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MOF nanozymes mediated acetylcholinesterase-free colorimetric strategy for direct detection of organophosphorus pesticides

Jiaxiang Xiao, ‡[a,b] Feng Shi, ‡[a] Ye Zhang,[a] Maoying Peng,[a] Jinming Xu,[a] Juan Li,*[a] Zhuo Chen,*[b] Zhanjun Yang*[a]

[a] *School of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, P.R. China*

[b]*Molecular Science and Biomedicine Laboratory (MBL), State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, Aptamer Engineering Center of Hunan Province, Hunan University, Changsha 410082, P.R. China*

Experimental

Materials and Reagents. Pirimiphos-methyl was purchased from Aladdin chemistry Co., Ltd. (Shanghai). Copper nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$) , Cobalt nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$), cetyltrimethyl ammonium bromide (CTAB, ($C_{16}H_{33}(CH_3)_3NBr$), 2-methylimidazole, anhydrous ethanol (C_2H_6O), disodium phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), hydrogen peroxide (H_2O_2), methylene blue (MB), potassium chloride (KCl), sodium chloride (NaCl), copper chloride ($CuCl_2$), cobalt chloride ($CoCl_2$), ferric chloride ($FeCl_2$) and ascorbic acid (Vc) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai). 3,3′,5,5′ -tetramethylbenzidine (TMB) and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) was purchased from America Acros. The phosphate buffer solution (PBS) was prepared by mixing 0.1 M Na_2HPO_4 and NaH_2PO_4 solutions and its pH was adjusted via H_3PO_4 or NaOH solution. The water used in the experiment was distilled water, and the other reagents were of analytical grade.

Apparatus. Ultraviolet-visible (UV-vis) experiments were completed with a UV-

2550 spectrophotometer (Beijing General Instrument Co., Ltd.). Scanning electron microscope (SEM) images were obtained from Hitachi S-4800 scanning electron microscope (Japan) with an acceleration voltage of 15 kV. Transmission electron microscope images were measured on a Tecnai 12 transmission electron microscope (TEM, Philips, the Netherlands), and the operating voltage was 120 kV. X-ray diffraction pattern (XRD) was measured on a D8 Advance polycrystalline X-ray diffractometer (Bruker AXS, Germany). X-ray photoelectron spectroscopy (XPS) data were recorded on an AXIS SUPRA+ X-ray photoelectron spectrometer (SHIMADZU, Japan). Electron paramagnetic resonance (EPR) spectroscopy measurements were carried out on a Bruker A300-10/12 (Germany).

Synthesis of Cu_XCo_{10-X} ZIF. Bimetal Cu_4Co_6 ZIF was synthesized in accordance with the previous report with modification. [S1] First, 96.6 mg (0.4 mmol) of $Cu(NO_3)_2{\cdot}3H_2O$, 174.6 mg (0.6 mmol) of $Co(NO_3)_2{\cdot}6H_2O$ and 7.5 mg of CTAB were dissolved in 10 mL purified water. Then, it was rapidly injected into 70 mL aqueous solution containing 4.54 g of 2-methylimidazole and kept intensely stirring for 1 h at room temperature. The precipitate (named as Cu_4Co_6 ZIF) was centrifuged and washed with ethanol for 3 times. At last, the Cu_4Co_6 ZIF was dried in vacuum oven overnight. For comparison, other Cu ratios of Cu_XCo_{10-X} ZIFs (X=0, 2, 8. X is the molar ratio of Cu versus the total Cu + Co molar amount multiplied by 10) were respectively synthesized by similar methods with different molar ratios of Cu to Co.

The POD-Like Activity of Cu_xCo_{10-x} ZIF. For comparison, the POD-like activity of Cu_XCo_{10-X} ZIF was explored by monitoring the UV-vis absorption spectra of the reaction solution. In Brief, 200 μL Cu_XCo_{10-X} ZIF (1 mg mL^{-1}) was mixed with 1.4 mL phosphate buffer solution (PBS, 0.1 M, pH=4.0). Next, 200 μL H_2O_2 (0.1 M) solution and 200 μL TMB (20 mM) was added to the above solution. After reacting for 15min at room temperature, the solution presented intense blue, whose absorption intensity was recorded by UV-2550. The optimization experiments of Cu_4Co_6 ZIF were also performed under different reaction temperatures (30, 35, 40, 45 and 50) or different pH (2, 3, 4, 5, 6, 7, 8 and 9). All the tests mentioned above were repeated three times.

The Steady-State Kinetic Analysis of Cu_4CO_6 ZIF. The kinetic experiments of

Cu_4CO_6 ZIF were performed via measuring the absorbance change at 652 nm by UV-2550. The experiments were carried out under different concentrations of TMB (0.1, 0.2, 0.4, 0.6, 0.7, 0.8 and 0.9 mM) or H_2O_2 (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM) with the rest of reaction conditions remaining certain and constant. Finally, the TMB and H_2O_2 concentration with the initial velocity were used to calculated the apparent kinetic parameters (K_m) according to Michaelis-Menten equation. [S2]

Verification of ·OH via Degradation of Methylene Blue. For verifying the generation of reactive oxygen species, the experiment of discoloration of methylene blue (MB) was carried out at room temperature. Typically, 30 μL MB solution (1 mg mL^{-1}) was dispersed in 1.75 mL PBS (0.1 M, pH=7.0). Next, 200 μL of Cu_4Co_6 ZIF (1 mg mL^{-1}) and 20 μL of H_2O_2 (10 M) were added to the solution, which was shaken severely. In order to avoid the photocatalytic degradation of MB, the mixture was placed in a dark room for 30 min reaction. The discoloration of MB was monitored utilizing UV-2550.

Colorimetric Assay for Pirimiphos-Methyl Detection. To verify the feasibility of the proposed colorimetric assay for pirimiphos-methyl detection, 200 μL Cu_4Co_6 ZIF (1 mg mL^{-1}) and 50 μL pirimiphos-methyl (120 μM) were mixed with 1.53 mL PBS (pH=4.0, 0.1 M) and then the mixture was incubated at 30℃, followed by adding 200 μL TMB (20 mM) and 20 μL H_2O_2 (10 M). After another 15 min of incubation, the absorption spectra were measured by UV-2550. Similarly, detection of pirimiphos-methyl was performed via adding different concentrations of pirimiphos-methyl to the mixture of PBS and Cu_4Co_6 ZIF, and the rest of experimental steps were conducted according to the methods mentioned above. In addition, the detection of different pesticides (including deltamethrin, dimethoate, dichlorvos and clorpyrifos) was carried out in the same experimental steps as above with the concentration unified to 150 nM in the solution.

X-ray Photoelectron Spectroscopy (XPS) Characterization of Cu_4Co_6 ZIF Nanozyme. As shown in Fig. S1A, the XPS full spectrum verified the co-existence of Cu, Co, C and N elements in Cu_4Co_6 ZIF, which was consistent with the experimental results of XRD. Further, according to the XPS spectrum of Co 2p (Fig. S1B), the peaks

at 780.04 eV and 795.58 eV were associated with the Co^{3+} and the peaks at 783.27 eV and 800.09 eV were assigned to Co^{2+}. In the Cu 2p XPS spectrum (Fig. S1C), the peaks at 934.7 eV and 954.4 eV were associated with the Cu^{2+}. Moreover, three new peaks located at 928.9 eV, 943.0 eV and 963.3 eV were observed in Fig. S1C, corresponding to satellite peaks of Cu^{2+} state.

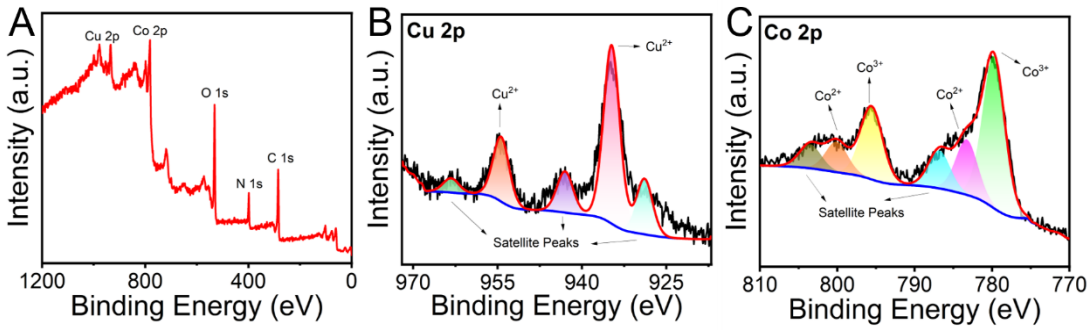

Fig. S1 The XPS spectra of (A) full spectrum, (B) Cu 2p and (C) Co 2p in Cu_4Co_6 ZIF.

Table S1. Michaelis-Menten constant (Km) for different catalysts.

Catalyst	K_m (mM)		Ref.
	TMB	H_2O_2	
ZIF-8	0.371	0.416	[S3]
ZIF-67	0.305	0.21	[S3]
CoZn ZIF	0.287	0.113	[S3]
HRP	0.263	0.318	[S3]
Cu_4Co_6 ZIF	0.150	0.022	This work

Table S2. Comparison of various methods for pirimiphos-methyl detection.

Method	Material	Liner range (nM)	LOD (nM)	Ref.
Colorimetric	Cu_4Co_6 ZIF	0.6-30, 30-3000	0.151	This work
Electrochemistry	CS-PVA NFM	0.1-80	0.2	S4
Electrochemistry	-	75-3000	114	S5
Fluorescence	Brij-35	-	9	S6
SERS	mPEG-SH-coated GNRs	-	132.6	S7

The practical application. The practical feasibility of the proposed analysis was evaluated by its application in real water samples. Using the standard addition method, the measured values of real sample system were calculated from the working curve. As shown in Table S3, the detected values corresponded well with the actual added values with the good recovery of 93.6-103.3%, indicating the acceptable practicability of the colorimetric detection platform based on Cu_4Co_6 ZIF nanozyme.

Table S3. The practical application of our assay for pirimiphos-methyl detection in actual samples.

Added (μM)	Measured (μM)	Recovery (%)	RSD (n=3)
0.015	0.0155	103.3	1.12
0.03	0.0286	95.3	1.15
0.1	0.094	94.0	2.64
0.5	0.468	93.6	2.33
1	0.955	95.5	1.90

The stability of the synthesized Cu_4Co_6 ZIF nanozyme. The stability of the synthesized Cu_4Co_6 ZIF nanozyme was assessed via comparing the activity variation of the long-term storage and the activity of the Cu_4Co_6 ZIF nanozyme from different batches. The relative catalytic activities of the five batches of Cu_4Co_6 ZIF did not differ by more than 7% (Fig. S2A). Furthermore, after being placed in the condition of dry and room temperature for 20 days, the peroxidase-like activity of the Cu_4Co_6 ZIF decreased by only 9.5% of its original activity (Fig. S2B). These results demonstrate that the Cu_4Co_6 ZIF material has good batch-to-batch reproducibility and can be stored for a long time.

The anti-interference performance of the constructed colorimetric analysis. The anti-interference performance of the constructed colorimetric analysis for detection of pirimiphos-methyl based on Cu_4Co_6 ZIF nanozyme was evaluated by observing the absorbance changes at 652 nm with the addition of the interferences such as ascorbic acid (Vc), Fe^{2+}, Co^{2+}, Na^{+} and K^{+}, in which the concentrations of the above interferences are same as pirimiphos-methyl (2 μM, as control group). Fig. S2C revealed that the absorbance change caused by other interfering components was remarkably lower than that of pirimiphos-methyl, indicating that the coexistence of

these interfering components had no significant effect on the detection.

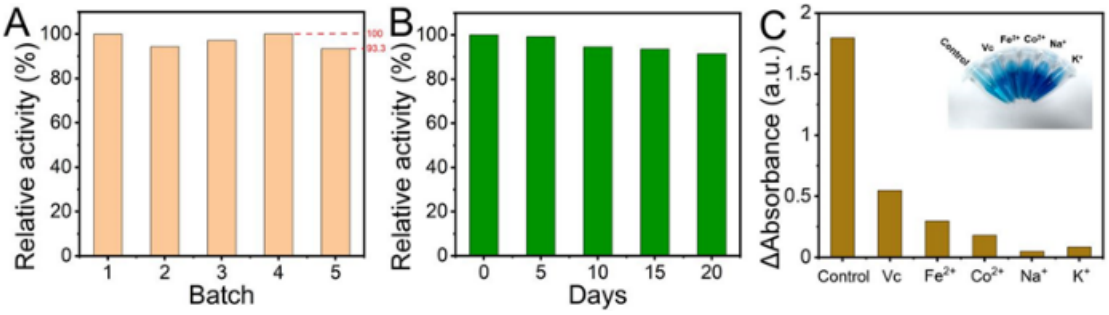

Fig. S2 The stability and anti-interference performance of Cu_4Co_6 ZIF. (A) The peroxidase-like activity of five different batches of Cu_4Co_6 ZIF. (B) The peroxidase-like catalytic activity of Cu_4Co_6 ZIF over time. The maximum absorbance was set to 100% (relative activity). (C) Control group was added with pirimiphos-methyl (2 μM) and the absorbance was collected at 652 nm ($\Delta A=A_0-A$, A_0 and A represent the absorbance of solution with and without pesticide or interfering components, respectively).

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