Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2022

Supporting Information for:

CuCoFe Layered Double Hydroxides as Laccase Mimicking Nanozymes for Colorimetric Detection of Pheochromocytoma Biomarkers

Feng-Wei Huang, Ke Ma, Xiu-Wen Ni, Sheng-Lin Qiao*, and Ke-Zheng Chen*

Lab of Functional and Biomedical Nanomaterials, College of Materials Science and Engineering, Qingdao University of Science and Technology (QUST), Qingdao, 266042, P. R. China.

* To whom correspondence should be addressed.
E-mail: qiaosl@qust.edu.cn; kchen@qust.edu.cn

Chemicals, Solvents and Materials

Reagents and solvents were used as received without further purification otherwise noted. Copper nitrate ($Cu(NO_3)_2 \cdot 3H_2O$), cobalt nitrate ($Co(NO_3)_2 \cdot 6H_2O$), and iron nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) were of analytical grade and obtained from Xilong Scientific Co., Ltd Guangdong, China). Sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), and hydrogen peroxide (H_2O_2, 30 wt.%) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Tris(hydroxymethyl)aminomethane (Tris-HCl) and 2-(N-Morpholino)ethanesulfonic acid (MES) monohydrate, 2,4-Dichlorophenol (2,4-DP), and 4-aminoantipyrine (4-AP), 4-(1-Hydroxy-2-(methylamino)ethyl)benzene-1,2 diolhydrochloride (epinephrine) were purchased from Aladdin Industrial Co. (Shanghai, China). Milli-Q water was used to prepare all the buffers and solutions.

Experimental

1. LDH preparation and characterization

1.1 Preparation of LDHzymes

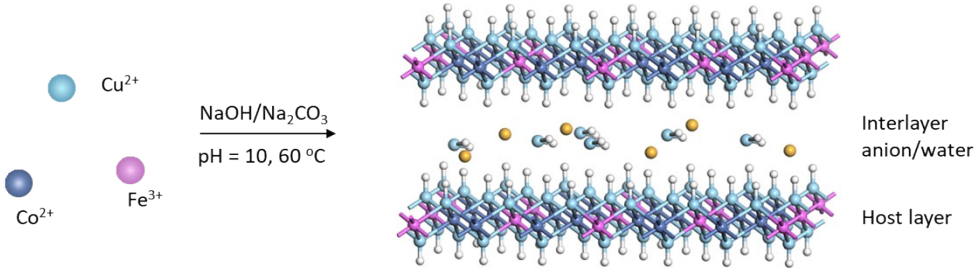


Figure S1. Scheme illustration of a typical fabrication procedure for CuCoFe-LDHzyme.

The LDHzymes were prepared by the co-precipitation method with carbonate interlayers. In a typical experiment, the mixed metal salt solution was first prepared (Table S1). Secondly, as for CoFe-LDH and CuCoFe-LDH, NaOH (0.12 mol) and Na_2CO_3 (0.01 mol) were dissolved in 60 mL of DI water to prepare the alkaline solution. The metal salt solution with 60 mL were drop-wisely added into the as-prepared alkaline solution in a 1000 ml three-neck flask. The pH value of previous miscible liquids was required to adjust to 10 by using NaOH or HCl solution (0.1 M) and the reaction temperature was required to keep at 60 °C during the synthesis process for 24 h. Then, the product was filtered and washed with a mount of deionized water with suction filtration in order to remove the impurity. Finally, the solid was dried at 70 °C and ground into powder. As for CuFe-LDH, NaOH (0.5 mol L^{-1}) and Na_2CO_3 (0.125 mol L^{-1}) were mixed in a 500 ml flask to prepare the alkaline solution. The metal salt solution and alkaline solution were drop-wisely added into a 1000 ml three-neck flask at room temperature with stirring for 30 min. The pH value was adjusted to 10 and the temperature was raised to 60 °C and aged for 24 hours. Finally, the products were filtered, washed with deionized water, dried at 70 °C and ground.

Table S1 The amount of each metal ion in the preparation of the catalyst

Material	$Cu(NO_3)_2 \cdot 3H_2O$	$Co(NO_3)_2 \cdot 6H_2O$	$Fe(NO_3)_3 \cdot 9H_2O$
CuCoFe-LDH	0.5 mol l^{-1}	0.25 mol l^{-1}	0.25 mol l^{-1}
CuFe-LDH	0.09375 mol l^{-1}	-	0.03125 mol l^{-1}
CoFe-LDH	-	0.25 mol l^{-1}	0.25 mol l^{-1}

1.2 Powder X-ray diffraction (XRD)

X-ray diffraction measurements were performed on an X-ray powder diffractometer (Rigaku D/MAX/2500PC). The diffraction intensity of Cu $K_{\alpha 1}$ radiation (wavelength 0.15405981 nm) was measured under 40 kV and 150 mA with a scan rate of 2° min^{-1} in a 2θ range between 3° and 70°. The internal spacings ($d_{(003)}$) of CuCoFe-LDHzyme was calculated by using Bragg's Law equation (nλ = 2dsinθ, n = 1):

$$d = \frac{0.15405981}{2sin\theta} nm \quad \text{(Equation S1)}$$

1.3 X-ray photoelectron spectroscopy (XPS)

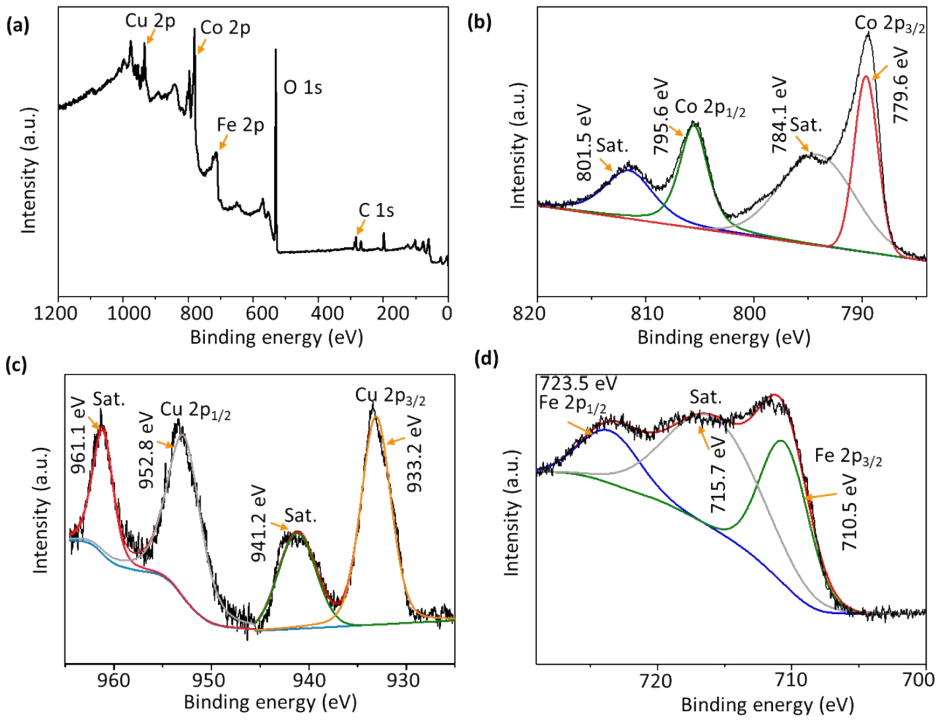


Figure S2. XPS spectra of CuCoFe-LDHzyme (a) survey, (b) Co 2p, (c) Cu 2p, (d) Fe 2p.

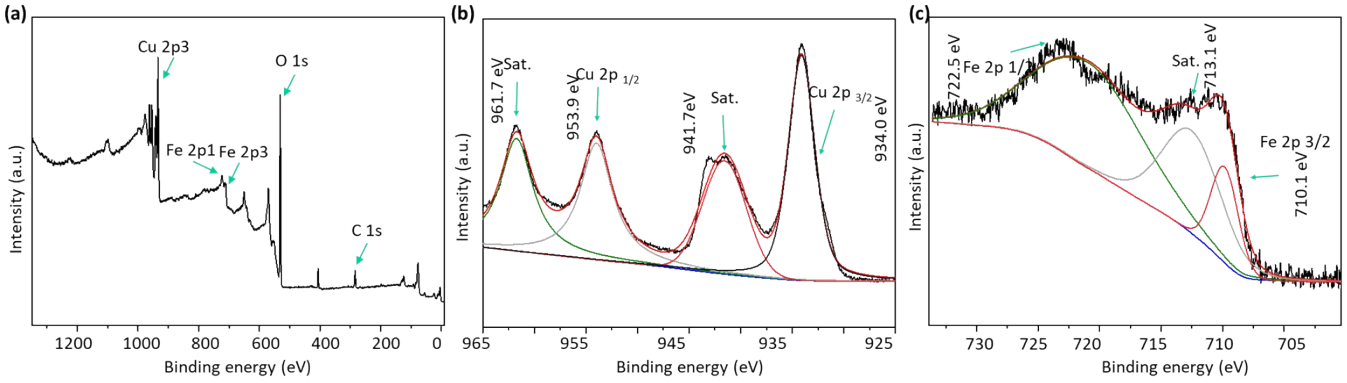


Figure S3. XPS spectrum of CuFe-LDHzyme: (**a**) survey, (**b**) Cu 2p, (c) Fe 2p.

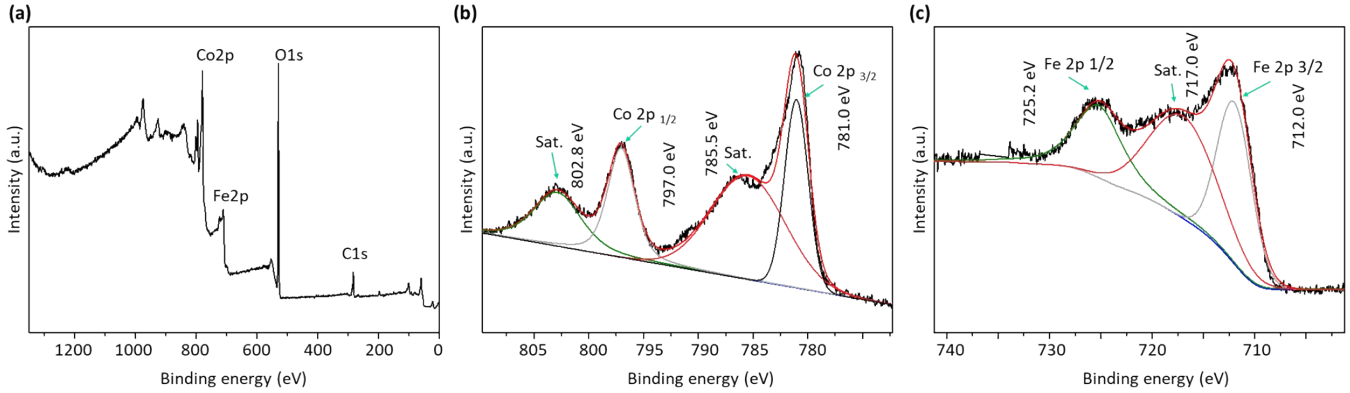


Figure S4. XPS spectrum of CoFe-LDHzyme: (**a**) survey, (**b**) Co 2p, (c) Fe 2p.

1.4 Fourier-transform infrared (FTIR) spectroscopy

Transmission FT-IR spectra were recorded on a Nicolet iS10 FTIR spectrometer (Thermofisher). CuCoFe-LDHzyme, CuFe-LDHzyme and CoFe-LDHzyme powders were grinding with potassium bromide (KBr) powder followed by pressing into a disk before observed in transmission mode.

1.5 Scanning electron microscopy (SEM)

The morphology of the synthesized LDHzymes was measured by using scanning electron microscopy (SEM). All samples were sputter coated with platinum (Pt) using an auto fine coater (JFC-1600, JEOL Ltd., Japan) and then imaged by using a JSM-6700F SEM + EDS system (JEOL Ltd., Japan) at an acceleration voltage of 8 kV.

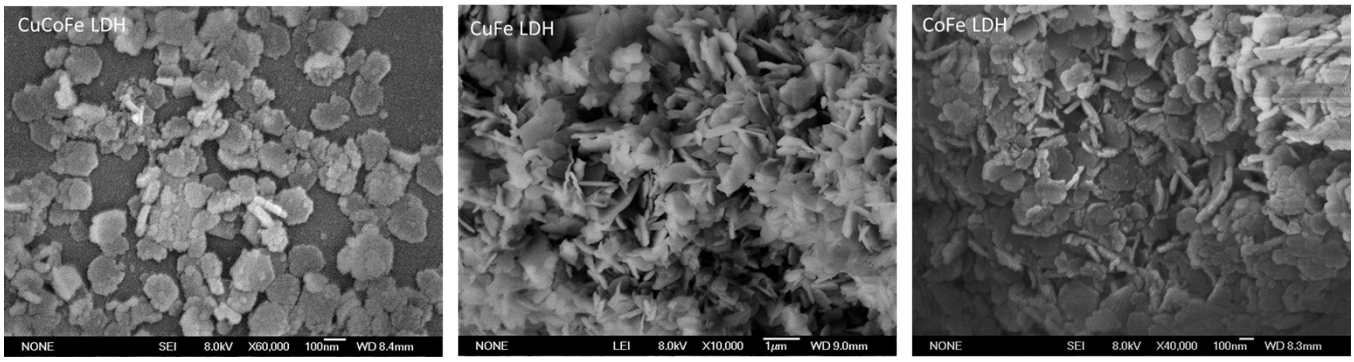


Figure S5. SEM images of CuCoFe-LDHzyme, CuFe-LDHzyme and CoFe-LDHzyme.

1.6 Dynamic light scattering (DLS)

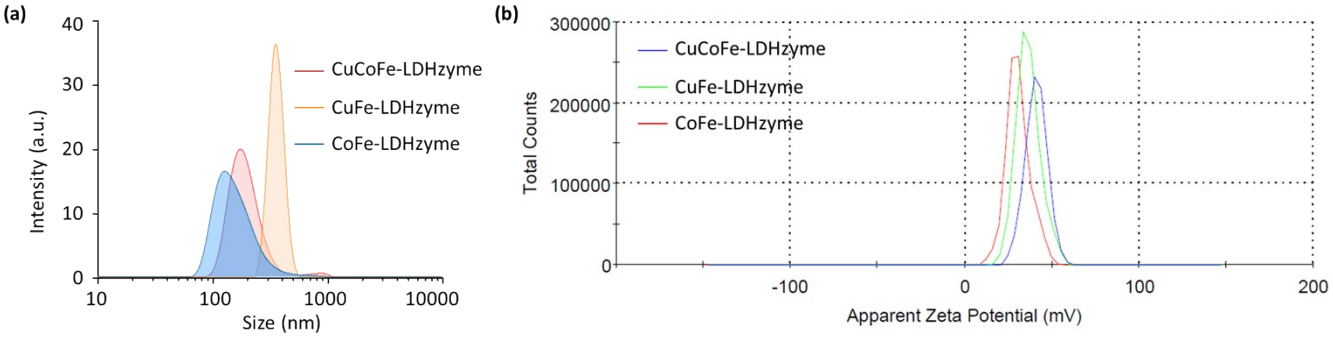
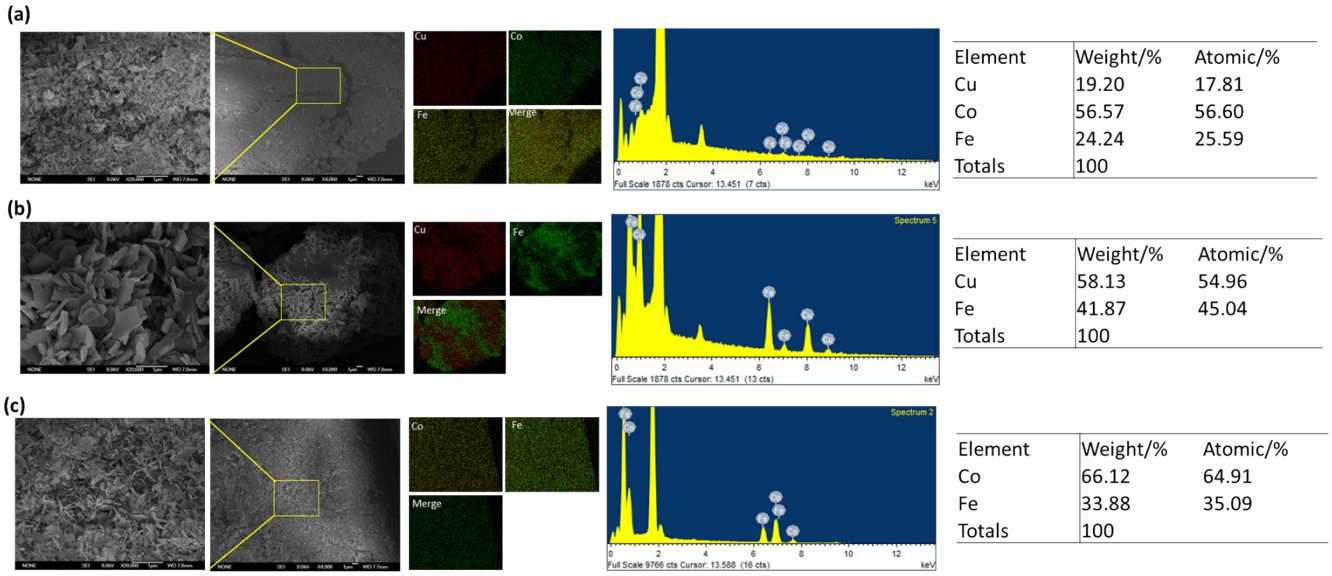


Figure S6. (**a**) DLS Sizes and (**b**) Zeta potentials of CuCoFe-LDHzyme, CuFe-LDHzyme, and CoFe-LDHzyme.



Element	Weight/%	Atomic/%
Cu	19.20	17.81
Co	56.57	56.60
Fe	24.24	25.59
Totals	100	

Element	Weight/%	Atomic/%
Cu	58.13	54.96
Fe	41.87	45.04
Totals	100	

Element	Weight/%	Atomic/%
Co	66.12	64.91
Fe	33.88	35.09
Totals	100	

Figure S7. EDX mapping of (**a**) CuCoFe-LDHzyme, (**b**) CuFe-LDHzyme, and (**c**) CoFe-LDHzyme.

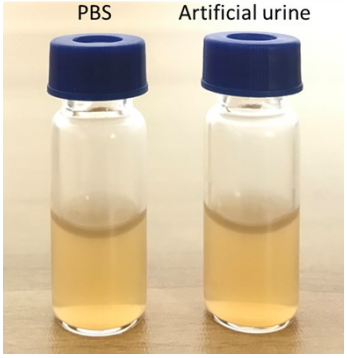


Figure S8. Negligible aggregation or sedimentation of the CuCoFe-LDHzyme in phosphate buffer saline (PBS) or artificial urine on standing unstirred for 24 h.

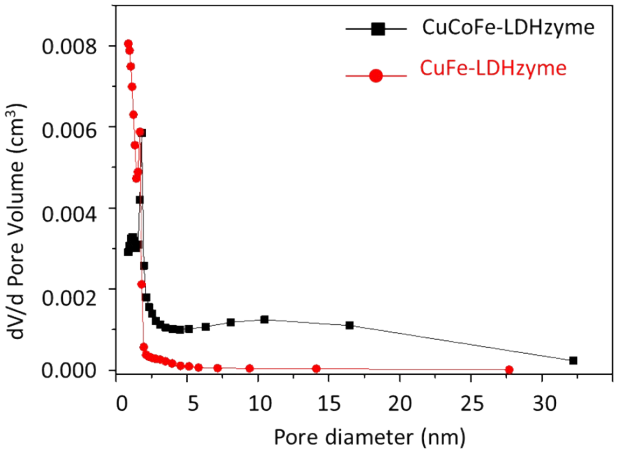


Figure S9. Corresponding pore-size distributions for CuCoFe-LDHzyme and CuFe-LDHzyme that obtained from the N_2 isotherm.

2. Evidences of laccase-mimetic property of CuCoFe-LDHzyme

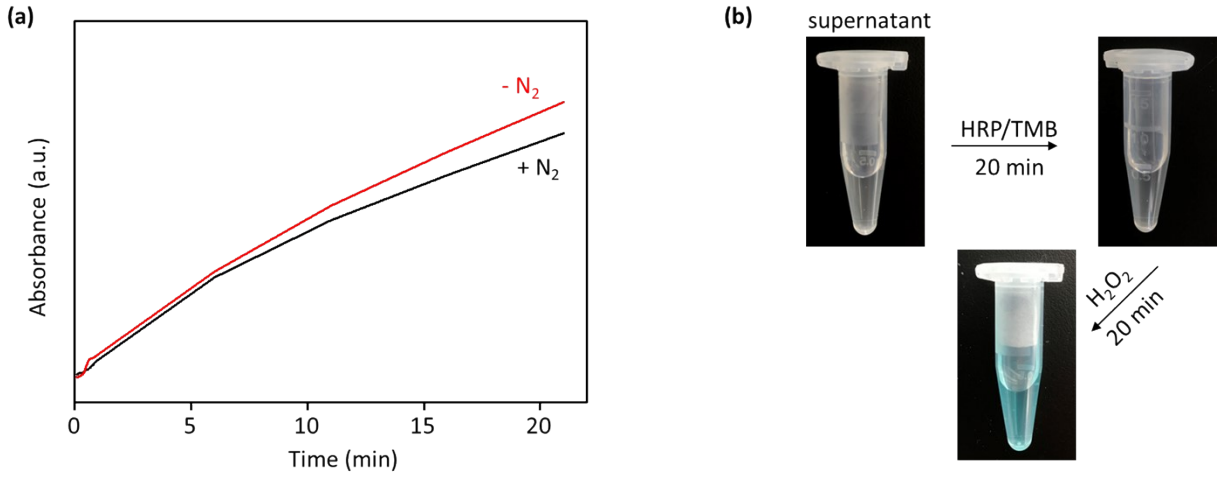


Figure S10. (**a**) Relative reaction velocity of the CuCoFe-LDHzyme catalyzed laccase reaction with/without nitrogen saturation. (**b**) Control experiment for confirming the absence of H_2O_2 as a byproduct during the laccase-like activity of CuCoFe-LDHzyme.

The laccase-like catalytic activity of CuCoFe-LDHzyme was verified through the color reaction between 2,4-dichlorophenol (2,4-DP) and 4-aminoantipyrine (4-AP). Briefly, an aqueous solution of 100 µl, 1 mg ml^{-1} 2,4-DP and 4-AP was mixed with 50 mM MES buffer (pH = 7.0, 1.5 ml total volume). Afterwards, ultrasonic dispersion CuCoFe-LDHzyme was introduced, and then reacted at 37 °C for 1.5 h. Finally, the absorbance was determined by UV-Vis spectrophotometer. The reaction kinetics was evaluated by adding 2,4-DP with different consistencies and 4-AP (1 mg ml^{-1}) into MES buffer (pH = 7.0, 1.5 ml) to react with CuCoFe-LDHzyme (1 mg ml^{-1}). These kinetic constants were acquired by the Michaelis-Menten equation:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

Where K_m was Michaelis constant; [S] was substrate concentrations; V_{max} was maximum reaction velocity.

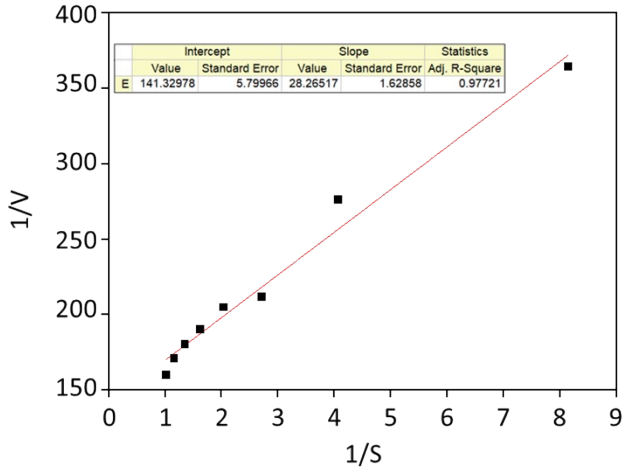


Figure S11. Lineweaver-Burk plot for CuCoFe-LDHzyme oxidizing 2,4-DP.

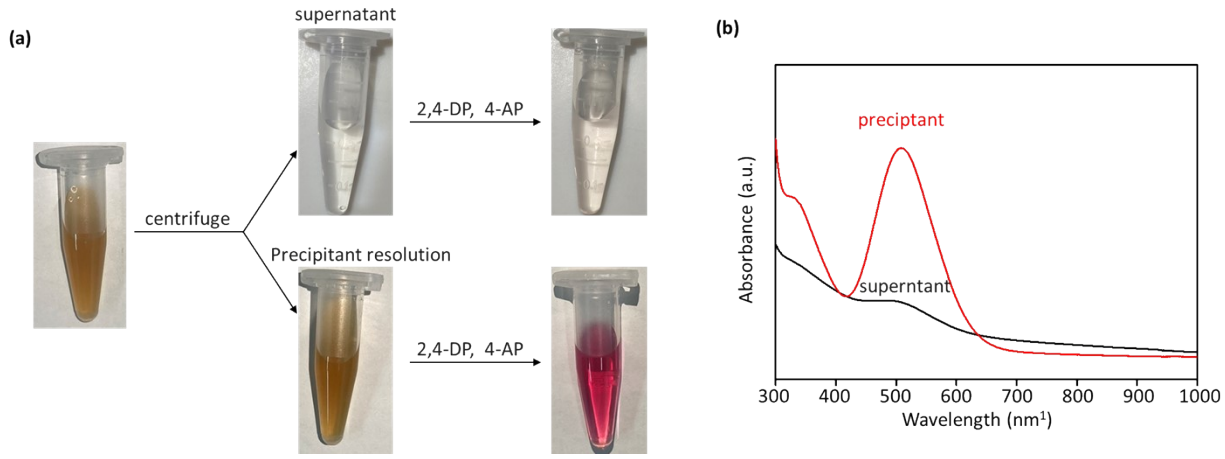


Figure S12. (**a**) the laccase-like activity of the supernatant and precipitate of the CuCoFe-LDHzyme dispersion in water. (**b**) UV-Vis absorption spectra of catalytic reaction catalyzed by the sediment and supernatant of aqueous dispersion using 2,4-DP and 4-AP as substrates.

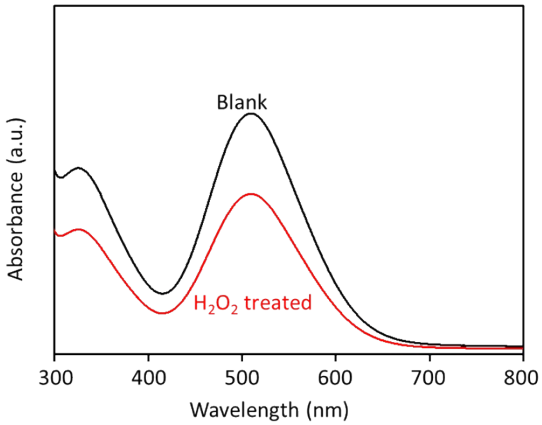


Figure S13. UV-Vis absorption spectra of catalytic reaction catalyzed by CuCoFe-LDHzyme and H_2O_2-treated CuCoFe-LDHzyme using 2,4-DP and 4-AP as substrates. The H_2O_2 aqueous solution (24.5 mM) was used to treat certain amount of CuCoFe-LDHzyme. The product was washed using water three times and then dried. Then CuCoFe-LDHzyme and H_2O_2-treated CuCoFe-LDHzyme with concentration of 1 mg m^{-1} were employed to performed the catalytic reaction using 2,4-DP and 4-AP as the substrates.

3. Catalytic mechanism of CuCoFe-LDHzyme

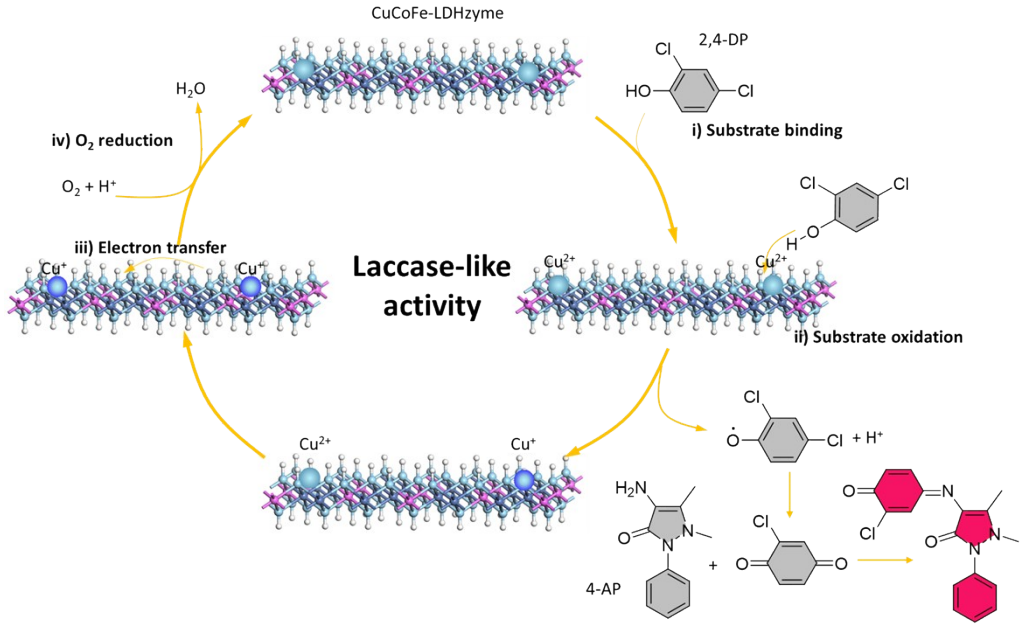


Figure S14. Schematic of the possible catalytic mechanism of CuCoFe-LDHzyme.

4. Catalytic activity assay

The catalytic activity of the CuCoFe-LDHzyme was determined using a typical chromogenic reaction of 4-AP and phenolic compound. Firstly, 200 μl 4-AP aqueous solution (1 mg ml^{-1}) and 200 μl 2,4-DP aqueous solution (1 mg ml^{-1}) were mixed in 1400 μl MES buffer (30 mM, pH 6.5). After that, 200 μl aqueous dispersion of CuCoFe-LDHzyme was added into the above mixture. The reaction solution was reacted at room temperature for 1 h. After centrifugation at 10,000 $r \cdot min^{-1}$ for 5 min, the absorbance data was collected using a UV-Vis spectrophotometer. For the detecting other phenolic compounds, 4-AP was replaced by these phenolic chemicals and used the same protocol.

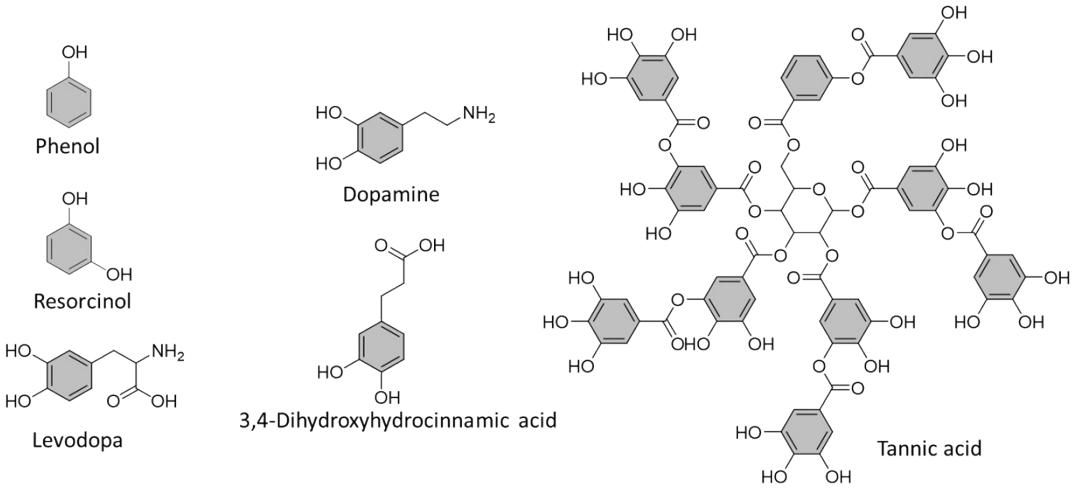


Figure S15. Chemical structures of six phenolic compounds.

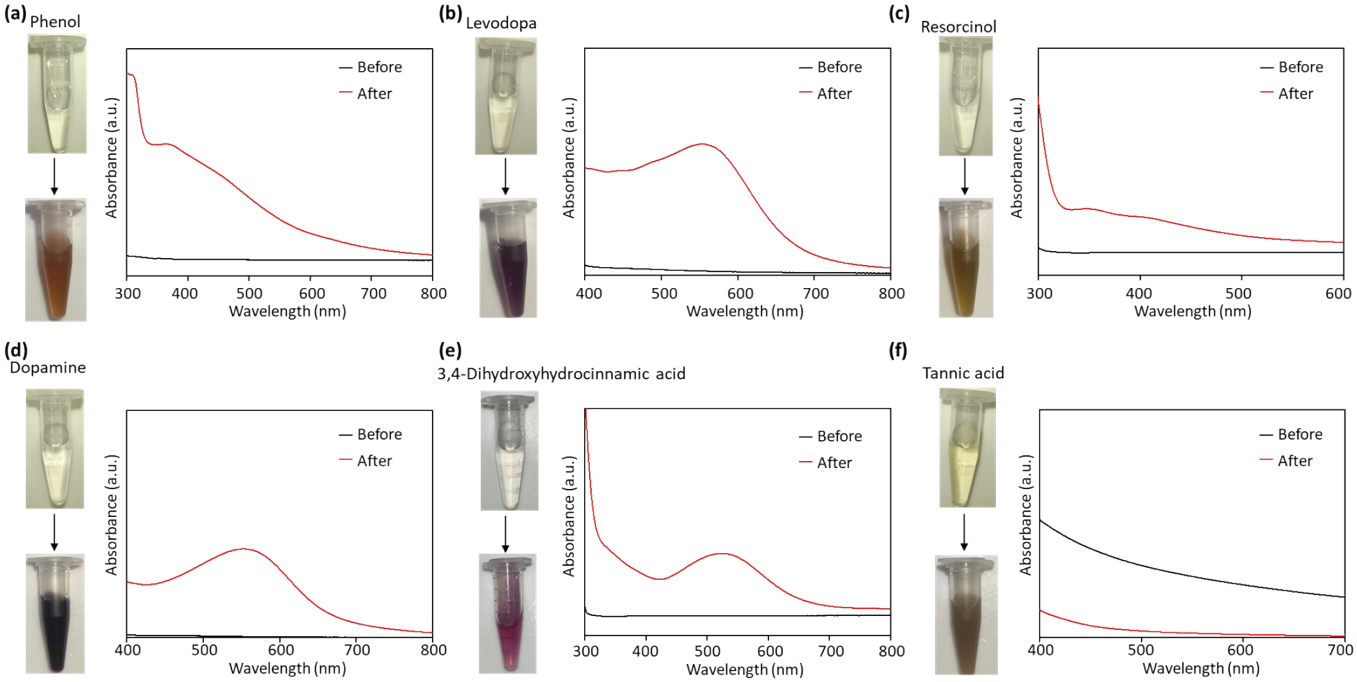


Figure S16. UV-Vis absorption spectra of catalytic reaction catalyzed by CuCoFe-LDHzyme using 4-AP and other kinds of phenolic compounds as substrates (**a**) phenol, (**b**) levodopa, (**c**) resorcinol, (**d**) dopamine, (**e**) 3,4-Dihydroxyhydrocinnamic acid, and (**f**) tannic acid.

5. The influence of pH, temperature and ion strength on the catalytic activity of CuCoFe-LDHzyme

To study the influence of pH on the catalytic activity of the CuCoFe-LDHzyme, 200 μl 4-AP aqueous solution (1 mg ml^{-1}) and 200 μl 2,4-DP aqueous solution (1 mg ml^{-1}) were mixed in 1400 μl buffer solutions with different pH (4-9) at room temperature. Subsequently, 200 μl aqueous dispersion of CuCoFe-LDHzyme was added into the above mixture and reacted for 1 h. After centrifugation at 10,000 $r \cdot min^{-1}$ for 5 min, the absorbance data was collected using a UV-Vis spectrophotometer. The temperature tolerance of CuCoFe-LDHzyme was studied using the similar procedure in MES buffer solution (30 mM, pH 6.5) with the reaction temperature changed from 25 to 90 °C. The ion strength tolerance of CuCoFe-LDHzyme was studied using the similar procedure in NaCl (0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 M) at room temperature. The results were normalized at pH 7, temperature 25 °C and ion strength 0 M as indicated with red dash line below.

For long-term storage stability, the LDHzymes were stored at room temperature for 180 days, and the relative activity was compared with the activity at first day. The reusability of CuCoFe-LDHzyme were studied for 7 cycles by centrifugation and washing using water to recycle the CuCoFe-LDHzyme for each reaction cycle.

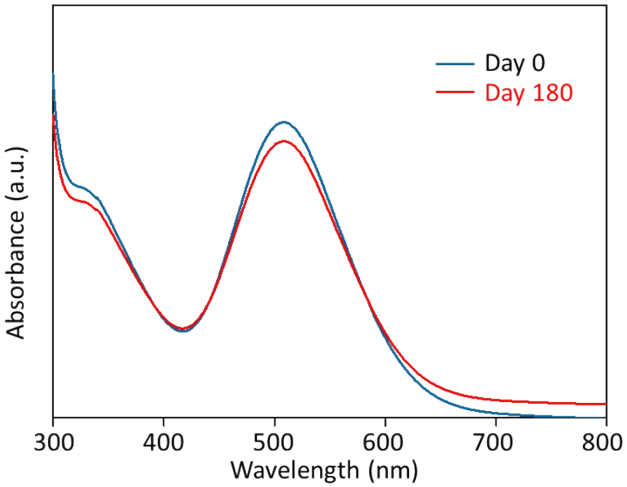


Figure S17. The long-term stability of CuCoFe-LDHzyme. The catalytic activity of CuCoFe-LDHzyme could be maintained (~93.5%) after 6 months of storage.

6. Detection of epinephrine

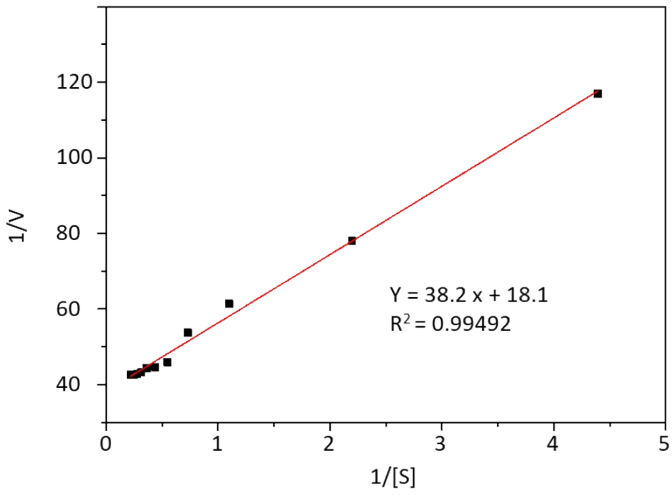
6.1 Limit of detection

For LOD assays, in centrifugation tube, CuCoFe-LDHzyme (300 µl, 5 mg ml^{-1}, in MES buffer) was mixed with epinephrine (1.2 ml, varying concentrations) and incubated at 37 °C for 90 min. The final concentration of CuCoFe-LDHzyme was 1 mg ml^{-1} and the final concentration of epinephrine sample corresponds to the concentration of 1, 10, 25, 50, 100 and 250 µM. After the incubation, the solutions were centrifugated (10,000 r min^{-1}, 3 min) to remove CuCoFe-LDHzyme, and the colored supernatants were collected and the absorbances at 485 nm was measured by using a spectrometer (Cary 5000, Agilent Technologies Inc.). Linear regression was used to obtain the slope (k) in the plot of $A_{485\ nm}$ *v.s.* epinephrine concentration. To calculate the LOD value, 11 blank signals of the spectrometer were recorded and their standard deviation (σ) was obtained. Then, the detection limit LOD is calculated with formula LOD = 3σ/k.

6.2 Smartphone-based analysis of epinephrine

A cheap portable device based on smartphone was developed to detect epinephrine. Epinephrine with different concentrations were mixed with 1 mg ml^{-1} CuCoFe-LDHzyme in MES buffer (1.5 ml total volume) for 1.5 h at 37 °C, respectively. Collecting the colorimetric images by the smartphone, then selecting multiple regions of the obtained images and converting to HSV (Hue, Saturation, Value) values *via* an open-source app *Color Pickers*. The parameter ΔV (difference of value between the absence and presence of a certain concentration of epinephrine) was associated with the concentration of epinephrine. Finally, the linear equations and correlation coefficients were presented.

6.3 Kinetic parameters of the CuCoFe-LDHzyme



Enzyme	K_m (µM)	V_{max} (µM min^{-1})	
CuCoFe-LDHzyme	473.4	26.2	This work
Laccase	410	6.41	Ref. 1

Figure S18. The kinetic parameters of the CuCoFe-LDHzyme were also calculated

6.4 Specificity of the CuCoFe-LDHzyme

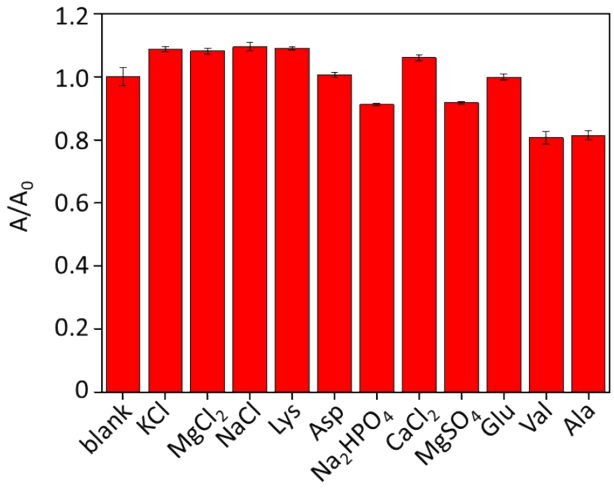


Figure S19. The interference study of several interfering substances to epinephrine detection.

Table. S2. Brief list of abbreviations

Sample	**Added epinephrine (μM)**	**Found (μM)**	Recovery (%)	RSD (%), n = 3
Urine	0	-	-	-
	10	11.4 ± 1.3	114	11.1
	25	25.8 ± 2.0	103	7.7
	50	50.0 ± 1.8	100	3.6
	100	102.5 ± 2.1	102	2.0

References

1. Xu, X.; Wang, J.; Huang, R.; Qi, W.; Su, R.; He, Z., Preparation of laccase mimicking nanozymes and their catalytic oxidation of phenolic pollutants. *Catalysis Science & Technology* **2021,** *11* (10), 3402-3410.