Supplementary Information

Microwave-Assisted Synthesis of Fe-based Single-Atom Nanozyme: A Colorimetric Approach to Detect Cr(VI)

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S1 Experiment Section

S1.1 Chemicals

Iron nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$), zinc chloride ($ZnCl_2$), 3,3',5,5'-tetramethyl-benzidine (TMB), isopropyl alcohol (IPA) and 2-methylimidazole(2-MI) were provided by Aladdin. Methyl alcohol, zinc nitrate hexahydrate ($Zn_2(NO_3)_2 \cdot 6H_2O$), ethanol, potassium chloride (KCl), muriatic acid, glacial acetic acid, 30% hydrogen peroxide, potassium dichromate ($K_2Cr_2O_7$), copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), sodium chloride (NaCl), anhydrous calcium chloride ($CaCl_2$) and magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) were obtained from Sinopharm Chemical Regent. 8-hydroxyquinoline (8-HQ), p-benzoquinone (PBQ), tryptophan (Trp), chromium chloride hexahydrate ($CrCl_3$) and manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$) were purchased from Macklin, Cadmium chloride hemihydrate ($CdCl_2 \cdot 2.5H_2O$) and anhydrous sodium acetate manufactured by Sinopharm Shanghai Test.

S1.2 Apparatus

Microscopic morphologies of samples were characterized by focused ion beam-scanning electron microscopy (FIB-SEM) (Thermo, USA) and transmission electron microscopy (TEM) (Thermo, USA). X-ray photoelectron spectroscopy (XPS) (Thermo, USA) and X-ray absorption fine structure spectroscopy (XAFS) were conducted to explore the elemental composition and their chemical states. The peroxidase-like mechanism of Fe-N-C was verified by an Electron paramagnetic resonance (EPR) (Bruker, Germany) spectrometer Ultraviolet-visible (UV-Vis) (SHIMADZ, Japan) measurement was used to monitor the absorbance changes during the mimic enzyme reaction process. Fluorescence (FL) (Edinburg, English) spectra was used to monitor the fluorescence changes during the mimic enzyme reaction process. Inductively coupled plasma optical emission spectrometer (ICP-OES) (Thermo, USA) was used to analyze Cr(VI) in environmental water samples.

S1.3 Enzyme mimicking activity of Fe-N-C

The oxidase-like catalytic activity of the Fe-N-C was assessed by the TMB color reaction. Briefly, 20 μL of Fe-N-C (2.5 mg/mL) and 50 μL of TMB (10 mM) were added to a 2mL centrifuge tube. This mixture was then diluted with 0.2 mol/L HAc-NaAc buffer (pH 4.5) to a final volume of 1 mL. The reaction was allowed to proceed for 5 min, and the absorbance at 652 nm was recorded. Similarly, the peroxidase-like catalytic activity of the Fe-N-C were investigated by adding 20 μL of H_2O_2 to the above reaction system while maintaining all other experimental conditions.

S1.4 Kinetic analysis

The experiments of kinetic parameters of the POD-like activity of Fe-N-C were carried out under their optimal conditions and the concentration of the corresponding substrate was changed in different enzymological active systems. The corresponding initial velocities were obtained through the operation of time scan and the results were calculated according to the Lineweaver-Burk equation:

$1/v = (K_m/V_{max}) \times (1/[C]) + 1/V_{max}$ (1)

Where v refers to the initial velocity, K_m and V_{max} are the Michaelis-Menten constant and the maximum reaction velocity, respectively, and C is the substrate's concentration[1, 2].

S1.5 Colorimetric detection of Cr(VI) in real samples

We selected Yangtze River samples to estimate the feasibility of Cr(VI) detection by the Fe-N-C colorimetric platform. The water source of the laboratory is taken as Yangtze River sample is treated with 0.2 μm water filter to take out impurities. In order to ascertain the Cr(VI) concentration, spiked concentrations of Cr(VI) (20 and 50 μM) were added to the above solution. The absorption spectra were recorded by the UV–visible after 5 min incubation at ambient temperature.

S2 Results and discussions

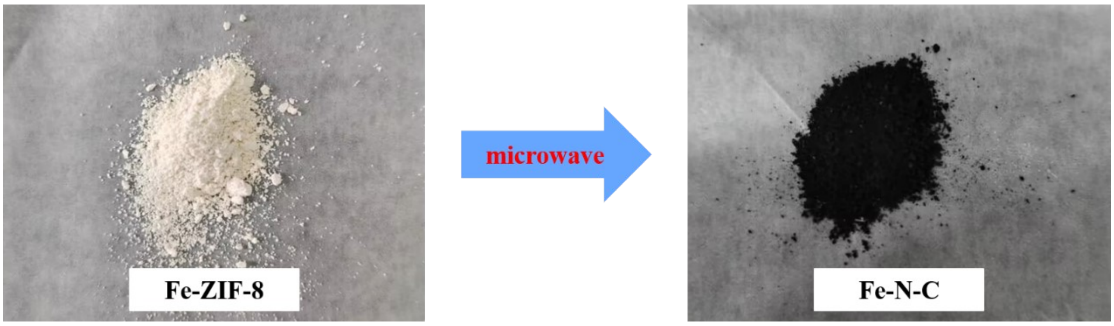

Fig. S1. Photographs of Fe-ZIF-8 and Fe-N-C.

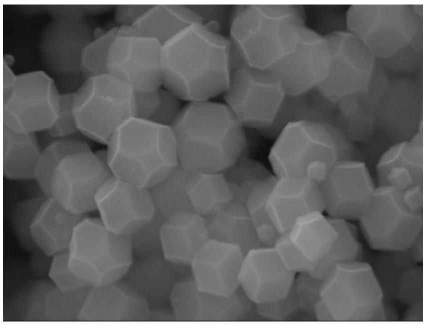

Fig. S2. SEM image of Fe-ZIF-8.

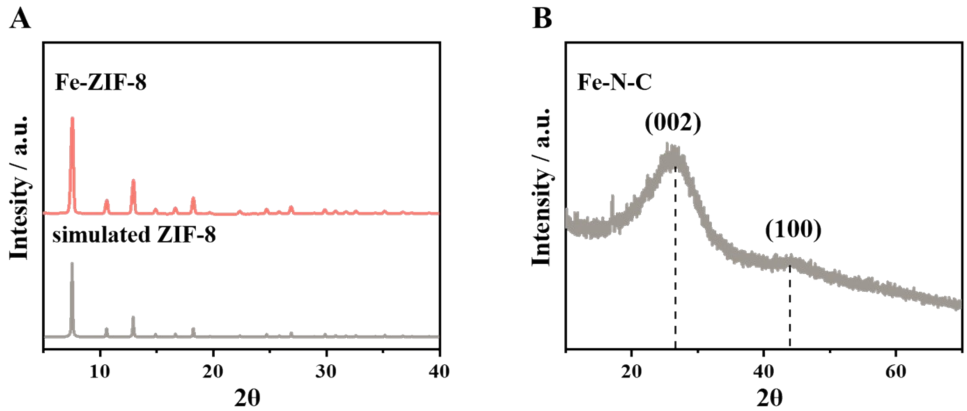

Fig. S3. Powder XRD patterns of (A) Fe-ZIF-8 and (B) Fe-N-C.

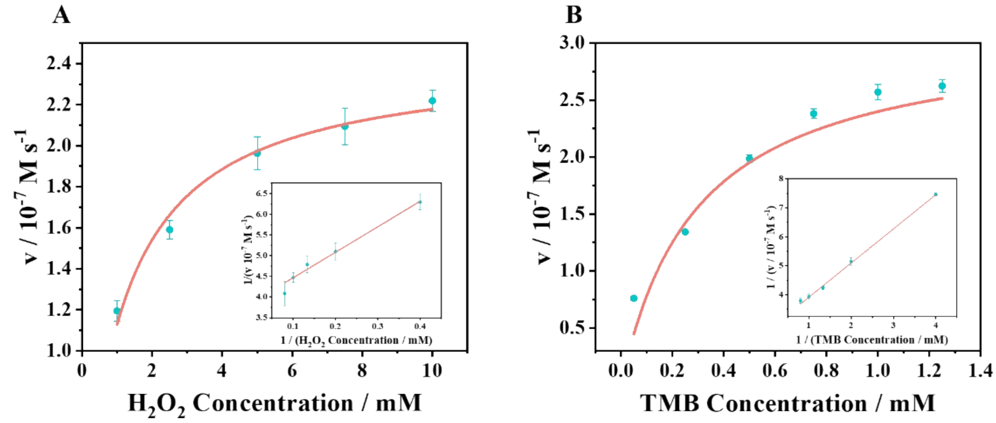

Fig. S4. (A) Kinetic curve and double-reciprocal plot of Fe-N-C with H_2O_2 as a substrate. (B) Kinetic curve and double-reciprocal plot of Fe-N-C with TMB as a substrate.

Table S1 Comparison of the kinetic parameters of the Fe-N-C and other nanozymes.

Catalyst	**Substrate**	**K_m** (**mM**)	**V_{max}** (**10^{-8} M s^{-1}**)	Ref.
HRP	H_2O_2	3.70	8.71	**3**
	TMB	0.43	10.00	
f-FeNC	H_2O_2	2.50	4.95	**4**
	TMB	0.15	3.61	
Fe-N-C	H_2O_2	12.2	116.00	**5**
	TMB	3.6	35.60	
Fe SAEs	H_2O_2	0.24	8.25	**6**
	TMB	3.92	58.80	
Mn_{SA}-N_3-C	H_2O_2	1200	63.00	**7**
	TMB	0.54	63.00	
Cu-N-C	H_2O_2	19.94	20.07	**8**
	TMB	3.76	75.05	
Fe-N-C	H_2O_2	1.15	24.28	This work
	TMB	0.29	31.13	

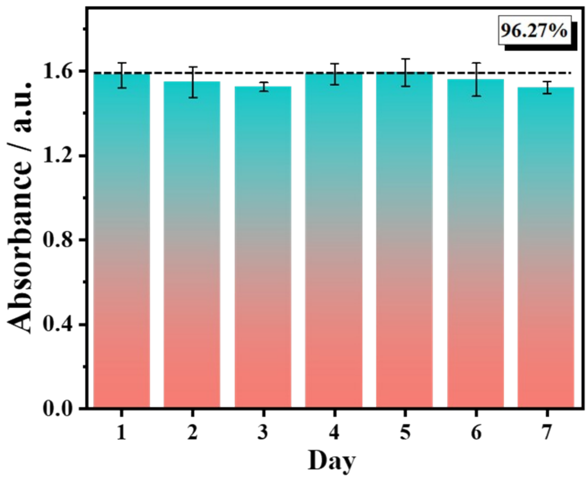

Fig. S5. POD activity of Fe-N-C in different days. (The ordinate is the absorbance of Fe-N-C/TMB/H_2O_2 system at the wavelength of 652 nm)

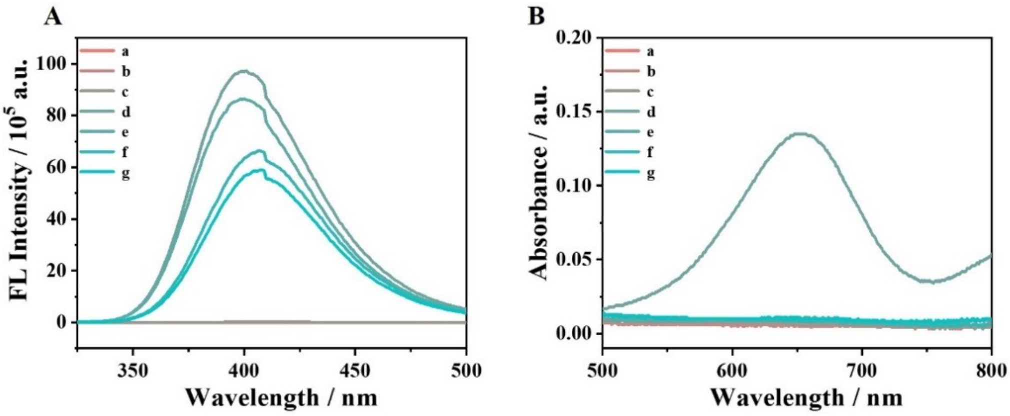

Fig. S6. (A) FL spectra and (B) UV spectra of a) H_2O_2, b) 8-HQ, c) H_2O_2+8-HQ, d) TMB, e) TMB+H_2O_2, f) TMB+8-HQ, and g) TMB+H_2O_2+8-HQ.

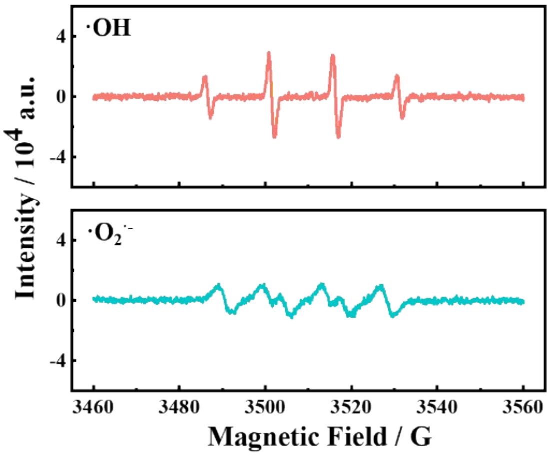

Fig. S7. EPR signals for $\cdot$OH and $\cdot O_2^{\cdot -}$ detection of TMB/H_2O_2/8-HQ system.

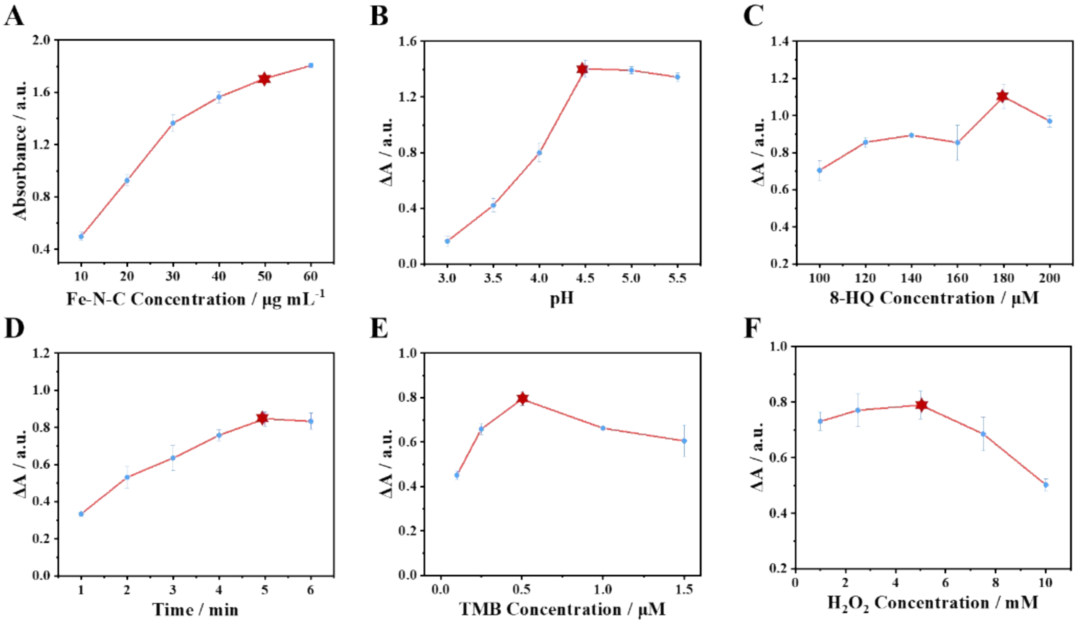

Fig. S8. The relationship between (A) Fe-N-C concentration, (B) pH value, (C) 8-HQ concentration, (D) illumination time, (E) TMB concentration, (F) H_2O_2 concentration and ΔA (ΔA=A_0-A, where A_0 and A respectively represent the absorbance at 652 nm of the TMB/H_2O_2/Fe-N-C system before and after adding 8-HQ).

Table S2 The comparison of properties of different methods for Cr (VI) detection based on different materials.

System	**Method**	**Linear range (μM)**	**LOD (μM)**	**Refs**
CD/PVA	Fluorescence	0~60	0.64	**9**
KCQDs@SBA-15	Fluorescence	0~100	0.866	**10**
PEI-AgNCs	Colorimetry	5~300	1.1	**11**
Cu-PyC MOF	Colorimetry	0.5~50	0.051	**12**
GO/AuNPs	Colorimetry	0.1~15	0.153	**13**
H-Fe-POP	Colorimetry	2~130	0.23	**14**
Fe_3O_4@MQDs	Colorimetry	0~60	0.26	**15**
Fe-N-C	Colorimetry	1~130	0.13	This work

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