Support Information

1. Materials and methods

1.1. Materials

FMOC-histidine, Hemin, DMSO, 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 3,3',5,5'-tetramethylbenzidine (TMB), 1,2-diaminobenzene (OPD), GOx was purchased from Macklin reagent Co., Ltd. (Shang-hai, China). $MnCl_2$ and $FeCl_3$ were purchased from Sinopharm Chemical Reagent Co. Ltd. Glucose, fructose, maltose, arabinose, xylose and galactose were purchased from Sigma-Aldrich, and the purity was greater than that 98%. All solutions prepared are derived from Wahaha purified water (China). All other chemicals were of the highest grade and used without further purification unless otherwise noted.

1.2. Instrumentation

Scanning electron microscopy (SEM) was conducted using Hitachi FE-SEM S-4800 at 3 kV. X-ray photoelectron spectroscopy (XPS) spectra were measured using Thermo ESCALAB 250Xi. Inductively coupled plasma spectrometer (ICP-OES) was measured by Agilent 725. The dissolved oxygen meter was operated with Mettler Toledo M400. UV–vis spectra were recorded on the UV-2700 spectrophotometer manufactured by Shimadzu (Japan).

1.3. Analysis of POD-like and CAT-like activity of Fe-His@Hemin

Fe-His@Hemin with POD-like activity can directly oxidize substrates in the presence of H_2O_2. The whole reaction system consists of 60 μL of 0.10 mg mL^{-1} Fe-His@Hemin, 50 μL H_2O_2 (1 mM) and 100 μL of 3 mM, TMB were added to 790 μL of 0.1M Tris-HCl buffer solution (pH 8.0). Finally, the mixed system was reacted at 25 °C for 10 min and the UV absorption was measured at 652 nm.

The CAT-like activity was measured by monitoring the production of O_2 with dissolved oxygen meter by decomposition H_2O_2. In brief, Fe-His@Hemin solution (40 μL, 1 mg mL^{-1}) and H_2O_2 (40 μL, 30% H_2O_2) were added to Tris-HCl buffer (0.1 M, pH 8.0, 920 μL). The content of O_2 was detected every 1 min for 10 min.

1.4. Steady-State Kinetic Analysis

Kinetic experiments were carried out in a reaction volume of 1.0 mL Tris-HCl

buffer (0.1 M, pH 8.0) containing nanozyme (60 μL, 0.1 mg mL^{-1}), H_2O_2 (50 μL, 1mM) and TMB (100 μL,3 mM). The solution mixtures were incubated at 25 °C for 10 min and then used for the absorbance measurement at a wavelength of 652 nm.

The kinetic parameters are determined by the following equations: $1/v = K_m /V_{max} \times (1/[S] + 1/K_m)$, where v is the initial velocity, V_{max} is the maximal reaction velocity, and [S] is the concentration of the substrate. K_m is the Michaelis–Menten constant, which indicates the enzyme affinity for the substrate.

1.5. One-step colorimetric detection of H_2O_2 and glucose based on the Fe-His@Hemin CAT-like

H_2O_2 detection was performed as follows: 60 μL Fe-His@Hemin (0.1 mg mL^{-1}), 100 μL TMB (3 mM) and 50 μL of different concentrations of H_2O_2 in 790 μL Tris-HCl buffer (0.1 M, pH 8.0) was incubated at 25 °C for 10 min and then used for the absorbance measurement at a wavelength of 652 nm.

Glucose detection was performed as follows: 50 μL GOx (5 mg mL^{-1}), 60 μL Fe-His@Hemin (0.1 mg mL^{-1}), 100 μL TMB (3 mM) and 200 μL of different concentrations of glucose in 590 μL HAc-NaAc buffer solution (0.1 M, pH 6.0) was incubated at 37 °C for 30 min and then used for the absorbance measurement at a wavelength of 652 nm.

1.6. Detection of glucose in human serum

The human serum utilized in this study was procured from the blood bank of the First Affiliated Hospital of Jilin University. All experiments were performed in accordance with the Guidelines “Declaration of Helsinki (as revised in 2013)”, and approved by the ethics committee at “the Institutional Ethics Committee of the First Affiliated Hospital of Jilin University”. Informed consents were obtained from human participants of this study. The collected serum was diluted 50-fold and the detection method of glucose was performed in accordance with 2.6 section (***One-step colorimetric detection of H_2O_2 and glucose based on the Fe-His@Hemin CAT-like***) and the absorbance of the solution at 652 nm were recorded by UV-vis spectroscopy.

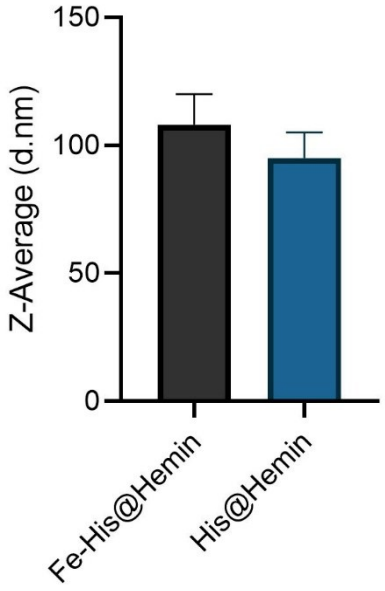

Fig S1. The hydrodynamic diameters of Fe-His@Hemin and His@Hemin detected by dynamic light scattering.

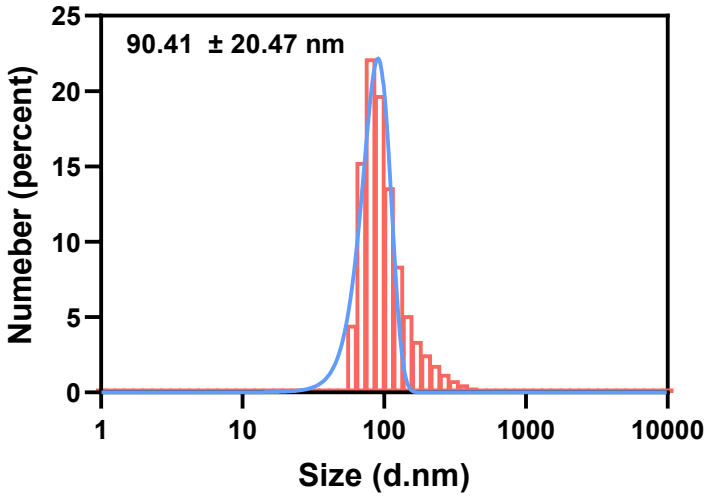

Fig S2. The particle size distribution diagram of Fe-His@Hemin nanozymes.

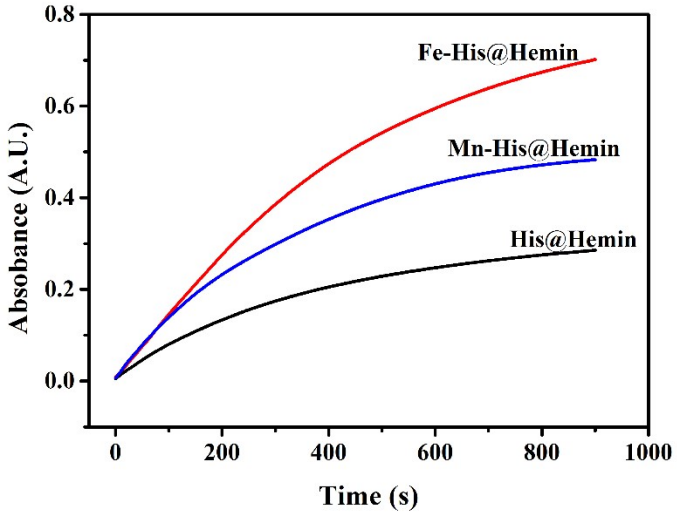

Fig S3. Time-dependent absorbance of Fe-His@Hemin, Mn-His@Hemin, His@Hemin at 652 nm.

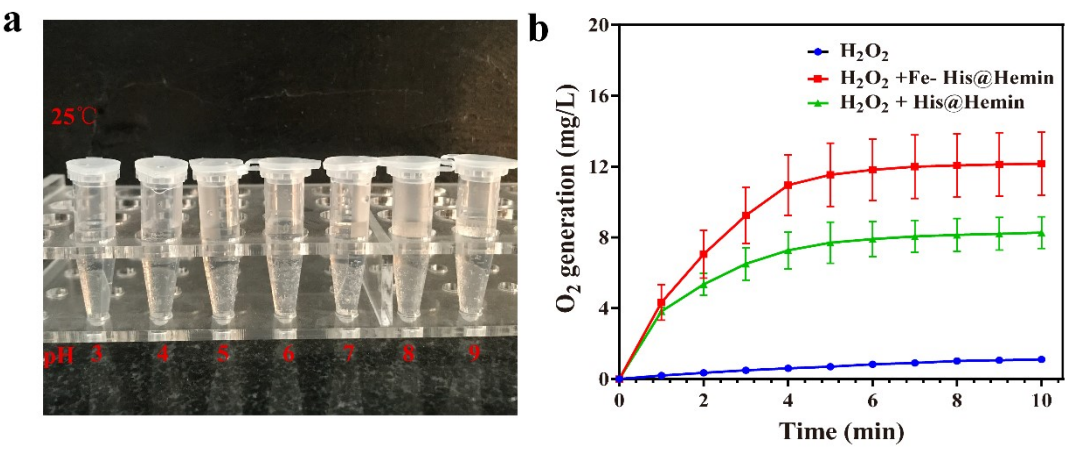

Fig S4. Investigate the catalase-like activity of Fe-His@Hemin. (a) Effects of different pH on catalase-like activities of Fe-His@Hemin at 25 ℃；(b) O_2 generation from H_2O_2 decomposition with H_2O_2+Fe-His@Hemin and H_2O_2+His@Hemin.

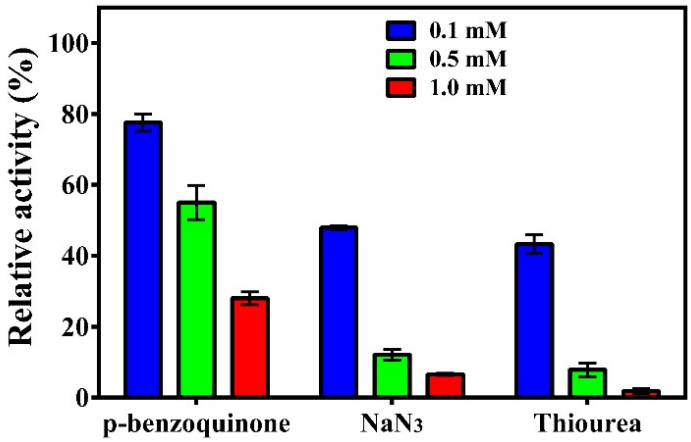

Fig S5. Effects of different concentrations of free radical traps on dual-enzyme activity of Fe-His@Hemin (Conditions: 6 μg/mL Fe-His@Hemin, 0.3 mM TMB, 0.1 M pH 8.0 Tris-HCl buffer, 10 min and 25℃). The error bars are the standard deviation of the third parallel sample.

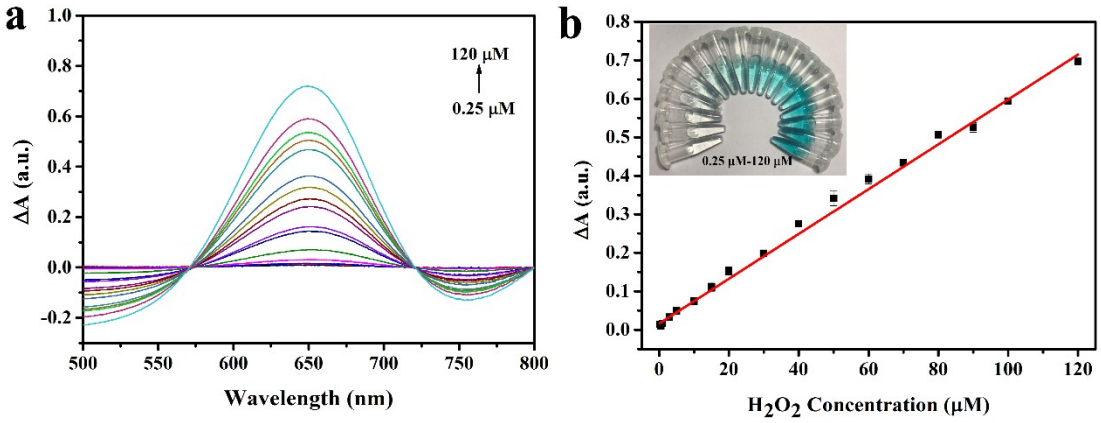

Fig S6. Sensitivity research of H_2O_2 colorimetric sensing platform based on Fe-His@Hemin. (a) The UV-vis absorption curves of Fe-His@Hemin and TMB with different concentrations of H_2O_2 (0.25-120 μM); (b) Linear plots of ΔA versus H_2O_2 concentration (Inset: corresponding color changes of 0.25-120 μM H_2O_2 in the reaction system). Error bars denote standard deviations based on three measurements.

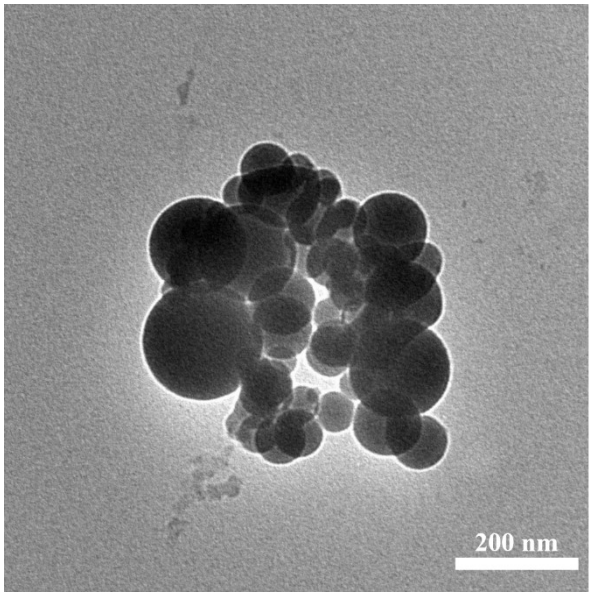

Figure S7. TEM image of Mn-His@Hemin. The scale bar is 200 nm. The sample exhibits a nanoparticulate aggregate structure with particle sizes ranging from approximately 80 to 150 nm.

Table S1. Comparison of the K_M and V_{max} values with other peroxidase mimetics.

Catalysts	K_M (mM)		V_{max} (M s^{-1})		Refs.
	H_2O_2	TMB	H_2O_2	TMB	
HRP	3.7	0.434	8.71×10^{-8}	1.0×10^{-7}	[1]
Hem-Au@apoHb	2.05	0.27	13.1×10^{-8}	13.6×10^{-8}	[2]
Hemin	4.84	2.74	4.69×10^{-8}	3.53×10^{-8}	[3]
Hemin@ZIF-8	1.96	0.38	8.11×10^{-8}	6.63×10^{-8}	[4]
AB-Hemin	1.71	0.68	9.05×10^{-8}	5.56×10^{-8}	[5]
Fe-His@Hemin	1.07	0.25	1.08×10^{-5}	1.24×10^{-6}	This work
Mn-His@Hemin	1.13	0.14	9.81×10^{-6}	5.08×10^{-7}	This work
His@Hemin	5.52	0.15	1.01×10^{-5}	6.12×10^{-7}	This work

Table S2. Comparision of different sensors for H_2O_2 determination

Materials	Linear rang(μM)	LOD (μM)	Method	Refs.
PtNP/g–C_3N_4	50-200	3.33	colorimetry	[6]
MXene@Fe_3O_4	0.4-12	0.15	colorimetry	[7]
Pd NPs	1-50	0.35	colorimetry	[8]
CAT@Fe-BTC	8-60	5.5	colorimetry	[9]
Fe@CNs	5-200	2.04	colorimetry	[10]
Fe-His@Hemin	0.25-120	0.16	colorimetry	This work

Table S3. Comparision of different sensors for glucose determination

Materials	Linear rang(μM)	LOD (μM)	Method	Refs.
ZnIrOx/ZnIrMOFs	2.66 -319	1.9	colorimetry	[11]
CuO NPs	10-100	5.06	colorimetry	[12]
CuFe@TA	3.03-363.6	2.59	colorimetry	[13]
NO_2-MIL-53(Cu)	0.5-300	2.6	colorimetry	[14]
N-CDs/Fe_3O_4	1-180	0.56	colorimetry	[15]
Fe-His@Hemin	0.5-400	0.25	colorimetry	This work

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