Supporting information

Development of a Zr/Fe-MIL nanozyme sensor integrated with user-friendly colorimetric device for glyphosate monitoring

Zeyan Zheng ^a, Kailong Li ^b, Shizhuo Wang ^a, Haojie Zhao ^a, Xuewei Yang ^a, Yajing Niu ^a, Mengyang Li ^a, Xueli Luo ^c, Zhonghong Li ^a, and Wenzhi Tang ^a, *

^a College of Food Science and Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China

b College of Forestry, Northwest A&F University, Yangling, Shaanxi 712100, China

c School of Food Science and Engineering, Ningxia University, Yinchuan, Ningxia 750021, PR China

* Corresponding author: tangwenzhi@nwsuaf.edu.cn

Table of Contents

Fig. S1. SEM image of Fe-MIL5
Fig. S2. The high-resolution XPS spectra of Zr3d in Zr/Fe-MIL6
Fig. S3. The high-resolution XPS spectra of Fe2p in Zr/Fe-MIL7
Fig. S4. The UV - vis spectra of reaction solutions, all reactions were performed in
Tris-HCl buffer (pH = 3.0)8
Fig. S5. UV-vis spectra of Zr/Fe-MIL in buffer, TMB, H_2O_2 and TMB + H_2O_2 + buffer
system9
Fig. S6. Effect of pH on the POD - like activity of Zr/Fe-MIL10
Fig. S7. Effect of temperature on the POD - like activity of Zr/Fe-MIL11
Fig. S8. (A) The relative activity of different scavengers on $Zr/Fe-MIL +TMB+H_2O_2$
(n = 3); (B) Illustration of the possible catalytic activity of Zr/Fe-MIL in the oxidation
of TMB with H_2O_2
Fig. S9. Optimization of (A) Fe: Zr; (B) buffer solution pH, (C) Zr/Fe-MIL
concentration, (D) TMB concentration and (E) H_2O_2 concentration for the GLP
detection (n = 3)
Fig. S10. Selectivity of the colorimetric sensor towards GLP in the presence of
interferents14
Fig. S11. The dynamic changes of the solution on the paper strip within 30 s15
Fig. S12. (A) The physical picture of the portable device; (B) The stability of the
portable device both indoor and daylight within 30 min16
Fig. S13. Establishment of the calibration curve for UPLC
Fig. S14. The EPR plots before and after the interaction between Zr/Fe-MIL and GLP.
Fig. S15. The steady-state kinetic curve and double reciprocal plot of Zr/Fe-MIL after
incubation with GLP
Fig. S16. SEM images before and after the interaction between Zr/Fe-MIL and GLP.
Fig. S16. SEM images before and after the interaction between Zr/Fe-MIL and GLP.

Fig. S18. The comparison of peroxidase inhibition effects of glyphosate on Fe-MIL and
Zr/Fe-MIL
Fig. S19. The high-resolution XPS spectra of N1s in Zr/Fe-MIL before and after GLP
capture
Fig. S20. (A) POD-like activity of Zr/Fe-MIL after five successive cycles; (B) The
oxidase-like activity of four different batches Zr/Fe-MIL; (C)The relative activity of
Zr/Fe-MIL in 15 days; (D) The POD activity changes of Zr/Fe-MIL were exposed to
light irradiation for 10 days24

Table S1. Comparison on reported methods for the determination	of GLP26
Table S2. The practical application of the UPLC standard method	in detecting GLP in
actual samples	27

Materials and reagents

All chemicals used in the study were commercially available and used directly without additional purification. Zr (NO₃)₄·5H₂O, 1,4-dicarboxybenzene, 3,3',5,5'tetramethyl benzidine (TMB), o-phenylenediamine (OPD), 2,2'-azino-bis (3ethylbenzthiazoline-6-sulfonic acid) (ABTS), tris (hydroxymethyl) methyl aminomethane (Tris) were obtained from Aladdin (Shanghai, China). N, N-dimethyl formamide (DMF) and sodium acetate were supplied by Chengdu Kelong Chemical Reagent Factory (Chengdu, China). Glucose (Glu), Leucine (Leu), phenylalanine (Phe), histidine (His), and Threonine (Thr) were obtained from Guangdong Chemical Regent Engineering-technological Research and Development Center (Shantou, China). Glyphosate (GLP), 2, 4-dichlorphenoxyacetic acid (2, 4-D), thiophanatemethyl (TM), carbendazim (MBC), and tartaric acid (TA) were purchased from Aladdin (Shanghai, China). NaCl, KCl, and FeCl₃·6H₂O were provided by Xilong Chemical Industry Co. Ltd. (Shantou, China). The food samples were purchased from a local supermarket (Yangling, China). Filter paper was bought from Cytiva (Uppsala, Sweden). Deionized water was used in all experiments.

POD activity of Zr/Fe-MIL

To ascertain the POD enzyme activity of material, 20 μ L of 1 mg mL⁻¹ materials (Zr/Fe-MIL, Fe-MIL and Zr-MIL) suspension was mixed with 755 μ L of 0.1 M Tris-HCl buffer (pH = 3.0). Subsequently, 25 μ L of TMB (20 mM) and 200 μ L of H₂O₂ (50 mM) were added to initiate the catalytic reaction. After 2 min, the absorbances were

measured at 652 nm. The effects of reaction temperature (20°C to 90°C) was investigated by performed the catalytic reaction in a water bath, and the effect of pH (2.5 to 4.5) was investigated by adjusting the pH of Tris-HCl buffer. All the experiments were repeated three times.

Parameter Optimization for GLP Assay

The pH of the buffer (2.5 - 4.5), concentrations of Zr/Fe-MIL (0.005 mg mL⁻¹ - 0.04 mg mL⁻¹), TMB (0.1 mM - 2.0 mM), and H₂O₂ (1 mM - 20 mM) were optimized. The absorbance of oxTMB at 652 nm ($\Delta A = A_0 - A$, where A_0 and A represent the absorbance in the presence and absence of GLP, respectively) was measured.

Specificity tests

To assess the selectivity of the Zr/Fe-MIL nanozyme-mediated sensing platform, Zr/Fe-MIL solution containing 100 - fold ions, 10 - fold amino acids, 5 - fold sugars, and equivalent pesticides concentrations of GLP were mixed, respectively, followed by incubating for 5 min in Tris-HCl buffer at room temperature. Subsequently, 20 mM TMB and 50 mM H_2O_2 were added and after 2 min A_{652} was monitored.

Detection of GLP in real samples

The concentration of GLP in real food samples (water, soybean, sorghum) was quantified using the standard addition assay. Water was collected from the laboratory. The pre-treatment of sorghum and soybean was pretreated using a modified QuEChERS method, referring to previous work ¹. Then, the extracted samples were tested with the strips and the portable reader.



Fig. S1. SEM image of Fe-MIL.



Fig. S2. The high-resolution XPS spectra of Zr3d in Zr/Fe-MIL.



Fig. S3. The high-resolution XPS spectra of Fe2p in Zr/Fe-MIL.



Fig. S4. The UV - vis spectra of reaction solutions, all reactions were performed in Tris-HCl buffer (pH = 3.0).



Fig. S5. UV-vis spectra of Zr/Fe-MIL in buffer, TMB, H_2O_2 and TMB + H_2O_2 + buffer system.



Fig. S6. Effect of pH on the POD - like activity of Zr/Fe-MIL.



Fig. S7. Effect of temperature on the POD - like activity of Zr/Fe-MIL.



Fig. S8. (A) The relative activity of different scavengers on Zr/Fe-MIL +TMB+ H_2O_2 (n = 3); (B) Illustration of the possible catalytic activity of Zr/Fe-MIL in the oxidation of TMB with H_2O_2 .



Fig. S9. Optimization of (A) Fe: Zr; (B) buffer solution pH, (C) Zr/Fe-MIL concentration, (D) TMB concentration and (E) H_2O_2 concentration for the GLP detection (n = 3).



Fig. S10. Selectivity of the colorimetric sensor towards GLP in the presence of interferents.



Fig. S11. The dynamic changes of the solution on the paper strip within 30 s.



Fig. S12. (A) The physical picture of the portable device; (B) The stability of the portable device both indoor and daylight within 30 min.



Fig. S13. Establishment of the calibration curve for UPLC.



Fig. S14. The EPR plots before and after the interaction between Zr/Fe-MIL and GLP.



Fig. S15. The steady-state kinetic curve and double reciprocal plot of Zr/Fe-MIL after incubation with GLP.



Fig. S16. SEM images before and after the interaction between Zr/Fe-MIL and GLP.



Fig. S17. pHpzc of Zr/Fe-MIL composite beads.



Fig. S18. The comparison of peroxidase inhibition effects of glyphosate on Fe-MIL and Zr/Fe-MIL.



Fig. S19. The high-resolution XPS spectra of N1s in Zr/Fe-MIL before and after GLP capture.



Fig. S20. (A) POD-like activity of Zr/Fe-MIL after five successive cycles; (B) The POD-like activity of four different batches Zr/Fe-MIL; (C)The relative activity of Zr/Fe-MIL in 15 days; (D) The POD-like activity changes of Zr/Fe-MIL were exposed to light irradiation for 10 days.

Table S1. Comparison on reported methods for the determination of GLP					
Sensor	Linear range (µg mL ⁻¹)	LOD (µg mL ⁻¹)	References		
CDs	0.003-0.338	0.101	2		
DNA-AgNCs	0.015-0.100	0.005	3		
MPH-Cu ²⁺	0.845-2.705	0.099	4		
Ponceau 4 R	0.170-15.210	0.023	5		
MQDs@CuNi	0-16.900	0.190	6		
MOF-Calix	0.422-7.608	0.380	7		
Zr/Fe-MIL	0.025-1.000	0.079	This work		

 Table S1. Comparison on reported methods for the determination of GLP

Samula	Spilled (up mI-l)	UPLC $(n = 3)$		
Sample	Spiked (µg mL ⁺)	Found ($\mu g m L^{-1}$)	Recovery (%)	RSD (%)
Water	2	2.1596 ± 0.0965	107.98	1.7546
	4	4.1249 ± 0.0756	103.12	1.2967
	8	7.9568 ± 0.1856	99.46	3.4261
Sorghum	2	2.0516 ± 0.0853	102.58	2.0532
	4	4.1345 ± 0.1264	103.06	1.9562
	8	8.1026 ± 0.1026	101.28	1.2635
Soybean	2	2.0536 ± 0.0756	102.68	2.0261
	4	3.9526 ± 0.1268	98.82	1.5699
	8	7.9234 ± 0.1062	99.04	2.1266

Table S2. The practical application of the UPLC standard method in detecting GLP in actual samples

References

- 1. X. L. Luo, G. L. Huang, C. X. Bai, C. Y. Wang, Y. Yu, Y. W. Tan, C. Y. Tang, J. Kong, J. H. Huang and Z. H. Li, *J. Hazard. Mater.*, 2023, 443.
- 2. Y. S. Yuan, J. Z. Jiang, S. P. Liu, J. D. Yang, H. Zhang, J. J. Yan and X. L. Hu, Sen. Actuators B Chem., 2017, 242, 545-553.
- Y. X. Yang, B. Ghalandari, L. Y. Lin, X. Sang, W. Q. Su, A. Divsalar and X. T. Ding, Food Chem., 2022, 367.
- Y. T. Liu, Q. Q. Zhang, S. Y. Yao, H. W. Cui, Y. L. Zou and L. X. Zhao, *J. Hazard. Mater.*, 2024,
 477.
- 5. B. C. Hsieh and Y. T. Li, Sen. Actuators B Chem., 2024, 401.
- Y. L. Guo, X. T. Li, P. Shen, X. C. Li, Y. H. Cheng and K. Chu, J. Colloid Interf. Sci., 2024, 661, 533-543.
- 7. C. X. Yu, F. L. Hu, J. G. Song, J. L. Zhang, S. S. Liu, B. X. Wang, H. Meng, L. L. Liu and L. F. Ma,

Sen. Actuators B Chem., 2020, 310.