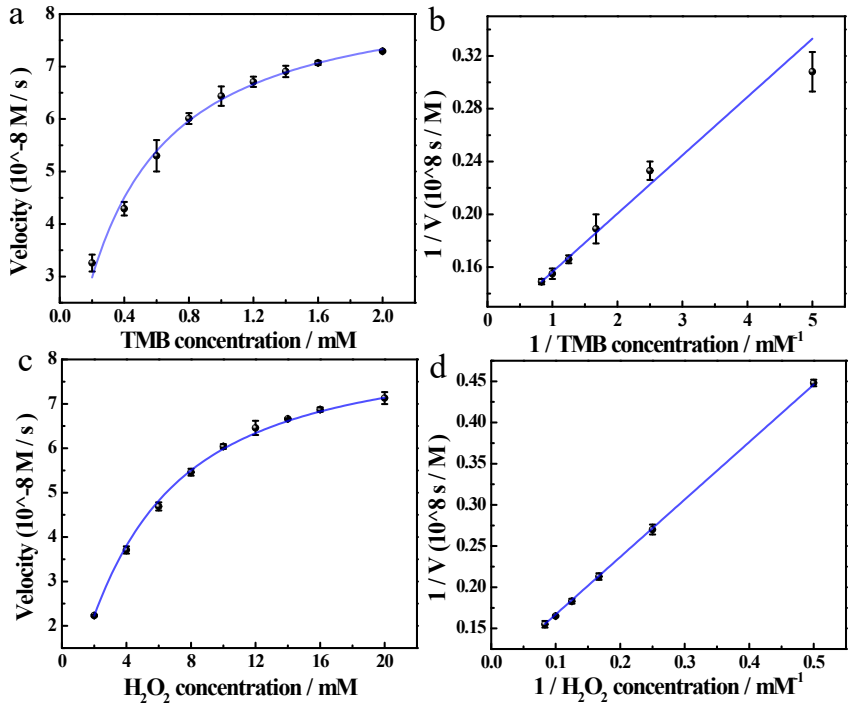


Fig. S7 Steady-state kinetic assays (a, c) and corresponding double reciprocal (Lineweaver-Burk) plots (b, d) of CuO/H-Gr.

8. Steady-state kinetic assays and corresponding double reciprocal (Lineweaver-Burk) plots of CuO

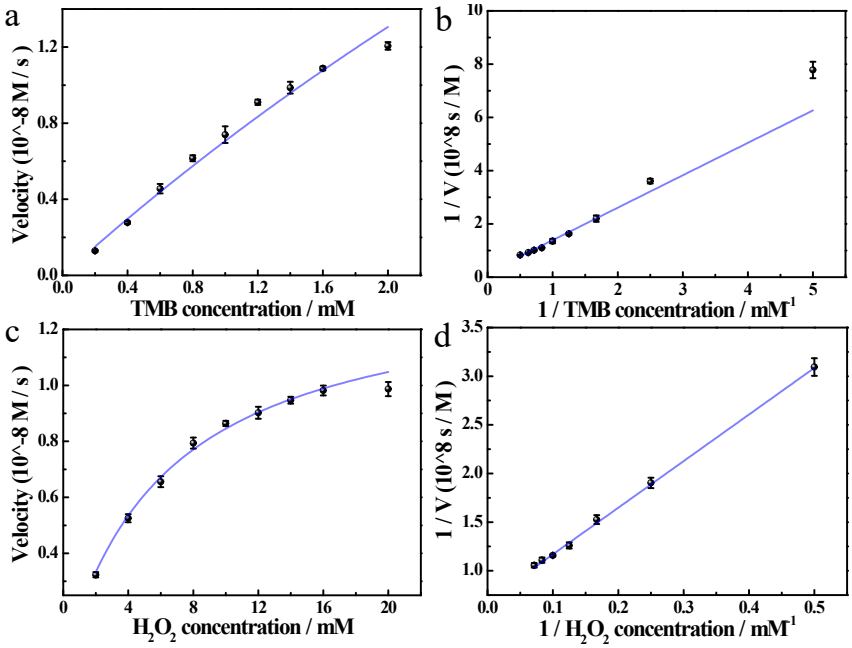


Fig. S8 Steady-state kinetic assays (a, c) and corresponding double reciprocal (Lineweaver-Burk) plots (b, d) of CuO.

9. The fluorescence spectrum

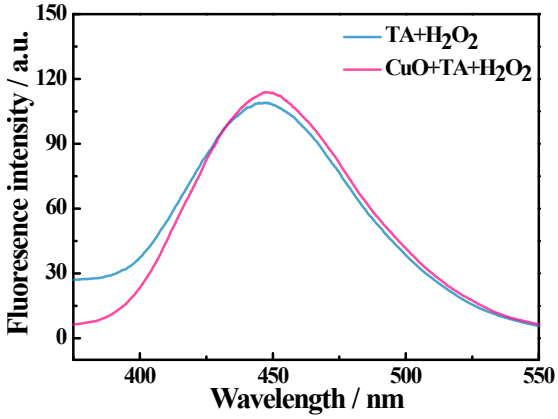


Fig. S9 The fluorescence spectrum of different systems.

10. The fluorescence spectrum

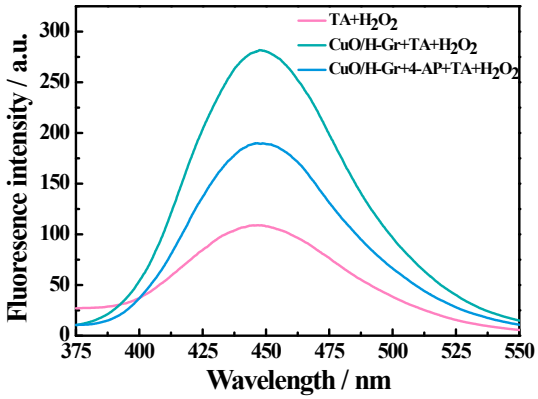


Fig. S10 The fluorescence spectrum of different systems.

11. DPV curves

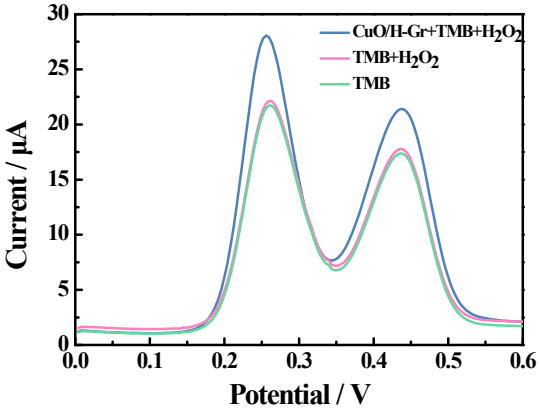


Fig. S11 The DPV curves of different systems.

12. Comparison of the proposed assay method with this work for 4-AP detection.

Table S1 Comparison of the proposed assay method with this work for 4-AP detection.

Method	Materials	Linearity range (μM)	LOD (μM)	References
Colorimetric method	Co_3O_4@Rh NC	1.70-105	0.680	1
	Mo_5N_6 Ns	1.00-80.0	0.560	2
	Au@Ag nanocrystals	1.00-70.0	0.640	3
	Ag NPs	0-85.0	0.320	4
	CuO/H-Gr	1.00-200	0.687	This work
Electrochemical method	D-h-BN/GCE	0.0100-30.0	0.00330	5
	Au/Pd/rGO	1.00-300	0.120	6
	BN-IL-WS_2/GCE	0.0100-50.0	0.00300	7
	ZnO/NPC/GCE	5.00-120	0.0140	8
	rGO/AuNPs/MWCTs	0.0500-25.0	0.00295	9
	Co_3O_4@g-C_3N_4/SPE	0.0500-780	0.0150	10
	N-rGO/CuO/ILCPE	0.100-800	0.0270	11
	CuO/H-Gr	0.0100-300	0.00756	This work

References

1 Q. Zhao, X. Zheng, L. Xing, Y. Tang, X. Zhou and L. Hu, *J. Hazard. Mater.*, 2021, **409**, 125019.
2 C. Xia, W. Huang, X. Kang, P. Chen, L. You and L. Guo, *Microchim. Acta*, 2022, **189**, 1.
3 T. Lin, Z. Li, Z. Song, H. Chen, L. Guo, F. Fu and Z. Wu, *Talanta*, 2016, **148**, 62-68.
4 S. Shaban, B-S. Moon and D-H. Kim, *Environ. Technol. Innov.*, 2021, **22**, 101517.
5 Y. Shen, H. Yan, H. Guo, Y. Long and W. Li, *Sens. Actuators B Chem.*, 2020, **303**, 127248.
6 H. Wang, S. Zhang, S. Li and J. Qu, *Talanta*, 2018, **178**, 188-194.
7 X. Li, Z. Niu, Z. Lin, Y, Qin, H. Huang, L. Wang, J. Zhang and Z. Xu, *Microchim. Acta*, 2019, **186**, 614.
8 S. Chen, R. Huang, Z. Jiao, D. Liao, J. Yu and X. Jiang, *Ecotoxicol. Environ. Saf*, 2020, **191**, 110194.
9 N. Dou and J. Qu, *Anal. Bioanal. Chem.*, 2021, **413**, 813-820.
10 P. Jahani, H. Beitollahi, F. Nejad, Z. Dourandish and A. Di Bartolomeo, *Nanotechnology*, 2022, **33**, 395702.
11 M. Poursoltani Zarandi and H. Beitollahi, *Microchem. J.*, 2022, **181**, 107726.