Supporting information

ZIF-derived Co_3O_4/Fe_2O_3 Dual-layer Hollow Nanoboxes as Bimetallic Nanozymes for Detection of Hydrogen Peroxide and Ascorbic Acid

Rongguan Lv [a], Mengting Shao [b], Yuan Xiao[b], Shufei Liu[b], Yongjun He[b,c], Jingjing Jiang[b*], Haiyan Yu[a], Fang Guo[d*], Ming Chen[b,e*], Guowang Diao[b,e]

a School of Chemistry and Environmental Engineering, Yancheng Teachers University, Yancheng 224000, P. R. China

b School of Chemistry and Materials, Yangzhou University, Yangzhou 225002, Jiangsu, P. R. China

c Jiangsu Jinshiyuan Latex Products Co., Ltd.

d School of Chemistry and Chemical Engineering, Yancheng Institute of Technology, Yancheng 224051, Jiangsu, P. R. China

e Jiangsu Provincial Key Laboratory of Green & Functional Materials and Environmental Chemistry

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# These authors contributed equally to this work

*Corresponding Authors E-mail address: jjj@yzu.edu.cn (J. Jiang); gfycit@163.com (F. Guo); chenming@yzu.edu.cn (M. Chen).

1 Chemicals and materials

Cobalt nitrate hexahydrate ($Co(NO_3)_2$❿$6H_2O$), $K_3[Fe(CN)_6]$, cetyltrimethylammonium bromide (CTAB), 2-methyimidazole (2-MIM), and ascorbic acid (AA) were purchased from Shanghai Chemical Corp. Glacial acetic acid (CH_3COOH), 3,3’,5,5’-tetramethylbenzidine (TMB), isopropanol (IPA), L-histidine (L-His), p-benzoquinone (p-BQ), terephthalic acid (PTA) and sodium acetate (NaAc) were purchased from Aladdin Biological Technology Co., Ltd. (Shanghai, China). Hydrogen peroxide (H_2O_2, 30%) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The vitamin C injection (0.5 g/5 mL) was purchased from Shanxi Nuocheng Pharmaceutical Co., Ltd (Shanxi, China). All of the experiments were conducted with ultrapure water.

2 Apparatus

Morphology was characterized using a Supra 55 SEM set at 5.0 kV, energy-dispersive X-ray spectroscopy (EDX, GENESIS, EDAX, USA), Transmission Electron Microscopy (TEM) at 200 kV, and High-resolution TEM. X-ray diffraction (XRD) data was collected using a graphite monochromator and Cu K radiation (λ= 0.1541 nm) on a D8 advance superspeed powder diffractometer (Bruker). Surface chemical species were identified using X-ray photoelectron spectroscopy (XPS) with the Thermo Escalab 250 instrument. The specific surface area was determined using the Brunauer-Emmett-Teller (BET) method (Autosorb-IQ surface area analyzer). Electron paramagnetic resonance (EPR) spectra were measured using an A300-10/12

(Bruker BioSpin GmbH).

3 Synthesis of Co-Fe PBA single-layer hollow nanoboxes (SLHNBs)

ZIF-67 NCs were dispersed in ethanol, and the solution was heated to 60 C. $K_3[Fe(CN)_6]$ and 0.1 mL of aqueous acetic acid (0.1 M) were combined in water to create a clear solution. This mixed aqueous solution was then rapidly injected into the heated ZIF-67 NCs dispersion, with continuous stirring for 1 hour. The resulting product was collected by centrifugation and washed three times with both water and ethanol.

4 Synthesis of Co_3O_4/Fe_2O_3 single-layer hollow nanoboxes (SLHNBs) and Co_3O_4 (SLHNBs)

Co-Fe PBA SLHNBs and ZIF-67 NCs were heat-treated at 350 C in air for 2h to obtain Co_3O_4/Fe_2O_3 single-layer hollow nanoboxes (SLHNBs) and Co_3O_4 (SLHNBs), respectively.

5 POD-like (peroxidase like) of Co_3O_4/Fe_2O_3 DLHNBs and its kinetic tests

The POD-like activities of Co_3O_4/Fe_2O_3 DLHNBs nanozymes were assessed through the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) to ox-TMB, facilitated by the nanoboxes in the presence of H_2O_2, resulting in a colorimetric reaction. The reaction was carried out in 1.86 mL of 0.1 M HAc-NaAc buffer (pH=4), with the addition of 20 μL of 0.5 mM TMB and 20 μL of 1 mM H_2O_2. Subsequently, 100 μL of a 1 mg/mL catalyst dispersion was introduced and mixed for 3 minutes. The UV-Vis absorption spectrum was then recorded at 652 nm. Optimal pH and temperature conditions for the catalytic oxidation of TMB by Co_3O_4/Fe_2O_3 DLHNBs were thoroughly investigated by comparing the UV absorption intensity of the catalysts at 652 nm.

The steady-state kinetic parameters, K_m and V_{max}, were determined by varying the concentration of TMB while maintaining a constant concentration of H_2O_2 (1 mM), or by varying the H_2O_2 concentration while keeping the TMB concentration constant at 0.5 mM. The change in absorbance at 652 nm over time was recorded to monitor the color change of the reaction in time scan mode.

The Lambert-Beer law is utilized to figure out the initial rate of reaction by Eq. (1):

$$V = k/\varepsilon b \quad (1)$$

($k = \Delta A/\Delta t$, $\varepsilon = 39{,}000\ M^{-1}\ cm^{-1}$, and $b = 1$ cm)[1]

By plotting the calculated initial rate versus substrate concentration, a Michaelis−Menten curve Eq. (2):

$$V = V_{max}\,[S]/(K_m + [S])^{[2]} \qquad (2)$$

(V_{max} = the maximum reaction velocity, [S] = the substrate concentration, K_m = the Michaelis constant)

6 Exploration of the catalytic reaction mechanism

Isopropanol (IPA), L-histidine (L-His), and p-benzoquinone (p-BQ) were employed to explore the catalytic mechanism of Co_3O_4/Fe_2O_3 DLHNBs displaying peroxidase-like activity. This investigation involved the trapping of hydroxyl radicals (•OH), singlet oxygen radicals ($^1O_2$), and superoxide radicals ($O_2^{\bullet-}$). Terephthalic acid (PTA) was used as a fluorescent probe within the Co_3O_4/Fe_2O_3 DLHNBs system to assess the presence of •OH. Fluorescence intensity was recorded for different catalysts concentrations using a fluorescence spectrophotometer F-7000 2014XHTM158. Furthermore, electron spin resonance (ESR) spectroscopy was performed to verify the production of •OH.

7 Detection of H_2O_2 and ascorbic acid based on nanozymes catalysis

A total of 100 μL of 0.05 mg/mL Co_3O_4/Fe_2O_3 DLHNBs and 20 μL of 0.5 mM TMB were added to 1.86 mL of acetate buffer (pH 4.0) and mixed thoroughly. Different concentrations of H_2O_2 (ranging from 0 to 1 mM) were then introduced, and the reaction was conducted for 3 minutes at 37 °C in a constant-temperature water bath. The absorbance at the maximum wavelength was measured to establish the linear relationship between H_2O_2 concentration and absorbance.

A mixture was prepared by combining 1.84 mL of acetate buffer (pH 4.0), 100 μL of 0.05 mg/mL Co_3O_4/Fe_2O_3 DLHNBs, 20 μL of 0.5 mM TMB, and 20 μL of 1 mM H_2O_2. Subsequently, 20 μL of ascorbic acid at varying concentrations (0-40 μM) was added to the solution, which was then incubated at 37 °C for 3 minutes. The absorbance was measured at 652 nm.

To assess the practical applicability of the colorimetric assay, we selected a commercial vitamin C injection for direct detection and commercial orange juice for spiked recovery experiments. The vitamin C injection was diluted to various concentrations for experimental analysis. For the juice samples, the sample is first subjected to centrifugation. Then, filtration was performed using a Macherey-Nagel filter membrane with a pore size of 0.22 μm. Subsequently, the solution undergoes activated carbon adsorption to remove natural pigment substances. Lastly, the solution was diluted 50 times with acetate buffer solution for the spiked recovery experiments. Each sample was analyzed in triplicate to ensure accuracy. To further validate the reliability of the colorimetric method, high-performance liquid chromatography (HPLC) was employed to determine the corresponding concentrations of ascorbic acid.

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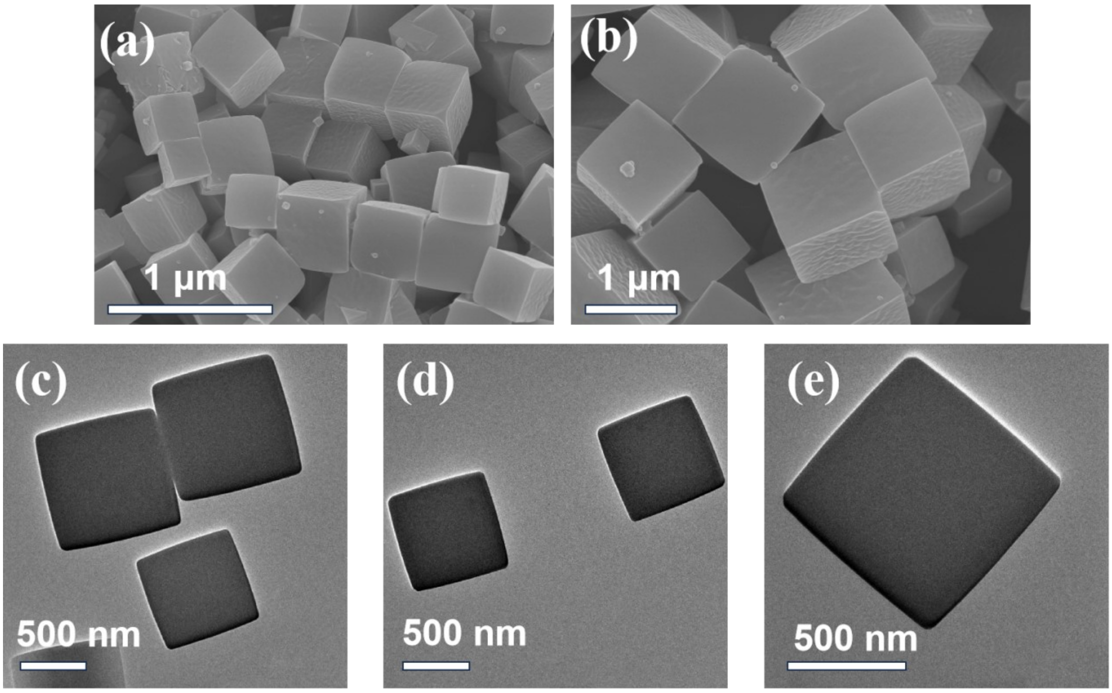

Figure S1 (a, b) SEM and (c-e) TEM images of ZIF-67 NCs

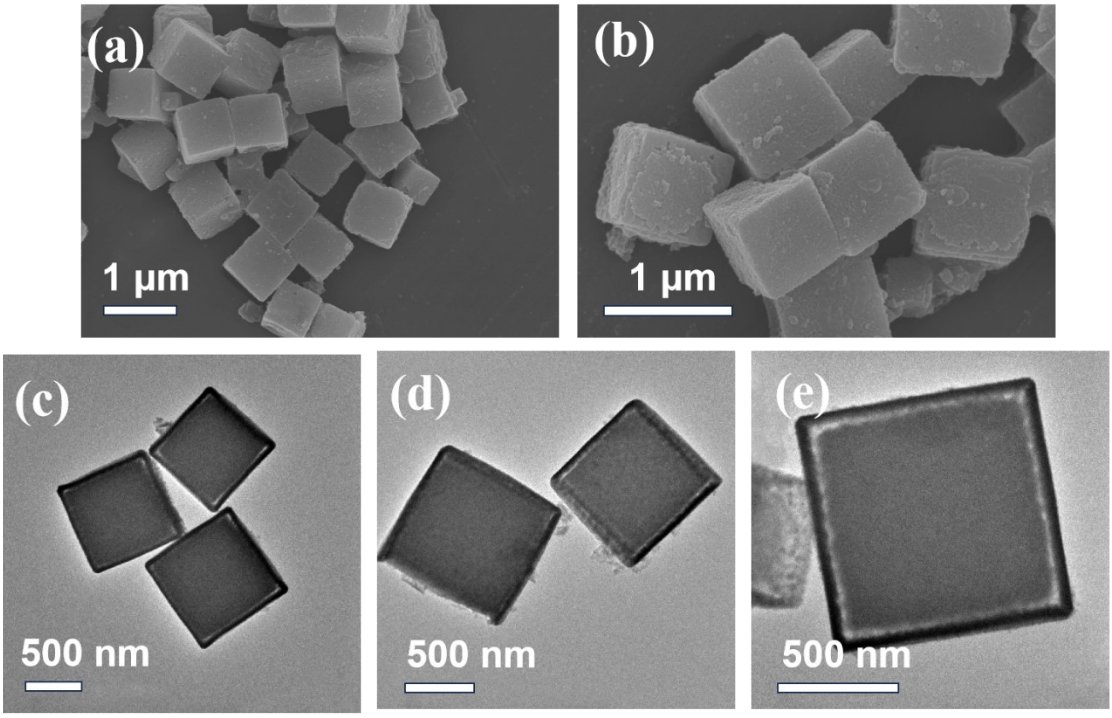

Figure S2 (a, b) SEM and (c-e) TEM images of ZIF-67@Co-Fe PBA CSNCs

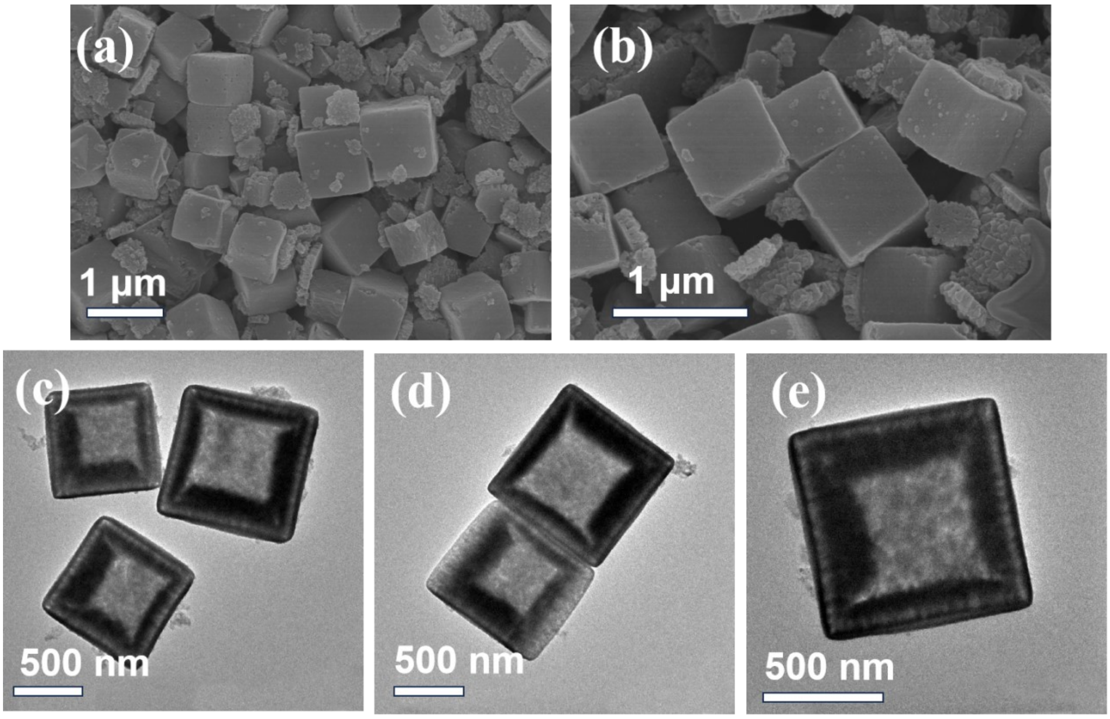

Figure S3 (a, b) SEM and (c-e) TEM images of Co-Fe PBA SLHNBs

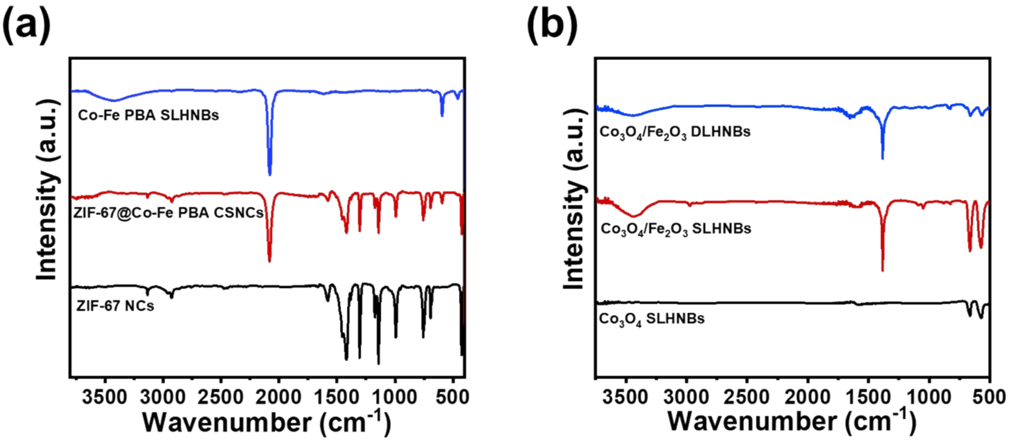

Figure S4 FTIR spectra (a) ZIF-67 NCs, ZIF-67@Co/Fe PBA CSNCs and Co/Fe PBA SLHNBs. (b) Co_3O_4 SLHNBs, Co_3O_4/Fe_2O_3 SLHNBs and Co_3O_4/Fe_2O_3 DLHNBs.

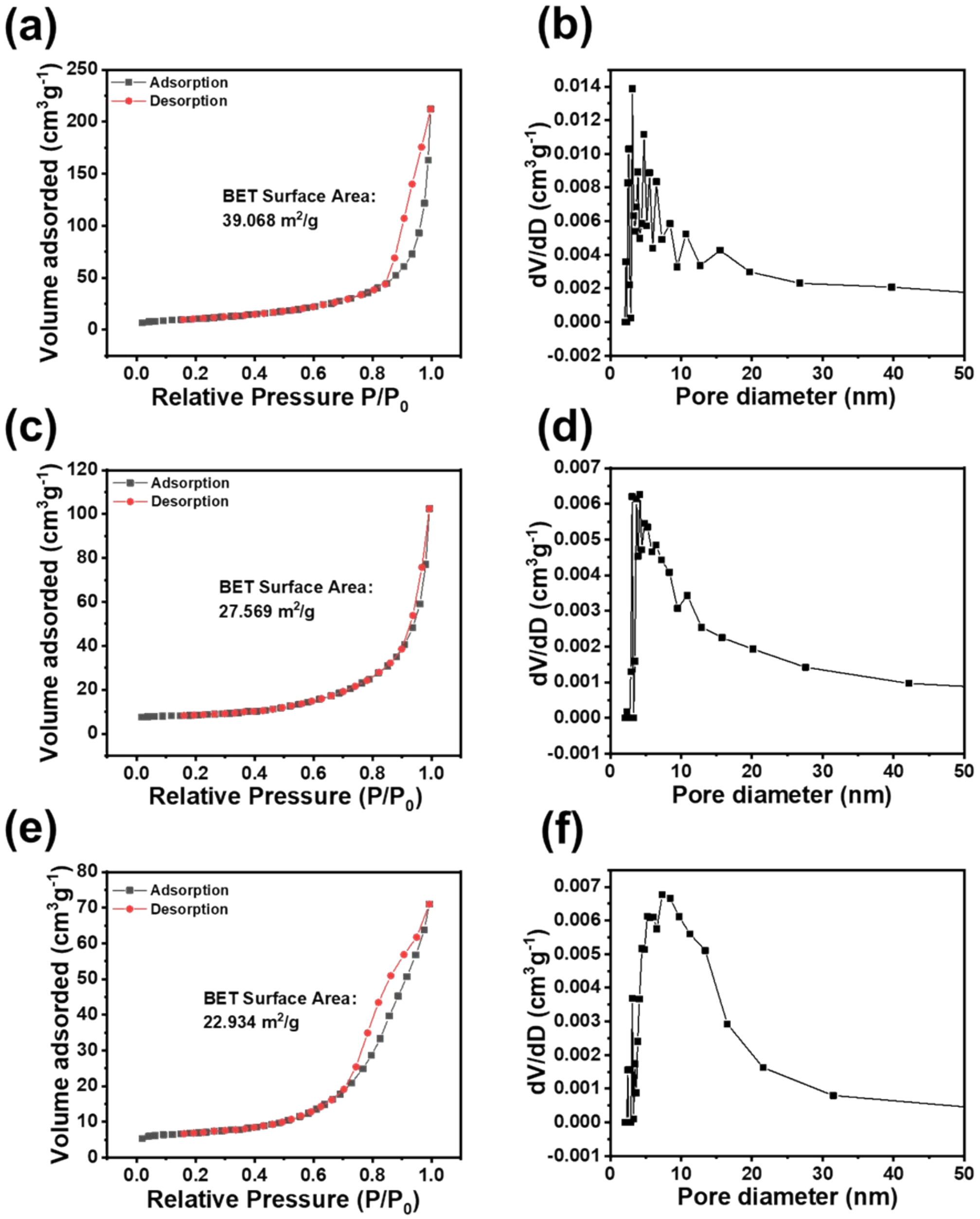

Figure S5 N_2 adsorption-desorption isotherms and pore-size distribution plot of (a, b) Co_3O_4/Fe_2O_3 DLHNBs, (c, d) Co_3O_4/Fe_2O_3 SLHNBs, (e, f) Co_3O_4 SLHNBs.

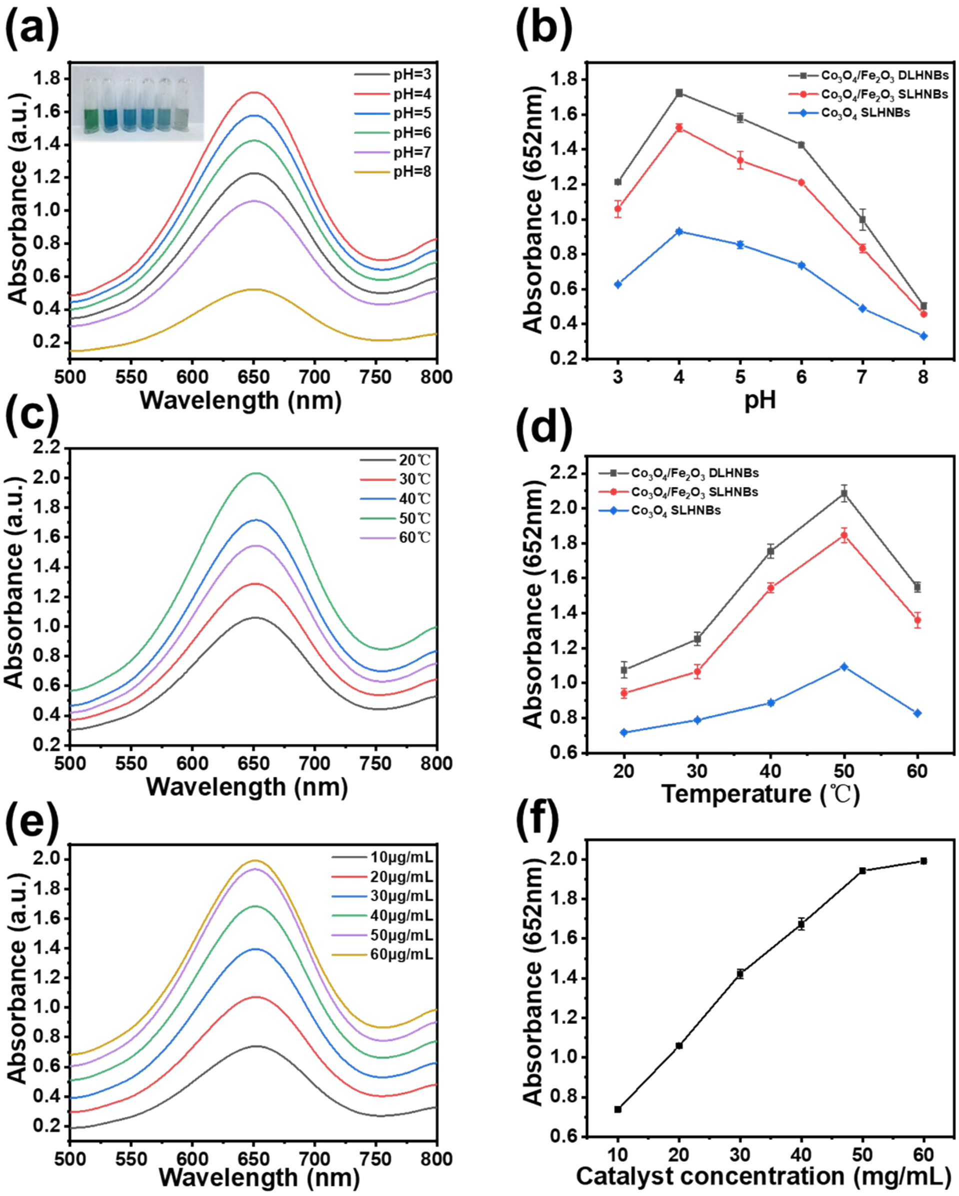

Figure S6 The effects of (a,b) pH, (c,d) temperature, (e,f) catalyst concentration on the peroxidase-like activities of Co_3O_4/Fe_2O_3 DLHNBs.

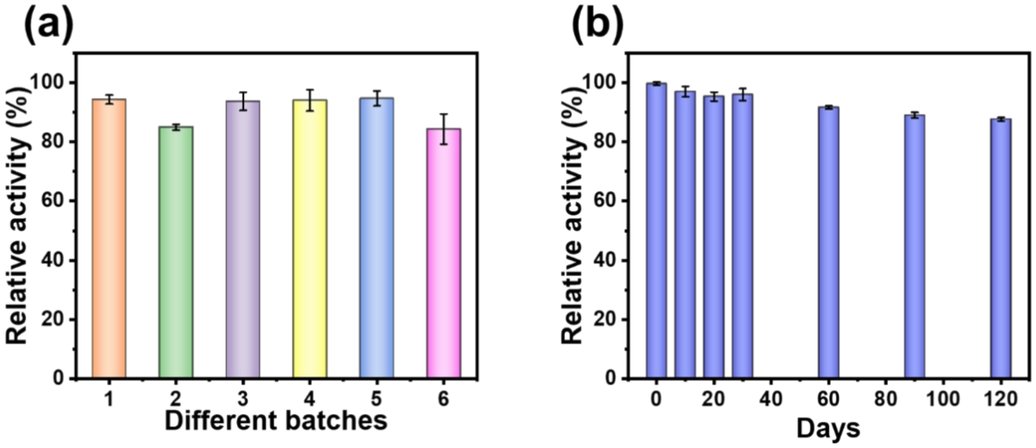

Figure S7 (a) The catalytic activity of six different batches Co_3O_4/Fe_2O_3 DLHNBs nanozymes. (b) The storage stability of Co_3O_4/Fe_2O_3 DLHNBs. The experimental condition for the Co_3O_4/Fe_2O_3 DLHNBs as a nanozyme are 100 μL of 0.05 mg mL^{-1} Co_3O_4/Fe_2O_3 DLHNBs, 20 μL of 1 mM H_2O_2 and 20 μL of 0.5 mM TMB mixed with 1.86 mL of acetatebuffer solution (pH 4) at 37 C. After 3 min, the absorbance of the solution was measured by a UV vis spectrophotometer at 652 nm.

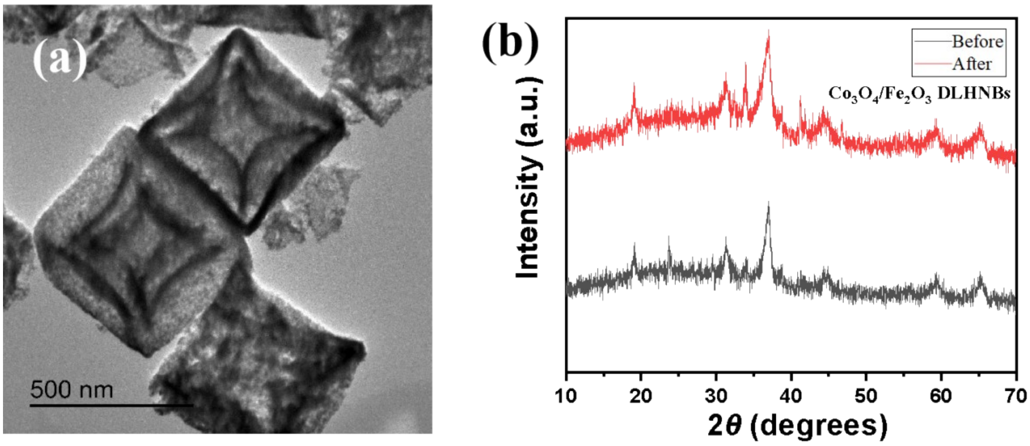

Figure S8 (a) TEM image and (b) XRD pattern of Co_3O_4/Fe_2O_3 DLHNBs after catalytic reaction.

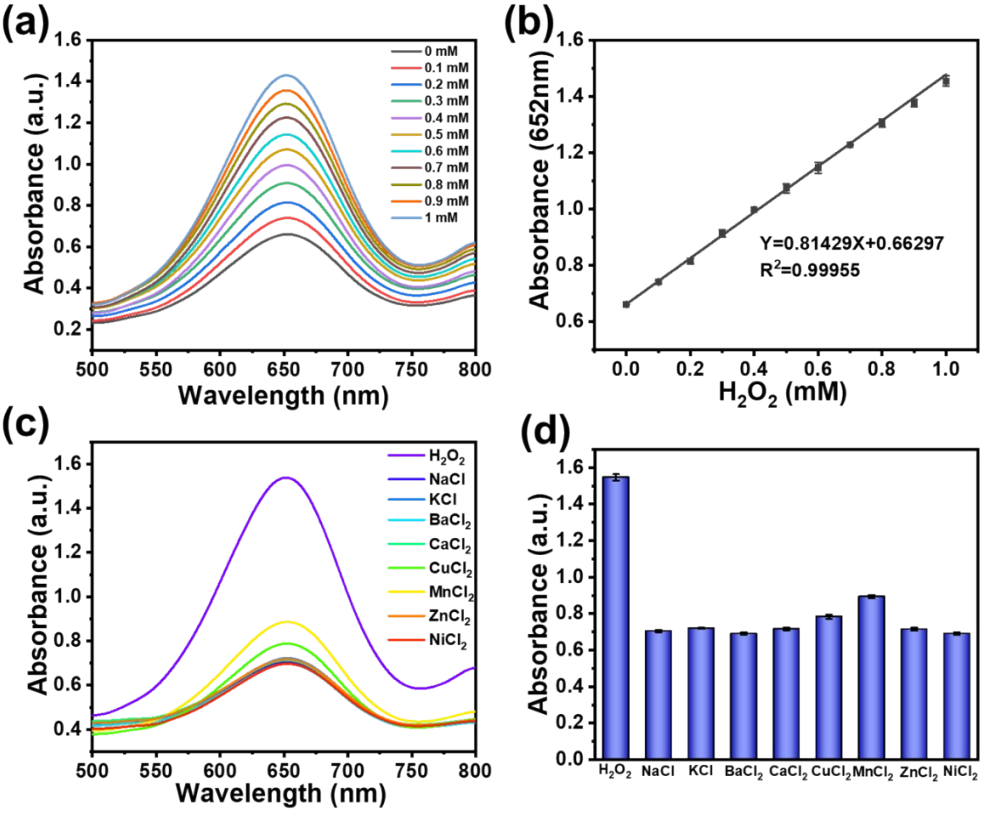

Figure S9 (a) UV-vis absorption spectra of TMB + Co_3O_4/Fe_2O_3 DLHNBs + H_2O_2 system with varied concentration of H_2O_2 and (b) fitted linear curve for H_2O_2. (c, d) The absorbance (652 nm) values caused by different species (1 mM H_2O_2, 10 mM NaCl, KCl, $BaCl_2$, $CaCl_2$, $CuCl_2$, $MnCl_2$, $ZnCl_2$ and $BaCl_2$).

Table S1. Comparison of K_m of different nanozymes.

Catalyst	Substrate	K_m [mM]	V_{max} [10^{-8} M s^{-1}]	Ref.
HRP	TMB	0.43	10.00	[1]
	H_2O_2	3.70	8.71	
Co_3O_4 NPs	TMB	0.037	6.27	[2]
	H_2O_2	140.07	0.12	
PCN-224-Fe/Co	TMB	0.26	26.35	[3]
	H_2O_2	8.94	35.34	
Fe_3O_4 MNPs	TMB	0.098	3.44	[4]
	H_2O_2	154	9.78	
Pal@Co_3O_4	TMB	0.0218	0.45	[5]
	H_2O_2	30.1	0.42	
Co/Fe-MOFs	TMB	3.51	7.63	[6]
	H_2O_2	5.37	2.71	
Co_3O_4 SLHNBs	TMB	0.398	13.9	This work
	H_2O_2	1.87	8.80	
Co_3O_4/Fe_2O_3 SLHNBs	TMB	0.254	21.2	This work
	H_2O_2	1.49	13.7	
Co_3O_4/Fe_2O_3 DLHNBs	TMB	0.194	24.1	This work
	H_2O_2	0.83	13.8	

Table S2. Comparison of linear range and LOD for H_2O_2 sensing with other mimetic peroxidase materials.

Materials	Linear range (μM)	LOD (μM)	Ref.
FePt-Au	20-700	12.33	[7]
Co_3O_4-MMT	10-100	8.7	[8]
Co_3O_4	50-25000	10	[2]
Cu/Fe_3O_4@FeOOH	10-400	7.5	[9]
ZIF-67/rGO	7.5-750	3.81	[10]
Fe-N-C SANs	10-600	4.36	[11]
Co_3O_4/Fe_2O_3 DLHNBs	100-1000	2.13	This work

Table S3. Detection of AA in Vitamin C injections using a colorimetric method based on Co_3O_4/Fe_2O_3 DLHNBs (*n* = 3).

		Colorimetry			HPLC	
Sample	Theoretical value (μg/mL)	Experimental value (μg/mL)	Recovery (%)	RSD(%)	Experimental value (μg/mL)	RSD(%)
1	unspiked sample	undetected	-	-	undetected	-
2	10.00	10.02±0.29	97.3-103.1	2.7	10.06 0.21	1.9
3	20.00	20.11±0.46	98.2-102.8	2.1	20.08 0.24	1.1
4	40.00	40.85±0.83	100.1-104.2	1.6	40.56 0.62	1.2

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