Supplementary Information

Highly active silver-based laccase-like nanozyme for colorimetric distinction and detection of phenylenediamine isomers

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1. Reagents and instrument

Sodium malate, 4-aminoantipyrine (4-AP), 2,4-dichlorophenol (2,4-DP), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), p-chlorophenol (p-CP), phenol (Ph), 3-methoxyphenol (3-MP), 2,4,6-trichlorophenol (2,4,6-TCP), bisphenol A (BPA), resorcinol (Res), 3-nitrophenol (3-NP), o-phenylenediamine (OPD), m-phenylenediamine (MPD), p-phenylenediamine (PPD), aniline (An), melamine (Mel), 4-chloroaniline (4-CA), diphenylamine (DPA), furfuryl alcohol, mannitol, p-benzoquinone, HCl, $NaNO_3$, Na_2SO_4, Na_2CO_3, CH_3COONa, KNO_3, $Mg(NO_3)_2$, $Ba(NO_3)_2$, $AgNO_3$, $Ca(NO_3)_2{\cdot}4H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$were purchased from Aladdin Co. Ltd. (Shanghai, China). *Trametes versicolor* laccase (≥ 0.5 U/mg) was purchased from Yuanye Biotechnology Co. Ltd. (Shanghai, China). Horseradish peroxidase (HRP) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich Trading Co. Ltd. (Shanghai, China). Hydrogen peroxide (H_2O_2) was obtained from Macklin Reagent Company (Shanghai, China). All chemical reagents were analytically pure and deionized water was used in the experiment.

The PhenomLE scanning electron microscopy (SEM) (Phenom, Netherlands) was used to photograph the morphology of AgMal. Transmission electron microscope (TEM) image of AgMal was acquired by Tecnai G2 F20S (FEI, USA). The elemental composition and chemical states of AgMal were analyzed using an AXIS SUPRA X-ray photoelectron spectrometer (XPS) (Shimadzu, Japan) equipped with a monochromatic Al Kα radiation (hν = 1486.6 eV). The functional groups of AgMal were determined through a Nicolet Is50 Fourier transform infrared (FT-IR) spectrometer (Thermo Fisher Scientific, USA). Ultraviolet-Visible (UV-Vis) absorption spectra were recorded by Buetta fluorescence and absorbance spectrometer (HORIBA, Japan).

2. Supplementary Figures and Tables

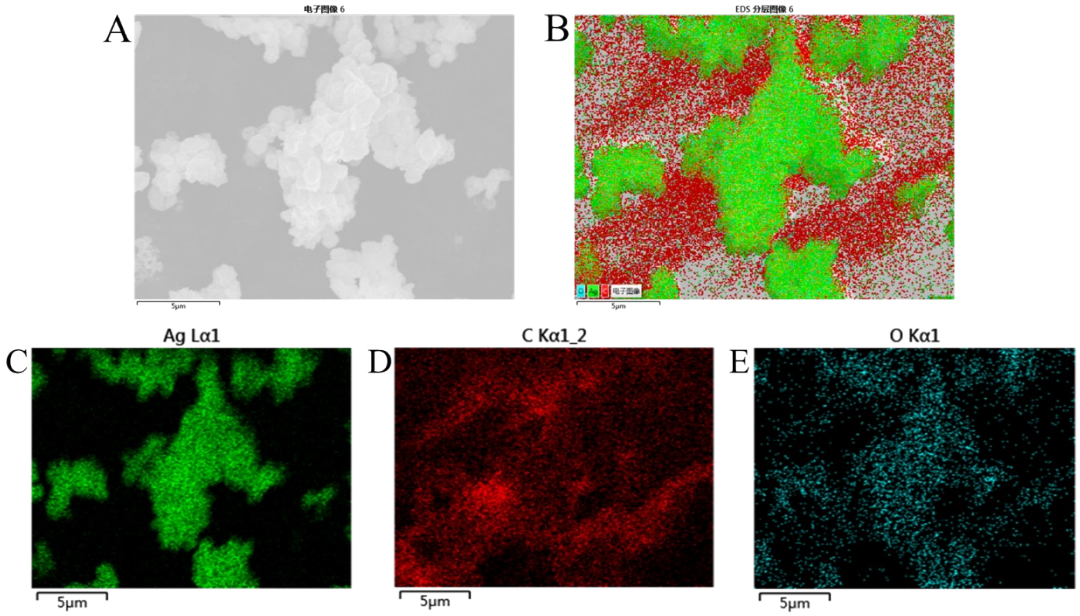


Fig. S1. (A) Electron image of AgMal; (B) EDS layered image of AgMal, corresponding element mapping image: (C) Ag; (D) C; (E) O.

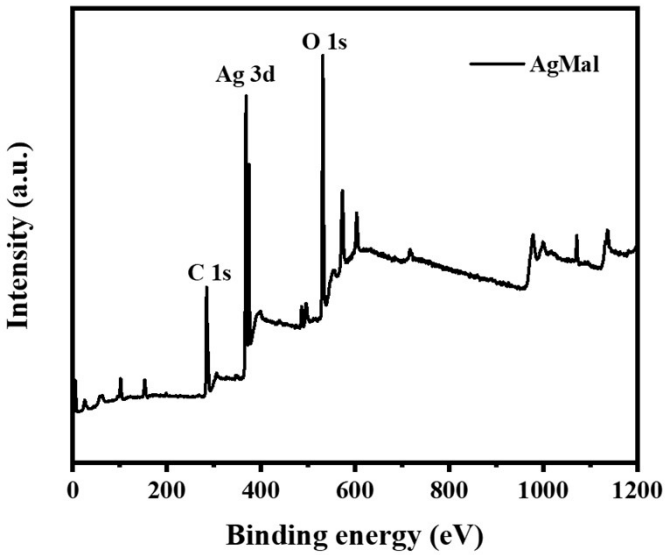


Fig. S2. XPS survey spectrum of AgMal.

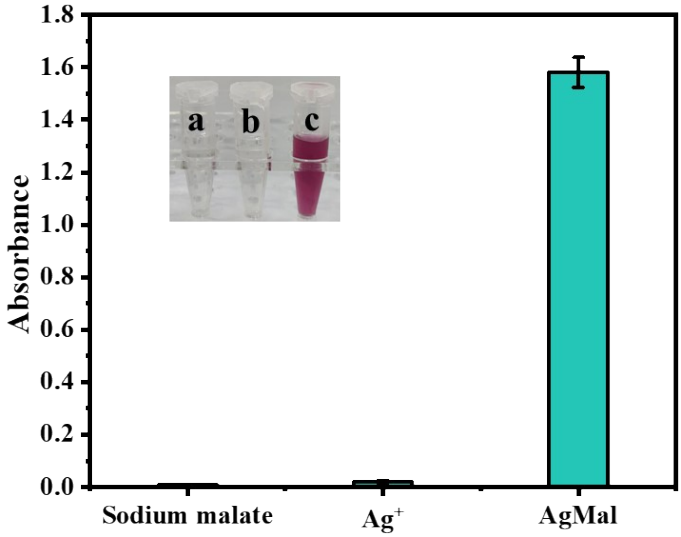


Fig. S3. Comparison of the laccase-like activity of sodium malate, Ag^+ and AgMal. Inset: a: sodium malate, b: Ag^+, c: AgMal.

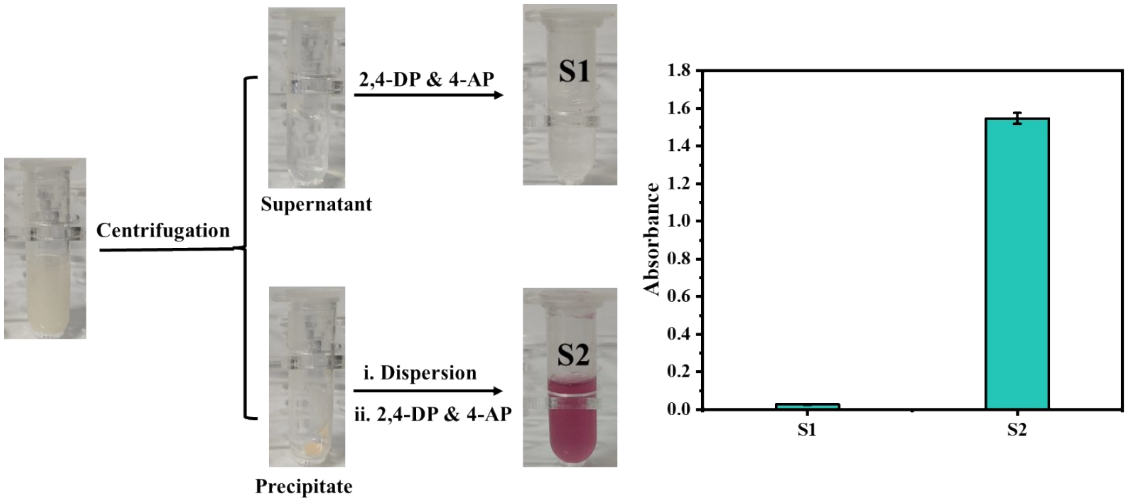


Fig. S4. Comparison of the laccase-like activity of the supernatant and precipitate separated from HEPES dispersion of AgMal.

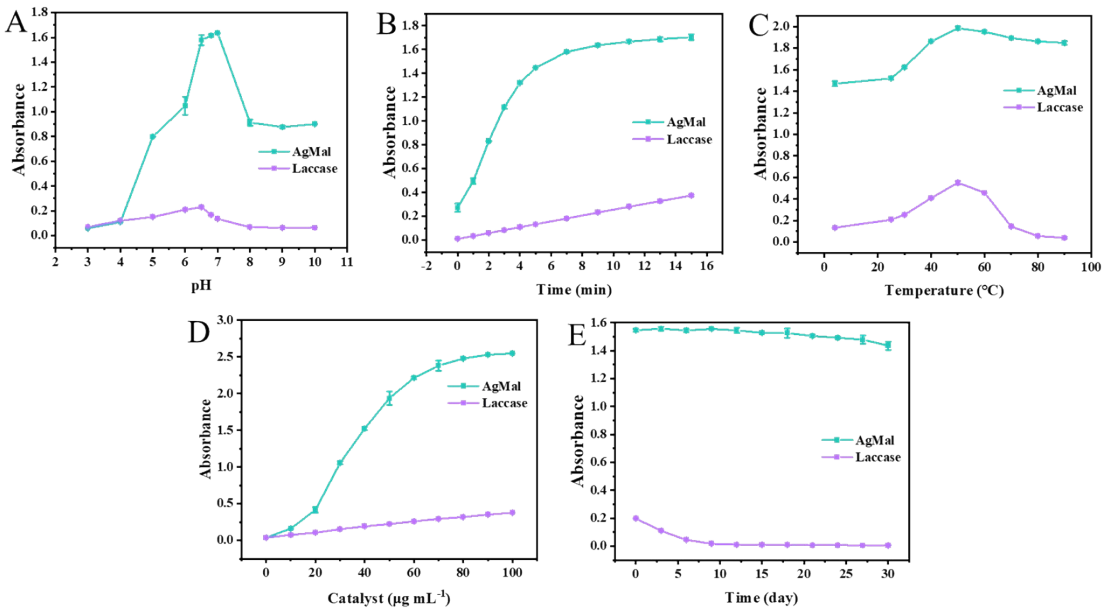


Fig. S5. Catalytic activity of AgMal and laccase at different (A) pH, (B) reaction time, (C) temperature, (D) catalyst concentration, and (E) storage time.

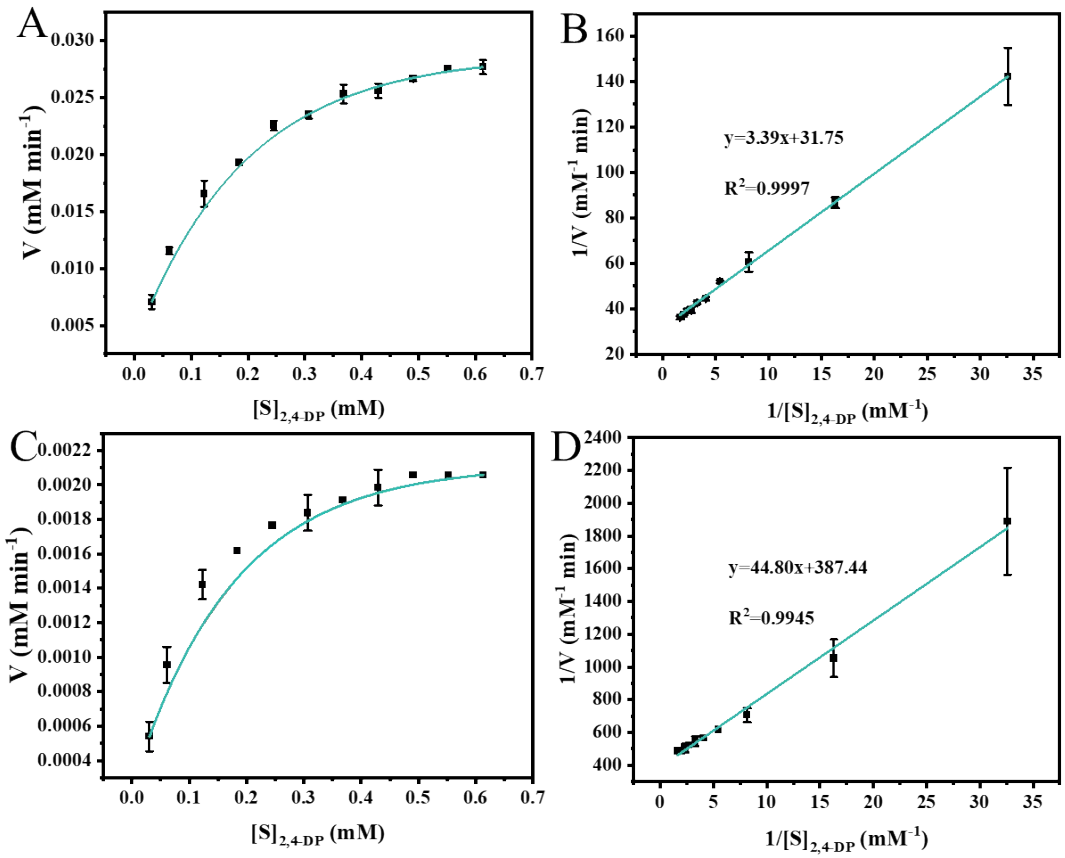


Fig. S6. Catalytic kinetic assay of AgMal and laccase: (A, C) Michaelis-Menten curve of AgMal and laccase; (B, D) corresponding double-reciprocal Lineweaver-Burk plot.

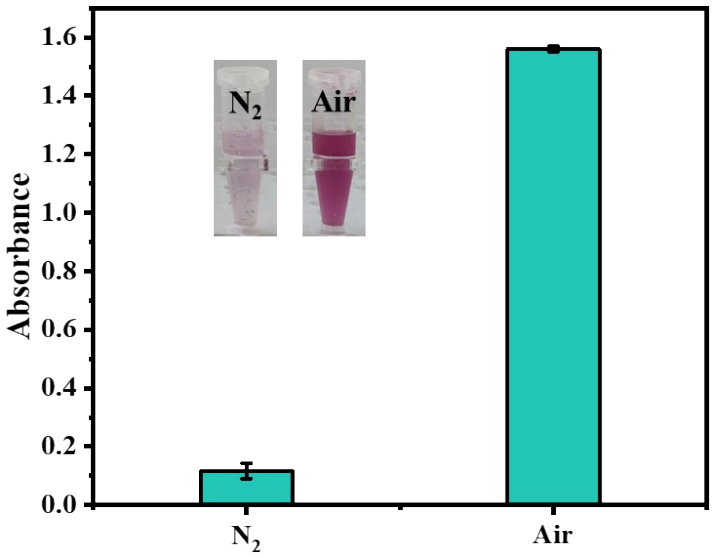


Fig. S7. Comparison of the catalytic activity of AgMal in N_2 and air reaction systems. Inset: pictures with corresponding colorimetric changes.

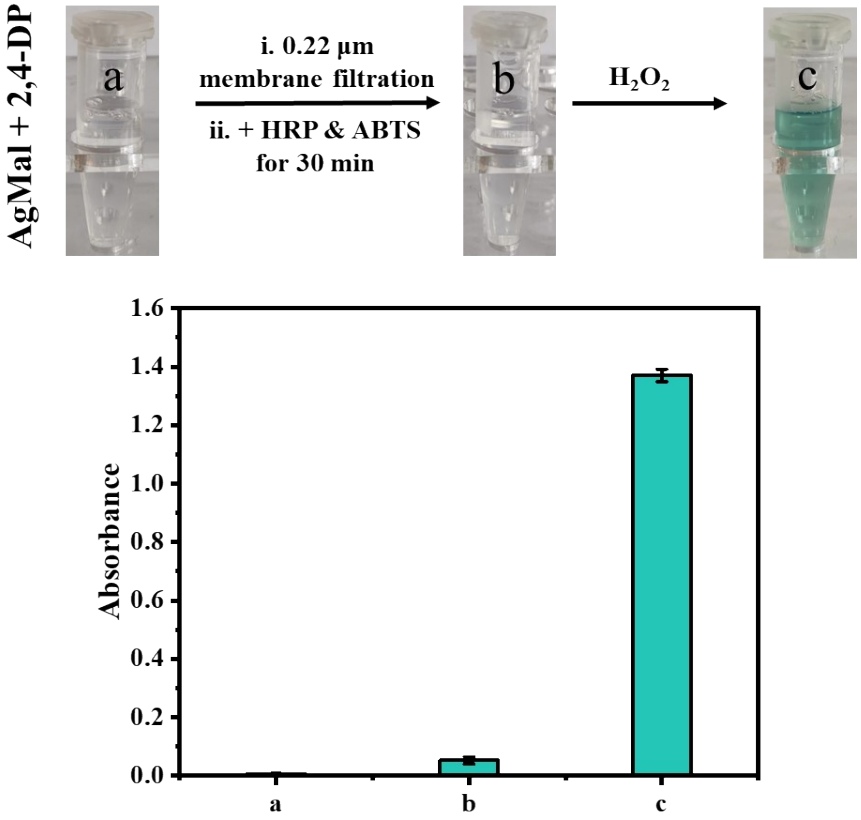


Fig. S8. Photograph a) the reaction solution after AgMal oxidizing 2,4-DP for 10min, b) the filtered solution after mixed with HRP and ABTS for 30 min, c) the solution after adding H_2O_2. Below is their corresponding absorbance at 414 nm.

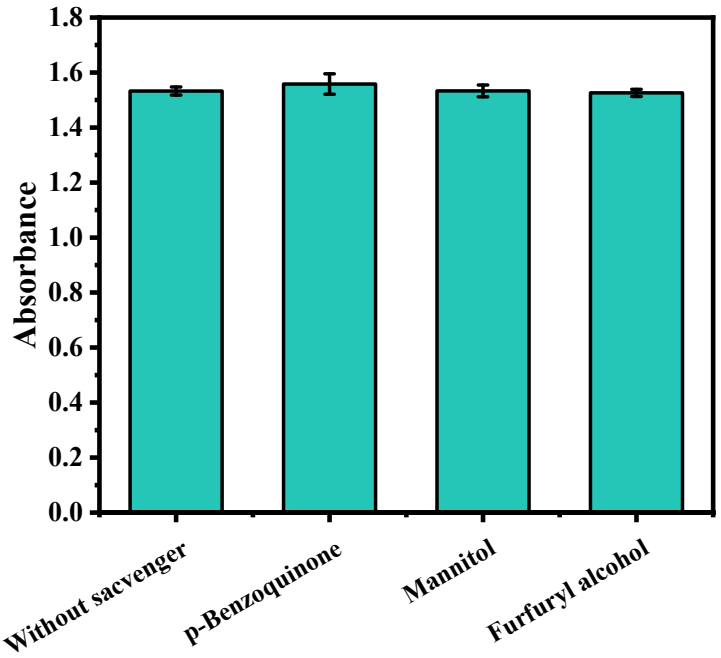


Fig. S9. Effect of different ROS scavengers (2 mM) on the catalytic oxidation of 2,4-DP by AgMal.

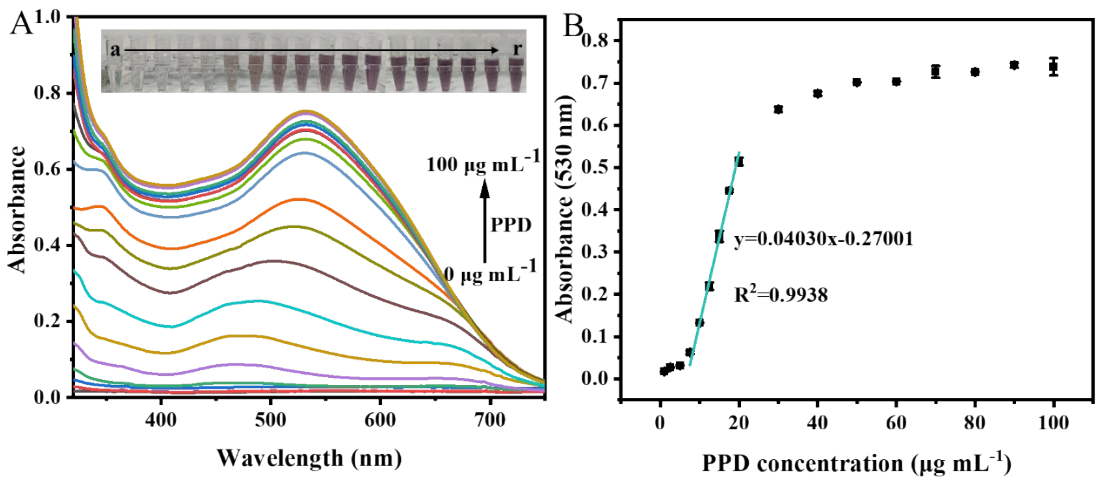


Fig. S10. (A) UV-Vis absorption spectra of different concentrations of PPD catalyzed by AgMal without 4-AP addition (Inset: the corresponding solution); (B) linear relationship between absorbance and PPD concentration.

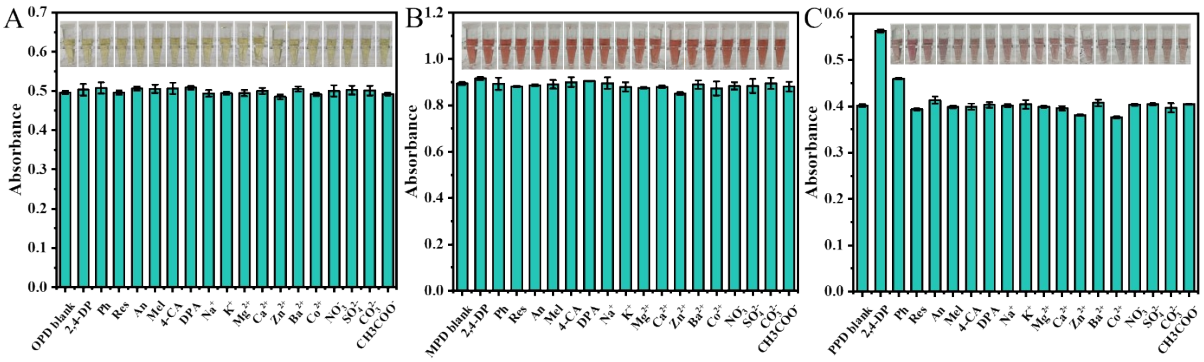


Fig. S11. (A) Solution color and absorbance of OPD + AgMal system at 418 nm in the presence of different interferential substances. (B) Solution color and absorbance of MPD + 4-AP + AgMal system at 482 nm in the presence of different interferential substances. (C) Solution color and absorbance of PPD + 4-AP + AgMal system at 496 nm in the presence of different interferential substances.

Table S1. Comparison of laccase-like nanozymes and natural laccase.

Category	Materials	K_m (mM)	V_{max} (mM/min)	Ref.
Laccase	Trametes versicolor	0.410	0.00641	1
	Trametes versicolor	0.149	0.00666	2
	Trametes versicolor	0.600	0.01721	3
	Trametes versicolor	0.116	0.00258	This work
Nanozymes	Cu/GMP	0.590	0.83000	4
	CH-Cu	0.420	0.00732	1
	I-Cu	0.172	0.02460	2
	ATP-Cu	0.207	0.00220	5
	Bpy-Cu	0.190	0.00148	6
	His-Cys-Cu	1.53	0.00285	7
	CMC-PtNPs	0.218	0.00678	8
	Tar-IrNPs	0.204	0.00540	9
	Fe_1@CN-20	0.078	0.00249	10
	Ce-MOF-808	0.130	0.00222	11
	Mn-GMPNS	0.350	0.05574	12
	Ag_2O NPs	0.289	0.07269	3
	Ag_3PO_4 NPs	0.449	0.03408	13
	AgMal	0.107	0.03150	This work

Table S2. Molecular information of various phenolic pollutants catalytic oxidation by AgMal and laccase.

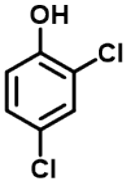
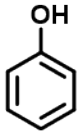
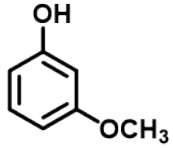
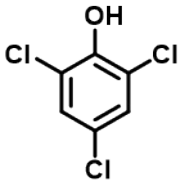
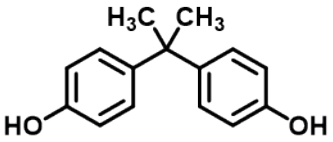
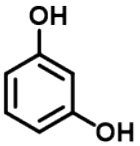
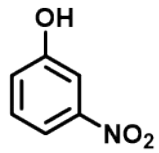
Phenolic compounds	Chemical formula	Molecular weight	Structural formula
2,4-Dichlorophenol	$C_6H_4Cl_2O$	163.00	
p-Chlorophenol	C_6H_5ClO	128.56	
Phenol	C_6H_6O	94.11	
3-Methoxyphenol	$C_7H_8O_2$	124.14	
2,4,6-Trichlorophenol	$C_6H_3Cl_3O$	197.45	
Bisphenol A	$C_{15}H_{16}O_2$	228.29	
Resorcinol	$C_6H_6O_2$	110.11	
3-Nitrophenol	$C_6H_5NO_3$	139.11	

Table S3. Comparison of different phenylenediamine isomers detection methods.

Methods	Analytes	Linear range (μM)	LOD (μM)	Ref.
MEKC	OPD	50.00–100.00	6.10	14
	MPD	50.00–100.00	9.90	
	PPD	50.00–100.00	19.70	
HPLC	OPD	4.62–184.95	2.59	15
	MPD	0.46–184.95	0.46	
	PPD	0.92–138.71	0.55	
Electrochemical analysis	OPD	-	-	16
	MPD	-	-	
	PPD	2.00–200.00	1.20	
SERS	OPD	0.10–1.00	0.03	17
	MPD	-	-	
	PPD	-	-	
Fluorescence detection	OPD	5.00–1200.0	1.50	18
	MPD	-	-	
	PPD	5.00–100.00 100.0–1000.0	1.00	
Colorimetric analysis	OPD	1.00–80.00	0.16	19
	MPD	-	-	
	PPD	-	-	
Colorimetric analysis	OPD	5.00–200.00	1.12	20

	MPD	-	-	
	PPD	2.50–700.00	1.91	
	OPD	4.60–46.00	0.17	
Colorimetric analysis	MPD	-	-	[9]
	PPD	11.50–184.00	2.30	
	OPD	1.00–90.00	0.32	
Colorimetric analysis	MPD	-	-	[21]
	PPD	3.00–70.00	0.53	
	OPD	4.62–115.60	0.47	
Colorimetric analysis	MPD	2.31–46.24	0.16	This work
	PPD	4.62–55.48	0.55	

Table S4. Detection of OPD in the real samples (n = 3).

Sample	**C_{OPD} in sample**	**Added (μg mL^{-1})**	**Found (μg mL^{-1})**	**Recovery (%)**	**RSD (%)**
Tap water	ND	1.00	0.93	93.00	1.85
		5.00	5.29	105.86	2.79
		10.00	9.74	97.40	4.55
Lake water	ND	1.00	1.04	104.31	4.05
		5.00	5.13	102.65	1.66
		10.00	10.29	102.92	3.22

ND: not detected.

Table S5. Detection of MPD in the real samples (n = 3).

Sample	C_{MPD} in sample	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	RSD (%)
Tap water	ND	0.50	0.50	100.76	6.85
		2.00	1.95	97.61	5.59
		4.00	3.85	96.33	0.99
Lake water	ND	0.50	0.52	103.32	1.53
		2.00	2.07	103.52	2.28
		4.00	4.08	102.11	2.13

Table S6. Detection of PPD in the real samples (n = 3).

Sample	C_{PPD} in sample	Added (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	RSD (%)
Tap water	ND	1.00	1.03	102.80	7.42
		3.00	3.20	106.81	3.72
		5.00	5.28	105.69	1.23
Lake water	ND	1.00	1.02	101.90	5.02
		3.00	3.15	104.89	2.61
		5.00	5.02	100.47	3.16

References

1. J. Wang, R. Huang, W. Qi, R. Su, B. P. Binks and Z. He, *Appl. Catal. B*, 2019, **254**, 452–462.
2. J. Wang, R. Huang, W. Qi, R. Su and Z. He, *J. Hazard. Mater.*, 2022, **429**, 128404.
3. L. Huang, Y. Tang, J. Wang, X. Niu, J. Zhou and Y. Wu, *Sens. Actuators B Chem.*, 2023, **391**, 134052.
4. H. Liang, F. Lin, Z. Zhang, B. Liu, S. Jiang, Q. Yuan and J. Liu, *ACS Appl. Mater. Interfaces*, 2017, **9**, 1352–1360.
5. H. Huang, J. Bai, J. Li, L. Lei, W. Zhang, S. Yan and Y. Li, *J. Mater. Chem. B*, 2019, **7**, 6508–6514.
6. M. Li, Y. Xie, L. Lei, H. Huang and Y. Li, *Sens. Actuators, B*, 2022, **357**, 131429.
7. X. Yan, H. Li, T. Wang, A. Li, C. Zhu and G. Lu, *Chem. Eng. J.*, 2022, **446**, 136930.
8. L. Yang, X.-Y. Guo, Q.-H. Zheng, Y. Zhang, L. Yao, Q.-X. Xu, J.-C. Chen, S.-B. He and W. Chen, *Sens. Actuators B Chem.*, 2023, **393**, 134165.
9. S. Hao, C. Fu, L. Zhou, Z. Guo and Q. Song, *J. Mater. Chem. B*, 2023, **11**, 2770–2777.
10. Y. Lin, F. Wang, J. Yu, X. Zhang and G.-P. Lu, *J. Hazard. Mater.*, 2022, **425**, 127763.
11. S. Liang, X.-L. Wu, J. Xiong, X. Yuan, S.-L. Liu, M.-H. Zong and W.-Y. Lou, *Chem. Eng. J.*, 2022, **450**, 138220.
12. Q. Tang, C. Zhou, L. Shi, X. Zhu, W. Liu, B. Li and Y. Jin, *Anal. Chem.*, 2024, **96**, 4736–4744.
13. X. Niu, H. He, H. Ran, Z. Wu, Y. Tang and Y. Wu, *Food Chem.*, 2023, **429**, 136903.
14. Y. Wu, F. Jiang, L. Chen, J. Zheng, Z. Deng, Q. Tao, J. Zhang, L. Han, X. Wei, A. Yu and H. Zhang, *Anal. Bioanal. Chem.*, 2011, **400**, 2141–2147.
15. S.-P. Wang and T.-H. Huang, *Ana. Chim. Acta*, 2005, **534**, 207–214.
16. N. S. Lawrence, E. L. Beckett, J. Davis and R. G. Compton, *Analyst*, 2001, **126**, 1897–1900.
17. P. Ma, F. Liang, D. Wang, Q. Yang, B. Cao, D. Song, D. Gao and X. Wang, *Microchim. Acta*, 2015, **182**, 167–174.
18. Y. Xia, K. Sun, Y.-N. Zuo, S. Zhu and X.-E. Zhao, *Chin. Chem. Lett.*, 2022, **33**, 2081–2085.
19. N. Li, Y. Gu, M. Gao, Z. Wang, D. Xiao, Y. Li, R. Lin and H. He, *Spectrochim. Acta Part A*, 2015, **140**, 328–333.
20. L. Lin, Y. Xiao, Y. Wang, Y. Zeng, Z. Lin and X. Chen, *Microchim. Acta*, 2019, **186**, 288.
21. B. Shi, Y. Su, L. Zhang, M. Huang, X. Li and S. Zhao, *Nanoscale*, 2016, **8**, 10814–10822.