Supporting Information for

Peroxidase Activity of a Cu-Fe Bimetallic Hydrogel and Applications for Colorimetric Detection of Ascorbic Acid

Xiao-Juan Wang [a], Yan Long [a], Chuan-Wan Wei [a*], Shu-Qin Gao [b], and Ying-Wu Lin [a,b*]

[a] School of Chemistry and Chemical Engineering, University of South China, Hengyang 421001, China; [b] Key Lab of Protein Structure and Function of Universities in Hunan Province, University of South China, Hengyang 421001, China.

Corresponding authors:

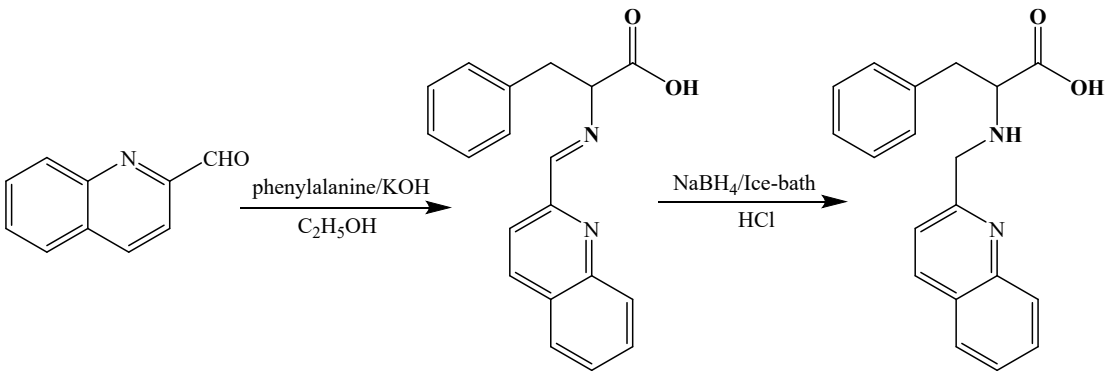
*E-mail: weichw@hotmail.com (C.-W. Wei); ywlin@usc.edu.cn (Y.-W. Lin)

Contents

L-phenylalanine (L-Phe), Quinoline-2-formaldehyde, sodium borohydride ($NaBH_4$), H_2O_2 (30%), Sodium hydroxide, acetic acid (HAc), sodium acetate (NaAc), Isopropyl alcohol (IPA), Tert-Butanol (TBA), 3,3,5,5-tetramethylbenzidine (TMB), ascorbic acid (AA), all other L-amino acids and saccharides were purchased from Aladdin Reagent (Shanghai). All chemicals were of analytical grade and used as received without further purification. Deionized water was used in all solutions and experiments.

The mass spectra were collected on G2-XS QTOF mass spectrometry. Fluorescence measurements were recorded on PerkinElmer LS 45, Hitachi fluorescence spectrometer. UV-vis absorbance curves were obtained on PerkinElmer Lambda 365. Scanning electron microscope (SEM) images and Transmission electron microscope (TEM) images were obtained on a TESCAN MIRA LMS and a FEI Titan microscope (America), respectively. The model of infrared spectrometer is Thermo Scientic Nicolet iS5 FT-IR.

The compound 2-QF was prepared following a literature procedure reported by our group,[1] and the synthetic route was shown in scheme 1. Mixing 2-QF (0.2 M, pH 9-10) and $CuSO_4$ solutions with the mole ratio of 2:1, green metallohydrogel (2-QF-Cu-G) was obtained through sonication treatment for about 10 min. Similarly, brown bimetallic hydrogel (2-QF-CuFe-G) was prepared by replacing $CuSO_4$ with the mixture of the same amount of $Fe_2(SO_4)_3$ and $CuSO_4$. The xerogels of 2-QF-Cu-G and 2-QF-CuFe-G were obtained through a facile freeze drying method. In addition, 2-QF-Cu@Fe suspension was obtained under similar conditions except for sonication treatment.



Scheme 1 The synthetic route of 2-QF.

1.4 Peroxidase-Mimetic Activity of 2-QF-CuFe-G metallohydrogels

Mixing 20 μL of 0.1 M TMB, 20 μL of 0.1 M H_2O_2 and 1940 μL of acetate buffer (pH 3.5), and 20 μL of 2-QF-CuFe-G (2 mg mL^{-1}) was added to the solution. The color change of the solution was observed and the UV-vis absorption spectra of ox-TMB were recorded. Meanwhile, the absorbances at 654 nm at different time were monitored. In addition, the contrast experiments (TMB, TMB+2-QF-Cu-G) were carried out at similar conditions.

1.5 Catalytic mechanism of 2-QF-CuFe-G metallohydrogels

Mixing 20 μL TMB (0.1 M), 20 μL H_2O_2 (0.1 M), 20 μL 2-QF-CuFe-G (2 mg/mL) and 940 μL acetate buffer (pH 3.5), and then 1 mL of 10 M •OH scavenger (isopropyl alcohol (IPA) or tert-

butyl alcohol (TBA)) was added to this solution, respectively. The colour change of the solution w as observed and the UV-Vis absorption spectrum of ox-TMB was recorded.

To further confirm the involvement of •OH radical, fluorescent spectrum of terephthalic acid (TA) in the presence of H_2O_2 and 2-QF-CuFe-G was carried out at λ_{ex} = 360 nm. 2-QF-CuFe-G (0-40 μL, 2 mg/mL) was added gradually to the mixing solution of 20 μL TA (0.1 M) + 20 μL H_2O_2 (0.1 M). The characteristic emission peak of 2-hydroxyterephthalic acid at 435 nm was detected.

1.6 Peroxidase-Mimetic Activity and Steady-state kinetic study of 2-QF-CuFe-G metallohydrogels

The absorbance value of ox-TMB was detected using the peroxidase-mimetic activity of 2-

QF-CuFe-G in the presence of H_2O_2. For this，20 μL of 0.1 M TMB , 20 μL of 0.1 M H_2O_2 and 20 μL of catalyst (2 mg/mL) were mixed with 1940 μL of acetate buffer (at pH 3.5). The quantitative activity was assessed using a UV–vis spectrophotometer where the absorbance value was collected at λ_{max} = 654 nm within 3 min.The substrate concentration, pH of the buffer, and reaction time were optimized. Furthermore, the reaction kinetics was studied using different concentrations of H_2O_2 and TMB.

The Michaelis−Menten constant was calculated using a Lineweaver−Burk plot as follows:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

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

1.7 Detection of Hydrogen Peroxide

The colorimetric detection of H_2O_2 was carried out as follows: 20 μL TMB (0.1 M) and 20 μL 2-QF-CuFe-G dispersion (2 mg/mL) were added into the acetate buffer solution (pH 3.5), after that different concentration of H_2O_2 over the range of 0-300 μM was added into the above solution and the mixing solution was incubated for 3 min at room temperature. Then, the absorbance at 654 nm was monitored by UV-vis spectroscopy. The LOD was calculated with the formula 3σ/s along with the S/N ratio of 3, where “s” denotes the slope of the linear curve and σ represents the standard deviation.

1.8 Detection of AA

20 μL H_2O_2 sample (0.1 M), 20 μL TMB (0.1 M) solution and 20 μL of 2-QF-CuFe-G dispersion (2 mg/mL) in acetate buffer solution (pH 3.5) with a final volume of 2000 μL were incubated for 3 min at room temperature, and blue color of ox-TMB was produced. And then various concentration of AA (0-100 μM) was added into the above blue color solution and the UV-vis absorbance spectrum was monitored. In a similar way, the interference experiment was performed by replacing ascorbic acid with other reducing agents such as KBr, KI, glucose, fructose, xylose, mannose, citric acid, oxalic acid and tartaric acid.

1.9 Detection of Ascorbic Acid in the Real Sample

The fresh fruits (including grape, watermelon, lemon, kiwifruit, orange) were brought from the supermarket and washed, and then 5 g of the edible part containing AA was weighed and triturated. Adding 5 mL ultrapure water to the treated fruits, insoluble substances in fruit juice were removed through centrifuging at 5000 rpm at 4℃ for 20 min. And then the juice after centrifugation was filtered through 0.45 μm polyether sulfone (PES) membrane. Adding 20 μL (1 g/mL) of different real samples to 1980 μL of blue color oxTMB solution, then UV-vis absorption spectra were recorded.

Error bars in various Figures (in the manuscript or in supporting information) represent standard deviations from three repeated expected experiments.

2. Supplementary Figures

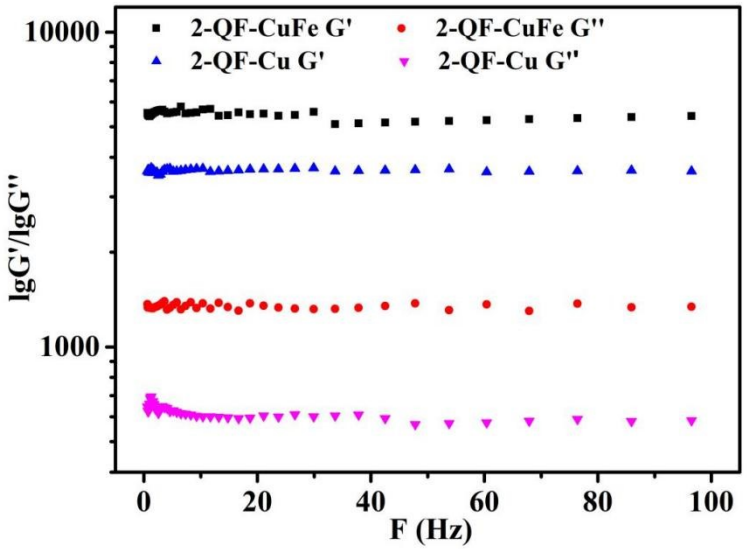


Fig. S1 The dynamic frequency scan of 2-QF-Cu-G and 2-QF-CuFe-G metallohydrogels.

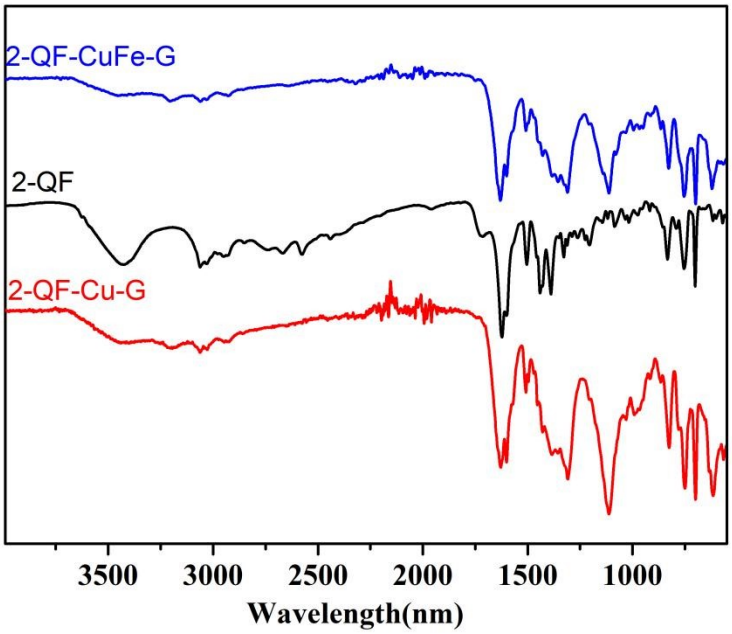


Fig. S2 The IR spectra of 2-QF powder and 2-QF-Cu-G, 2-QF-CuFe-G xerogels.

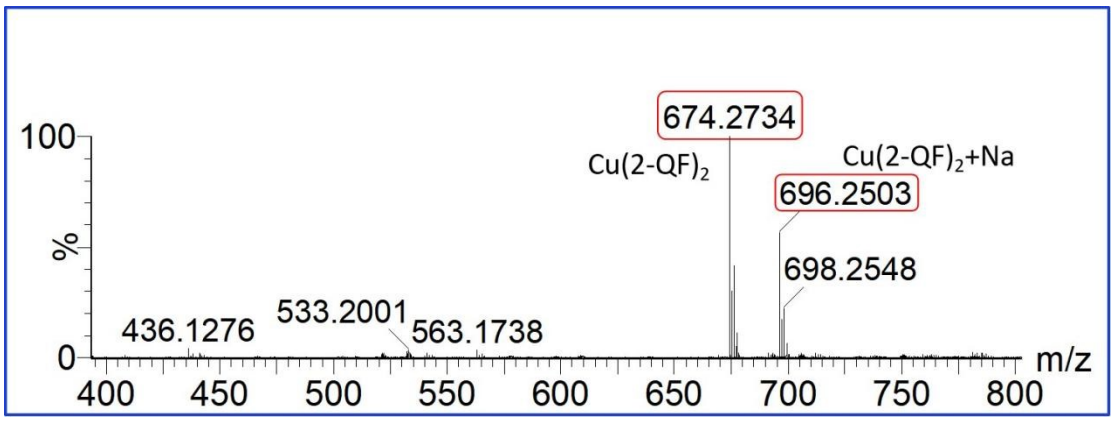


Fig. S3 ESI-MS spectra of 2-QF-Cu complex.

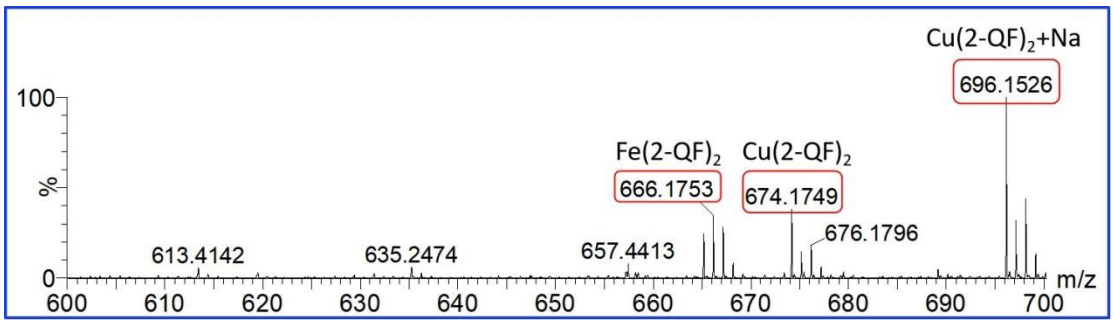


Fig. S4 ESI-MS spectra of 2-QF-Cu and 2-QF-Fe complex (diluted 2-QF-Cu-G and 2-QF-CuFe-G metallohydrogels).

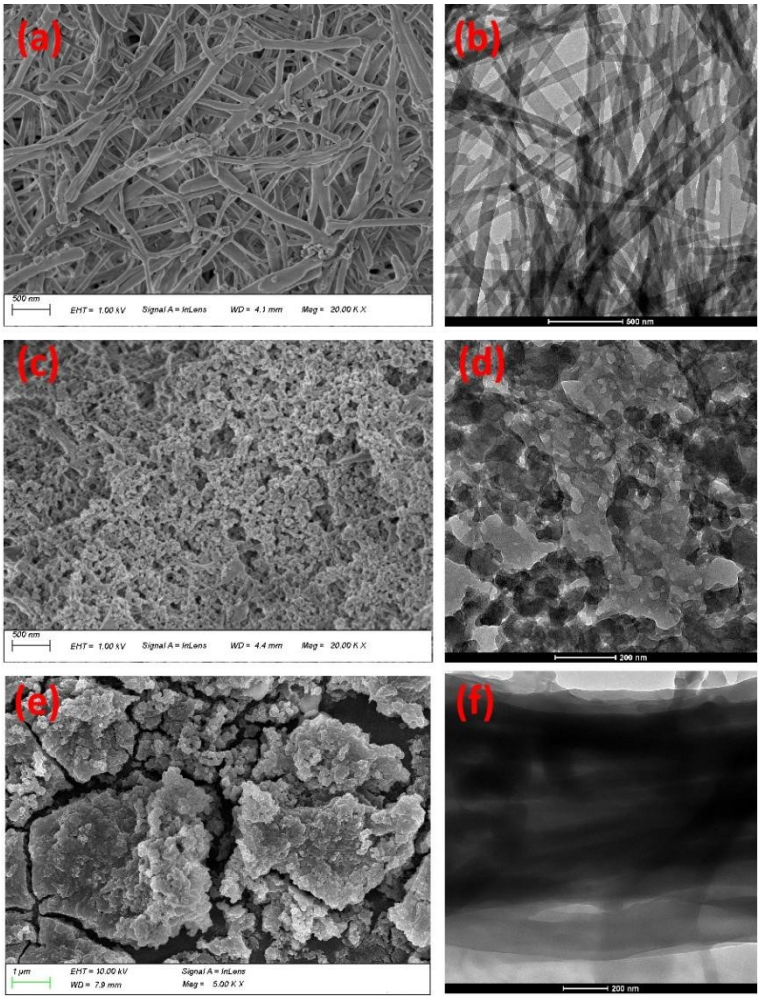


Fig. S5 SEM and TEM images of 2-QF-Cu-G (a,b), 2-QF-CuFe-G (c,d) xerogels and (e,f) the freeze-dried powder of 2-QF-Fe@Cu suspension.

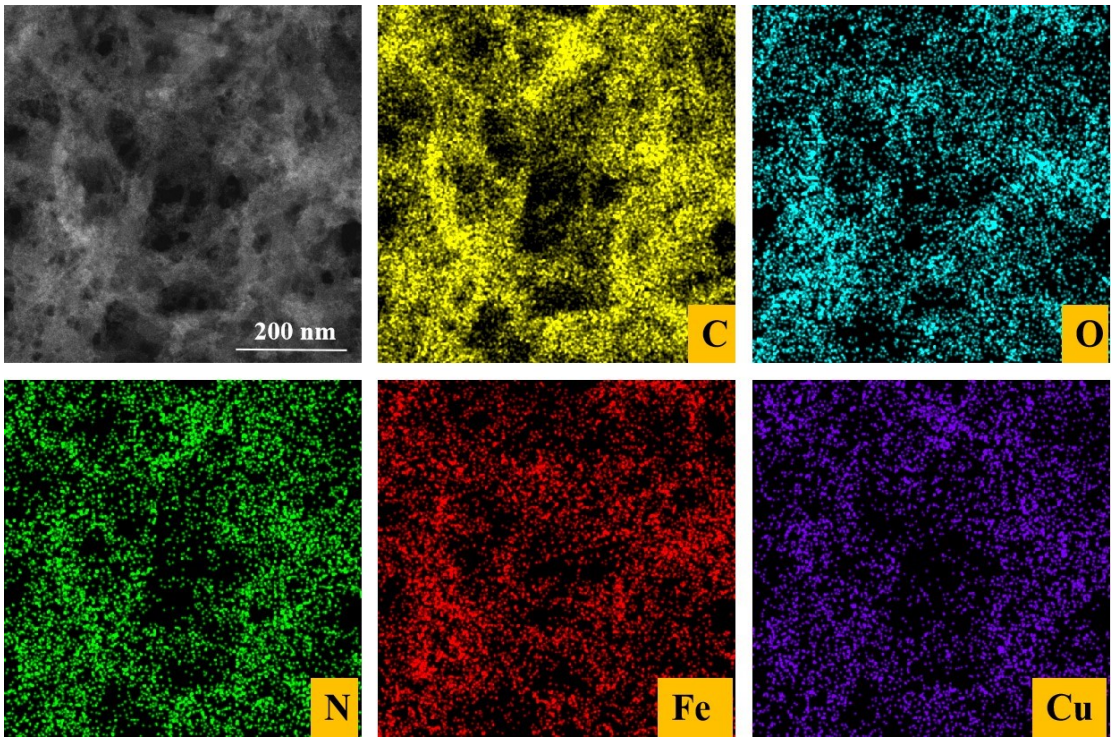


Fig. S6 Elemental mapping of C, O, N, Fe, and Cu of 2-QF-CuFe-G xerogel.

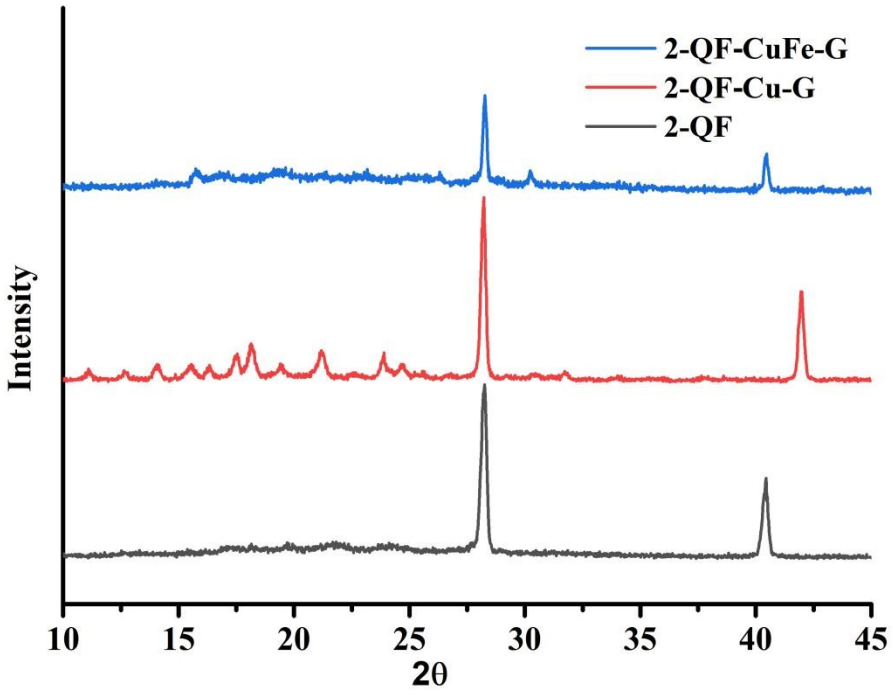


Fig. S7 XRD pattern of 2-QF powder and 2-QF-Cu-G, 2-QF-CuFe-G xerogels.

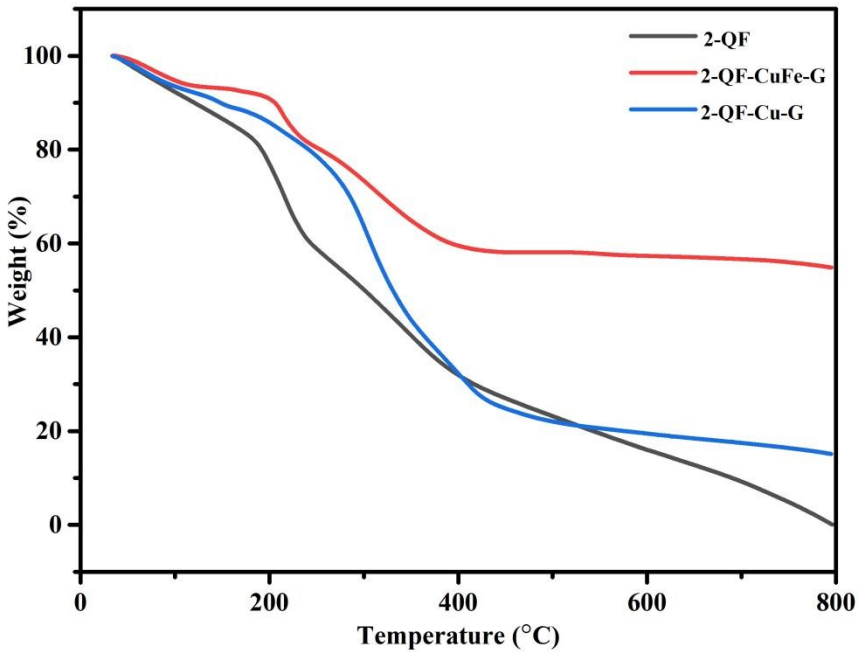
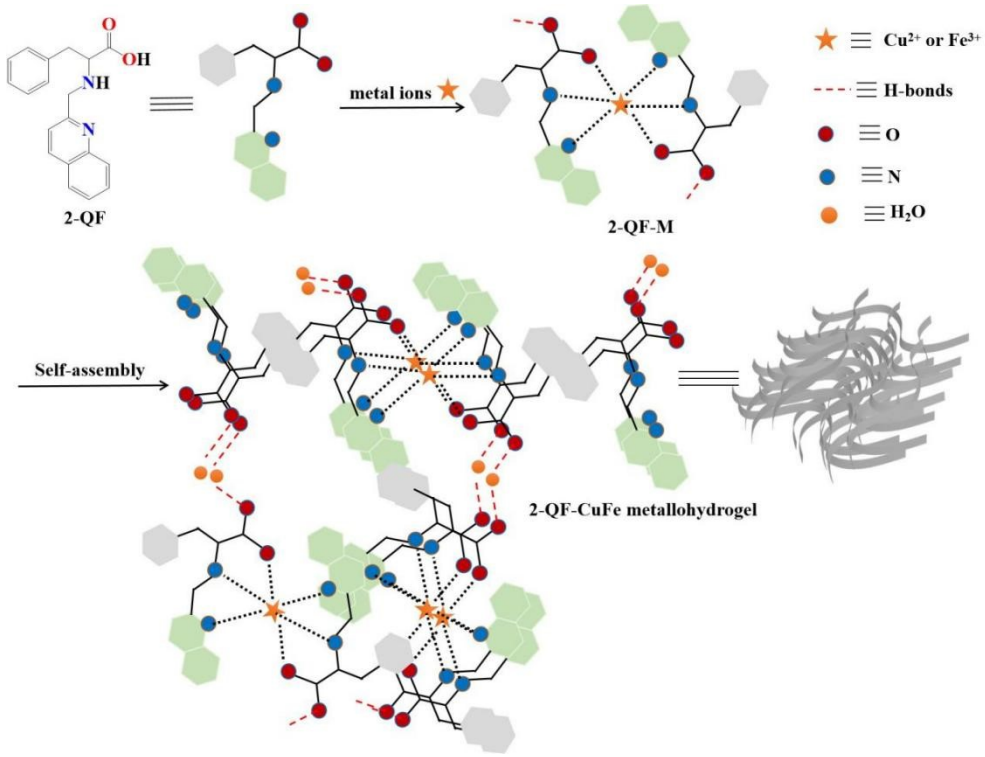


Fig. S8 Thermogravimetric analysis (TGA) thermogram of 2-QF powder and 2-QF-Cu-G and 2-QF-CuFe-G xerogels.



Scheme 2 The assembling mechanism and schematic representations of the probable formation procedure for 2-QF-CuFe metallohydrogel.

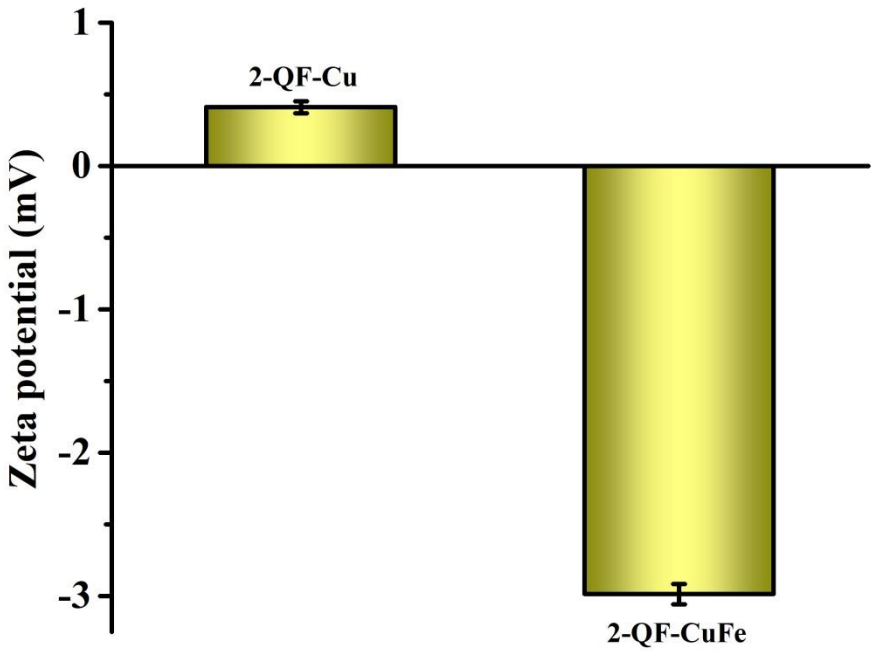


Fig. S9 Zeta potential analysis of 2-QF-Cu and 2-QF-CuFe nanoenzyme.

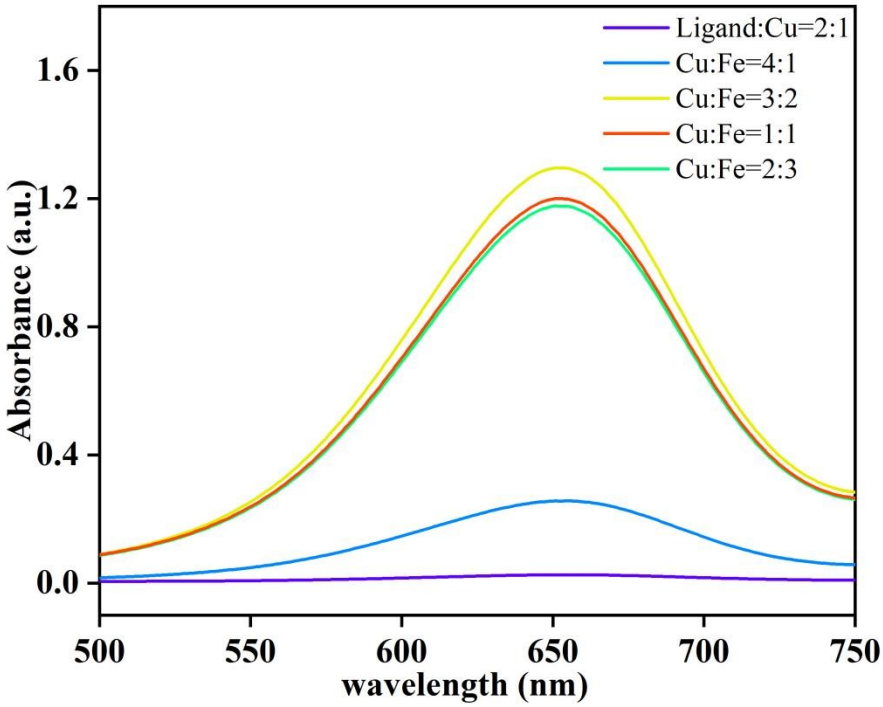


Fig. S10 The UV-vis absorbance spectra of the mixture of TMB (1 mM), H_2O_2 (1 mM), and 2-QF-CuFe-G (20 μg/mL) with different Cu/Fe mole ratios.

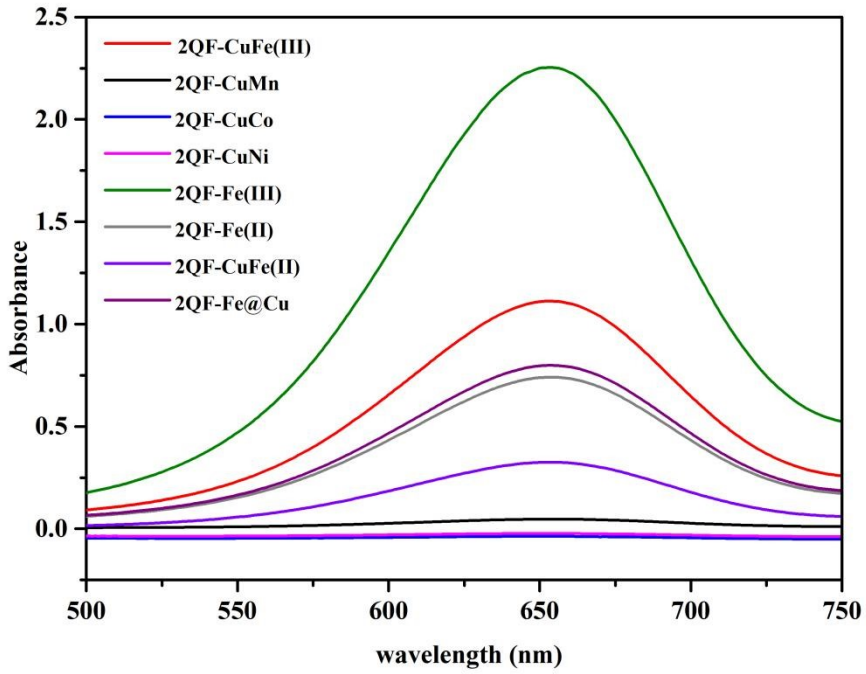


Fig. S11 The UV-vis spectra of TMB+H_2O_2+2-QF-CuFe(Ⅲ)-G, TMB+H_2O_2+2-QF-CuFe(Ⅱ), TMB+H_2O_2+2-QF-CuMn(Ⅱ), TMB+H_2O_2+2-QF-CuCo(Ⅱ), TMB+H_2O_2+2-QF-CuNi(Ⅱ), TMB+H_2O_2+2-QF-Fe(Ⅲ), TMB+H_2O_2+2-QF-Fe(Ⅱ), TMB+H_2O_2+2-QF-Cu@Fe.

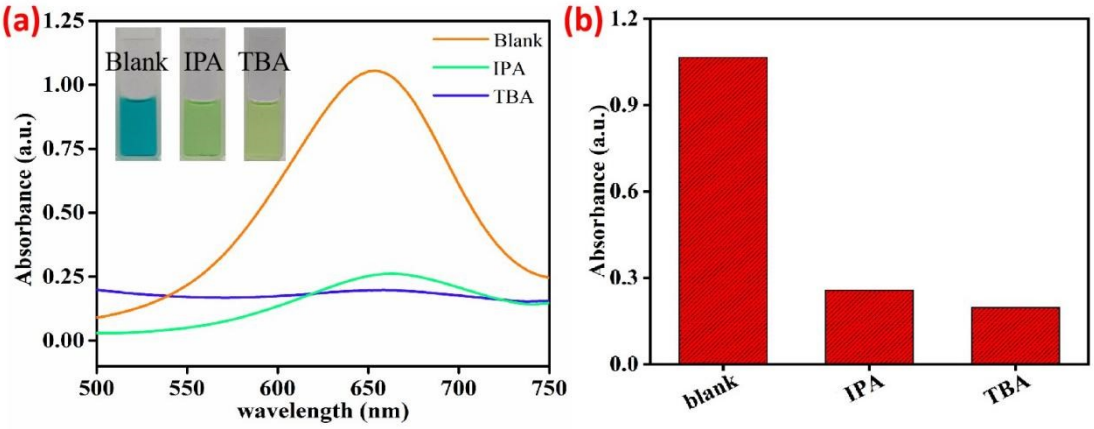


Fig. S12 The UV-vis spectra (a) and histogram (b) of TMB+H_2O_2 +2-QF-CuFe-G (Blank), Blank+IPA, Blank+TBA. The experiments were performed using 1 mM TMB, 1 mM H_2O_2 with 20 μg/mL 2-QF-CuFe-G metallohydrogel in HAc-NaAc buffer (pH=3.5). C(IPA)=C(TBA)=5 M.

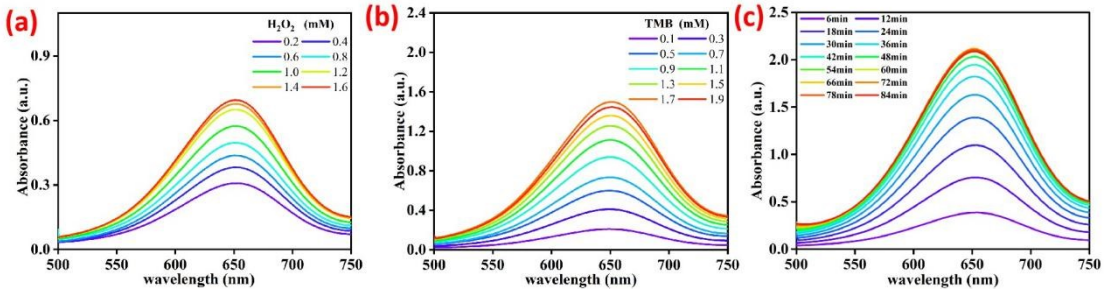


Fig. S13 The UV-vis spectra of peroxidase-like activity of 2-QF-CuFe-G with different (a) H_2O_2 concentrations, (b) TMB concentrations, (c) time. The experiments were performed using 20 μg/mL 2-QF-CuFe-G metallohydrogel in HAc-NaAc buffer (pH=3.5).

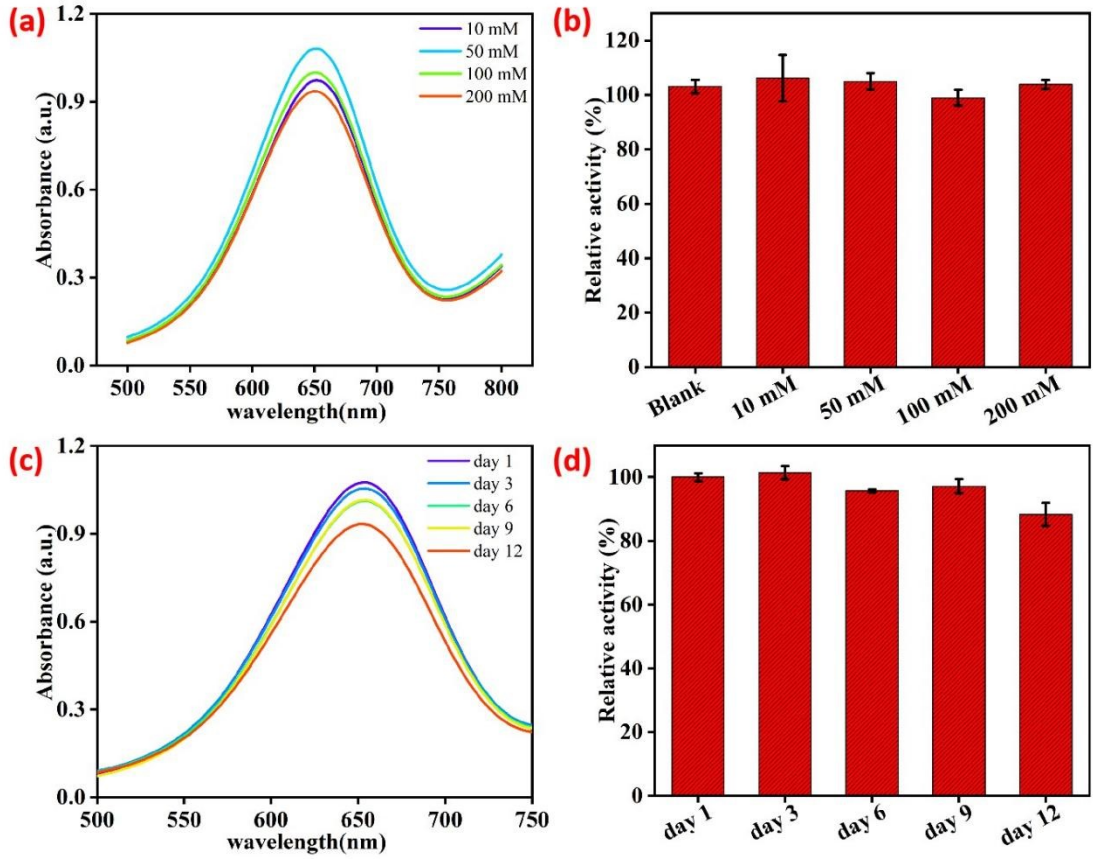
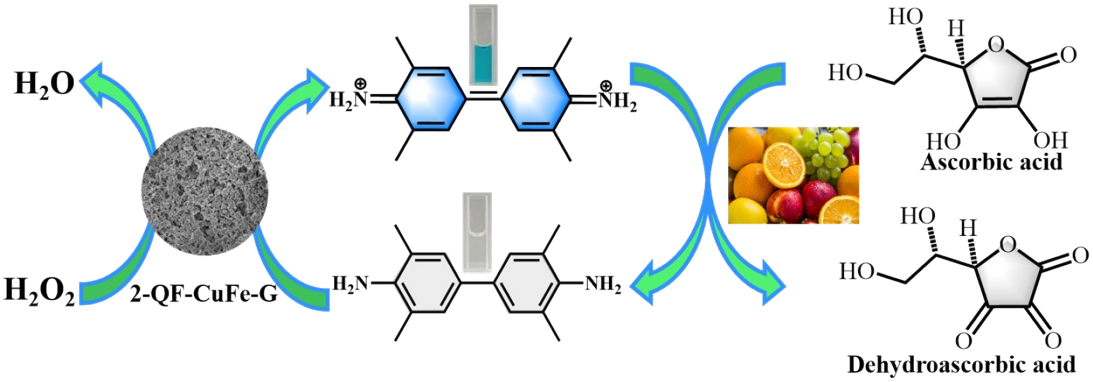


Fig. S14 Catalytic stability of 2-QF-CuFe-G metallohydrogel with different (a) (b) NaCl concentrations, (c) (d) storage time.



Scheme 3 Schematic representation of the colorimetric detection of ascorbic acid using 2-QF-CuFe-G metallohydrogel.

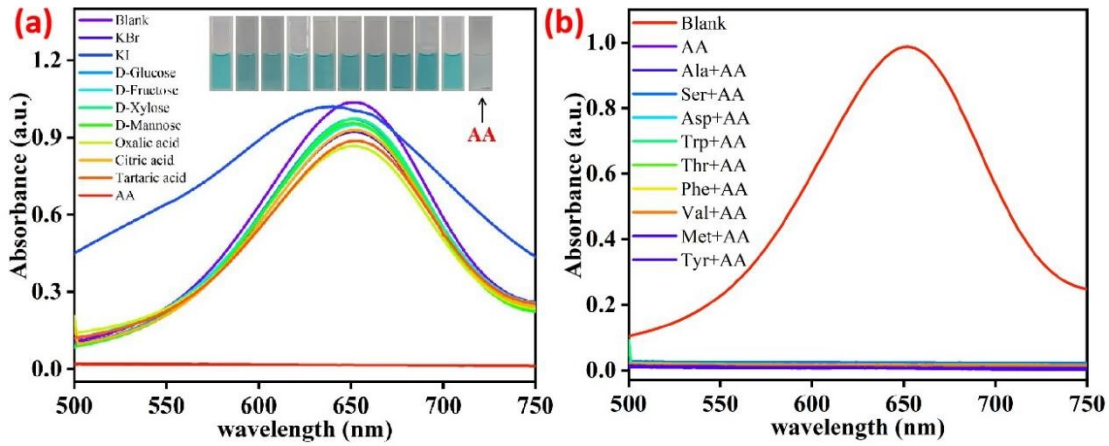


Fig. S15 UV-vis spectra of ox-TMB in the presence of (a) various reducing agents, (b) AA and AA+various amino acids. ([TMB] =1 mM, [H_2O_2] =1 mM, [Cat] = 20 μg/mL, NaAc-HAc buffer pH =3.5, [AA] = 50 μM, [other reducing agents] = 500 μM, [other amino acids] = 500 μM)

References

1. H.-X. Wang, C.-W. Wei, X.-J. Wang, H.-F. Xiang, X.-Z. Yang, G.-L. Wu and Y.-W. Lin, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2021, **250**, 119378.