Supporting Information for

Fe-N-C oxidase-mimicking nanozymes for discrimination of antioxidants and detection of Hg^{2+}

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1. Materials and Instruments

Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), 2-Methylimidazole (2-MeIM), terephthalic acid (TA), 3,3',5,5'-tetramethylbenzidine (TMB), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid ammonium salt) (ABTS), o-phenylenediamine (OPD), glutathione (GSH), cysteine (Cys), ascorbic acid (AA), iron trichloride hexahydrate ($FeCl_3 \cdot 6H_2O$), and tryptophan were obtained from Sigma-Aldrich (USA). Methanol and ethanol were purchased from Alfa Aesar Sinopharm Chemical Reagent Co., Ltd. (China).

Transmission electron microscopy (TEM) were obtained on a JEM-2100Plus field emission electron microscope.The X-ray diffraction (XRD) patterns were performanced on a Bruker D8 Advance with Cu Kα radiation. X-ray photoelectron spectra (XPS) were carried out on a VG Thermo Scientific K-Alpha. The electron spin resonance (ESR) spectroscopy was measured by a Bruker ESR5000. Raman spectra were measured by HR Evolution. All UV-vis spectra were from the UV-8000 spectrophotometer. The fluorescence spectrum was conducted by an RF-6000 Fluorospectrophotometer.

2. Oxidase-mimicking activities

2.1 Oxidase-like activity of Fe-N-C SAzymes

In detail, the reaction solution contains 20 μL of Fe-N-C SAzymes (0.25 mg/mL), 50 μL of TMB (5 mM), and 930 μL of acetate buffer (pH 3.2, 0.2 M). After being incubated for 20 min, the absorption spectra were recorded.

2.2 Calculation of kinetics constants and specific activity

The kinetics constants (V_{max}, K_m) were obtained by Michaelis-Menten equation:

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

Where K_m is the Michaelis constant, V is the initial reaction velocity, and V_{max} is maximal reaction velocity, [S] is the TMB concentration.

The specific activity (SA) of the nanozyme was calculated by the following equations[1]:

$$b_{nanozymes} = \frac{V}{\varepsilon \times l} \times \left(\frac{\Delta A}{\Delta t}\right)$$

$$a_{nanozyme} = b_{nanozymes}/[m]$$

where $b_{nanozyme}$ is the catalytic activity of nanozyme expressed in units. V is the total volume of reaction solution (μL). ε is the molar absorption coefficient of TMB (39000 M^{-1} cm^{-1}). l and A is the path length of light traveling in the cuvette (cm) and the absorbance, respectively. $\Delta A/\Delta t$ is the initial rate of change. $a_{nanozyme}$ is the SA expressed in units per milligram (U mg^{-1}) nanozymes, and [m] is the weight (mg) of Fe in nanozyme of each assay.

2.3 Colorimetric detection of AA, GSH, or Cys

20 μL of Fe-N-C SAzymes, 50 μL of TMB, 50 μL of different concentrations of AA, GSH, or Cys, and 880 μL of acetate buffer (pH 3.2, 0.2 M) were mixed and incubated for 20 min.

2.4 Colorimetric detection of TAC in Vitamin C, GSH in serum.

20 μL of Fe-N-C SAzymes, 50 μL of TMB, 50 μL of Vitamin C solution (0.5 mg/mL), and 880 μL of acetate buffer (pH 3.2, 0.2 M) were mixed and incubated at room temperature for 20 min.

20 μL of Fe-N-C SAzymes, 50 μL of TMB, 50 μL of different concentrations of GSH, 10 μL of serum (Attenuated 1000 times), and 870 μL of acetate buffer (pH 3.2, 0.2 M) were mixed and incubated for 20 min.

2.5 Colorimetric detection of Hg^{2+}

20 μL of Fe-N-C SAzymes, 50 μL of TMB, 50 μL of Cys solution (2 mM), 50 μL of Hg^{2+} solution, and 830 μL of acetate buffer (pH 3.2, 0.2 M) were mixed and incubated for 20 min.

2.6 Electron spin resonance experiment

In a typical measurement of •OH radicals, 50 μL of 0.25 mg mL^{-1} Fe-N-C SAzymes aqueous solution was added to 900 μL of buffer solution. Subsequently, 50 μL of 0.5 M DMPO was introduced into the mixture. After 10 minutes, the reaction solution was extracted using a quartz capillary tube for electron paramagnetic resonance (ESR) testing. For the measurement of $\bullet O_2^-$ radicals, the acetate buffer solution was replaced with methanol solution. For the measurement of $^1O_2$ radicals, TEMP was used as the radical scavenger, while all other conditions were kept the same.

3. Computational details

The DFT calculations were obtained using the Vienna Ab initio Simulation Package (VASP). The valence cutoff energy, convergence thresholds for the electronic structure, and forces were set to 440 eV, 1.0×10^{-6} eV, and 0.01 eV/Å, respectively. Use standard Monkhorst Pack grid sampling for structural optimization at gamma point and $3 \times 3 \times 1$. A vacuum layer as large as 15 Å was used along the c direction.

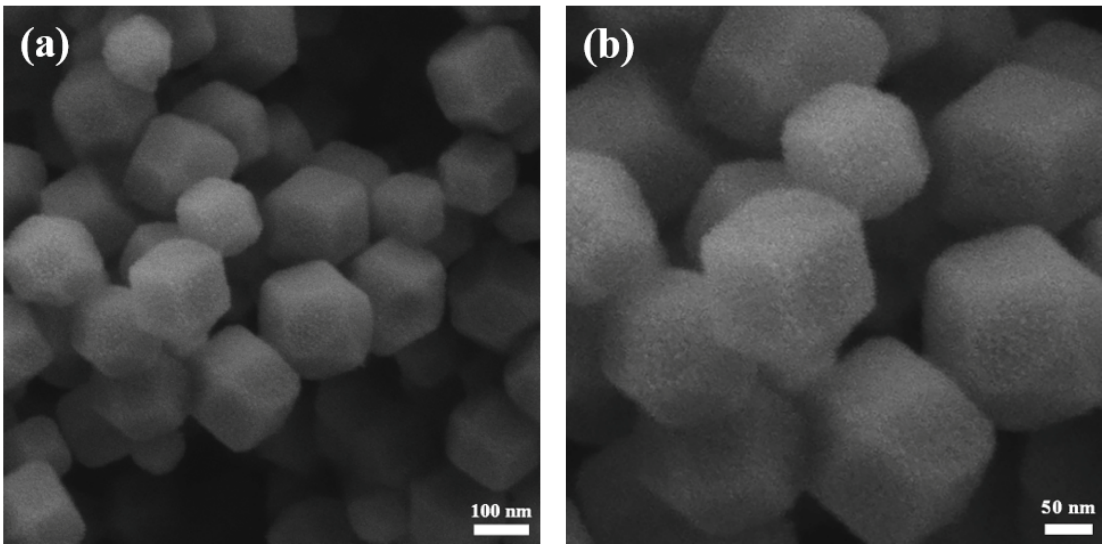

Fig. S1 The SEM images of NC at different magnifications.

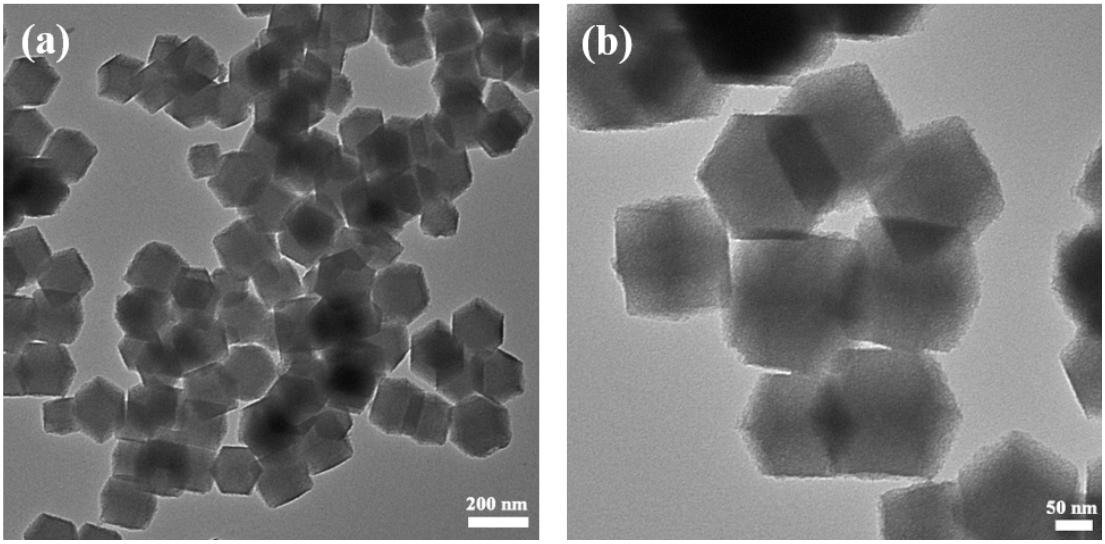

Fig. S2 The TEM images of NC at different magnifications.

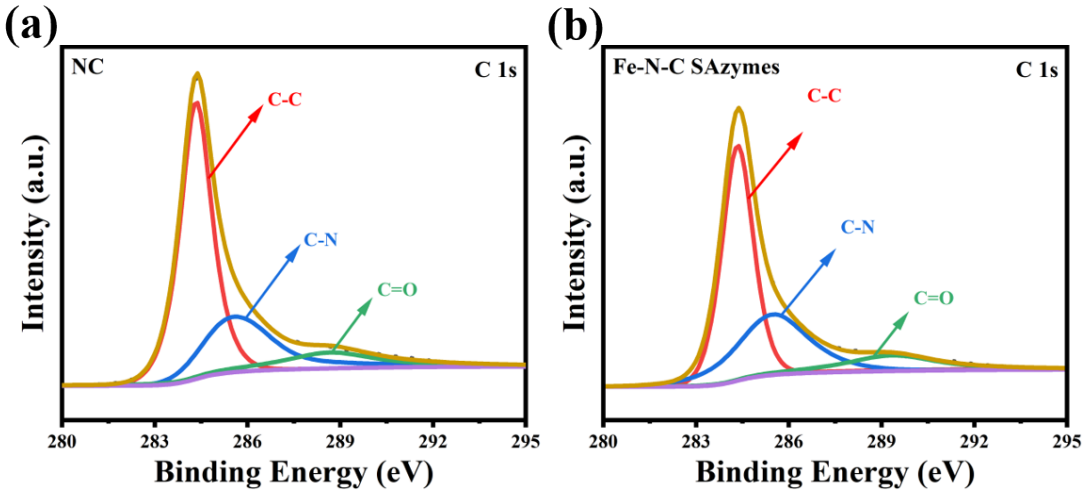

Fig. S3 XPS analysis of C 1s patterns: (a) NC and (b) Fe-N-C SAzymes.

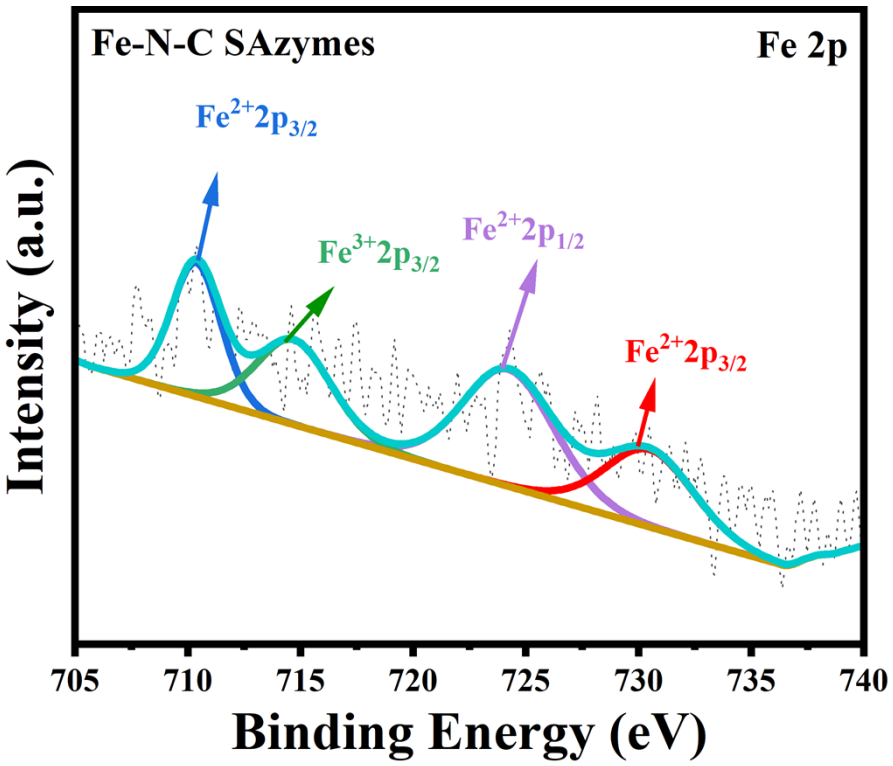

Fig. S4 XPS analysis of Fe 2p patterns of Fe-N-C SAzymes.

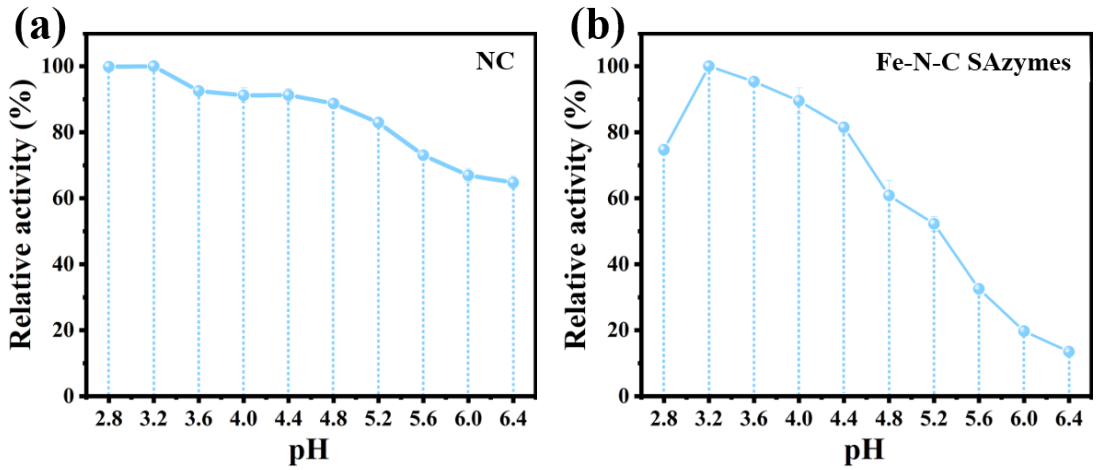

Fig. S5 The relative activity of oxidase in Nanozyme-TMB reaction system for different pH: (a) NC and (b) Fe-N-C SAzymes.

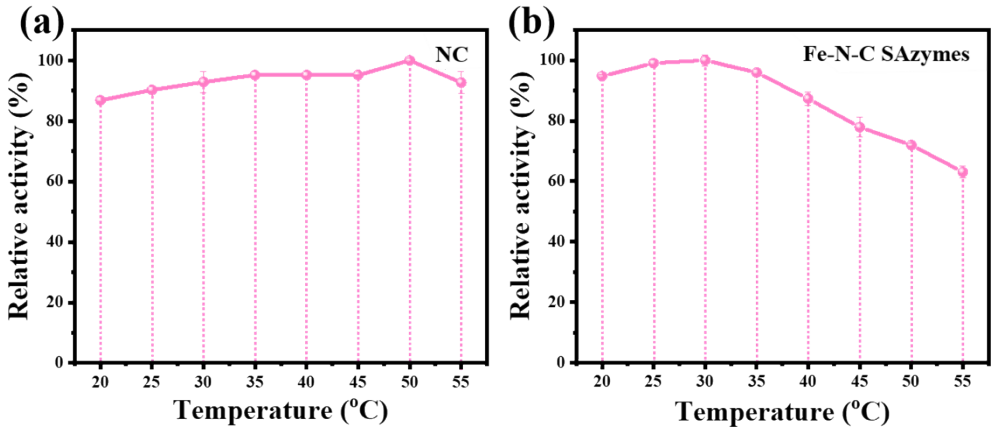

Fig. S6 The relative activity of oxidase in Nanozyme-TMB reaction system for different temperature: (a) NC and (b) Fe-N-C SAzymes.

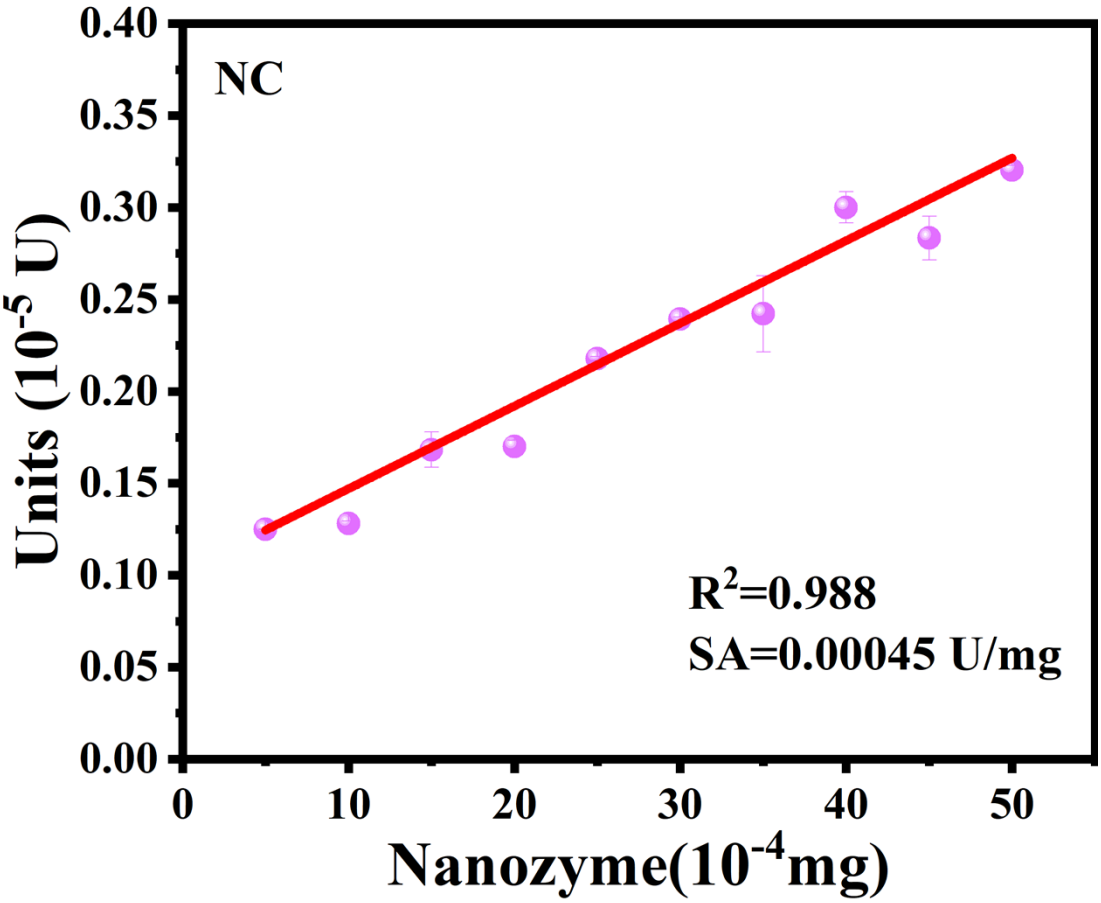

Fig. S7 The specific activity of NC using TMB as substrate.

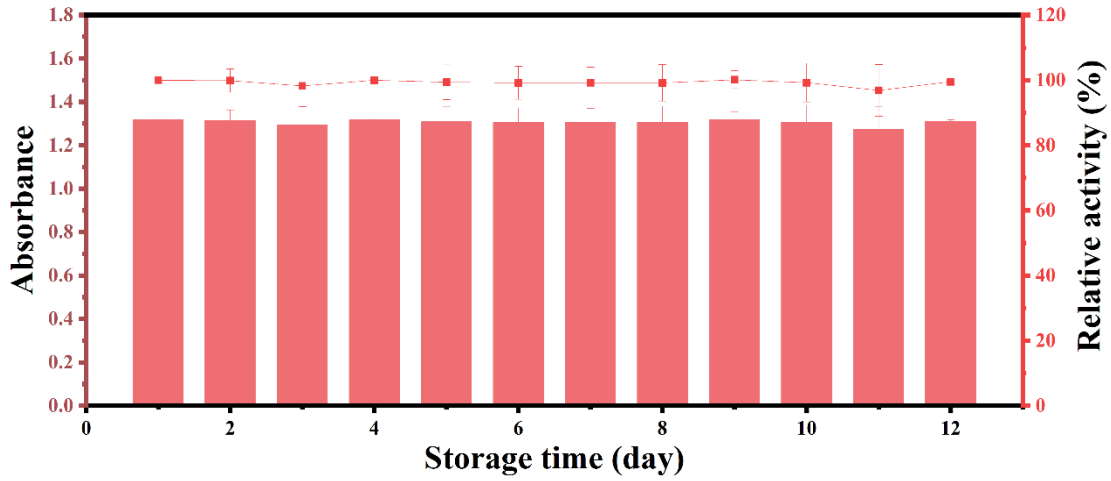

Fig. S8 Long-term stability of Fe-N-C nanozymes in 12 days.

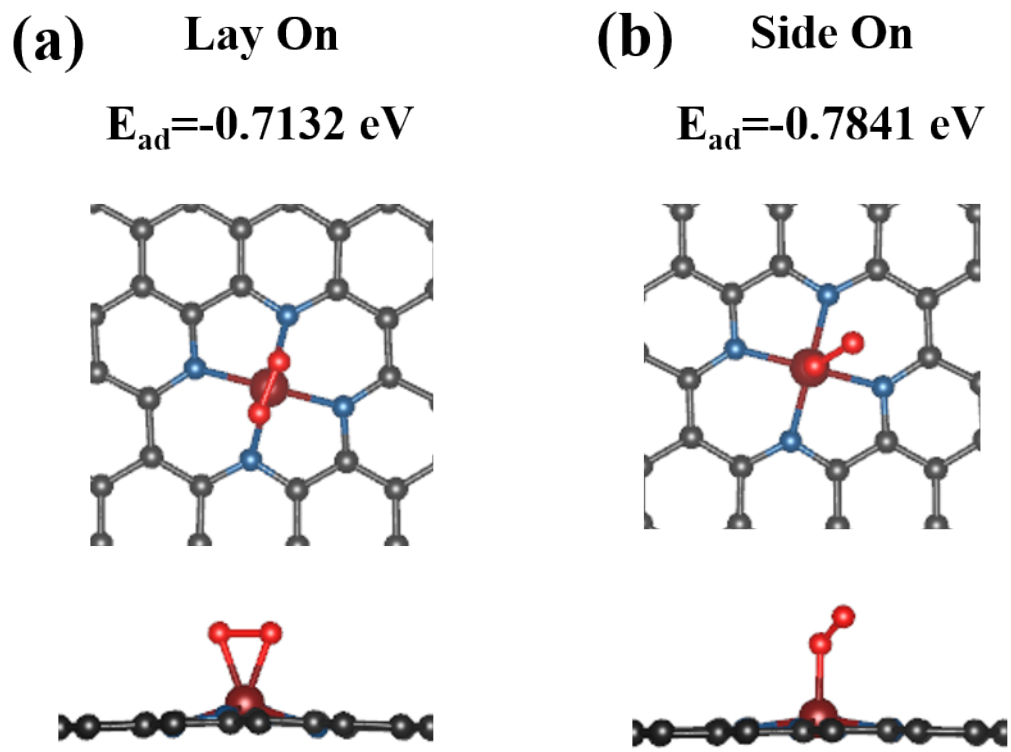

Fig. S9 Structural models of the oxidase-like nanozymes adsorbing oxygen in (a) Lay on and (b) "side on" mode.

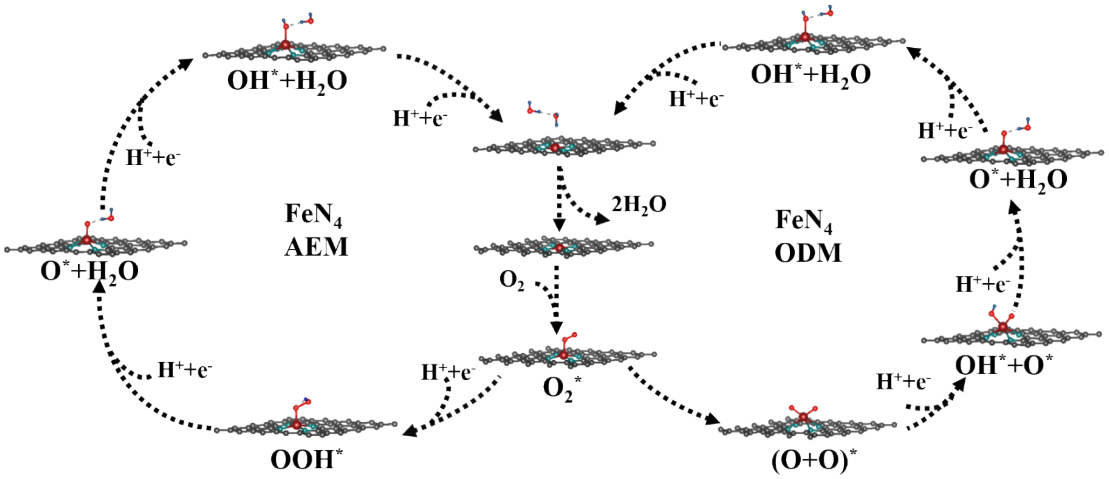

Fig. S10 The schematic diagram of oxygen reduction pathways following AEM (left) and ODM (right).

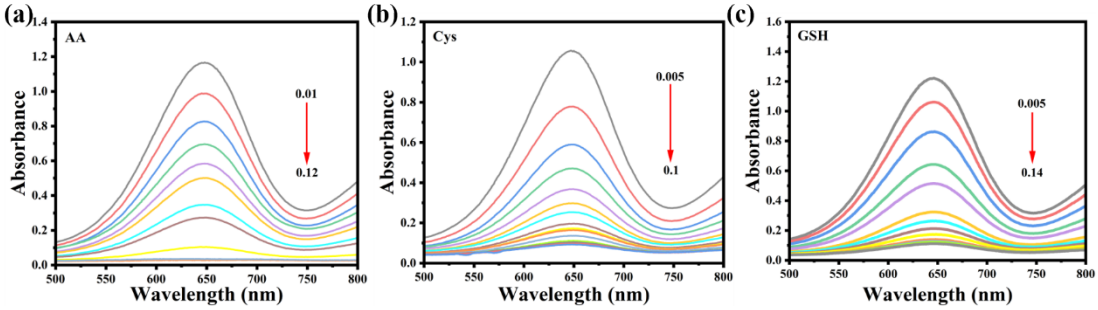

Fig. S11 (a) UV-vis absorption spectra of AA, Cys, and GSH with different concentrations.

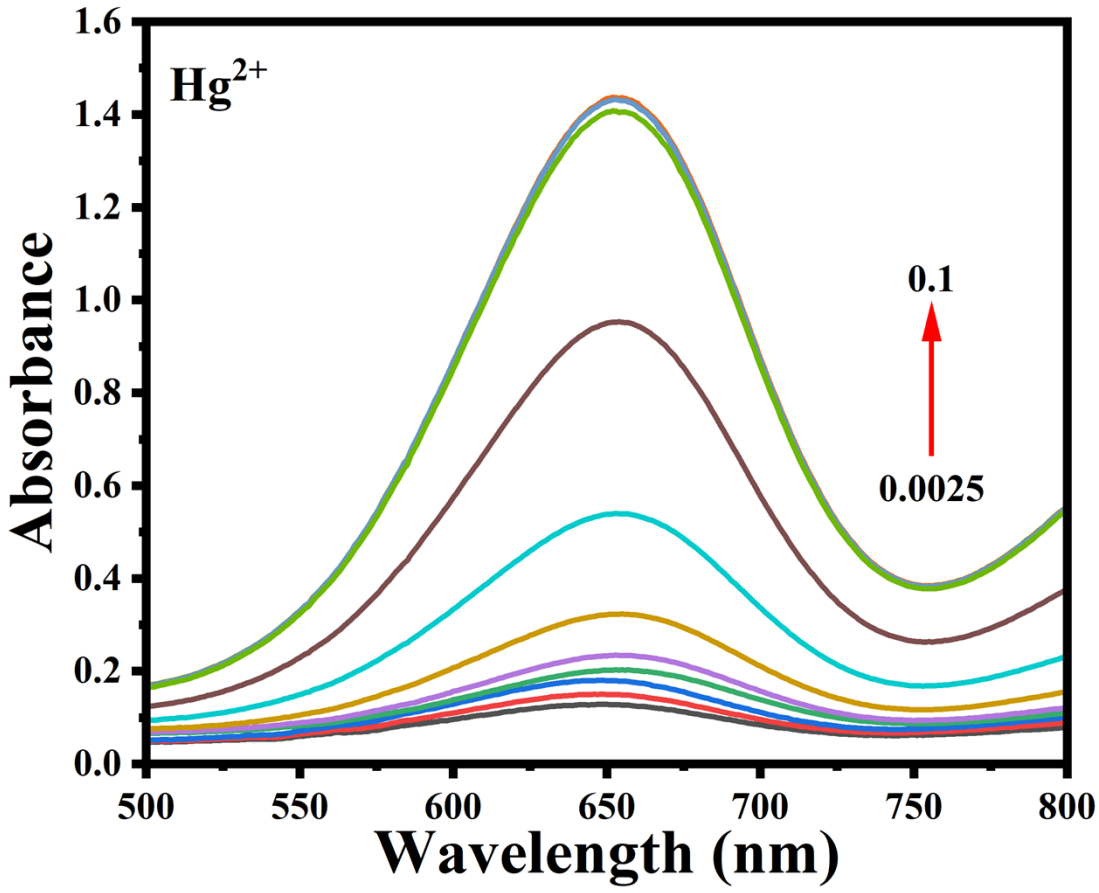

Fig. S12 UV-vis absorption spectra of Hg^{2+} with different concentrations

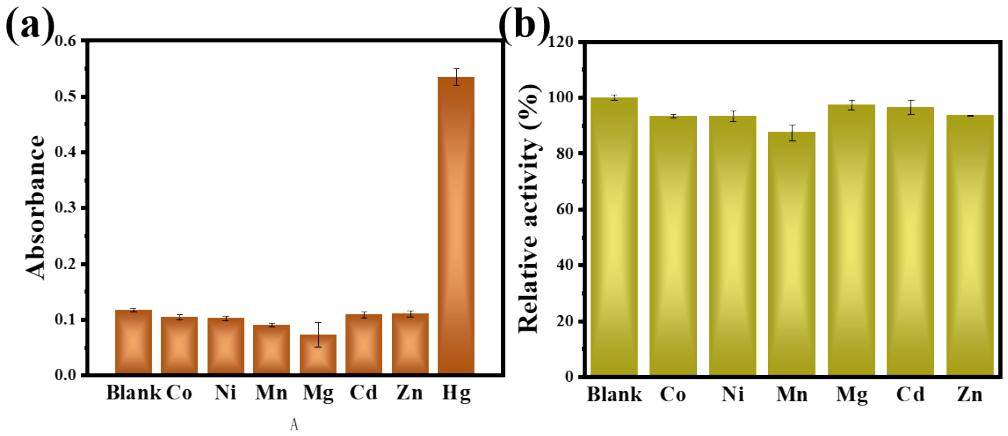

Fig. S13 Absorbance value of sensing system at 652 nm after adding metal ion (a); The relative activity after adding possible interference (b).

Table S1. Kinetic parameters comparison of Fe-N-C SAzymes and other oxidase-like nanozymes.

Nanozyme	K_m(mM)	V_{max}(10^{-7} M s^{-1})	Reference
Fe-N-C SAzymes	1.81	0.000601	[2]
Fe SAEs	2.13	0.225	[3]
Fe-N-C	0.253	0.4136	[4]
Fe/NPC	0.59	1.274	[5]
Fe-N-C-400	0.269	3.38	[6]
Fe-N-C-500	0.230	1.33	
Fe-N-C SAzymes	0.212	1.19	This work

Table S2 The analytical performance comparing for determination of AA.

Method	Material	detection range （μM）	LOD （nM）	Ref.
Fluorescence	MIL-53(Fe)	0.3-100	150	[7]
Colorimetric	Fe_4Mo_8Na	0-750	1070	[8]
Colorimetric	$FePO_4$@GO	250-75000	1250	[9]
Colorimetric	PtNi/NCFs	1-20	940	[10]
Fluorescence	R-CDs& PDA	0.5-30	280	[11]
Colorimetric	Fe-N-C SAzymes	0-110	0.98	This work

Table S3 The analytical performance comparing for determination of Cys.

Method	Material	Detection range (μM)	LOD (nM)	Ref.
Electrochemical	Ag@rGO	0.1-470	57	[12]
Colorimetric	PVP-AuNP	1-50	200	[13]
Fluorescence	PYR-CG	2-10	88	[14]
Colorimetric	CoO/Co–Try-GQD	0.05-2	32	[15]
Colorimetric	TAnc-Mnx-y	8.26-90.86	2280	[16]
Colorimetric	Fe-N-C SAzymes	0-45	0.194	This work

Table S4 The analytical performance comparing for determination of GSH.

Method	Material	detection range (μM)	LOD (nM)	Ref.
Colorimetric	$BaTiO_3$	0.5-20	200	[17]
Fluorescence-	NSCQDs@MSN	5-900	1600	[18]
Colorimetric		20-460	7000	
Colorimetric	FeS_2	0.2-35	150	[19]
Colorimetric	1Al/MIL-100(Fe)	0.01-1000	2.2	[20]
Electrochemical	Cu@BCNNTs/GCE	0.5-120	24	[21]
Colorimetric	Fe-N-C SAzymes	10-100	1.146	This work

Table S5. The analytical performance comparing for determination of Hg^{2+}.

Material	Method	LOD (nM)	Reference
$MnFe_2O_4$@Cys	Electrochemical	208	[22]
P(DHB-a-DHBDT-g-PST) vesicles	Fluorescent	53	[23]
HS-CQDs	Fluorescence	12	[24]
OV-ZnO	Electrochemical	23	[25]
P-CQDs	Fluorescence	52.5	[26]
red-emitting fluorescence probe (rhodamine and isophorone units)	fluorescent	122	[27]
TPE-Hg	fluorescent	754	[28]
Fe-N-C SAzymes	colorimetric	9.290	This work

Table S6. Determination of Hg^{2+} in Lake and Tap water

Samples	Spiked (nM)	Proposed method (nM)	Recovery (%)	RSD (%, n=3)
Lake water	0.5	0.469	93.834	0.5
	0.9	0.905	100.53	3.5
	1.1	1.120	101.82	1.7
Tap water	0.6	0.618	103.02	3.6
	0.9	0.934	103.86	2.0
	1.1	1.157	105.18	0.7

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