Electronic Supplementary Material

Determination of bisulfite in food by Etch-Cu-HCF nanozyme with enhanced polyphenol oxidase-like activity

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Chemicals. Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was purchased from Xilong Scientific Co., Ltd. (Shantou, China). Ferrous cyanide trihydrate ($K_4[Fe(CN)_6] \cdot 3H_2O$) and citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) were purchased at Beijing Beihua Fine Chemical Co., Ltd. (Beijing, China). 25-28% ammonia, 2,4-Dichlorophenol (2,4-DP), and 4-Aminoantipyrine (4-AP) were purchased by Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Other reagents were obtained from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). All chemicals were analytically pure grade and had not been further purified.

Instrumentation. Ultraviolet spectrophotometer (UV-8000S) from SHIMADZU (Japan) served to record the ultraviolet absorption spectra; the PHS-3C pH meter with an E-201F electrode of Chengdu Reach Analytical Control Instrument Company is used to detect the pH of solutions; X diffraction spectrometer (D8 advance) was purchased from Bru (Beijing) Technology Co., Ltd. to record XRD spectra; Fourier near-infrared spectrometer (IRPRESTIGE-21) was purchased from SHIMADZU (Japan) to record IR spectra.

Synthesis of Cu-HCF nanozyme. Copper Prussian blue analogs were synthesized by adjusting according to the literature.[S1] 0.1 mmol potassium ferricyanide monohydrate and 0.3 g citric acid monohydrate were dispersed in 20 mL of water as solution A, and 0.2 mmol copper sulfate pentahydrate was dispersed in 10 mL of water as solution B. Solution B was then added dropwise to solution A while stirring vigorously with a magnetic rotor and kept stirring for 24 h after the end of the dropwise addition. The reaction products were collected by centrifugation at 10000 rpm/min for

5 min, and washed with pure water three times, and the copper Prussian blue analogue Cu-HCF was dispersed. The Cu-HCF nanozyme was dispersed in pure water and stored in a refrigerator at 4 ℃.

Study of ammonia erosion time on the polyphenol oxidase-like activity of Etch-Cu-HCF nanozyme. In this experiment, 0.4 mL of deionized water, 0.3 mL of Tris-HCl buffer (100 mM, pH 8.5), 0.1 mL of 2,4-DP (0.1 mg/mL), and 0.1 mL of 4-AP (1 mg/mL) were added sequentially. Finally, 0.1 mL of Etch-Cu-HCF nanozyme (0.1 mg/mL) with different erosion times (0, 0.5, 1, 2, 3 h) were added to form different reaction systems. These different reaction systems were reacted at 25 °C for 1 h, and then the UV absorbance values at 510 nm were measured and recorded.

Optimization of experimental conditions for the detection of sulfite ions by Etch-Cu-HCF nanozyme. The detection of bisulfite by Etch-Cu-HCF nanozyme was divided into three steps. The pH and temperature were optimized for each step separately.

Temperature optimization for the first step. In a typical reaction, Tris-HCl (100 mM, pH 8.5, 0.3 mL), deionized water (0.37 mL), Etch-Cu-HCF nanozyme (1 mg/mL, 0.03 mL), 2,4-DP (0.05 mg/mL, 0.1 mL) and 4-AP (1 mg/mL, 0.1 mL) were added sequentially to centrifuge tubes at 30, 40, 50, 60 and 70°C incubated for 30 min. The UV absorbance values of these reaction systems at 510 nm were measured and recorded.

The reaction time optimization for the first step. In a typical reaction, Tris-HCl (100 mM, pH 8.5, 0.3 mL), deionized water (0.37 mL), Etch-Cu-HCF nanozyme (1

mg/mL, 0.03 mL), 2,4-DP (0.05 mg/mL, 0.1 mL) and 4-AP (1 mg/mL, 0.1 mL). The UV absorption values at 510 nm for the above reactions were measured and recorded at 40 °C for 10, 20, 30, 40, and 50 min.

Temperature optimization for the second reaction step. Tris-HCl (100 mM, pH 8.5, 0.3 mL), deionized water (0.37 mL), Etch-Cu-HCF nanozyme (1 mg/mL, 0.03 mL), and 2,4-DP (0.05 mg/mL, 0.1 mL) were reacted at 40°C for 30 min, followed by the addition of sodium bisulfite (250 μM, 0.1 mL) and incubated at 25, 30, 40, 50 and 60 °C for 10 min, followed by the addition of 4-AP (1 mg/mL, 0.1 mL) and incubation at room temperature for 1 h. The UV absorption values of the reaction system at 510 nm were then measured and recorded.

The reaction time optimization for the second step. Tris-HCl (100 mM, pH 8.5, 0.3 mL), deionized water (0.37 mL), Etch-Cu-HCF nanozyme (1 mg/mL, 0.03 mL), and 2,4-DP (0.05 mg/mL, 0.1 mL) were sequentially added to centrifuge tubes and kept at 40 °C for 30 min. Subsequently, sodium bisulfite (250 μM, 0.1 mL) was added and kept at 25 ℃ for 10, 20, 30, 40, and 50 min, then 4-AP (1 mg/mL, 0.1 mL) was added and kept at room temperature for 1 h. The UV absorption values of the reaction system at 510 nm were then measured and recorded.

Temperature optimization for the third step. Tris-HCl (100 mM, pH 8.5, 0.3 mL), deionized water (0.37 mL), Etch-Cu-HCF nanozyme (1 mg/mL, 0.03 mL), 2,4-DP (0.05 mg/mL, 0.1 mL) were added sequentially to the centrifuge tube and reacted at 40 ℃ for 30 min. Then sodium bisulfite (250 μM, 0.1 mL) was added and kept at 25 ℃ for 10 min, and 4-AP (1 mg/mL, 0.1 mL) was added and kept at 25, 30, 40, 50, and 60

℃ for 1 h. The UV absorbance values at 510 nm of the reaction system were then measured and recorded.

The reaction time optimization for the third step. Tris-HCl (100 mM, pH 8.5, 0.3 mL), deionized water (0.37 mL), Etch-Cu-HCF nanozyme (1 mg/mL, 0.03 mL), 2,4-DP (0.05 mg/mL, 0.1 mL) were added sequentially to the centrifuge tube and reacted at 40 ℃ for 30 min, and then Sodium bisulfite (250 μM, 0.1 mL) was added and kept at 25 ℃ for 10 min, then 4-AP (1 mg/mL, 0.1 mL) was added and kept at 25 ℃ for 40, 60, 80, 100 and 120 min, respectively. The UV absorbance values at 510 nm of the reaction system were then measured and recorded.

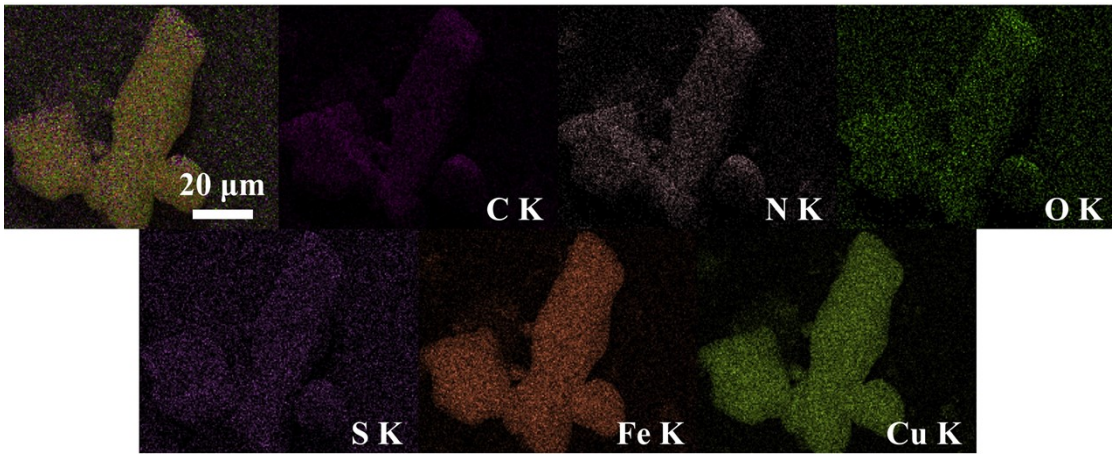


Figure S1. Elemental distribution of the Cu-HCF nanozyme.

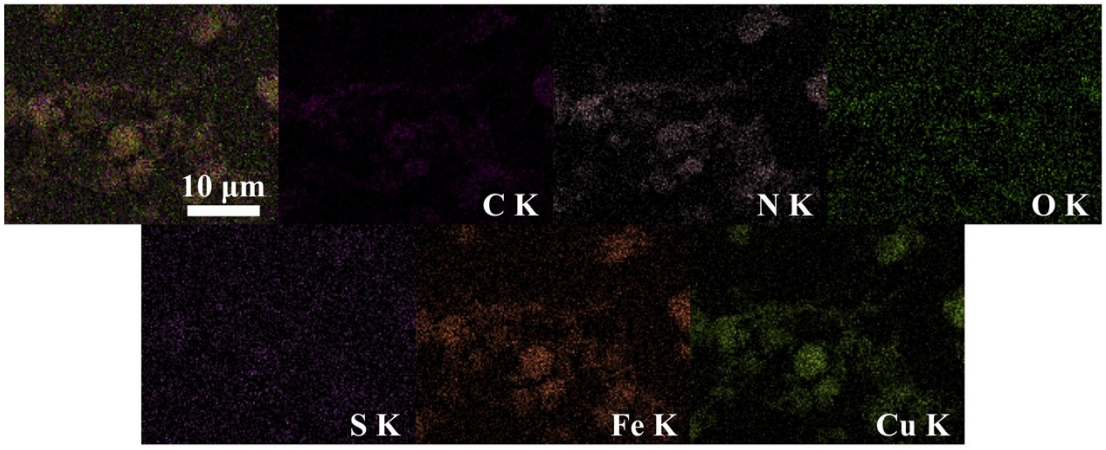


Figure S2. Elemental distribution of the Etch-Cu-HCF nanozyme.

Table S1. Results of the Elemental Content in Samples Analyzed by ICP-OES.

Elemental Content (mg/L)				
Samples	K	Cu	Fe	Cu/Fe ratio
Cu-HCF	20.6773	5.9915	2.6308	2.3
Etch-Cu-HCF	5.7309	1.2502	0.3493	3.6

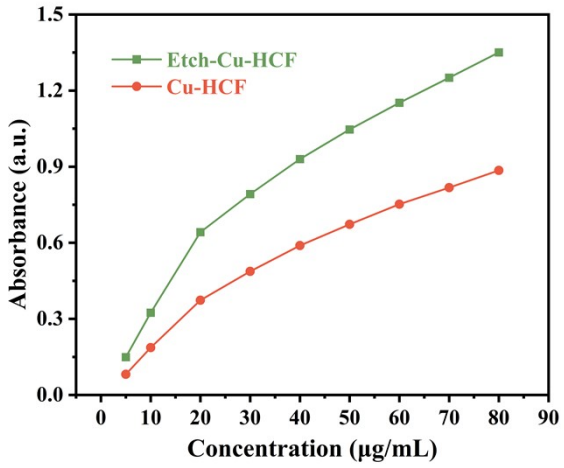


Figure S3. Comparison of the polyphenol oxidase-like activity of Etch-Cu-HCF nanozyme and Cu-HCF nanozyme by varying the catalyst concentration for the same reaction time.

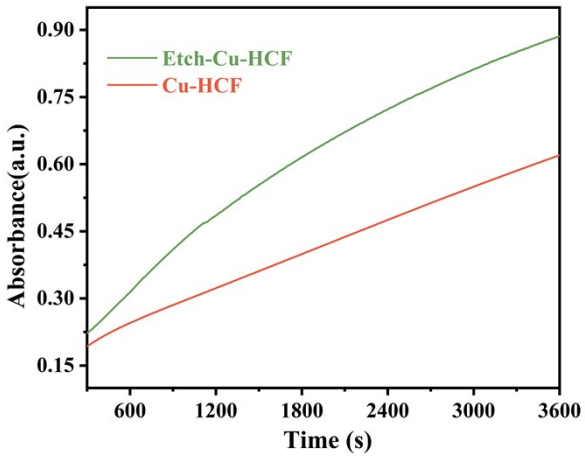


Figure S4. Comparison of the polyphenol oxidase-like activity of Etch-Cu-HCF

nanozyme and Cu-HCF nanozyme by varying the reaction time with the same catalyst concentration.

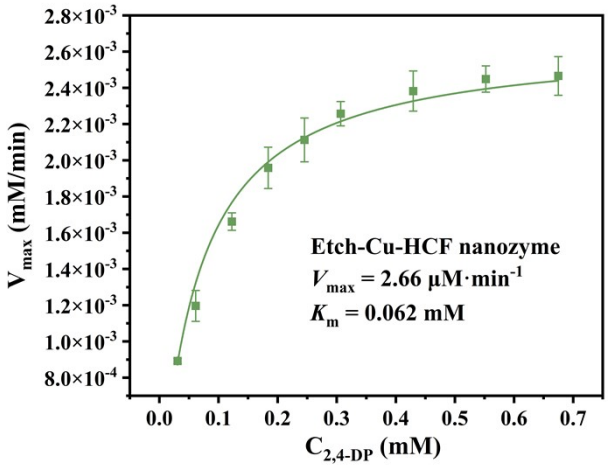
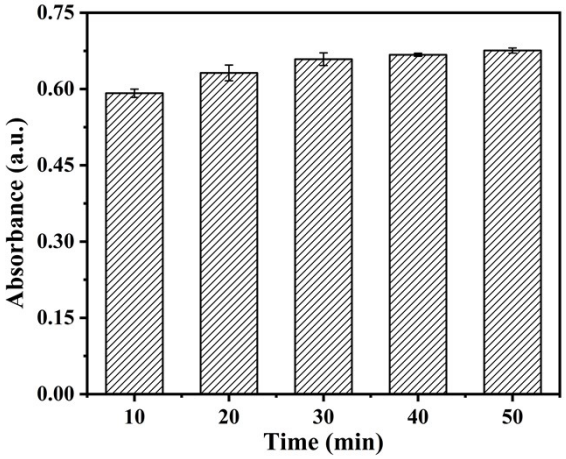


Figure S5. Reaction kinetics of Etch-Cu-HCF nanozyme.

Table S2. Comparison of kinetic parameters of polyphenol oxidase-like activity of Etch-Cu-HCF with other catalysts.

Catalysts	K_m (mM)	V_{max} (μM min^{-1})	Reference
Cu-Cys NLs	0.14	1.44	[S2]
Ce-MOF-808	0.13	2.22	[S3]
Cu FMA	0.45	3.43	[S4]
BSA-Cu	0.12	4.00	[S5]
Bpy-Cu	0.19	1.48	[S6]
CH-Cu	0.42	7.30	[S7]
Cu-HCF	0.06	1.33	[S8]
Natural PPO	0.40	2.34	[S8]
Etch-Cu-HCF	0.06	2.66	This work



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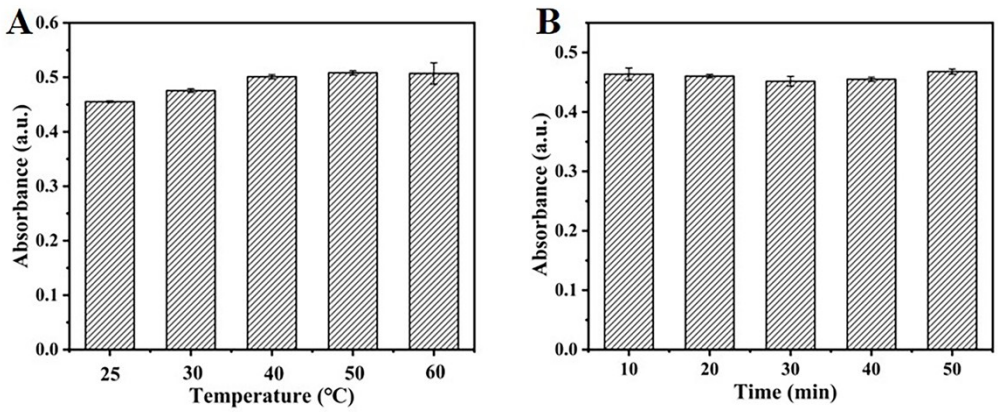
116 **Figure S6.** First step reaction time optimization for detecting bisulfite.



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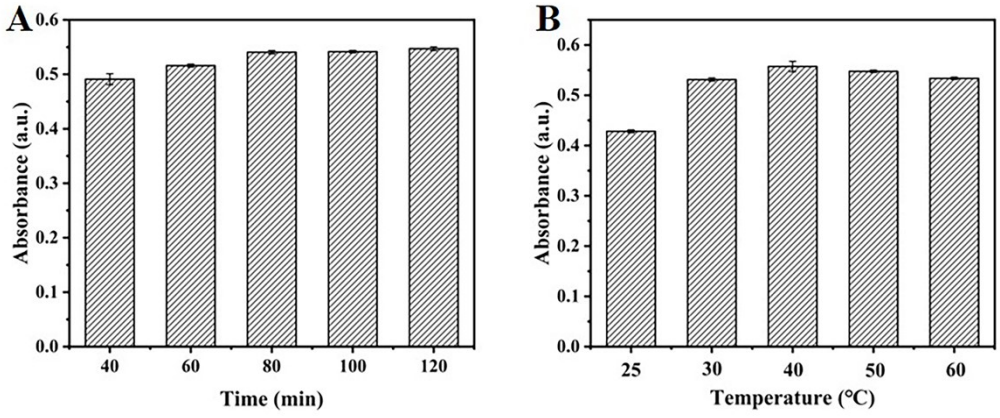
118 **Figure S7.** Optimization of the second step of the detection of bisulfite. (A)

119 Temperature. (B) Time.



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121 **Figure S8.** Optimization of the third step of the detection of bisulfite. (A) Time. (B)

122 Temperature.

Table S3. Comparison of different sensors for bisulfite.

Sensor	Analytical method	Linear range (μM)	LOD (μM)	References
CoHCF/BLPE	Electrochemistry	4-128	1.74	[S9]
Probe 1	Fluorescence	10-150	0.37	[S10]
Ir1@MSNs-NH2	Phosphorescence	10-140	0.80	[S11]
CM–BA	Spectrofluorometry	10-35	0.11	[S12]
ASHTI	Spectrofluorometry	0-60	0.27	[S13]
CyR	Spectrophotometry	10-160	11.50	[S14]
Etch-Cu-HCF	Spectrophotometry	0-50	0.47	This work
Paper sensor	Smartphone Photo	0-50	1.67	This work

Supplemental references

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