

Supporting Information

CoFe₂O₄ nanozyme mediated chemiluminescence imaging-colorimetric dual-mode sensor for monitoring ascorbic acid

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MATERIALS AND APPARATUS

Materials and reagents. Chitosan (CS) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Luminol and p-iodophenol (PIP) were purchased from Acros (Belgium) and Alfa Aesar Ltd. (China), respectively. Trisodium citrate, cobalt nitrate hexahydrate, 2-methylimidazole, ethanol, hexadecyl trimethyl ammonium bromide (CTAB), hydrogen peroxide (H_2O_2), methylene blue, potassium chloride, sodium chloride, cobalt chloride, ferrous chloride, potassium ferricyanide, and dimethyl sulfoxide were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). 3,3',5,5'-tetramethylbenzidine (TMB) was purchased from Aladdin Reagent (Shanghai) Co., Ltd.

Apparatus. Chemiluminescence (CL) imaging signals were recorded by FluorChemE imaging system (Protein Simple Co., America). Ultraviolet-visible (UV-vis) experiments were carried out on UV-2550 Spectrophotometer (Shimadzu Co., Japan). Scanning electron micrographs (SEM) were obtained with a Hitachi S-4800 scanning electron microscope (Japan) at an acceleration voltage of 12 kV. Transmission electron microscope images were measured on a Tecnai 12 transmission electron microscope (TEM, Philips, the Netherlands), and the operating voltage was 120 kV. X-ray diffraction (XRD) patterns were measured on a D8 Advance polycrystalline X-ray diffractometer (Bruker AXS, Germany).

EXPERIMENTAL SECTION

Steady-state kinetics analysis of $CoFe_2O_4$ nanozyme. Other conditions were consistent, and the steady-state kinetics of $CoFe_2O_4$ nanozyme were detected by

adjusting TMB concentrations or H_2O_2 concentrations in the reaction system. According to the Michaelis-Menten equation, the dynamical parameters are calculated separately.

Methylene blue degradation. 20 μL of methylene blue (2 mg/mL) was dispersed in 1.76 mL of phosphate buffer solution (0.1 M, pH=7.4). Then 200 μL of CoFe_2O_4 (1 mg/mL) and 20 μL of H_2O_2 (10 M) were added to the solution. Avoid light for 6 h to reduce the interference of photodegradation of methylene blue. Finally, the absorbance of the solution at 800 nm to 500 nm was detected by UV spectrophotometer.

Preparation of CL imaging sensor arrays. The slides were soaked in a solution of sulfuric acid and hydrogen peroxide in a volume ratio of 7:3, allowed for 8 to 12 h, rinsed with water, and left to dry. Then, the slides were immersed in a 1% GPTMS/toluene solution overnight, rinsed with toluene and ethanol in turn, and dried to make epoxy-silanized slides. 48 microwells in 4*12 format were printed on glass slides by using screen printing technology and stencils. 1 mg of CoFe_2O_4 was dissolved in 1 mL of deionized water, mixed with an equal volume of 1.0 wt% chitosan solution, and shaken well. 5 μL of the well-mixed solution was dropped into the CL micropores and dried naturally.

Peroxidase-like activity of CoFe_2O_4 in the presence of different concentrations of

AA.

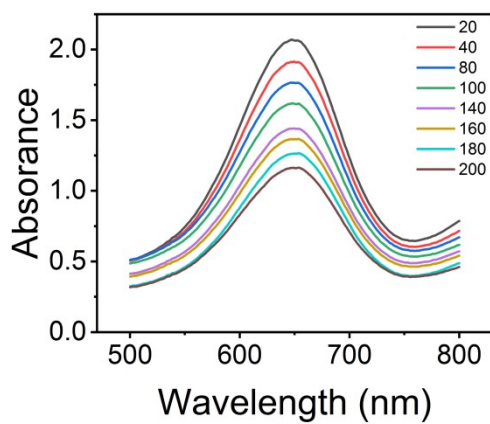


Fig. S1 UV-vis spectra of TMB+ CoFe_2O_4 + H_2O_2 +AA (20-200 μL , 2 mM)

Stability of the sensor based on CoFe₂O₄ nanozyme.

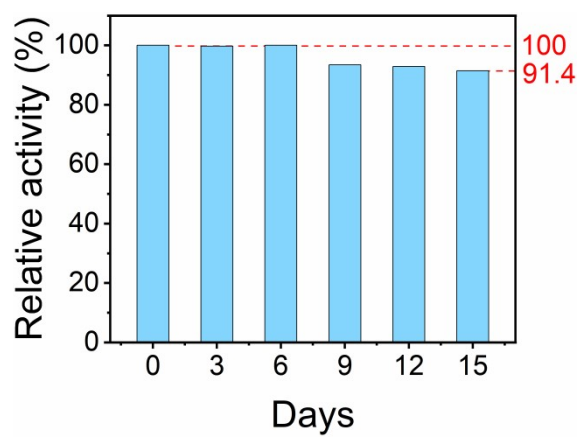


Fig. S2 Stability of the sensor based on CoFe₂O₄ nanozyme for 15 days of storage.

Table S1. Comparison of kinetic parameters of various nanozymes.

Sample	Substrate	K_m (mM)	V_m ($\times 10^{-8}$ M/s)	Ref.
HRP	TMB	0.434	10	S1
	H ₂ O ₂	3.7	8.71	
Fe ₃ O ₄ /GONRs	TMB	5.68	3.71	S2
	H ₂ O ₂	0.15	5.56	
PtFe@Fe ₃ O ₄	TMB	0.213	5.447	S3
	H ₂ O ₂	53.55	1.078	
MA-Hem/Au-Ag	TMB	2.39	1.42	S4
	H ₂ O ₂	2.71	11.4	
Fe-PCN-222	TMB	0.78	30.6	S5
	H ₂ O ₂	1.49	4.50	
CoFe ₂ O ₄	TMB	0.28	64.98	This work
	H ₂ O ₂	0.31	2.31	

Table S2. Various sensing strategies for detecting AA.

Methods	Detection range (μM)	Detection limit (μM)	Ref.
Electrochemical	220-211	28	S6
Fluorescence	350-70	200	S7
LSPR	10-200	25.78	S8
Fluorescence	50-1000	41.94	S9
CL imaging	40-160	25	This work
Colorimetric	20-200	18	This work

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