

Electronic Supplementary Information

For

A Two-dimensional Coordination Polymer with High Laccase-like activity for Sensitive Colorimetric Detection of Thiram

Songxue Han,^{a,b} Lili Xu^{b,c}, Youxing Fang^{b*}, and Shaojun Dong^{a,b,c*}

a. College of Chemistry, Jilin University, Changchun 130012, China

b. State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

c. University of Science and Technology of China, Hefei 230026, China

*Corresponding author: fangyx@ciac.ac.cn; dongsj@ciac.ac.cn

Experimental section

Reagents

L-phenylalanine (F), edetate disodium (EDTA), sodium phosphate dibasic (Na_2HPO_4), and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Sigma-Aldrich. Copper (II) chloride dihydrate (CuCl_2), tetramethylthiuram disulfide (thiram), sodium hydroxide (NaOH), 2,4-dichlorophenol (2,4-DP), 4-aminoantipyrine (4-AP), sodium chloride (NaCl), potassium chloride (KCl), fructose, sucrose, leucine, potassium carbonate (K_2CO_3) were bought from Aladdin (Shanghai, China). All materials were used as received. Milli-Q water was used to prepare all the buffers and solutions.

Apparatus

Scanning electron microscopy (SEM, ZEISS MERLIN Compact) was performed to test the morphology of the catalysts. X-ray diffraction (XRD, Bruker, Germany) patterns were obtained by a D8 ADVANCE diffractometer. X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-Alpha) was carried out to determine the elemental composition and valence state of the materials. UV absorption spectra were obtained by a Varian Cary 50 UV-Vis absorption spectrometer. The lateral distance and thickness range were detected by atomic force microscopy (AFM, Veeco, Nanoscope V, Bruker, CA, USA). The corresponding energy dispersive X-ray spectroscopy (EDS) elemental analysis were characterized by a JEM-2010 operating at 200 kV.

Experimental

Preparation of F-Cu:

The F-Cu was prepared by a simple method at elevated temperature according to the previous reports¹. Two equivalents of L-phenylalanine (10 mM) alkaline solution (containing NaOH (10 mM)) were sonicated for 15 min. While heating in a 60 °C water bath, one equivalent CuCl₂ (5 mM) aqueous solution was slowly added while stirring and reacted for 20 min. Thin sheet-like crystals were spontaneously formed at the liquid–air interface as well as within the solution. The nanozymes were then collected by centrifugation at 5000 rpm for 5 minutes, washed three times with pure water and ethanol, and finally redissolved in pure water.

Laccase-like activity assays:

The laccase-like activity of F-Cu nanozymes was investigated by the color reaction of phenolic substrate with amino antipyrine (4-AP). In PBS buffer (50 mM, pH 7.4, 600 µL) containing 0.1 mg/mL 4-AP and 0.1 mg/mL 2,4-DP as substrates, a laccase-like activity assay was performed using F-Cu nanozymes. The absorbance of the system was measured at 510 nm after reacting at 60 °C for 10 min.

Detection of thiram:

The laccase-like activity of F-Cu nanozymes was evaluated by the catalytic oxidation of phenols. To generate a standard curve, F-Cu nanozymes (6 mg/mL, 100 µL) were mixed with varying concentrations of thiram (0-0.8 mM) were mixed in PBS buffer (50 mM, pH 7.6, 600 µL). Subsequently, 2,4-DP (1 mg/mL, 100 µL) and 4-AP (1 mg/mL, 100 µL) solutions were added to the mixture to form a solution of 1 mL. The absorbance was measured at 510 nm after allowing the reaction to proceed at 60 °C for 10 minutes.

Subsequently, the effects of pH, temperature, reaction time, and substrate concentration on enzyme activity were systematically studied. To demonstrate the selectivity of thiram detection, various potential interferences at the same concentration were tested under identical detection conditions, including sodium chloride (NaCl), potassium chloride (KCl), fructose, sucrose, leucine, potassium carbonate (K₂CO₃), edetate disodium (EDTA). Briefly, 1 mL of the reaction solution consists of F-Cu nanozymes (1 mg/mL, 100 µL) and interferences (1 mM, 100 µL) as well as 2,4-DP (1 mg/mL, 100 µL), 4-AP (1 mg/mL, 100 µL) solutions and PBS buffer (600 µL).

Analysis of thiram in practical samples:

Water samples were collected from South Lake and Yitong River in Changchun City for practical application testing. These samples were spiked with different

concentrations of thiram (0.5, 2.5, and 5 μM) to prepare test solutions. The thiram concentrations in these prepared samples were then measured following the previously described protocol.

Supplementary figures and tables

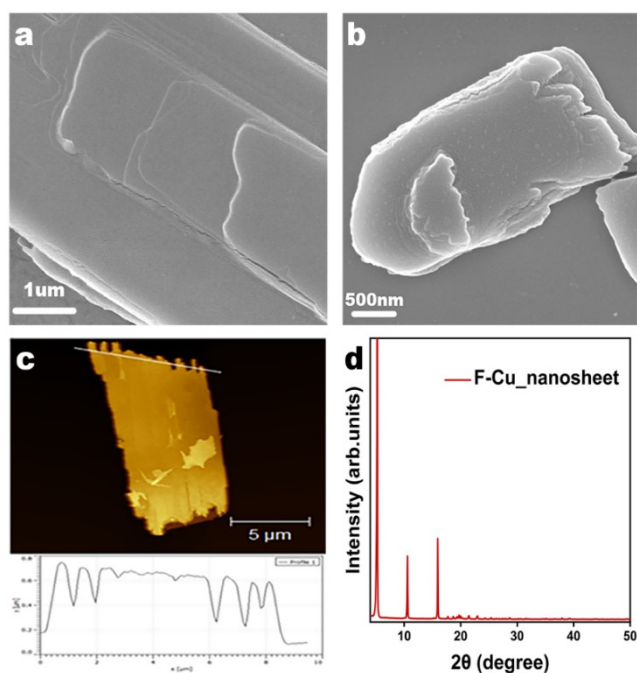


Figure S1 (a), (b) SEM micrographs of F-Cu nanosheets. (c) AFM image of an F-Cu 2D layered crystal with a height profile along the indicated line (inset). (d) XRD patterns of experimental 2D nanosheets.

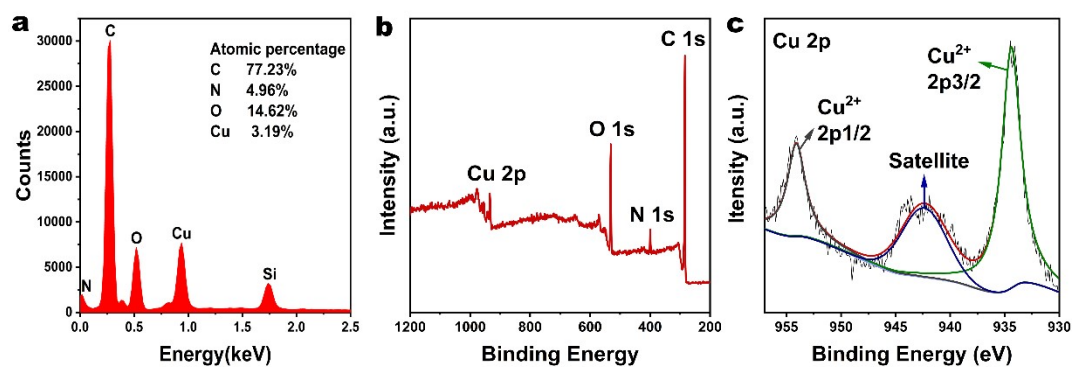


Figure S2 (a) EDX spectra of F-Cu nanosheets in the absence of thiram. (b) Survey XPS spectra of F-Cu nanosheets. (c) Cu 2p XPS spectrum and peak fitting by XPS peak-differentiating-imitating analysis of F-Cu nanosheets.

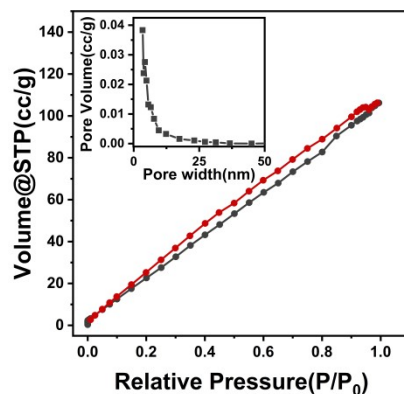


Figure S3 N_2 adsorption/desorption isotherm curve and pore-size distribution (inset) of F-Cu.

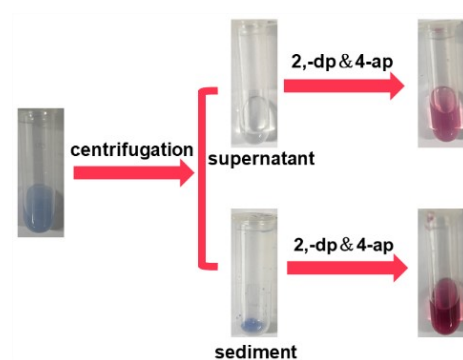


Figure S4 Control experiment for comparing the laccase-like activity of the precipitated F-Cu and the supernatant.

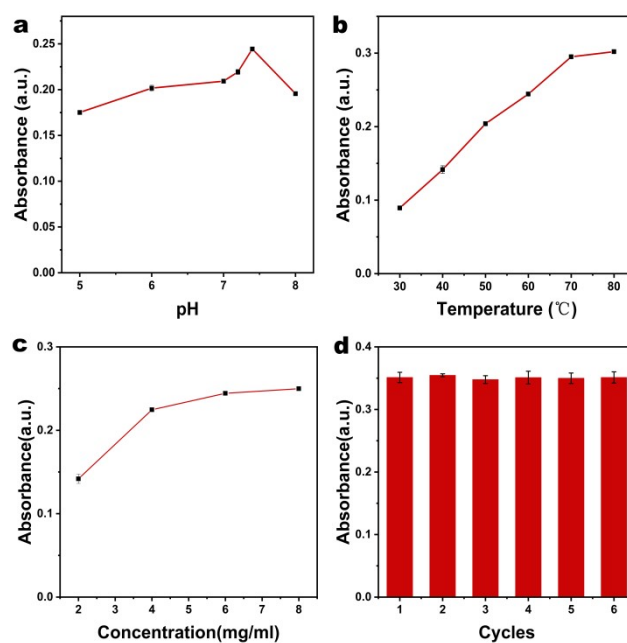


Figure S5 Influence of (a) pH, (b) temperature, (c) F-Cu concentration, (d) cycles on the laccase-like activity of F-Cu nanozymes.

Table S1 Comparison of performance of different thiram probing strategy.

NO.	Detection method	Linear range (μM)	LOD (μM)	Reference
1	High performance liquid chromatography	0.29-62	0.09	2
2	Fluorescence assay	2.08–10.40	0.42	3
3	Colorimetric assay	0.2-0.5	0.13	4
4	Fluorescence assay	2.1-33.3	0.457	5
5	Surface enhanced Raman spectroscopy	0.0416-0.416	0.015	6
6	Voltammetry	5-50	2.23	7
7	Colorimetric assay	0-7.5	0.0845	This work

Table S2 Detection of thiram in actual sample.

Sample	Added (μM)	Expected (μM)	Detected ^a (μM)	Recovery ^b (%)	RSD ^c (n=3)
Lake Water	0.5	0.5	0.63	117	2.42
	2.5	2.5	2.53	101.2	2.40
	5	5	5.92	118.4	1.93
River Water	0.5	0.5	0.59	118	1.57
	2.5	2.5	3.03	121.2	1.43
	5	5	6.09	121.8	2.37

^a Average of three detection.^b Recovery = (Detected value / Expected value) \times 100^c RSD (Relative Standard Deviation) = (Standard Deviation / Average of three detection) \times 100

References

1. P. Makam, S. S. R. K. C. Yamijala, V. S. Bhadram, L. J. W. Shimon, B. M. Wong and E. Gazit, *Nat Commun*, 2022, 13, 1505.
2. K. Charoenkitamorn, O. Chailapakul and W. Siangproh, *Talanta*, 2015, 132, 416–423.
3. Z. Tang, Z. Chen, G. Li and Y. Hu, *Analytica Chimica Acta*, 2020, 1136, 72–81.
4. C. Zhang, H. Ren, X. Jiang, G. Jia, Z. Pan and Y. Liu, *Nanomaterials*, 2022, 12, 2152.
5. F. Wang, Z. Li, H. Jia, C. Miao, R. Lu, S. Zhang and Z. Zhang, *Food Chemistry*, 2023, 409, 135328.
6. W. Zhai, M. Cao, Z. Xiao, D. Li and M. Wang, *Foods*, 2022, 11, 3597.
7. X. Wei, C. Liu, Z. Li, Q. Sun, X. Zhang, Y. Li, W. Zhang, J. Shi, X. Zhai, D. Zhang and X. Zou, *Chemosphere*, 2022, 307, 135960.