Electronic Supplementary Information (ESI) for

Colorimetric detection of methotrexate leveraging the halogen peroxidase-mimicking activity of Bi_2WO_6 nanoflowers

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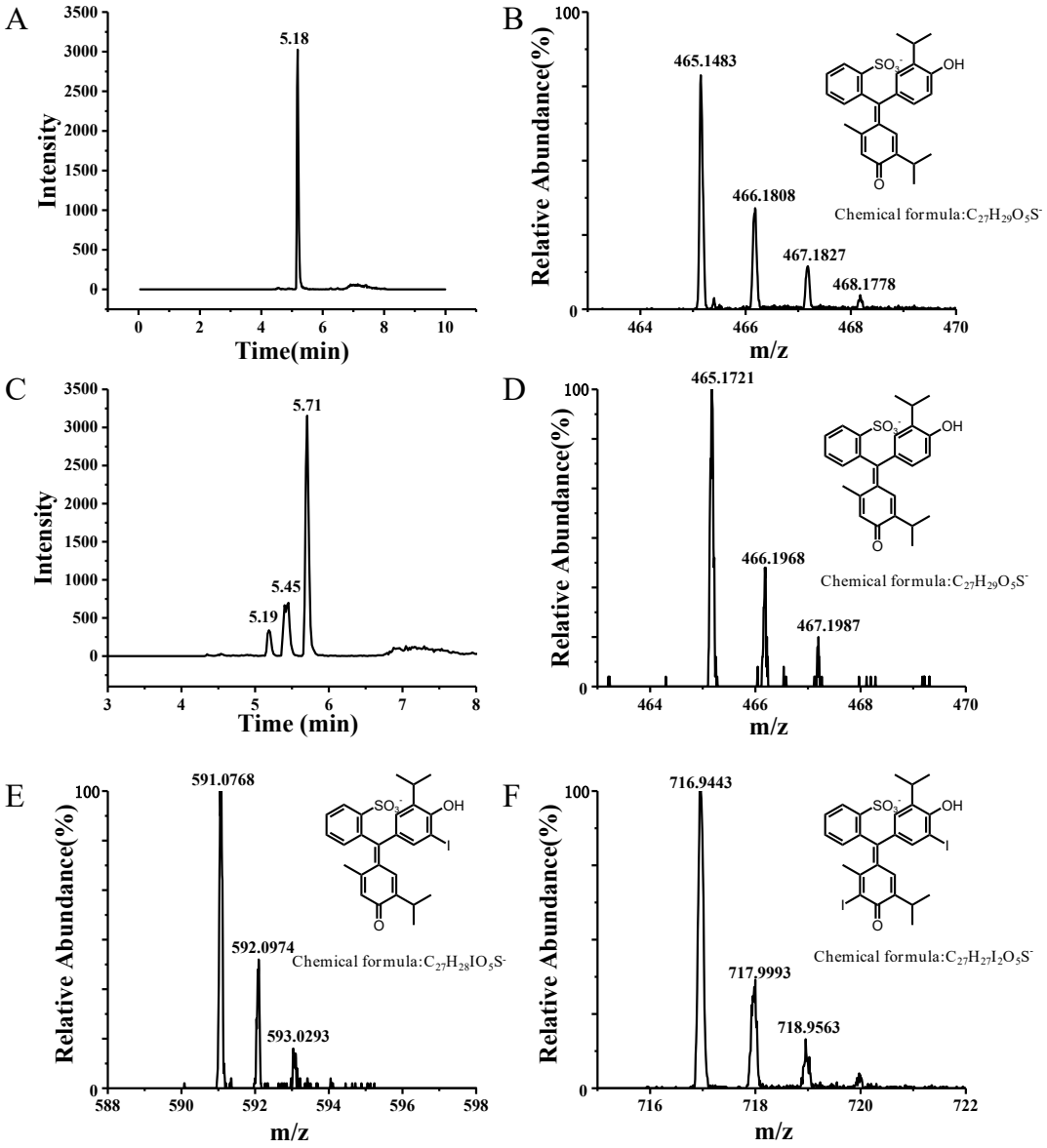

Fig.S1 The catalytic effect of Bi_2WO_6 on substrates as a mimic of halogen peroxidase: (A,B) Chromatogram and mass spectrum before the iodination reaction of TB. (C-F) Chromatogram and mass spectrum after the iodination reaction of TB.

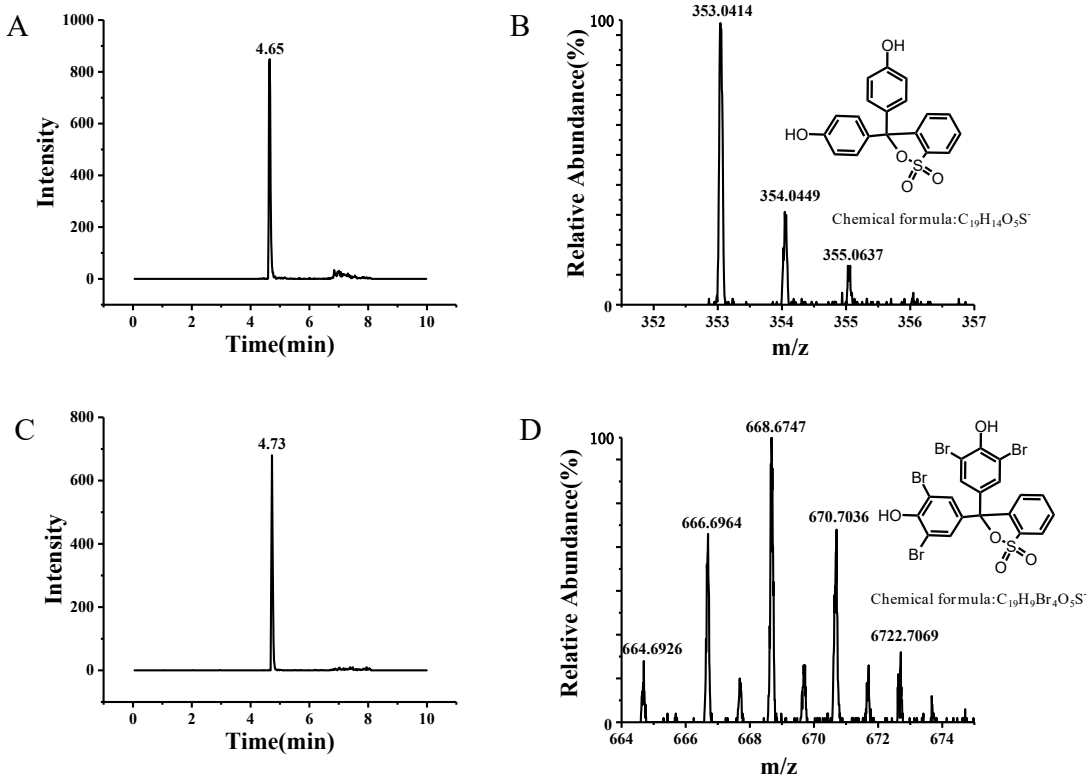

Fig.S2 The catalytic effect of Bi_2WO_6 on substrates as a mimic of halogen peroxidase: (A,B) Chromatogram and mass spectrum before the bromination reaction of PR. (C,D) Chromatogram and mass spectrum after the bromination reaction of PR.

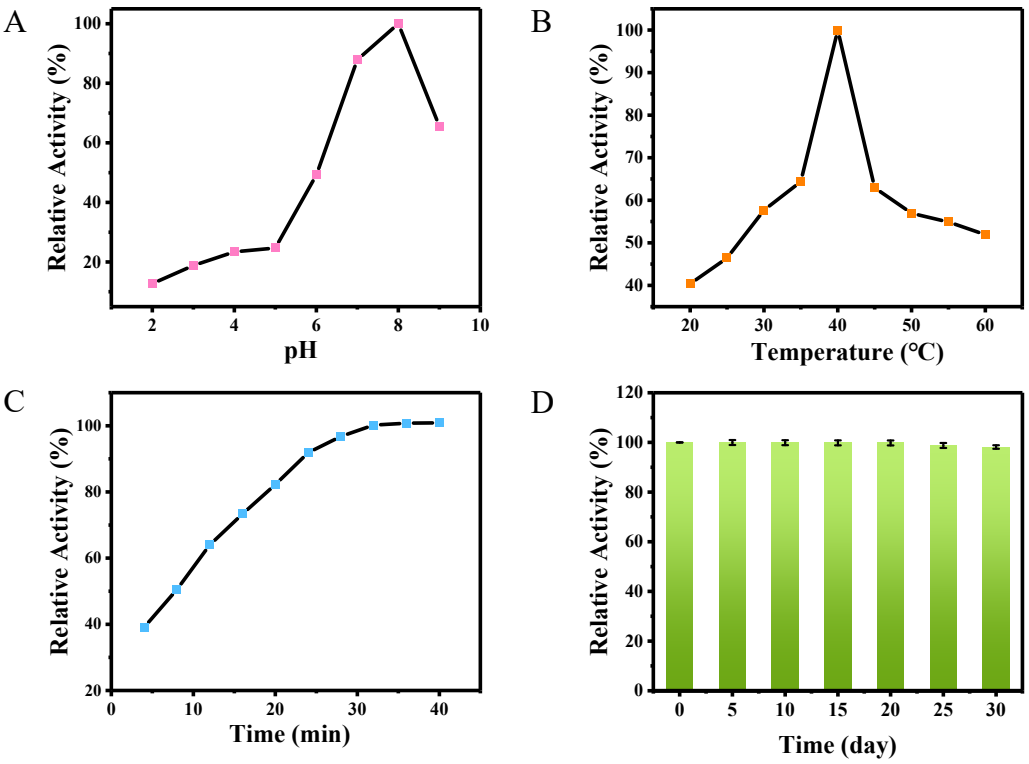

Fig.S3 The relative catalytic activity of Bi_2WO_6 iodoperoxidase in the presence of TB as a substrate vary with (A) reaction pH, (B) reaction temperature, and (C) reaction time, (D) over different storage time.

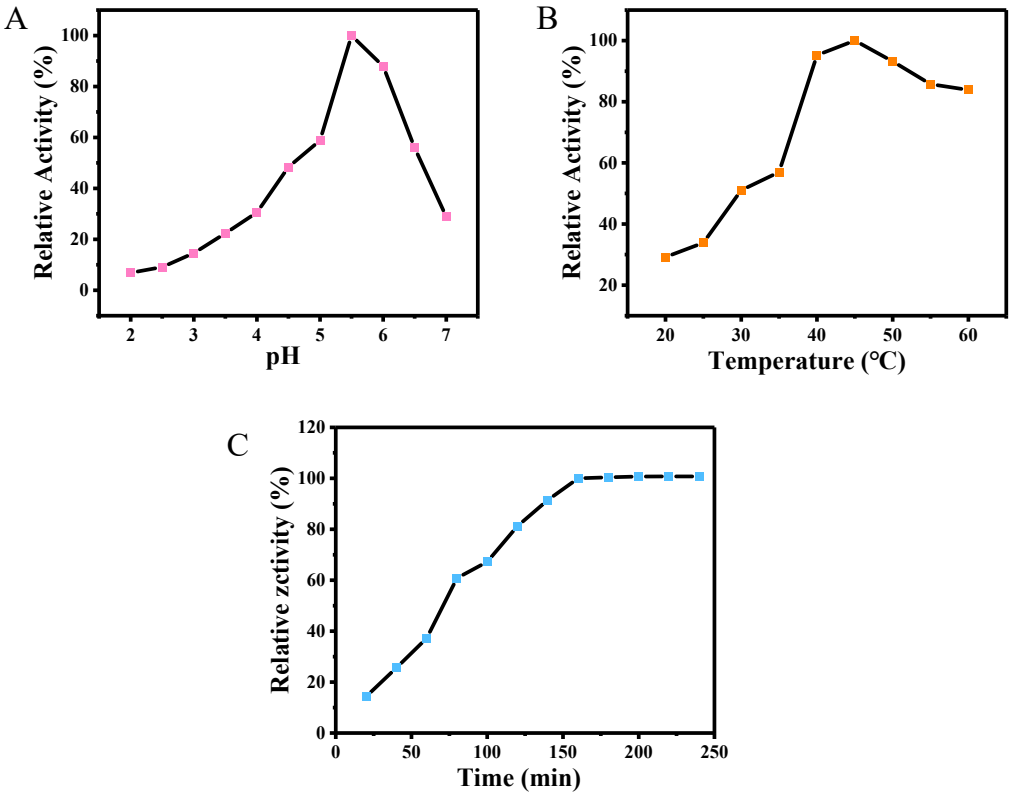

Fig.S4 The relative catalytic activities of Bi_2WO_6 bromoperoxidase in the presence of PR as a substrate changes with (A) reaction pH, (B) reaction temperature, and (C) reaction time.

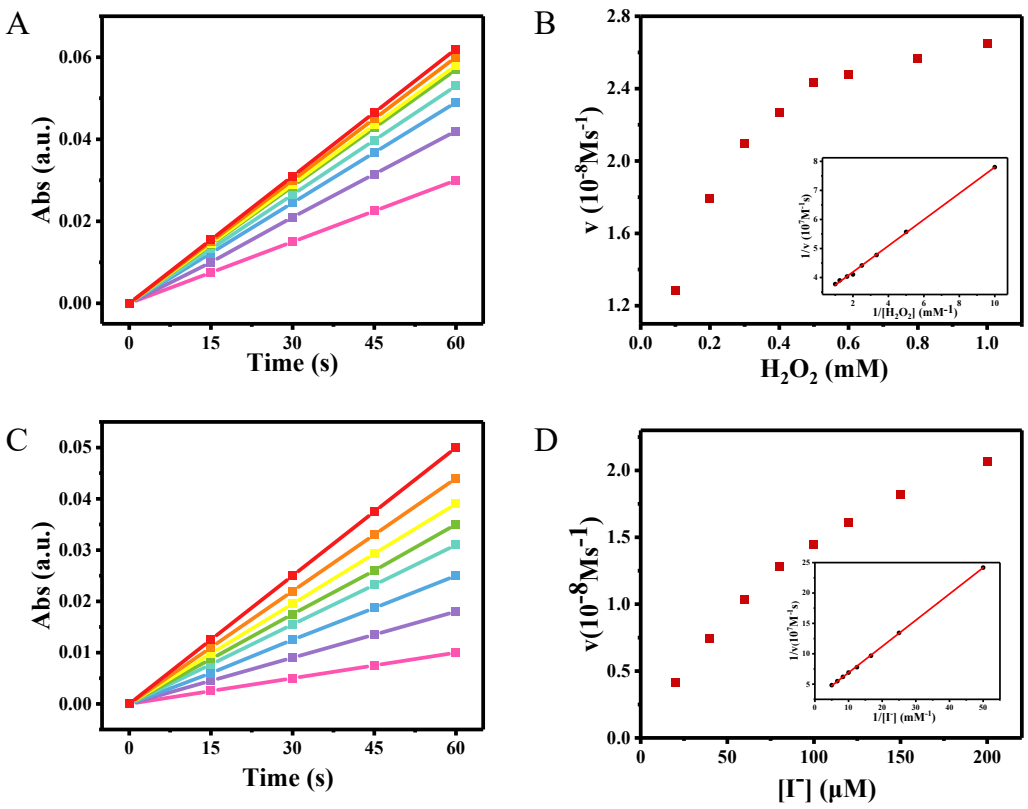

Fig.S5 The absorbance at 620 nm of Iodoperoxidase mimetic enzyme the nanoenzyme catalytic system in the presence of different concentrations of (A) H_2O_2 or (C) KI; The steady-state kinetic analysis of the Bi_2WO_6 nanoenzyme catalytic reaction in the presence of different substrates (B) H_2O_2 or (D) KI (the inset is the Lineweaver-Burk plot).

Table S1 Comparison of the kinetic parameters between Bi_2WO_6 and other reported nanomaterials

Materials	**Substrate**	**K_m (mM)**	**V_{max} (nM/s)**	**Reference**
CuO	H_2O_2	0.556	26.5	[1]
CeO_2-X nanorods	H_2O_2	0.261	1.667	[2]
Bi_2Te_3	H_2O_2	16.593	0.417	[3]
W-UiO	H_2O_2	0.555	0.317	[4]
CeMOF	H_2O_2	0.1	4	[5]
Bi_2WO_6	H_2O_2	0.137	30.4	This work
	I^-	0.214	169.6	

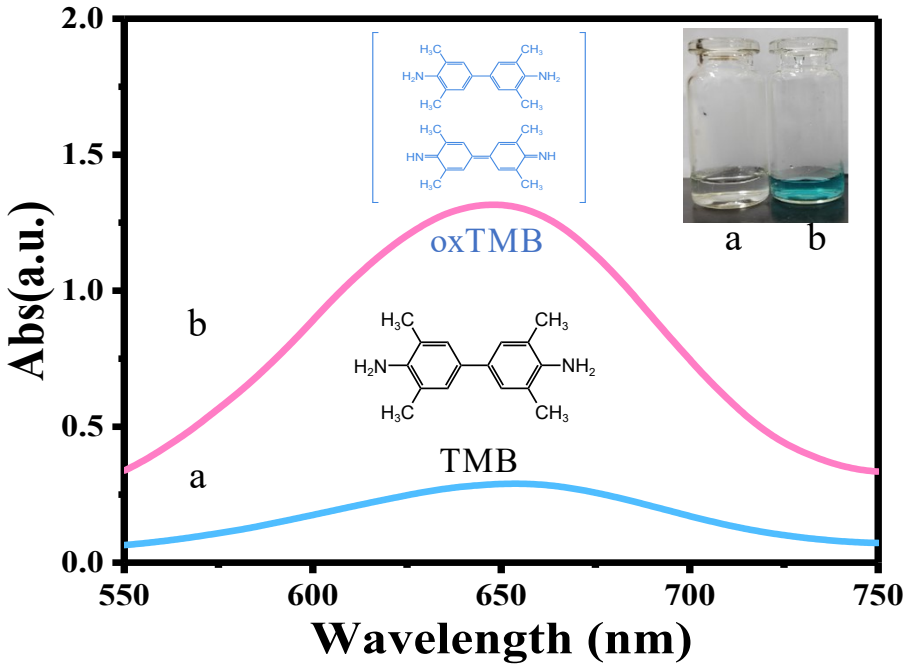

Fig.S6 UV-Vis spectra of oxTMB at different solutions. (a) Bi_2WO_6 + H_2O_2 + KI+MTX; (b) Bi_2WO_6 + H_2O_2 + KI (the concentration of Bi_2WO_6 is 0.1 mg/mL, TMB is 0.05 mM, KI is 4.0 mM, H_2O_2 are 2.0 mM and MTX is 100 μM).

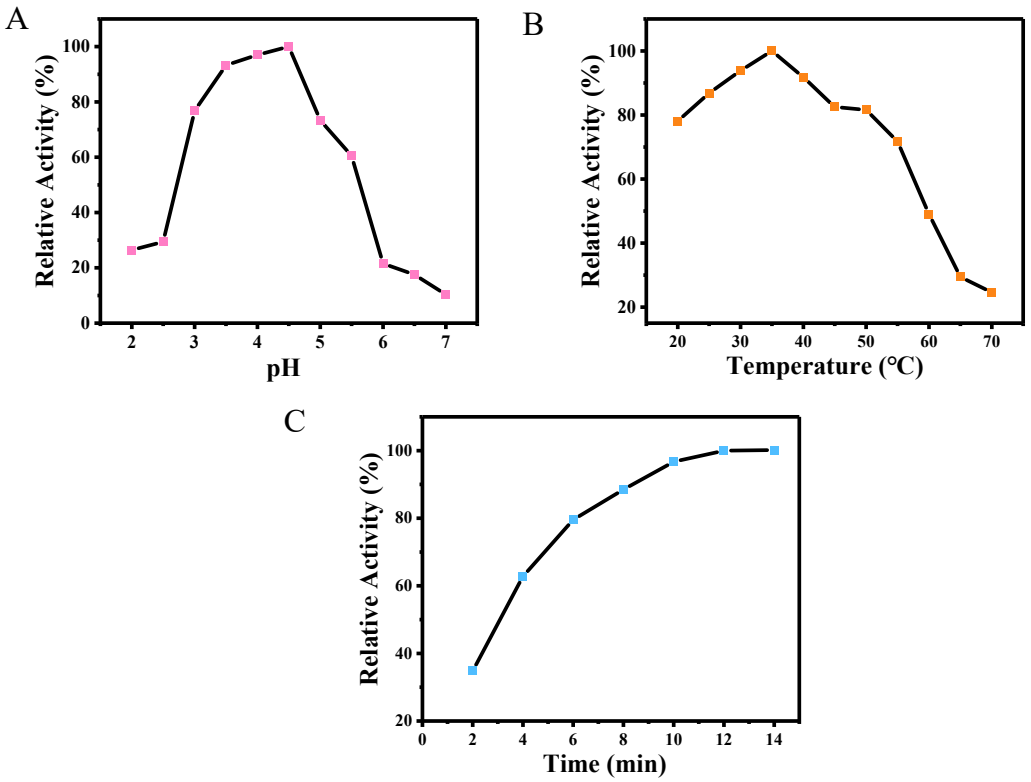

Fig.S7 The relative catalytic activities of Bi_2WO_6 iodoperoxidase change with (A) reaction pH, (B) reaction temperature, and (C) reaction time under the condition that TMB was used as the substrate.

Table S2 Comparison with other reported methods for MTX detection.

Method	**Linear Range (μM)**	**LOD (μM)**	**Referenc**
Fluorescence	2.9~117.4	0.95	[6]
Fluorescence	1~300	0.33	[7]
SERS	0~100	2.36	[8]
Electrochemistry	5-75	1.98	[9]
SERS	5~150	2.1	[10]
Colorimetry	1-100	0.31	This work

Table S3 Recovery of MTX in serum samples.

Sample number	**Spiked (μM)**	**Detected (μM)**	**Recovery (%)**	**RSD (%)**
1	1.0	1.25±0.04	125.0	4.0
2	10	10.49±0.36	104.9	3.6
3	20	22.39±0.66	119.5	3.5
4	50	47.86±1.31	95.0	2.6
5	75	80.00±1.38	106.0	1.8
6	100	105.9±1.22	105.9	1.2

References

1. L. Wang, J. Hou, S. Liu, A. J. Carrier, T. Guo, Q. Liang, D. Oakley and X. Zhang, *Sens. Actuators B: Chem.*, 2019, **287**, 180-184.
2. K. Herget, P. Hubach, S. Pusch, P. Deglmann, H. Gotz, T. E. Gorelik, I. A. Gural'skiy, F. Pfitzner, T. Link, S. Schenk, M. Panthofer, V. Ksenofontov, U. Kolb, T. Opatz, R. Andre and W. Tremel, *Adv. Mater.*, 2017, **29**, 1603823.
3. S. S. Kulkarni, D. K. Tong, C. T. Wu, C. Y. Kao and S. Chattopadhyay, *Small*, 2024, **20**, e2401929.
4. W. Wei, L. Qiang, L. Jinyang, L. Yunhong, W. Ronggen, L. Yilan, H. Xiaobing and W. Ning, *Chem. Eng. J.*, 2021, **431**, 133483.
5. Y. Cheng, L. Liang, F. Ye and S. Zhao, *Biosensors (Basel)*, 2021, **11**, 204.
6. P. Zuo, J. Liu, H. Guo, C. Wang, H. Liu, Z. Zhang and Q. Liu, *Anal. Bioanal. Chem.*, 2019, **411**, 1647-1657.
7. X. Wei, X. Si, M. Han and C. Bai, *Molecules*, 2022, **27**, 2118.
8. A. Subaihi, D. K. Trivedi, K. A. Hollywood, J. Bluett, Y. Xu, H. Muhamadali, D. I. Ellis and R. Goodacre, *Anal. Chem.*, 2017, **89**, 6702-6709.
9. I. N. Qureshi, A. Tahira, K. Aljadoa, A. M. Alsalme, A. A. Alothman, A. Nafady, A. Karsy and Z. H. Ibupoto, *J. Mater. Sci. Mater. Electron.*, 2021, **32**, 15594-15604.
10. Y. Göksel, K. Zor, T. Rindzevicius, B. E. Thorhauge Als-Nielsen, K. Schmiegelow and A. Boisen, *ACS Sens.*, 2021, **6**, 2664-2673.