Supporting Information

for

Hierarchical MOF-on-MOF Structured Dual-Functional Nanozyme for Enhanced Catalysis and Dual-Channel Detection of α-Glucosidase

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Materials and instruments

4,4'-Bipyridine (4,4'-bipy), Ethanol (EtOH), Copper(II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$), polyvinylpyrrolidone (PVP), dimethylimidazole (2-MI), ceric ammonium nitrate ($(NH_4)_2Ce(NO_3)_6$), 4-aminoantipyrine (4-AAP), 2,4-dichlorophenol (2,4-DP), 3,3',5,5'-tetramethylbenzidine (TMB), α-glucosidase (α-Glu), phenyl-α-D-glucopyranoside (Phenyl-α-Glc), phenol, ascorbyl glucoside (AAG), ascorbic acid (AA), ampliflu red (AR), acetonitrile (ACN) were purchased from Aladdin (Shanghai, China). All the reagents used were of analytical purity without further purification.

TEM images and EDS mapping were captured on JEOL-JEM-F200. Zeta potential was measured on Malvern Zetasizer (UK). X-ray Photoelectron Spectroscopy was measured on Thermo Fisher-K-Alpha U.S.A. The absorbance and fluorescence of sensing system were collected on Spectra Max iD3 Multi-Mode Microplate Reader. Electron Paramagnetic Resonance (EPR) spectra was collected on Bruker-Magnettech ESR 500. X ray diffraction spectra were collected on Bruker D8 Discover.

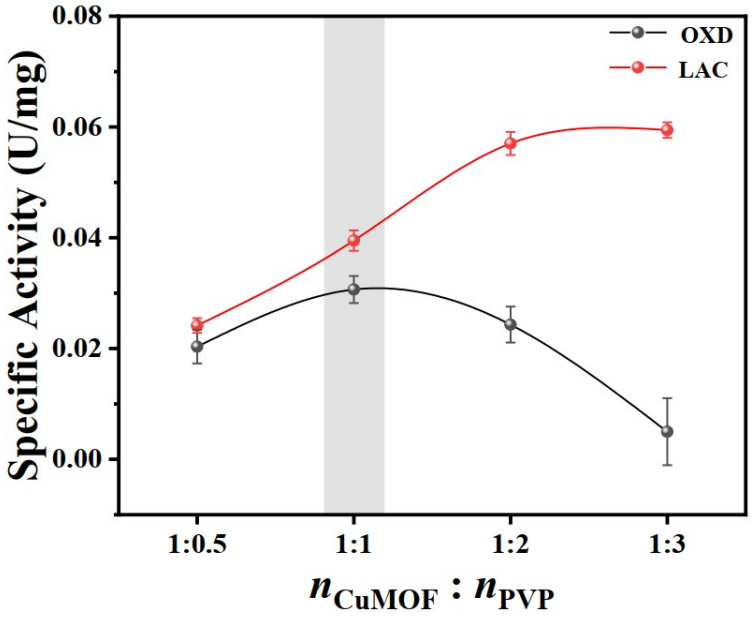

Fig. S1 The screening of optimal CuMOF and PVP ratio.

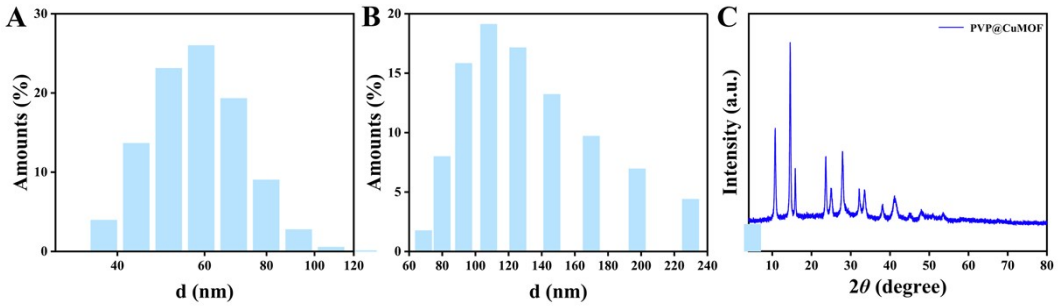

Fig. S2 (A) The DLS of CuMOF. (B) The DLS of CeMOF. (C) The XRD pattern of PVP@CuMOF.

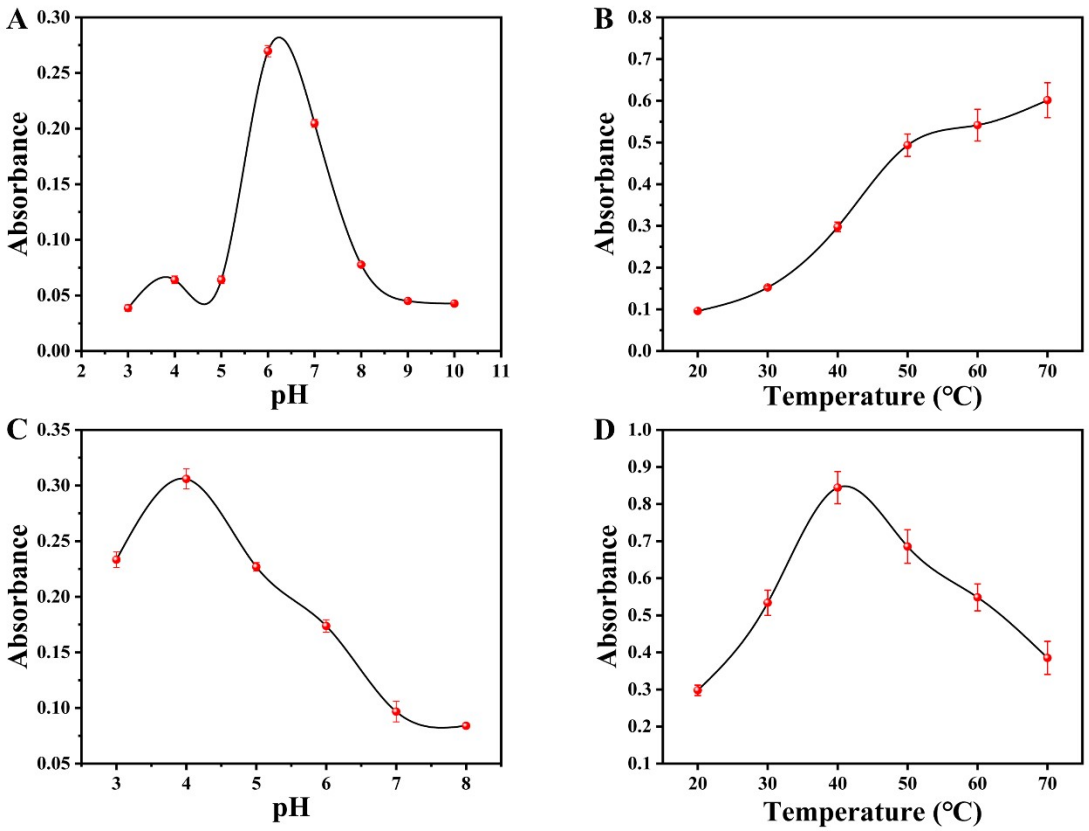

Fig. S3 (A) The screening of optimal pH for LAC-like activity. (B) The screening of optimal temperature for LAC-like activity. (C) The screening of optimal pH for OXD-like activity. (B) The screening of optimal temperature for OXD-like activity.

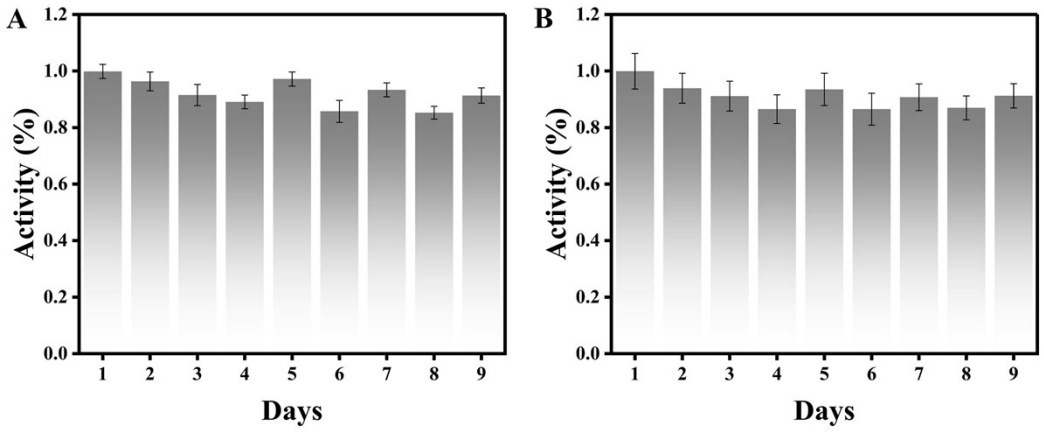

Fig. S4 The OXD-like (A) and LAC-like (B) storage stability of CeM-ON-CuM.

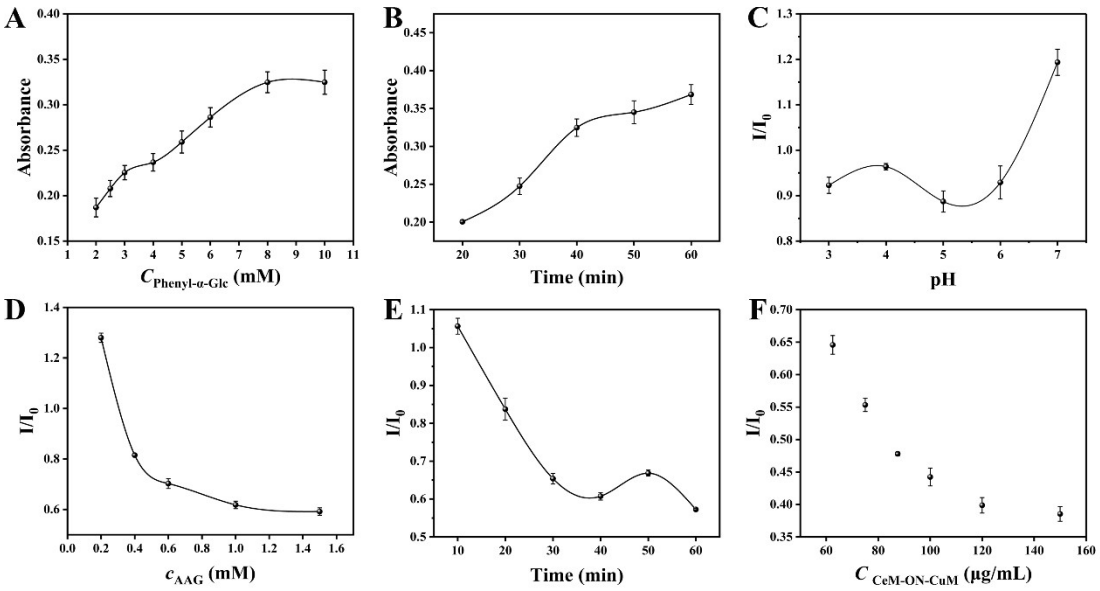

Fig. S5 (A) The screening of optimal Phenyl-α-Glc concentration. (B) The screening of optimal reaction duration for Phenyl-α-Glc hydrolysis. (C) The screening of optimal pH for AAG hydrolysis. (D) The screening of optimal AAG concentration for AA production. (E) The screening of reaction duration for AAG hydrolysis. (F) The screening of optimal CeM-ON-CuM concentration for the fluorescent sensing.

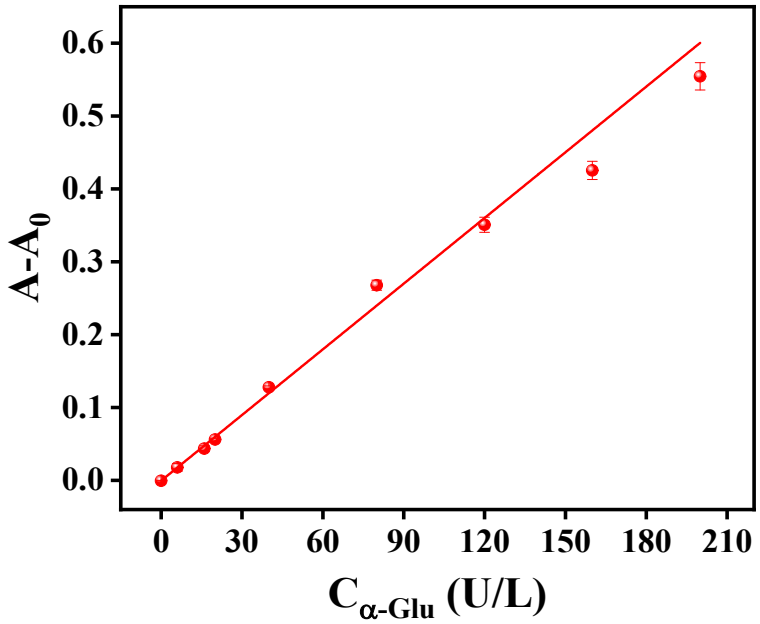

Fig. S6 The standard cure of pNPG based α-Glu sensing method.

Table S1 Comparison of kinetic parameters of OXD-like and LAC-like activity.

Materials	**Substances**	**K_m (mM)**	**V_m (10^{-8} M s^{-1})**	**Ref**
Ru SA/GFs	2,4-DP	0.324	3.43	[1]
Laccase	2,4-DP	0.40	6.35	[2]
Cu-GMP	2,4-DP	0.13	4.20	[3]
Cu-GMP+HBT	2,4-DP	0.12	6.5	[3]
Adenine phosphate-	2,4-DP	2.5	3.7	[4]
Bpy-Cu	2,4-DP	0.21	6.85	[5]
Fe-88A@CeO_2/CDs	TMB	0.69	24.28	[6]
Fe-88A@CeO_2	TMB	0.74	11.12	
WSe_2	TMB	0.12	1.67	[7]
$MoSe_2$	TMB	0.29	2.28	
CeM-ON-CuM	2,4-DP	0.87	6.15	This work
	TMB	0.54	2.89	

Table S2 Comparison between our methods and reported methods on α-Glu detection

Materials	**Methods**	**Linear range (U/L)**	**LOD (U/L)**	**Ref**
BSN-CQDs	fluorescent	10–5000	3	[8]
CoOOH NFs	colorimetric	10–400	4.8	[9]
N,B-CDs	fluorescent	10-1000	3	[10]
CuNCs@ZIF-8	fluorescent	5-500000	3	[11]
GSH-AuNCs	fluorescent	10-100	5.5	[12]
oxygen deficient MoO_3-x	colorimetric	10-1000	0.08	[13]
Cu-N-C SAzyme	colorimetric	0.25-8	0.09	[14]
Ag_3PO_4	colorimetric	0.01–60 U/L	0.005	[15]
Ce-ON-CuM	fluorescent	0.6-200	0.35	This work
	colorimetric	0.2-160	0.16	This work

Table S3 Colorimetric and fluorescent dual-mode detection of α-Glu in human serum.

	Added (U/L)			Found (U/L)			Recovery (%)			RSD (%)		
	F.L	Abs.	Ref.	F.L.	Abs.	Ref.	F.L.	Abs.	Ref.	F.L.	Abs.	Ref.
1	--	--	--	--	--		--	--		--	--	
2	40	40	40	40.22	38.86	41.28	100.6	97.2	103.2	4.00	2.81	5.6
3	60	80	60	62.07	82.77	69.99	103.5	103.5	116.7	6.07	1.08	0.1
4	80	100	80	84.01	119.1	88.55	105.0	119.1	103.9	4.12	0.46	0.44

References

1. R. Wang, X. Ma, E. M. Hamed, B. Cao, L. Wang, S. F. Y. Li and Y. Zhu, *Sensor. Actuat. B: Chem.*, 2025, **426**.
2. L. Yang, X.-Y. Guo, Q.-H. Zheng, Y. Zhang, L. Yao, Q.-X. Xu, J.-C. Chen, S.-B. He and W. Chen, *Sensor. Actuat. B: Chem.*, 2023, **393**.
3. X. Wang, K. Li, Y. Wang, Q. Han, X. Chen, H. Huang and Y. Li, *Chemical Engineering Journal*, 2025, **523**.
4. T.-Q. Chai, G.-Y. Chen, L.-X. Chen, J.-L. Wang, C.-Y. Zhang and F.-Q. Yang, *Analytica Chimica Acta*, 2023, **1279**.
5. M. Li, Y. Xie, D. Song, H. Huang and Y. Li, *Talanta*, 2023, **252**.
6. K. Liu, H. Zhu, F. Shi, J. Li, X. Li, Z. Lai, H. Li, H. Zeng, Z. Yang and H. Pang, *Adv. Sci.*, 2025, **12**.
7. J. Wang, M. Shen, F. Meng, X. Han and M. Zhang, *Chemical Engineering Journal*, 2025, **512**.
8. C. Yin, M. Wu, T. Liu, L. Fu, Q. Sun, L. Chen and N. Niu, *Microchemical Journal*, 2022, **178**.
9. D. Ma, J. Ge, A. Wang, J. Li, H. Yang, W. Zhai and R. Cai, *J Mater Chem B*, 2023, DOI: 10.1039/d2tb02580a.
10. S. Huang, E. Yang, J. Yao, Y. Liu and Q. Xiao, *Microchimica Acta*, 2018, **185**.
11. W. Huang, Y. Lu, N. Yao, X. Zhang, N. Wang and Y. Liu, *Sensor. Actuat. B: Chem.*, 2024, **404**.
12. W. Zhang, X. Jiang, Y. Wu, J. Jiang, X. Liu, Y. Liu, W. Wang, J. Lai and X. Wang, *Microchemical Journal*, 2022, **183**.
13. F. Zhang, J. Liu, Z. Chen, E. Wang, C. Li, J. Cheng, J. Shen and Z. Xu, *Analytica Chimica Acta*, 2024, **1293**.
14. Y. Jiang, Z. Chen, Y. Yuan, L. Tian, C. Dong, W. Shen, J. Wei, S. Wang, Y. Yang and J. Ge, *Materials Today Chemistry*, 2024, **41**.
15. D. Wu, N. Hu, J. Liu, G. Fan, X. Li, J. Sun, C. Dai, Y. Suo, G. Li and Y. Wu, *Talanta*,

2018, **190**, 103-109.